

Heat loss from colonies of bumblebees: mechanisms and consequences

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## Thesis Abstract

Pollinating insects provide ecosystem services worth billions of dollars globally and the pollination of crop plants is vital for food security. Declines in pollinating insects have been associated with a variety of anthropogenic drivers of change. With a global population expected to reach nine billion by 2050 a better understanding of the relationship between agroecosystems and pollinators is required to maintain food security. Bumblebees are important pollinators that utilise efficient thermoregulation to develop their colonies. Heat loss by convection, conduction and radiation are potential obstacles to thermoregulation. Insulation limits heat loss and so behaviours of bumblebees that mitigate heat loss could have high adaptive significance.

This thesis aimed to identify the relative importance of convection, conduction and radiation as mechanisms of heat loss from bumblebee colonies. Secondly, model the impacts of shortfalls in incubation on colony fecundity.

Microcolonies of six orphaned worker bumblebees were used to the effect of different types of insulation of thermoregulation. Sugar consumption and average brood temperature were measured in order to determine the effects of different types of insulation, which allows insight into the mechanisms of heat loss in bumblebee nests. Developmental and demographic models were used to investigate the relationship between brood temperature on developmental time and colony fecundity in bumblebees.

The results presented here show that heat loss by radiation is likely to be a small obstacle to thermoregulation in bumblebee nests. In contrast, insulation to reduce heat loss by convection and conduction resulted in higher average brood temperatures with no difference in syrup consumption compared to uninsulated boxes. Modelling revealed that queen production is highly sensitive to relatively small drops in average brood temperature. For example, a one degree reduction in average brood temperature from approximately 27.2°C to 26.2°C could result in an 11.4% reduction in queen production.

The impact of nest thermoregulation on queen production highlights the adaptive significance of nesting behaviours, both the initial nest site choice by the queen and insulation behaviours of workers. The results of the model on the effect of temperature on colony fecundity demonstrate the mechanism by which shortfalls in incubation could reduce colony fecundity and result in declines in bumblebee populations.

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## Equation List

**Eq. 1. –**

$$\text{Heat Loss} = P_{CV} + P_{CD} + P_R$$

Mechanisms of heat loss. Page 24.

**Eq. 2. –**

$$I = P_{CV} + P_{CD} + P_R$$

Incubation effort is equal to heat loss in a nest at stable temperature. Pages 24, 29.

**Eq. 3. –**

$$J^* = \epsilon \sigma T^4$$

The Stefan-Boltzman equation of radiant heat emitted from a surface. Page 25.

**Eq. 4. –**

$$qx \propto -\lambda \frac{dt}{dx}$$

Fourier's law of convective heat transfer. Page 27.

**Eq. 5.**

$$J^* = \sigma \epsilon A(T_1^4 - T_2^4)$$

Stefan-Boltzman model of heat exchange between two bodies. 34.

**Eq. 6. –**

$$P = \frac{V^2}{R}$$

Ohms law power law. Power dissipated across a resistor as a function of potential difference and resistance. Page 35.

**Eq. 7. –**

$$r = aT(T - T_1)(T - T_2)^{1/2}$$

Briere model of insect in the form presented in Shi et al. (2016). Page 56.

**Eq. 8. –**

$$\text{Fecundity Detriment} = 100 \times \left(1 - \frac{r}{r_{max}}\right)$$

Developmental rate effect on fecundity detriment. Page 59.

# Chapter 1: The Relevance of Heat Transfer in Bumblebee Nests

## Introduction

Bumblebees have been studied by naturalists for centuries and the nature of their life cycle and societal organisation has intrigued and inspired generations. Today bumblebees are also appreciated for the value of the pollination services they provide to both natural and agricultural ecosystems. Bumblebees are generalist pollinators that form connections with a diverse range of plant species, and therefore have an important role in maintaining floral networks (Memmott, Waser and Price, 2004). Declines in bumblebees, as well as other insect taxa, are well documented (Vogel, 2017). Research in recent years has identified several drivers of bumblebee declines; pesticides, parasites and loss of floral resources being identified as key factors (Goulson *et al.*, 2015).

Bumblebees evolved in the Palearctic approximately 30 MYA (Hines, 2008), adapting to life in boreal climates and survival in the tundra ecosystems of the North America. Since then they have expanded their ranges and diversified. There are now approximately 250 species of bumblebee stretching in distribution from within the Arctic Circle (*Bombus polaris*) (Richards, 1973) to the straits of Magdalen at the tip of South America (*Bombus dahlbomii*). Within such a large latitudinal distribution, bumblebees inhabit a wide variety of ecosystems, experiencing a broad range of climatic conditions.

Generally speaking the colony growth period for bumblebees begins in early spring and ends in late summer or autumn. For most temperate species, this period will be around six months. Bumblebees are for the most part eusocial, colony forming insects and at peak size, a bumblebee colony can contain anywhere between 40 and 2000 workers, depending on the species. Bumblebees are able to generate their own metabolic heat in their flight muscles (Surholt *et al.*, 1990), which is an important aspect of their physiology, since it allows them to forage at low ambient temperatures. Additionally, their ability to generate heat allows bumblebees to socially control the temperature of their brood through incubation (Gardner, Foster and O'Donnell, 2007). This is an important aspect of the ecophysiology of bumblebees which has been extensively studied. However, further work is still required to better understand growth and survival in bumblebee colonies.

## The Colony Cycle

Bumblebee colonies occur, for the most part, annually. New colonies are established in spring when a queen, who mated at the end of the previous summer, emerges from hibernation (Vogt *et al.*, 1994). She has spent the winter in a state of diapause and now, sensing the warmer weather, has awoken. During the first few weeks the queen will search for a nest site. Although nesting preferences vary between species, this first decision of the queen will influence the colony for the entirety of the season. Once the new queen has selected her nest site, she will gather nectar and pollen. She will form a brood clump of wax secreted from between her abdominal plates and a layer of pollen, onto this she lays her first brood of worker eggs (Heinrich, 1979c). During this initial phase, she must balance her time between foraging and caring for the brood, whereby, during the day, she will leave the nest to forage, building a store of nectar and pollen that she will feed on, while she incubates her brood through the night. After a few weeks this first brood of workers will emerge and begin carrying out tasks such as foraging, incubating and brood care (Woodard *et al.*, 2013). As the colony grows in size, the queen continues to lay with increasing frequency and no longer leaves the nest to forage. Towards the end of the summer, the queen begins to lay diploid eggs that can develop into gynes (unfertilised queens) and haploid eggs that develop into drones (males), once the founding queen has begun to lay haploid eggs she cannot revert to laying diploid eggs (Duchateau and Velthuis, 1988). The drones will leave the colony soon after emerging to search for a mate. Gynes linger in the colony longer to feed and develop fat stores, then also leave to find a mate (Votavová *et al.*, 2015). Once mated, the fertilised gynes must search for a safe place to hibernate through the winter, before emerging the following spring to establish their colonies. The remaining workers may survive a little longer after the founding queen dies, coming out to forage on warm winter days however they will not survive through to the next summer.

The reproductive success of a bumblebee colony is measured by the number of new queens (gynes) and males (drones) that are produced. Worker bees are also able to lay eggs that develop into males, which has been observed to happen both before and after the queen's death (Owen and Plowright, 1982). The sex ratio in bumblebees appears to be generally male-biased and this may be in part due to the males contributed by egg-laying workers (Owen and Plowright, 1982). Colony size can vary between colonies of the same species and smaller colonies may only produce males or may produce no new reproductives at all (Owen, Rodd and Plowright, 1980). More research is required to understand what factors influence the bumblebee colony cycle and how they affect fecundity.



## The Economic Value of Bumblebees and their Place in Modern Agricultural Landscapes

As keystone pollinators, bumblebees provide valuable ecosystem services. There have been substantial declines in insect biomass in the past 27 years, even in protected areas (Hallmann *et al.*, 2017). Global declines in pollinating insect communities are of increasing concern. The value of pollination services to crop plants by insects globally has been estimated at €150 billion and insect pollinated crops are worth five times more per ton than non-pollinated crops (Gallai *et al.*, 2009). In order to develop more sustainable agricultural practises a better understanding of the factors influencing bumblebee populations is required.

As of 2017, the total utilised agricultural area of the UK was 17.5 million hectares (*Farming Statistics Provisional crop areas , yields and livestock populations At June 2014 - United Kingdom*, 2018). Taking the total area of the UK to be approximately 24.25 million hectares would mean that over 70 % of the UK is agricultural land. The intensification of agriculture in the twentieth century has correlated with an increase in the rate of pollinator extinctions, peaking at 3.4 species per decade between the late 1920s and late 1950s (Ollerton *et al.*, 2014). The potential drivers for these extinctions include changes to farming processes, including increased use of crop protection products, reductions in wild flower abundance and the introduction of parasites and disease. Although the rate of species loss has slowed, worrying declines in insect biomass persist.

Key factors associated with insects declines include:

- i. Climate

A wide range of taxonomic groups are experiencing range shifts due to climate change (Hickling *et al.*, 2006). In some instances this has led to losses of species, since their climate envelope no longer overlaps with appropriate habitats. A study of Irish bumblebees found that the majority of rare or declining species were either late emerging or specialist feeders and that in general, bumblebees had experienced a westward range shift, likely due to land use change (Fitzpatrick *et al.*, 2007). This westward range shift is atypical of the poleward range shifts in many taxa that have been associated with recent climate change (Chen *et al.*, 2011). Models have demonstrated the potential for changes in climate and land use to drive changes in bumblebee distributions in the future (Marshall *et al.*, 2018). This is interesting as it appears a warming climate is not yet directly driving changes in bumblebee distributions in the UK.

The impact of climate change on bumblebee abundance and distributions is unclear. Although in the UK there have been no detected shifts in latitudinal distribution, studies of alpine bumblebees in Switzerland have suggested an uphill shift. Studies of *Bombus alpinus* used

habitat assessments to quantify how distributions of suitable habitat have changed since 1984, finding a 479 m uphill shift in the distribution of habitats (Biella *et al.*, 2017). *B. alpinus* is a cold adapted species and therefore, potentially more susceptible to poleward or altitudinal range shifts than the temperate species found in the UK. Broader impacts of climate change on ecosystems could have more subtle effects on bee health and abundance in the UK. Research conducted in the USA has identified the impact of long term changes in floral resource phenology on bumblebee abundance. The study found that over a period of eight years, changes in floral resource phenology, driven by climate change, was a key factor determining inter annual bee abundance (Ogilvie *et al.*, 2017). Changes in climate may have the potential to directly influence bumblebee population distributions in the future, so far warming temperature have only had indirect effects on bumblebee populations.

## ii. Farming

Modern agricultural practices have changed the landscape of the UK dramatically since the industrial revolution. With over 70 % of the United Kingdom having been converted to agriculture, the potential for agroecosystems to influence bee populations is high. Farming practices have developed, with increases in agricultural intensity required to meet the calorific demands of an expanding population. This has impacted pollinators in a variety of ways, but in general agricultural environments do not support successful bumblebee colonies compared to more urbanised areas (Samuelson *et al.*, 2018). The main reason for this may be that agricultural landscapes tend to have poor floral resources for bumblebees. A good bumblebee habitat must provide nectar and pollen resources from early spring to late summer in order to support successful colonies. Stewardship schemes have encouraged the planting of nectar and pollen rich wild flower mixtures at field margins in an effort to provide longer term floral resources (Carvell *et al.*, 2004). Nectar availability in the UK varies considerably through the year, blooms in May and July provide the highest nectar availability and there are periods in during March and late summer (August/September) where nectar availability falls considerably below the estimated daily sugar requirement of bumblebee colonies in the same landscape (Timberlake, Vaughan and Memmott, 2019). Other studies in the UK have shown that field margins increase bumblebee abundance in July and August when sown specifically with nectar and pollen rich flowers (Pywell *et al.*, 2006). There is probably variability in the timing of blooms year on year depending on climate patterns, however, it is clear that provision of floral resources by field margin planting does not provide a consistent supply of nectar. Some crop plants provide nectar resources for pollinating insects, but these mass flowering crops provide a short burst of high nectar availability. Studies of *Bombus terrestris* colonies placed in agricultural landscapes with mass flowering oil seed rape noted that although early colony growth was increased, there was no increase in the amount of sexual offspring produced

(Westphal, Steffan-Dewenter and Tschardt, 2009). Bumblebees do not store large amounts of nectar in the way honey bees do, so these bursts of high nectar availability do not provide for the colony for the rest of the season. Nectar resources must be spatially and temporally accessible within an agricultural landscape to facilitate successful pollinator communities.

### iii. Pesticides

The impact of climate change and anthropogenic activity on bumblebee populations globally remains an area of active research. It is clear that the additive effect of multiple stressors have resulted in bee declines in the past and will continue to do so in the future (Goulson *et al.*, 2015). What remains to be understood is the precise nature of these interactions. Pesticides or crop protection products are applied to crops in various ways. Insecticide pesticides aim to kill pest insects but their presence in the environment can affect non-target insects such as pollinators, this has been an area of significant research in recent years and the potential of these compounds to cause declines in wild populations remains an area of active research.

Neonicotinoid pesticides have been implicated in the decline of bees in the UK (Goulson *et al.*, 2015). Studies have demonstrated that exposure to neonicotinoids can suppress brood production in small groups of orphaned workers (Laycock *et al.*, 2012). Laycock *et al.* (2012) also found that ovary development in egg-laying workers was only affected at the highest dose, demonstrating a mechanism by which exposure to neonicotinoid pesticides could reduce fecundity. Reductions in brood production at lower doses may then have been driven by behavioural effects instead of direct physiological changes in the ovaries. Further studies on the effect of neonicotinoid exposure on queenright colonies (bumblebee colonies where the queen is alive) demonstrated that exposure to field-realistic concentrations of neonicotinoids reduced queen production significantly (Whitehorn *et al.*, 2012). Although these studies present evidence that neonicotinoids have the potential to cause declines in bee populations, there are studies that fail to demonstrate such strong effects. A study of low level, field realistic chronic exposure on learning behaviours and fecundity in microcolonies of *B. terrestris* failed to identify any significant effect of both pesticide exposure and infection by parasite on their test responses (Piiroinen *et al.*, 2016). The full impact of neonicotinoids on bumblebee populations under field conditions remains somewhat uncertain. Importantly, the interaction between factors such as pesticide application and floral resource density on pollinator colony dynamics is unknown. However as has been discussed, it is clear that agricultural environments remain relatively inhospitable to bumblebee populations.

## The Effect of Temperature on Insect Development

Rearing temperature is a key variable that can affect the rate of development in insects (Wigglesworth, 1939). At a biochemical level, temperature influences membrane permeability and the kinetics of enzymatic reactions that are crucial for growth and development. These principles can be used to inform biophysical models of insect development (Sharpe and DeMichele, 1977). As temperature increases, the rate of collisions between enzyme and substrate increases, which increases the rate of biochemical reactions within certain bounds. Most non-linear models of insect development describe a maximum and minimum developmental temperature, where, outside of these boundaries, development ceases (Lamb, 1992). As temperature approaches the maximum and minimum boundaries, the change in development rate is non-linear, which is due to physiology and not to genetic variability (Lamb, 1992). Linear degree day models can be used to predict the emergence of some insect species based on soil temperature data (Gage and Haynes, 1975). However, this approach cannot be applied to bumblebees since they are able to regulate their nest temperature relatively independent of the ambient temperature.

There are several intermediate morphological stages in the development of bumblebees; eggs develop into larvae before pupation, which is followed by the emergence of adults. Bumblebees exhibit caste size dimorphism, whereby queens are generally larger than workers and males (Plowright and Jay, 1977). When reared at a constant temperature, larvae destined to become new queens (gynes) grow for longer in the larval and pupa stages before emergence (Cnaani, Schmid-Hempel and Schmidt, 2002). A study of larval and pupal development in several species of bumblebees characterised 20 distinct stages of development using eye and cuticle pigmentation patterns (Tian and Hines, 2018). Additionally, they reared bumblebees in two constant temperature treatments, 32 °C and 29 °C and found that development time in all castes was increased at the lower temperature, even more so in the workers caste, where average development time across small, medium and large workers was approximately 14% longer at 29 °C. Although this study does not present a full temperature versus growth curve, the findings illustrate the potential for shortfalls in incubation resulting in a decrease in brood temperature, to cause significant delays in growth and development.

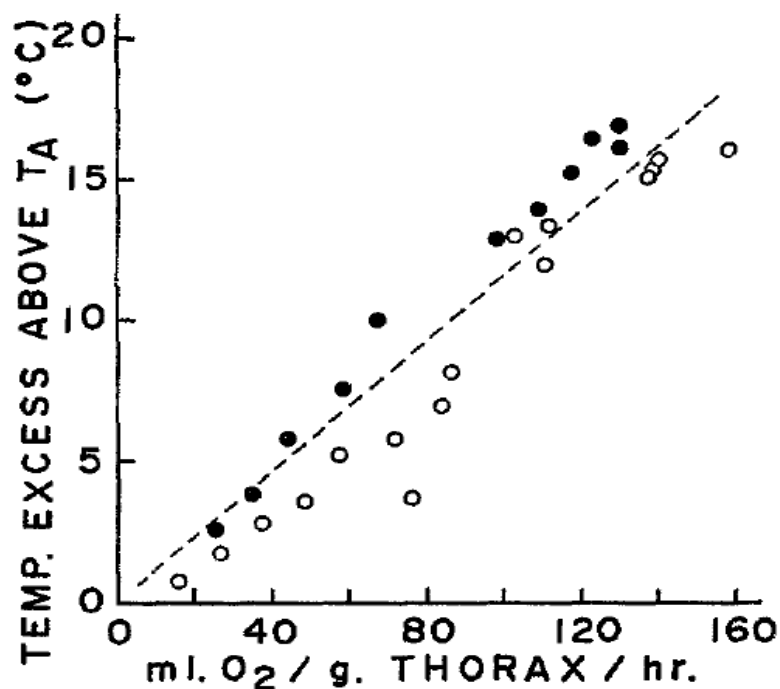
## Thermoregulation of the Brood in Bumblebee Nests

The temperature of brood in bumblebee nests can be actively regulated via two main mechanisms: non-flight thermogenesis (NFT) and fanning. During NFT, heat is generated by the stimulation of the dorsolateral and dorsoventral flight muscles within the thorax (Esch, Goller and Heinrich, 1991). The flight muscles are mechanically uncoupled from the wings during this process, so that heat can be generated by muscle contractions without the wings beating. During NFT, the stimulation of the muscles is distinctly different to that during flight. During flight, stimulation of the opposing sets of flight muscles is myogenic, the contraction of one muscle group stretches the other causing it to contract (Ikeda and Boettiger, 1965). However, during NFT, flight muscle contractions are neurogenic and action potentials directly correlate with activation of the flight muscles and an increase in body temperature (Heinrich and Kammer, 1973). The rate of nervous stimulation during NFT is at least 40 Hz (Mulloney, 1970). At this rate of stimulation, the muscles will be held in state of almost complete tetanus. NFT is used to generate heat for both pre-flight warm up and brood incubation (Esch, Goller and Heinrich, 1991). Warm haemolymph from the thorax is directed into the abdomen which the incubating bee presses against the brood. The ventral surface of the bumblebee abdomen is largely hairless, which allows for efficient transfer of heat to the brood (Heinrich, 1976).

The metabolic cost of NFT is demonstrated by observations of incubating *Bombus vosnesenskii* queens (Heinrich, 1974). The metabolic rate of the incubating queens has a negative correlation with ambient temperature, so that as ambient temperature increases, their metabolic rate decreases. A plausible explanation of this is that less additional heat energy is required to maintain the brood at an optimum temperature when ambient temperatures are warmer. Importantly **Fig. 1.** shows that an increase in metabolic rate associated with incubation, correlated with an increase in the brood temperature, independent of fluctuations in ambient temperature. What remains unclear is whether the metabolic rate of a whole colony follows the same trend as an individual incubating queen, and to what extent the addition of worker bees influences the efficiency of incubation.

In colonies of *B. polaris*, queens lay a very large first brood of up to 16 workers and once these emerge, nest temperature is controlled near 35 °C with little variation. However, prior to the emergence of workers, the brood temperature periodically cools as the queen leaves the nest to forage (Richards, 1973). The homeostatic control of brood temperature observed in bumblebees may be a result of variable response threshold amongst the worker bees. The principle of variable response threshold is based on the idea that amongst a population of insects, some will be more sensitive to a stimulus and some will be less sensitive. These

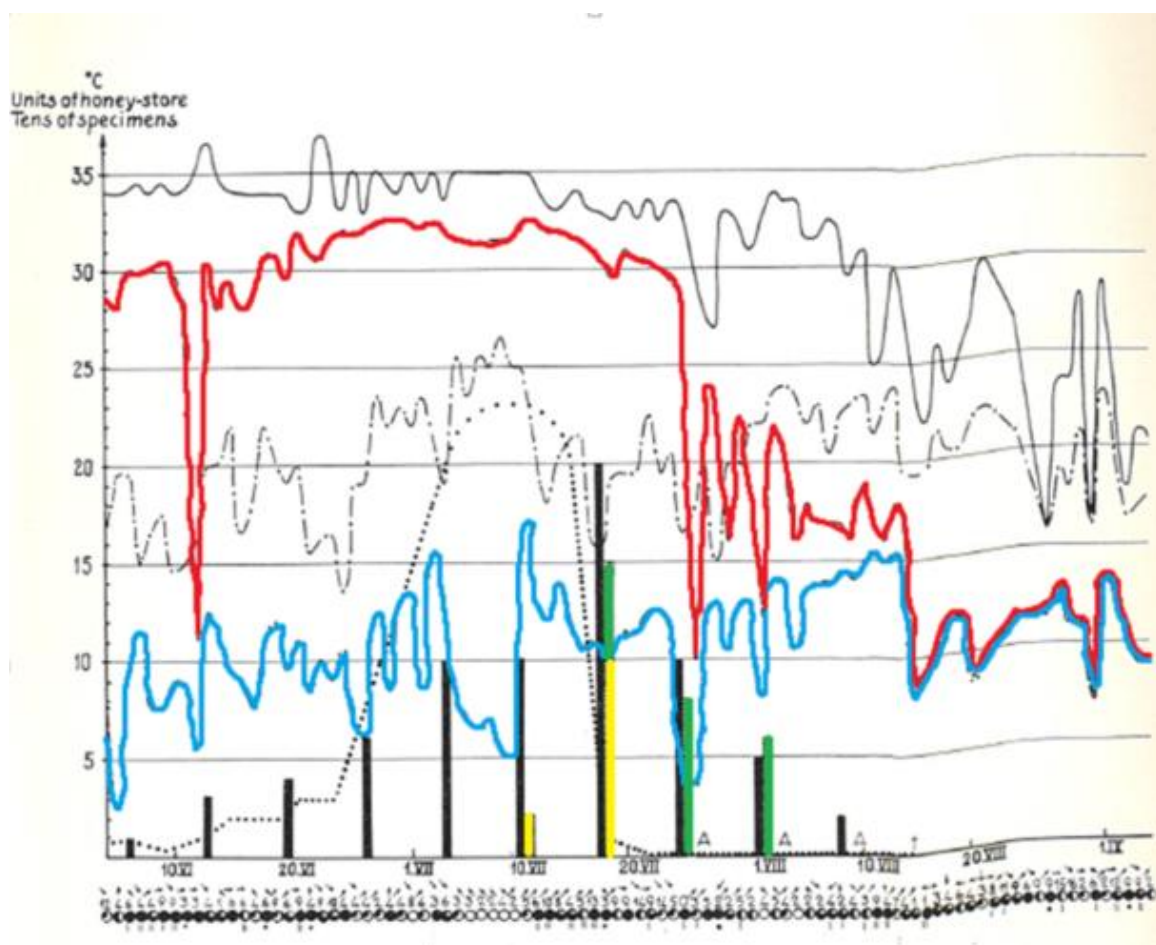
thresholds are reinforced by action or inaction, so that sensitive individuals become more sensitive to the stimulus the more often they respond and those that do not respond become increasingly less sensitive (Theraulaz, Bonabeau and Deneubourg, 1998). In theory this should allow a more subtle control of a parameter around the optimum, opposed to a digital flicker that would occur if all individuals responded at the same stimulus intensity. Response thresholds have been measured in *B. terrestris* in response to nest climate stimuli. Studies observing fanning behaviour in response to both increasing concentrations of CO<sub>2</sub> and increasing temperature demonstrated that bumblebee colonies have variable response thresholds amongst their workers and that that between colonies, the peak response for these stimuli varied (Weidenmüller, 2004). Worker bees have a key role in regulating brood temperature, both by the generation of heat and ventilation of the nest. Brood temperature can be moderated within bumblebee nests homeostatically due to the variable response thresholds amongst the population of worker bees.



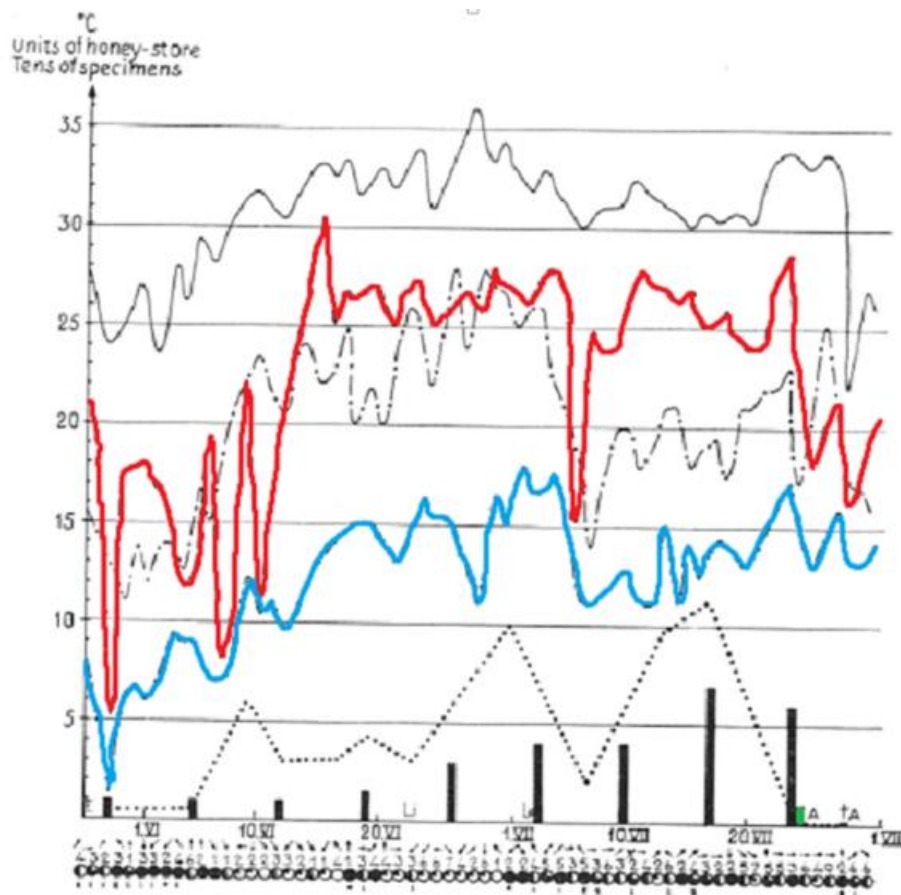
**Fig. 1. Brood temperature (degrees above ambient) at a range of metabolic rates of *Bombus vosnesenskii* queens.** Open and closed points show observations of two queens. Reproduced from Heinrich (1974).

## Average Brood Temperature Influences Colony Fecundity

Little work has studied the direct impact of rearing temperature on the production of gynes and drones over the length of a colony season. However, large-scale field studies on the domestication of bumblebees for commercial pollination gathered nest data for many variables. Several colonies of different bumblebee species in agricultural areas of Sweden and the data he recorded gives some insight into success and failure in bumblebee colonies (Hasselrot, 1960).



**Fig. 2. Observations of a 'successful' *B. terrestris* colony in Sweden.** Max nest temperature (red) and max air temperature (blue) recorded in a nest of *Bombus terrestris* over the course of one summer. Black bars show worker number, green shows males (drones) and yellow shows new queens (gynes). Gyne and drone production peaks in early August and by September the colony has died off, and there is no detectable incubation within the nest. Reproduced from Hasselrot (1960).



**Fig. 3. Observations of an 'unsuccessful' *B. terrestris* colony in Sweden.** Max nest temperature (red) and max air temperature (blue) over the course of the summer season in a colony of *Bombus terrestris*. Black bars show worker numbers, green shows drones (males) and yellow shows new queens (gynes). Reproduced from Hasselrot (1960).

The difference in reproductive output between these two colonies highlights how average brood temperature could influence reproductive success. The optimum developmental temperature for bumblebees may vary between species, however, observations across a variety of species demonstrate that successful colonies maintain their brood between 28 °C and 32 °C (Richards, 1973; Vogt, 1986; Schultze-Motel, 1991). Additionally, when *Bombus terrestris* workers are reared in relatively warm conditions (26 °C), they do not maintain their brood much above 31 °C, suggesting that this is near the optimum developmental temperature (Livesey *et al.*, 2019). The colony in **Fig. 2.** appears to sustain their nest temperature near optimum for the majority of the season, reaching a peak worker number of 200 and



successfully produces nearly 100 gynes before it is invaded by parasites. Conversely, the colony in **Fig. 3.** sustained its brood temperature below optimum for the majority of the growth period, reaching a peak worker size of 70 workers and producing no gynes.

There is some evidence to suggest that brood cells located more centrally in the brood cluster grow to a larger body size before emergence. When thermocouples were placed against individual brood cells in a colony of *Bombus pascorum* (Barrow and Pickard, 1985) it was observed that mean larval temperature was positively correlated with larval diameter and that there was a negative temperature gradient from the centre to the outside of the brood clump. It has also been found that larval cells located closer to the centre of the brood clump are fed more often and grow larger than those located on the periphery (Couvillon and Dornhaus, 2009), possibly because larval cells on the periphery are neglected by nurse bees. Although queen larvae are fed more often and achieve a larger peak size than most workers, it is unlikely that feeding rate or regime causes differentiation between castes. Instead, caste appears to be determined early in development where physiological differences act as feeding cues for worker bees (Pereboom, Velthuis and Duchateau, 2003). The size of worker bees at emergence may be determined by both the feeding and temperature regime they are exposed to and brood temperature can vary depending on the position of brood cells within the nest.

## Landscape Influence on Colony Growth and Development

It has generally been assumed that a larger colony has a higher reproductive output by number. This has been studied by different methods across a range of environments and species. In the UK, colonies of *B. terrestris* were placed in urban, semi-urban (village) and agricultural environments and found that in urban and semi-urban environments, the colonies grew to a larger size by worker number, producing more numerous reproductives (Samuelson *et al.*, 2018). In northern California, studies of *Bombus vosnesenskii* colonies found that semi-natural habitats promoted colony growth and that colony growth rate was the most important parameter predicting queen production (Crone and Williams, 2016). A further study in mid-western USA observed *Bombus impatiens* colonies, assessing the role of a variety of landscape scale effect on growth and reproduction (Spiesman *et al.*, 2017). Here, findings reported that in a nectar rich landscape, floral dominance facilitated larger more productive colonies, that is to say, a landscape with sufficient nectar supplied mainly by a few species was most profitable for bumblebee colonies. This was most likely because less time was spent learning a broad variety of flower types.

The role of mass flowering plant species in provisioning bee colonies is likely to be time specific. For example, mass flowering oil seed rape (canola) was found to increase worker numbers in colonies of *B. terrestris* located in Germany, although, it did not affect the number

of reproductives produced by the colony (Westphal, Steffan-Dewenter and Tschardtke, 2009). Oil seed rape flowers in spring and therefore, the provision of such a resource in the early stages of colony development, when foraging and storage capacity is limited, means this resource cannot be exploited to boost fecundity. Later, mass flowering species such as red clover, which flowers from late spring right through to early autumn, have been found to increase queen and male densities (Rundlöf *et al.*, 2014). This study counted new queens (gynes) and males that were netted in an area of rich forage, however, they may have been produced by colonies a substantial distance away. Rich forage resources could localise reproductives produced over a wide area and so counts of new queens in areas of rich forage may result in over estimates of average colony fecundity . In agricultural areas, declines in nectar availability, caused by mass flowering crops going to seed, could cause significant shortfalls in nectar availability for the rest of the season. Measurement of individual colony reproductive success in a variety of landscapes is required to understand the impact of changing resources on colony fecundity.

This evidence supports the idea that nectar rich landscapes support larger colonies that produce more new queens, demonstrating that reductions in floral resources could result in declines in the number of bumblebee colonies year-on-year. However, a clear mechanistic link between a lack of floral resources and reductions in colony fecundity has yet to be demonstrated.

## Foraging Fuels Incubation and Growth of the Colony

In order to sustain the incubation effort and to provide nutrients for the growing colony, foraging worker bumblebees must collect nectar and pollen from flowers in the surrounding landscape. Bumblebees are central place foragers and therefore, must always return to and forage from the colony (Cresswell, Osborne and Goulson, 2000). This means that their available foraging patch is constrained by how far it is economical to fly for a given resource. Foraging bumblebees collect nectar in their honey crop, which is an expanding sac located in the abdomen of the bee (Heinrich, 1979a). Once the foraging bees have returned to the nest, the nectar is unloaded into honey pots for storage. Pollen can also be stored and used with wax to build or directly provisioned to larvae (Free and Butler, 1959). Compared to honey bees, bumblebees are able to leave the nest to forage in far more inclement conditions. Hence, bumblebees do not hold large quantities of nectar in reserve.

Pollen grains are caught on the body hairs of the bee when it enters a flower and are then brushed off the body and formed into balls which are attached to the corbiculae on the hind

legs. Pollen provides protein essential for growth and development of workers and reproductives (Sutcliffe and Plowright, 1988). Pollen protein content is highly variable, ranging between 2.5 % and 61 % (Roulston, Cane and Buchmann, 2000). Due to this, bees may visit some plant species preferentially for their high protein pollen and others for better nectar reward. Both nectar and pollen are required for a growing bumblebee colony and the demand for each will change as the colony develops. Amongst foragers there may be some specialisation between nectar and pollen foraging, for example, pollen foraging trips are often shorter (Brian, 1952).

The rate at which resources can be returned to the colony is limited in part by the availability of resources in the surrounding landscape. It may sometimes be profitable to fly further from the nest to exploit a rich resource, however this incurs time and energy costs while travelling to and from the foraging site. *B. terrestris* workers have been observed to forage up to 800 m from the nest, with the majority foraging within 100 m (Wolf and Moritz, 2008). Another study using radar to track bees found that although the mean foraging distance was close to 275 m, bees frequently foraged past the radar horizon of 500 m (Osborne *et al.*, 1999). Foraging patch size is determined by the maximum distance it is possible to forage at profit given the increased travel cost. Forage patch size is most likely solely dependent on resource richness of an environment, richer environments should effectively support more colonies each with smaller patches. However, due to the length of the colony season, floral resource availability is likely to be highly dynamic; a rich landscape in spring may bring many colonies into close competition later in the season if nectar resources become scarcer.

Some assume that bees would tend to forage closer to the nest by preference, stating that the energetic cost of flight makes foraging from flowers beyond a certain distance unprofitable (Dramstad, 1996). However, it is important to consider that by increasing the foraging radius from the nest, the available area becomes larger in proportion with the square of the foraging radius, thus a bee that is willing to fly twice as far has an area four times as large in which it can seek the most rewarding food sources. Bumblebees fly at between 11-20 km hr<sup>-1</sup> (Heinrich, 1979a), with an assumed average flight speed of 15 km hr<sup>-1</sup>, and therefore, flying an additional kilometre in each direction adds eight minutes to a foraging trip. For a trip to be profitable, the reward rate of a patch of forage must increase with distance from the hive. In this manner, some plant species will only be profitable at close proximity to the nest. Ambient air temperature also has a significant effect on the profitability of plants species (Heinrich, 1979b). Bees must maintain the temperature of their flight muscles during bouts of foraging and at cooler air temperatures the cost of doing this is higher(Heinrich, 1979b).

Although foraging is essential for the growth of the colony, when resources become scarce, the additional time spent foraging reduces the colonies capacity for incubation, as well as other brood care tasks, which could result in a detriment in overall colony fecundity. A study comparing the foraging efficiency of *B. terrestris* colonies situated in both nectar-rich and nectar-poor environments found that foragers in a poor landscape made longer foraging trips and returned with much lower nectar rewards (Westphal, Steffan-Dewenter and Tscharrntke, 2006). Nectar availability will change within a landscape over the length of the colony season. In April, when colonies are still fairly small, average nectar availability may be near 10 kg ha<sup>-1</sup>. However by July, when the colony is at its largest, nectar availability may have increased to 20 kg ha<sup>-1</sup> (Baude *et al.*, 2016), a suitable landscape must provide appropriate nectar resources for the length of the colony season. A nectar poor environment could have limited incubation both by partial starvation and by reducing the number of worker bees available to incubate. Workers begin foraging depending on synergistic effect of multiple cues. Foraging workers have been shown to use colony nutritional status as well as pheromonal signals from other workers to initiate foraging, perception of both nectar stocks and colony demand allow for more efficient use of time, when nectar stores are high, workers will not initiate foraging even in response to applied pheromonal cues (Molet *et al.*, 2008). This suggests that foraging is controlled mainly by demand, via perception of stock levels and that bees will not continue to forage when stocks are perceived to be high, even if there is still a nectar profit to be made.

A recent study has shown robustly that colonies of *B. terrestris* perform much better in urban environments than agricultural ones (Samuelson *et al.*, 2018). They measured success by a variety of metrics including queen production, parasite invasion, and worker numbers. They found a clear benefit to colonies situated in city and village environments compared to agricultural landscapes, attributing this to the more barren nature of agricultural areas in terms of nectar resource. Meeting energy demands is crucially important for a bumblebee colony. Entering starvation due to a shortage of carbohydrates can have many negative influences on colony development. Colonies of *Bombus occidentalis* and *Bombus melanopygus* demonstrated several responses to short term starvation; colonies that entered starvation stopped brood incubation and, as a consequence, average brood temperature reduced during the period of starvation, after reintroduction of food supplies, incubation activity and brood temperature recovered (Carter & Dill, 1991). However, as has been discussed, the consequent reductions in brood temperature can have longer term impacts on colony and worker fitness as well as the rate of colony development.

## Division of Labour Enables more Efficient Thermoregulation

Amongst social insect species, a division of labour has been observed on a variety of scales. In early work, bumblebee workers were simply classified as foragers or house bees (Richards, 1946), and further observations noted that a proportion of workers appear redundant (Free, 1955). Task specialisation in social organisms is known as polyethism (Röseler and van Honk, 1990). Within social insects, polyethism has been observed at a relatively fine scale in ants, identifying both task specialisations amongst workers and a division between active and inactive individuals. Additionally it was demonstrated that removal of active workers caused inactive workers to take up tasks making up for the loss in the work force (Charbonneau, Sasaki and Dornhaus, 2017). The degree to which colony size influences the division of labour is unclear, however studies comparing colony size to the degree of labour division in wasps found that species with larger colony sizes contained workers with more complex individual behaviours, and that larger colonies operate more efficiently (Karsai and Wenzel, 1998). Here the authors conclude that species with smaller colony sizes adopt a jack-of-all-trades approach to task specialisation, and that larger colonies can accommodate more specialised workers. In a bumblebee colony that can grow from a founding brood of eight workers to several hundred at the end of the season, the degree of polyethism may develop through the season as the colony expands.

The benefit of division of labour to nest thermoregulation is clear; incubators stay home and maintain the brood temperature while foragers leave the nest to gather resources. The value of redundancy in the workforce is perhaps less clear, although its ubiquity amongst many species suggest it confers a relevant degree of fitness. The uptake of tasks by worker ants and the subsequent recovery of colony function after damage shows a clear benefit to the initial investment in production of a reserve workforce. However, there is a trade-off to be made, increasing the degree of redundancy in the workforce may protect against short term pressures such as damage from predation and acute cold temperatures. However, the cost of maintaining inactive workers may become a hindrance to the colony by placing too strong a demand on foraging resources. Simulations modelling the colony dynamics of ants have demonstrated that colonies with increased variability in response threshold persisted longer than those with limited variability (Hasegawa *et al.*, 2016). The redundant workers in this simulation were those with particularly high stimulus response thresholds that prohibited them from undertaking work in all but the most extreme stimulus intensities. The nature of redundant workers in this simulation may be different to those observed by Charbonneau, since in this study they identified a redundant component to the workforce by the uptake of work by previously inactive individuals. Whether the removal of active workers caused a change in perceived stimulus intensity that was responsible for the response in inactive workers, or

whether previously inactive workers changed or lowered their individual response thresholds after detecting a loss in worker number from the colony is unclear. It may also be likely that the degree of worker redundancy increases with colony size in the same way that task specialisation does (Karsai and Wenzel, 1998), so that in species with small peak colony sizes, the investment in redundant workers may be inefficient. However, it is clear that the sociality of bumblebees allows more homeostatic control of nest conditions, even in a relatively dynamic environment. Redundancy in the work force may allow for both increased foraging effort and increased incubation effort during particularly cool periods, however the degree of buffering may be limited in smaller colonies, so nest insulation could be more consequential in mitigating shortfalls in incubation.

## Nest Site Selection Varies Between Species and Environments

Since maintaining the brood at an appropriate temperature is so important for the development of a bumblebee colony, the selection of a nest site by queens could have significant implications for the colony, as the properties of the nesting substrate could have a strong influence on the retention of heat within the colony. Soil temperature data from June 2017 at the Exeter Airport weather station shows that even on the hottest day the soil temperature at 10 cm depth was 22 °C and that a 3-5 °C drop in temperature was common overnight (*Met Office Integrated Data Archive System*, 2018). In this location a soil nesting bumblebee colony would face an incubation challenge of at least 10 °C during the day, which demonstrates that even in relatively warm conditions there could still be a significant demand for incubation for soil nesting bees.

Preference of nesting site varies between species and is likely dependant to some degree on habitat and season length. In the UK, ground nesting species *B. terrestris*, *Bombus lapidarius* and *Bombus lucorum* show preference for habitats containing banks when searching for nest sites, whereas, species such as *Bombus pascorum*, *Bombus hortorum* and *Bombus ruderarius* show a preference for nest sites in tussock-type vegetation (Kells and Goulson, 2003). The tree bumblebee, *Bombus hypnorum*, which is an invasive species to the UK, has expanded its distribution since first being detected in the UK in 2001 (Goulson and Williams, 2001). *B. hypnorum*'s preference for nesting in elevated cavities means it is often found in the roofs of houses as well as trees (Prŷs-Jones, 2014). Cavity nesting bumblebee species similar to *B. hypnorum* have a lower critical thermal limit than ground nesting bees (Hamblin *et al.*, 2017), meaning they are less adapted to withstand high temperatures. This does not seem to be limiting their population growth and range expansion.

In contrast, the arctic bumblebee species *B. polaris* forms its nests in balls of dried plant matter suspended in the grass or shrubs of the tundra (Pape, 1983). This allows the nest to be warmed by the sun and avoids it being waterlogged by the melting permafrost. *B. polaris* nests are small in both size and worker number, due to the very limited time they have to produce a successful colony in the short arctic summer. Nest site preference is a key behavioural trait that is varied between species and the range of nesting strategies highlights how thermal stress, both high and low, could be mitigated by nest site choice. *B. polaris* and *B. hypnorum* will face different thermal challenges due to both the climates they inhabit and their nesting preference. For example, *B. polaris* is likely to experience much cooler ambient temperatures, and so nest construction and insulation behaviours may be of greater significance, whereas, *B. hypnorum* may be more likely to experience acute high temperature stress on hotter days. Therefore, thermoregulation by fanning to cool the brood may be more important. The significance of different traits in mitigating thermal stress could vary depending on the environment the nest is in.

There has been some suggestion that queens are more likely to establish nests in areas with floral resources that facilitate high rates of nectar collection and energy supply (Suzuki *et al.*, 2009). This may be more common in species that operate over shorter colony growth seasons, as nectar resource distributions may be less dynamic. Additionally, there are some reports of queens returning to their maternal nest sites. A study observing marked queens noted that some returned to the maternal nest site and at least one laid eggs, however a colony was not successfully established (Pomeroy, 1981). If suitable forage is available late in the season then new queens may over winter in close proximity to their maternal nests, therefore to see them searching for nest sites in these areas the following spring is not surprising. It is generally assumed that most bumblebee nest sites are not reused the following spring.

The choice of nest site has significant implications for the success of the colony. A naturally well-insulated nest will require comparatively less incubation effort to maintain optimum brood temperature. Reduced incubation demand allows a colony to inhabit landscapes with poorer floral resources, while still growing and producing gynes and drones. The adaptive significance of thermally efficient nesting behaviours has yet to be examined, this study aims to further understand the nature of heat loss from bumblebee nests and how it may impact colony growth.

## Heat Transfer in Bumblebee Colonies

The importance of temperature for insect development is clear, the variability in nesting behaviours between species highlights how the challenge of maintaining an optimum temperature may be managed. Very little work has investigated heat transfer within bumblebee colonies. However, Schultze-Motel (1991) was able to quantify change in heat loss from a bumblebee colony by placing it in calorimetry box, where rates of heat loss were recorded to be between 0.3 W and 1.4 W. In order to further understand what factors influence the retention of heat within in a bumblebee nest a basic understanding of the physics of heat transfer is necessary.

When an object is maintained at an elevated temperature in a cooler environment, the rate at which it is losing heat energy must be equal to the rate at which energy is being supplied to it. Applying this to a bumblebee colony can help us understand how the colonies incubation effort and carbohydrate requirement can be influenced by the ambient temperature and management of heat loss.

Energy in the form of heat can be transferred from a warmer object to cooler surroundings by three mechanisms; convection, conduction and radiation. The total rate of heat loss by an object will be equal to the sum of these three terms so that:

$$\text{Heat Loss} = P_{CD} + P_{CV} + P_R \quad \text{Eq. 1.}$$

Where  $P_{CD}$ ,  $P_{CV}$  and  $P_R$  denote the rate of heat loss in Watts by an object to its surroundings by conduction, convection and radiation respectively. In a bumblebee colony where the brood is maintained above the surrounding ambient temperature, an assumption can be made that the rate that heat energy is being lost is equal to the rate that it is being generated by incubating bees. So that –

$$I = P_{CD} + P_{CV} + P_R \quad \text{Eq. 2.}$$

Where  $I$  denotes the incubation effort of the colony in Watts. This equation demonstrates that any reductions in the rate of heat loss by these three mechanisms will result in a reduced demand for incubation. A reduction in the rate of heat loss may benefit the colony in several ways, by enabling the brood to be maintained at an optimum temperature or by freeing up worker bees for other tasks, a more thermally efficient nest should be beneficial to colony growth and fecundity. A better understanding of each mechanism of heat loss will allow the design of experiments that can demonstrate their relative significance in bumblebee nests.



## Heat Loss by Radiation

Energy in the form of radiation is given off by materials depending upon their temperature and emissivity. In the same way radiation can be absorbed by an object depending on its colour and temperature. The effect of colour on the absorption and emittance of radiation is quantified by a property called emissivity. Emissivity values are between 0 and 1, where a perfect black body would have an emissivity score of 1. The rate at which thermal energy is radiated into the environment is dependent upon the temperature and emissivity of an object.

Imagine a closed system in which there is a hot object, the system is at a cooler temperature. The rate at which the hot object loses energy in the form of radiation is described by the Stefan-Boltzman equation (Weast and Astle, 1983).

$$J^* = \epsilon\sigma T^4$$

**Eq. 3.**

Where  $J^*$  denotes the rate that energy is radiated,  $\epsilon$  denotes the emissivity of the object,  $\sigma$  denotes the Stefan-Boltzman constant and  $T$  denotes the absolute temperature difference between an object and its surroundings. The rate at which energy is lost from an object is proportional to the temperature difference between the object and the system. The Stefan-Boltzman constant links the absolute temperature difference and the emissivity of an object, resolving to the rate of thermal radiation in  $W m^{-2}$ . The Stefan-Boltzman constant or the constant of proportionality  $\sigma = 5.670373 \times 10^{-8} Wm^{-2} K^{-4}$  (Weast and Astle, 1983).

## Heat Loss through Convection

Convective heat transfer is the movement of heat energy away from an object by a fluid (either liquid or gas). There are two types of convective heat transfer and both may be relevant to bumblebees. Forced convection is the active movement of a fluid across a surface. For example, bees exhibit fanning behaviours when brood temperatures are too high, this circulates air and lowers the temperature of the brood surface (Weidenmüller, Kleineidam and Tautz, 2002). The second type of convective heat loss is known as natural or free convection. Whereby heat is transferred from an object to the fluid surrounding it, this drives a circulation in the fluid due to changes in density related to increases in temperature.

Natural convection is most relevant to bumblebee colonies under a cold challenge, where the warm brood will lose heat to the surrounding air within the nest. For ground nesting species, such as *B. terrestris*, the size of the air space in which the nest is located will affect the rate of

free convection. The rate of heat transfer is proportional to the temperature difference between the brood and the air within the nest, whereby a smaller nest cavity will have a smaller mass of air that will be warmed at a faster rate, however, as the air warms, the rate of heat transfer from the brood will reduce. Bumblebees often form wax canopies over their brood comb (Holland and Bourke, 2015), which could help trap a warm layer of air above their brood and therefore, insulate the brood. The insulation effects of canopy building behaviours is clear, since bees have been found to deconstruct the canopy in order to improve airflow and consequently, reduce temperature, when exposed to high ambient temperatures (Holland and Bourke, 2015).

Forced convection becomes more relevant for bumblebee colonies when ambient temperature exceeds the optimum limit, and so in response to this, workers begin to fan, in order to increase air circulation within the nest (Westhus *et al.*, 2013). Fanning increases the rate of air circulation over the brood surface, increasing the rate of heat transfer. However, the magnitude of the temperature difference between the brood surface and air still has an important effect on the rate of heat transfer. Under heat stress, a fanning response may include directional fanning towards the nest entrance in an attempt to circulate the nest with cooler air from outside, as has been observed in honeybees (Junge, 2006). Some nest sites may be naturally well-insulated, for example a rodent's burrow will protect the nest from cooling in the wind – a form of forced convection. Other species may be limited to forming nests on the soil surface, insulating them with organic matter such as lichens and mosses (Pape, 1983).

## Heat Transfer through Conduction

Conduction refers to the transfer of heat energy through solid materials in contact. In bumblebee nests, the brood comb structure consists of brood cells which may be eggs, larvae or pupae, which will be sites of incubation, where thermal energy is transferred to the system (Kelemen and Dornhaus, 2018). The heat energy generated by incubating worker bees at these points will be conducted to the rest of the comb structure; in mature bumblebee nests this may be between 10 cm and 15 cm across. Further conduction of heat away from the brood will occur where the comb structure contacts the outside of the nest, which may be soil in the case of ground nesting species. When both the nest and ambient temperature are held constant, the conduction of heat through the nest is deemed to be steady-state. In steady-state conduction a gradient of temperature exists across an object. In a nest, this would be from the hottest brood cells to the outside surface of the nest. Across the nest a gradient of temperature is established, but at any cross-sectional point, the temperature does not change with time.

Fouriers law describes the rate of heat flow through a material based on the thermal gradient across it so that:

$$qx \propto - \lambda \frac{dt}{dx}$$

**Eq. 4.**

Where **qx** is the heat flux through an object in  $Wm^{-2}$ , **λ** represents the thermal conductivity coefficient of the material and **- dt/dx** represents the thermal gradient across the object from hot to cold, hence its negative term (Sneddon, 1976). In steady–state, conduction heat energy is always transferred from hot to cold, from a high energy state to a low energy state (Sneddon, 1976).

Bumblebees form brooding structures from wax, secreted from in-between their abdominal plates. The brooding comb is constructed using this wax and consists of honey pots and brood cells joined normally into one continuous structure. Although incubation efforts are concentrated on the brood cells, either eggs or larvae (Kelemen and Domhaus, 2018) as these structures form part of a larger mass thermal energy will be conducted between them and a gradient of heat flow will always be established from the centres of incubation to the peripheries.

## Conclusion

Bumblebee colonies operate in an envelope of time and temperature, where the parameters of that envelope change depending on their environment. Maintenance of an optimum brood temperature increases the rate of colony growth (Hasselrot, 1960) . The generation of heat within the nest is metabolically costly (Heinrich, 1974) so floral resources are required within the landscape to supply nectar sugars. The thermal properties of a nest could have a significant influence on the ability of a colony to avoid shortfalls in incubation. Nesting characteristics and traits vary between bumblebee species (Kells and Goulson, 2003), most likely due to the nuisances of the environments that they inhabit. A more in-depth understanding of the nature of heat transfer within bumblebee colonies is necessary to understand the adaptive significance of nesting behaviours.

## Thesis Aims

In order to develop a better understanding of heat transfer in bumblebee nests this thesis has the following aims.

- A) Determine whether radiant heat loss has a relevant effect on the thermal efficiency of bumblebee microcolonies.
- B) Determine the extent to which insulation improves the thermal efficiency of bumblebee microcolonies.
- C) Assess the potential for shortfalls in incubation to reduce bumblebee colony fecundity over the length of a colony season.

## Chapter Two: The importance of radiant heat loss on the energy budget of bumblebee colonies *Bombus terrestris*

### Abstract

Declines in bumblebees are of increasing concern, due to their important role in the pollination of both crop plants and wild ecosystems. Such declines, have been linked to changes in land use and subsequent loss of floral resources, but to better understand the impacts of these factors, a more complete energy budget for bumblebee colonies must be established. Bumblebees are nectarivores, they acquire the majority of their nutritional requirement from the nectar of flowering plants. The heating of a bumblebee nest is crucial for colony development, survival and fecundity. Generating heat is metabolically costly and is a drain on nectar stores. Heat is lost from the nest by convection, conduction and radiation, but details of heat loss in bumblebee colonies are poorly understood.

To establish the relative importance of radiative heat loss, measurements of surface emissivity were used to model radiative heat loss from bumblebee brood structures. Additionally, two radiant barrier treatments were tested by measuring average brood temperature and syrup consumption in small groups of orphaned workers, termed microcolonies.

Modelling indicated that the maximum rate of radiative heat loss possible in microcolonies may be equivalent in power to the heat generated by two incubating worker bees (approximately 70 mW). However, empirical testing revealed no detectable effects on brood temperature and syrup consumption between a reflective (more insulating) and absorbent (less insulating) radiative barrier treatment.

Convection, conduction and radiation are obstacles of thermoregulation in bumblebee nests. Quantifying the energy budgets of bumblebee colonies will lead to a better understanding of their requirements for floral resources. The relative importance of radiative heat loss may be fairly small, meaning the majority of heat is lost through convection and conduction. Combining laboratory-based studies of bumblebee nesting physiology with field observations of colony locations and temperature relationships may lead to a better understanding of bumblebee population sustainability in various landscapes.

## Introduction

### Mechanisms of Heat Loss to the Nesting Substrate

The rate at which heat energy is lost to the surrounding environment determines the incubation effort required to maintain the brood at an optimum temperature. Energy in the form of heat is transferred between materials by three mechanisms; convection, conduction and radiation. For a bumblebee colony to remain at a stable temperature the heat energy transferred to the brood from incubating bees must equal the total rate of heat loss to the surrounding environment. So that:

$$I = P_{CD} + P_{CV} + P_R$$

**Eq. 2.**

Where **I** denotes the rate of heat energy input to the brood by incubating worker bees. **P<sub>CD</sub>**, **P<sub>CV</sub>** and **P<sub>R</sub>** denote the rate of heat energy lost to the surrounding environment by conduction, convection and radiation, respectively.

Thermal energy can be transferred by conduction when materials with a temperature differential touch. In a bumblebee colony the brood is often slightly elevated above the floor of the nest raised on wax pillars, or built on top of older brood structures (Free and Butler, 1959). While there may be several reasons for this, such as allowing workers better access to the brood or reducing parasite ingress, the reduction in the surface area of the brood that is in contact with the cooler nesting substrate will potentially limit the rate of heat loss by conduction.

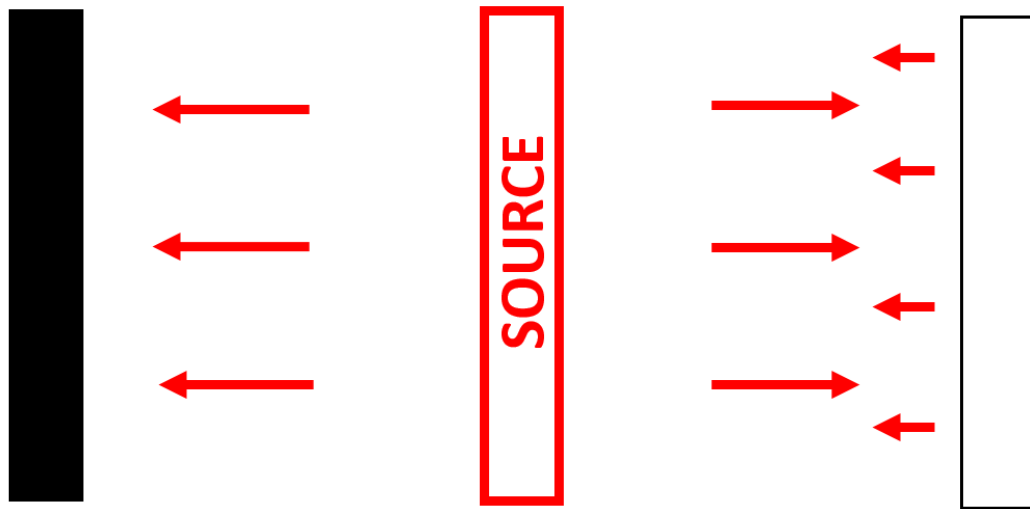
Loss of thermal energy via convection involves the transfer of kinetic energy from molecules in a solid matrix to molecules in a fluid, at the boundary layer interface across a temperature gradient. The rate of convective heat loss is determined by a combination of factors. As in all heat exchanges, the rate of heat loss is proportional to the temperature difference between the two materials. In addition, the surface area over which the air flows will also proportionally affect cooling, that is a larger surface area to volume ratio will increase the rate of heat loss. Bumblebee brood structures are often highly irregular, due to honey pots and other structures which increase rugosity (surface roughness), which could increase the rate of cooling. The rate of convection will be dependent on how well-directed the incubation effort is, as brood cells may be clustered together and only occupy a small proportion of the nest structure, thus reducing the surface area of the brood being maintained at an elevated temperature. The final factor affecting convective cooling is the heat transfer coefficient of the surface material, which is specific to different materials. For example, human skin has a coefficient of approximately

4.5 W m<sup>-2</sup> K<sup>-1</sup> (de Dear, Arens and Zhang, 1996), however, the coefficient of bumblebee brood structures is yet unknown.

## Heat Loss by Radiation

The rate that energy is radiated from the surface of an object depends on its temperature and emissivity. Emissivity is the property of a surface that influences how much heat energy is emitted in the form of radiation. Similarly radiation can be absorbed by an object depending on its emissivity and temperature. The effect of colour on the absorption and emittance of radiation is quantified by emissivity. Emissivity values range between 0 and 1. An ideal absorber would have an emissivity score of 1, commonly referred to as a perfect black body. The rate at which thermal energy is radiated into the environment is dependent upon the temperature and emissivity of an object, this is described by the Stefan-Boltzmann equation presented in chapter one.

In the context of a bumblebee colony, the warm brood is surrounded by a cooler environment. Brood lump area changes as the colony develops and honey pots are added. Although heating is focused on the brood (Kelemen and Dornhaus, 2018), some heat will be conducted throughout the rest of the nest architecture. Heat energy will be lost by radiation from the brood lump depending on the difference between the surface temperature and the ambient temperature of the nest cavity. In modern building insulation, a radiant barrier, usually a reflective material, is added to the insulation material, **Fig. 4.** illustrates how materials with high emissivity values (black) absorb the majority of the radiant energy incident upon them while materials with lower emissivity values reflect heat energy. Similarly, the surface properties of the nesting cavity could influence the net rate of radiative heat loss to the substrate. The magnitude of radiant heat loss from bumblebee nests could influence the ability of the incubating workers to sustain the brood at an optimum temperature.



**Fig. 4.** Schematic demonstrating how black objects absorb heat in the form of radiation, while white object reflect radiation back.

### Aims and Objectives of the Study

The principle aim of this study is to determine whether heat loss by radiation makes up an ecologically significant proportion of the total heat loss from bumblebee microcolonies by:

- A)** Measuring the emissivity of bumblebee brood cells and modelling the degree to which of radiative heat loss contributes to the total heat loss from bumblebee brood.
- B)** Testing for a difference in heat retention due to differential radiative properties of two surface treatments, at power levels relevant to bumblebees.
- C)** Testing for effects of radiant barrier insulation on brood temperature and carbohydrate demand in bumblebee microcolonies.



## Methods

### Definitions

**Microcolonies** – Small softwood boxes that house six orphaned worker bumblebees, previously removed from a queenright colony obtained from a commercial supplier. When worker bees are separated from a queenright colony and housed together with access to pollen and nectar they begin laying eggs and exhibit brooding behaviours (Klinger *et al.*, 2019). This makes them a useful system to study many aspects of the biology of social insects.

**Queenright** – A bumblebee colony where the founding queen is still alive and laying new workers, queens or drones.

### Bee Provenance and Husbandry

Throughout the experiment, queenright colonies of *Bombus terrestris audax* were obtained from commercial suppliers; Biobest, Westerlo, Belgium and Koppert B.V., Berkel en 142 Rodenrijs, Netherlands. Softwood microcolony boxes (16x16x5 cm) were provisioned with an 8 g pollen ball on one side of the box and syrup filled feeder tubes on the other. Microcolonies were established with six orphaned worker bumblebees. Microcolonies were placed in a controlled temperature room (CTR) (25 °C +/- 2 °C), with an approximate humidity of 40 %, to establish before being moved to a cooler cabinet. While housed in the CTR the microcolonies were further insulated with foil-covered pieces of cardboard and felt blankets. Bees were fed *ad libitum* with sugar syrup (Attraker, Koppert, Rodenrijs, Netherlands).

### Treatment Design – White and Black Boxes

In order to test the effects of radiant barriers on heat retention, the interior surfaces of microcolony boxes were painted with either white acrylic paint (Winsor & Newton Galeria White Acrylic, London, UK) or black acrylic paint (Crawford & Black, The Works, Coleshill, UK). White surfaces are expected to have a relatively low emissivity value and would therefore reflect more radiant heat energy back towards the brood compared to a matte black surface, which would be expected to absorb a higher proportion of the heat energy radiated towards it.

### Measuring Brood Emissivity

In order to estimate the magnitude of radiative heat loss from incubated brood cells an accurate estimate of the emissivity of pollen and brood cell wax was required. To determine the emissivity of the brood cells, brood lumps that had been extracted from queenless microcolonies of *Bombus terrestris* were used. Brood lumps were removed from microcolonies that had been brooding for approximately 10 days so that both eggs and larvae were present.

To determine whether emissivity values varied within a temperature range relevant to bees, emissivity values were measured between approximately 20 °C and 40 °C as follows.

The brood lump was removed from each microcolony and a thermocouple was embedded into the surface of a brood cell. A brood cell was defined as either containing eggs or larvae, no pupae were used. Emissivity was calculated by adjusting the emissivity setting on a thermal camera (Testo 870, Testo , Alton, UK) until the thermocouple embedded in the surface of the brood cell was recording the same temperature as the thermal image on the camera. The thermal imaging camera records surface temperature to +/- 2 °C, the thermometers used to record the surface temperature are accurate to +/- 0.1 °C.

In order to vary the temperature of brood cells, heat was applied using a hair dryer for approximately 90 s, so that the surface temperature reached approximately 40 °C. The heated pollen ball was then placed on a sheet of polystyrene and the thermocouple was attached to the brood cells surface. Measurements were taken as the pollen ball cooled to room temperature so that emissivity measurements were obtained approximately for  $\Delta T$  0-20 °C.

## Modelling Radiative Heat Loss

In order to model the magnitude of heat exchange in microcolonies, a form of the Stefan-Boltzman equation that describes the exchange of radiant energy between two identical bodies was used. To estimate the rate of heat radiant heat transfer at the surface of the brood lump the following equation can be used:

$$J^* = \sigma \epsilon A(T_1^4 - T_2^4)$$

**Eq. 5.**

The terms in this form of the Steffan-Boltzman equation are the same as **Eq. 3**, with the addition of  $T_1$  which is the surface temperature of the pollen ball in degrees Kelvin and  $T_2$ , the surface temperature of the surrounding box. It is assumed that the estimate for the emissivity of brood lump surface is similar to the emissivity of the interior surface of the soft wood boxes (López *et al.*, 2013). In order to make approximations on the magnitude of heat transfer, I set the temperature of the nest box ( $T_2$ ) to 293.15 K (20 °C) and generated estimates for brood lump temperatures between 293.15 K and 303.15 K (20-30 °C).

## Heat Retention in Black and White Boxes

In order to test the effect of colour on heat retention in black and white microcolony boxes, 22  $\Omega$  resistors were pressed gently into the surface of an 8 g pollen ball and capped with a further 0.2 g of pollen dough. A circuit consisting of three of these resistors in parallel loops allowed three boxes to be tested simultaneously. Temperature measurements were taken with a K-type thermocouple imbedded in the centre of the pollen ball and a Digitron 22T digital thermometer. The power output of the resistors was varied by changing the voltage across them according to Ohm's power law:

$$P = \frac{V^2}{R}$$

**Eq. 6.**

To assess the insulation effect of the two paint colours, pollen ball temperatures were measured and compared relative to an unheated control. Boxes were maintained at each voltage for between 2-3 h to allow the temperature to equilibrate. The equilibrium temperature of boxes was measured over a range of resistor power outputs.

The effect on radiative heat transfer was tested using power outputs between 40 mW and 200 mW. The individual power output of an incubating worker bee is approximately 35 mW (Livesey *et al.*, 2019), so the range of power outputs tested represents the potential incubatory power output of between one to six bees.

## Establishment of Microcolonies

Initially, workers were separated from queenright colonies of *Bombus terrestris* before being chilled and inserted into either black or white microcolony boxes. Each box was established with 6 workers bees and a clear plastic lid was installed. In order to allow microcolonies to begin brooding prior to the experiment, they were left to establish in a temperature controlled room (25 °C +/- 2 °C). After 3-5 days, temperature measurements and observations determined whether the bees had laid eggs and were incubating their brood clump. Microcolonies that did not show evidence of brooding were removed from the experiment at this stage. So that the internal surface treatment completely surrounded the brood lumps, the plastic lids of microcolonies that had successfully started brooding were replaced with wooden lids, which had been painted with either black or white acrylic to match the condition and secured with screws.

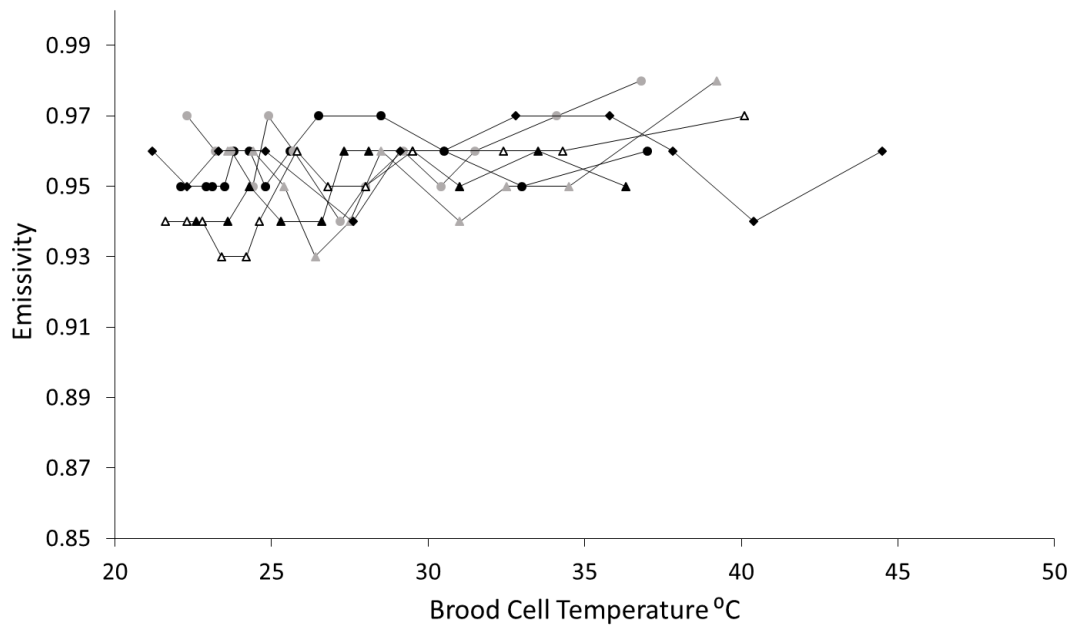
## Observations of Brood Temperature and Feeding Rate

Bumblebee colonies generally maintain their brood temperature between 28-32 °C, below this temperature range, bees incubate their brood and generate heat within the nest (Vogt, 1986; Schultze-Motel, 1991) . In order to detect differences in the demand for carbohydrates or average brood temperature as a result of the radiant barrier treatments, the microcolonies were kept at an ambient temperature substantially below that which they would normally incubate their brood to. A temperature of 21 °C was selected, subjecting the bees to a temperature challenge of approximately 10 °C .To this end, the microcolonies were transferred to a climate controlled cabinet (Snijder Economic Delux, Tilburg, The Netherlands) set at 21 °C and 40 % humidity. The position of black and white boxes within the cabinet was randomised. In order to record the effect of internal surface colour on average brood temperature, measurements of the brood clump temperature were taken with a digital thermometer (Digitron 2022T, Port Talbot, UK) three times a day for at least 10 days. In order to determine whether changes in radiant heat exchange with the nesting box could affect the syrup demand of the colony, feeding rate was recorded by net changes in the weight of microcolony syrup feeders daily. In order to compare the effect of each treatment, the brood temperature and average syrup consumption rate was recorded for the 10 days spent in the climate controlled cabinet, at approximately 21 °C ambient temperature.

## Results

### Brood Lump Emissivity

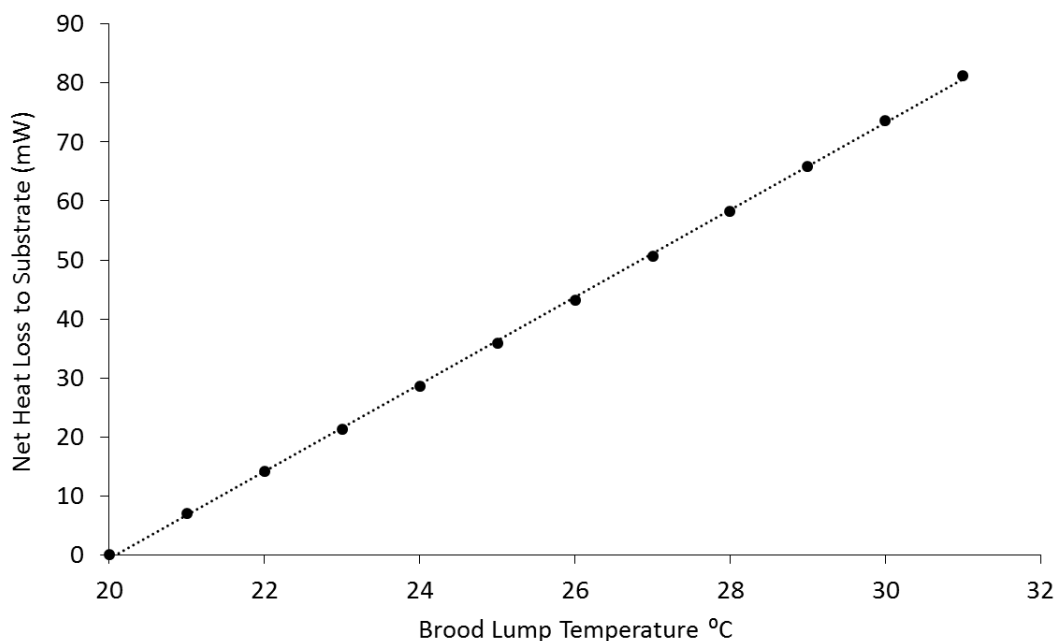
Emissivity of brood lumps was observed to be approximately 0.96, at a range of temperatures between approximately 20-40 °C, with standard error = +/- 0.0021 (N = 6). There was no difference in emissivity between pollen balls (ANOVA,  $F_{5,66} = 2.3$ ,  $P > 0.05$ ).



**Fig. 5.** Emissivity of brood lumps removed from six different microcolonies as they cooled to room temperature after heating. Each marker represents a separate brood lump removed from a microcolony (N = 6).

## Modelled Radiative Heat Loss from Microcolony Brood Lumps

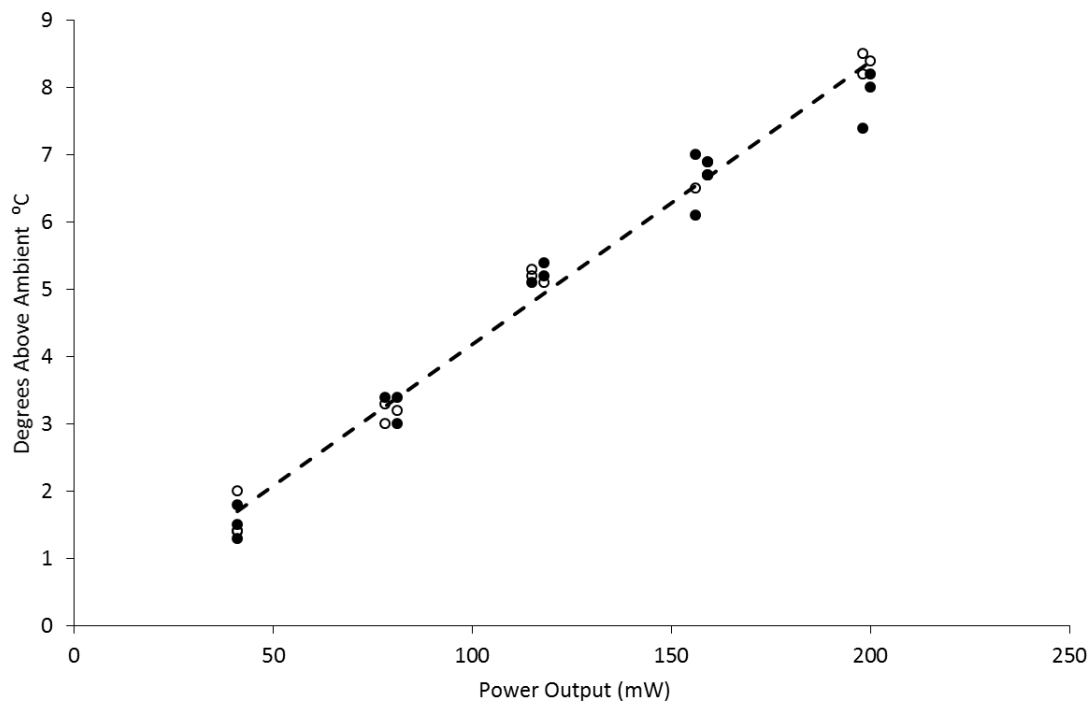
An empirical estimate of brood cell emissivity ( $\epsilon = 0.96$ ) to parameterise the Stefan-Boltzmann equation (**Eq. 3**) for the rate of heat loss by a brood lump through radiation. The model was solved for an object with the surface area of the same as the experimental brood lumps, surrounded by a surface with equal emissivity and absolute temperature 293.15 K (20 °C), allowing comparison with other work conducted on laboratory reared microcolonies. As the heat loss from the object to the surrounding environment is dependent on the absolute temperature of each surface. Net heat loss is the difference in radiative energy emitted by the brood lump and the radiant energy the brood absorbs after it has been reflected back from the interior surface of the box. Trend line has equation  $y = 7.3908x - 148.19$ .



**Fig. 6.** A model of the maximum magnitude of radiative heat loss for the 8 g pollen balls used in the experiments. The Stefan-Boltzmann equation was used with measurements of brood cell emissivity to calculate the magnitude of heat loss over a range of relevant temperature differentials.

## Electrical Simulation of Microcolony Heating

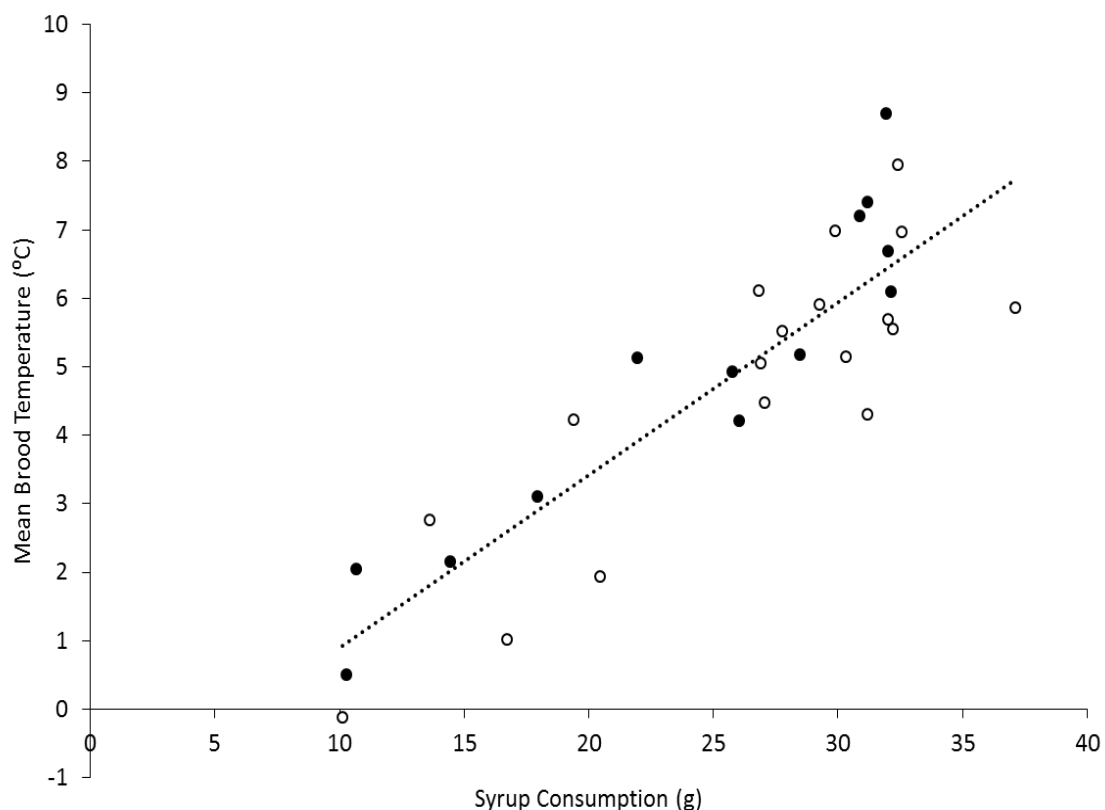
Analysis of covariance was performed in SPSS, finding that interior box colour was not a statistically significant contributor to box equilibrium temperature over a range of bumblebee-realistic power outputs (ANCOVA,  $F_{1,26} = 1.963$ ,  $P > 0.05$ ). Power output was a significant predictor of equilibrium temperature (ANCOVA,  $F_{10,26} = 176.8$ ,  $P < 0.05$ ). A trend line is fitted to a data set containing both black and white boxes and has equation  $y = 0.0432x - 0.1376$ ,  $R^2 = 0.9754$ .



**Fig. 7. Equilibrium temperatures of black and white boxes at a range of power outputs.** Closed points indicate pollen balls housed in a black painted box and open points indicate a white painted box. Boxes reached equilibrium between two and three hours after the power supply was connected. Equilibrium temperatures were recorded as degrees above ambient, ambient temperature measurements were taken from a pollen ball in an unpainted and unheated box.

## Mean Brood Temperatures and Total Syrup Consumption In Black and White Microcolony Boxes.

Box colour was not a statistically significant contributor to average temperature (ANCOVA,  $F_{1,28} = 3.714$ ,  $P > 0.05$ ). A univariate analysis demonstrated that syrup consumption was a significant predictor of average temperature across boxes regardless of colour (ANCOVA,  $F_{1,28} = 111.167$ ,  $P < 0.05$ ). A trendline was fitted to a data set combining both black and white boxes and has equation  $y = 0.2523x - 1.6241$ ,  $R^2 = 0.7723$ . Based on this equation and solving for  $\Delta T = 0$ , the non-thermogenic metabolic cost of six worker bees is approximately 0.65 g of syrup per day which accounted for a quarter of syrup consumption in the hottest boxes. The cost in syrup per additional degree of brood temperature was found to be approximately 0.38 g degree<sup>-1</sup> day<sup>-1</sup>. The sugar content of the syrup fed to the bees was between 60-70 % by weight. Using an average sugar content of 65 %, daily sugar demand per additional degree of brood temperature was estimated to be 0.247 g deg<sup>-1</sup>.



**Fig. 8. Mean brood temperature and total total syrup consumption in microcolonies over 250 hours.** Syrup Consumption was scaled to 250 h of exposure to experimental conditions to allow comparisons between different groups. Mean brood temperature was recorded relative to a pollen ball in an unpainted control box without any bees present. Open points represented white painted boxes and closed points represent black painted boxes.



## Discussion

### Relevance of Radiative Heat Transfer to the Energy Budget of Incubating Bees.

Using the estimate of brood lump emissivity (**Fig. 5.**), models indicate that the radiative heat loss from a bumblebee brood will only be consequential providing the brood is maintained at a temperature which is significantly above the ambient temperature within the nest, and if the surrounding material is both a strong absorber and conductor of thermal energy. In the microcolony system, bees that maintained their brood temperature near 30 °C in a 21 °C cabinet experienced a radiative heat loss of up to 70 mW (**Fig. 6**). In early trials of the radiative barrier insulation experiment, the ambient air temperature within the box was also measured and suggested that even in warm boxes, the ambient nest temperature was near that of the cabinet (21 °C), demonstrating that the brood itself was subject to a 10 °C challenge.

One limitation of this model is the assumption that the substrate is highly conductive, meaning that even though the surface is absorbing energy, the surface temperature does not change. This highlights an important point regarding heat transfer to the nesting substrate, whereby in reality, the surface of the nesting substrate would absorb energy and therefore increase in temperature. This increase in temperature would reduce the net radiant heat transfer from the brood to the substrate. After a while, an equilibrium would be established depending on the conductivity of the substrate. A poorly conductive substrate would establish an equilibrium at a higher temperature, whereas a highly conductive substrate would transfer heat energy away faster, maintaining a cooler surface temperature and hence a larger net rate of radiative heat loss from the brood. This is important for understanding how heat is transferred to the soil in ground nesting insects, where dry soils may be much less conductive and so will have better insulation properties for both conductive and radiative heat loss.

The model of radiative heat loss, generated from empirical measurements of brood cell emissivity, shows that the magnitude of radiative heat loss within the microcolony system is small, but not biologically insignificant. A brood lump at 30 °C would have a radiant heat loss of approximately 70 mW. Previous studies have estimated the power output of an incubating worker bumblebee to be 35 mW (Livesey *et al.*, 2019). Assuming that in the hottest boxes all six bees are incubating, the total incubatory effort would be approximately 210 mW, based on the assumption that all bees are incubating all of the time. So, in a microcolony of six bees maintaining a constant brood temperature of 30 °C and a substrate temperature of 20 °C, where the magnitude of heat loss is equal to the magnitude of incubation effort, radiative heat loss could account for over 30 % of the colonies thermogenic effort. However, this is based

on the internal surface of the nesting structure or substrate remaining at 20 °C, and therefore, to fully understand the magnitude of radiative heat loss, further knowledge of the conductivity of nesting substrates is required.

The brood structure in wild bumblebee nests will have a significant effect on how heat is transferred between the nest and its surroundings. Bumblebee nests consist of a highly corrugated comb structure that incorporates brood cells or larvae and eggs with pupae cells and honey pots for nectar storage, which intersperse the comb and are often manufactured from old pupae cells. Although bees target brood cells for incubation, heat is naturally transferred to the surrounding honey pots. A recent study has highlighted how temperature of the comb structures, both brood and non-brood structures, vary across the surface of the comb. They found that, in bumblebee nests kept at an ambient temperature of 20 °C, both brood and non-brood structures reduced in average temperature with increasing distance from the centre of the nest, whilst brood cells at the periphery were maintained at higher temperatures than non-brood structures. Average temperatures varied from 30 °C, at the centre of the nest, to 22 °C, at the periphery, across an 8 cm radius (Kelemen and Dornhaus, 2018). This demonstrates that the majority of the brood comb is maintained above ambient temperature and so will radiate heat. The superficial structure of brood comb may also act to conduct heat away from centres of incubation where there is brood. Even if only the brood surface area is considered, radiative heat loss may still be relevant, however further analysis of comb structure in mature nests may be necessary to produce more accurate estimations of radiative heat loss.

### Internal Surface Treatment Did Not Predict Power Demand or Syrup Consumption

There was no detectable difference in syrup consumption or average temperature between the radiative barrier treatments in the microcolony experiment (**Fig. 7.**). The difference in infrared reflectivity between treatments may not have been as strong as initially assumed and so, although modelling demonstrates the potential magnitude of radiant heat loss, it was not detectable amongst the biological noise. Variability in the relationship between feeding rate and temperature could have been caused by a number of factors. Honey pot food stores were not measured at the end of the experiment, this may have added a small amount of variation to the results as some boxes may have had a stronger tendency to store food than others, however over the length of the experiment any residual syrup in honey pots would be likely be negligible compared to the total consumption.

## Syrup Consumption Predicts Average Brood Temperature

Syrup consumption is a strong predictor of brood temperature and the relationship between the two is linear (**Fig. 8.**). This finding has important consequences for understanding colony scale energy budgets. The sugar syrup fed to the bees had a sugar content of approximately 65 % by weight, which is considerably higher than most floral nectars. For example, the nectar from fireweed (*Chamerion angustifolium*), a common forage for bumblebees in North America contains approximately 33 % sugar by weight (Heinrich, 1979b). Climate conditions can have a large influence on the sugar content of nectars, for example, sugar content in the nectar of heather can reach up to 60 % provided there is sufficient rainfall prior to flowering (Enkegaard, Kryger and Boelt, 2016). The high sugar concentration in the syrup should not have affected the outcome of the experiments, since even if a lower concentration of sugar had been used, the total foraging time would likely be negligible due to the proximity of the food source to the brood.

The microcolonies in this experiment were exposed to a thermal challenge of approximately 10 °C below optimum, assuming an optimum brood temperature of 31 °C. The range of incubation responses for boxes that were deemed to be actively brooding varied from < 1 to 9 °C and this positively correlated with increased syrup consumption. The non-zero intercept of the syrup consumption-vs-brood temperature relationship demonstrates that there is an underlying energetic cost of sustaining worker bees and incubation adds a further demand for energy on top of this.

Whilst there was a large variation in total syrup consumption between microcolonies in the experiment, microcolonies that fed more sustained a higher average brood temperature. There are several possible explanations for the variance in feeding rate and incubation performance. Workers in some microcolonies could have had higher incubation response thresholds, that is, the perceived stimulus intensity required for an individual to start a task (Theraulaz, Bonabeau and Deneubourg, 1998). Individuals with higher incubation response thresholds may not have incubated at all during the experiment and so would require less caloric intake, which would explain the correlations between low syrup consumption and low average brood temperature observed in some boxes. Alternatively, microcolonies that fed and incubated less may have been less socially integrated. When a group of orphaned workers are established in a microcolony one of the workers normally establishes dominance, her ovaries develop and she begins to lay eggs (Klinger *et al.*, 2019). Meanwhile, the other worker bees begin to undertake tasks as they normally would in a queenright colony. In some microcolonies the strength of social organisation may be weaker meaning that some of the workers may not undertake colony tasks.

## Nectar Availability and Incubation Demand Could Affect Foraging Dynamics

To understand how the energetic demands of incubation in microcolonies could affect foraging dynamics, estimates of ‘rich’ and ‘poor’ environments can be used to assess colony viability based on experimentally derived estimates of thermal efficiency. In these estimations, the sugar content of nectar is assumed to be the same in the rich and poor environments, so that the only variable is the rate at which nectar can be returned to the colony. The sugar content of nectar is assumed to be 65 %, the same as the syrup fed to the bees during the experiment.

On average, microcolonies in the experiment required 0.247 g of sugar in order to maintain their brood each additional degree above the ambient temperature. A poor environment has forage consisting of *Monarda fistulosa*, with each foraging trip yielding 67 mg of sugar (Cruden, *et al.*, 1984). Trip duration in a poor environment is 82 minutes (Westphal *et al.*, 2006). A rich environment has forage consisting of *Delphinium nelsonii*, with each foraging trip yielding 110 mg of sugar (Hodges, 1985). Trip duration in the rich environment is 62 minutes (Westphal *et al.*, 2006).

Environment	Average trip duration (min)	Average nectar yield per trip (mg)	Forage hours per degree of brood temperature (h)
Poor ( <i>Monarda fistulos</i> )	82	67	7.7
Rich ( <i>Delphinium nelsonii</i> )	62	110	3.6

**Table 1.** Estimates of additional foraging time per degree of brood temperature based on observations of forage trip duration and nectar yield in comparatively rich and poor environments.

In this example, bees nesting in a poor environment require almost twice the foraging effort to achieve each additional degree of brood temperature compared to bees in the rich environment (**Table 1**). Although the microcolonies in the experiment may be poorly insulated compared to naturally occurring bumblebee nests, it is clear that both the thermal efficiency of the nest and the profitability of the landscape have a key influence on colony success. Under electrical heating, microcolonies required nearly 200 mW to maintain a brood lump 8 °C above ambient temperature. This is equivalent to the incubation effort of six to seven bees, so even in a rich environment this would be unachievable by a small colony of bees under realistic conditions, since it leaves no workers free to forage.

This 'running-cost' proportion of the energy budget may become larger depending on the degree of worker redundancy within the colony. Within the study of social insects, ideas of social organisation and division of labour are well-established. In studies of labour division, observations of an inactive caste of workers are common, for example in Free's (1955) study, worker bumblebees were classified into three roles; foragers, house bees (these exhibited behaviours that could also be called brood care) and bees that did not appear to take on either task. Importantly, the division of labour was found to be equal between the three categories. Redundant workers serve several functions within a colony. During periods of increased stress the reserve work force can be mobilised to maintain stasis within the colony, for example, during acute periods of cold, redundant workers with higher response thresholds may begin to incubate in order to maintain the brood at a suitable temperature (Gardner, Foster and O'Donnell, 2007). Additionally, models show that redundant workers allow colonies to persist for longer, by replacing fatigued or deceased workers (Hasegawa *et al.*, 2016).

### Electrical Simulation Indicates an Incubation Power Output of More than 30 mW per Bee.

The hottest boxes in the bee experiment averaged between 8 °C and 9 °C above ambient. The electrical simulation experiment shows that this requires a power output of approximately 200 mW (**Fig. 7.**). Assuming in the hottest box all bees were incubating almost all of the time (food was *ad libitum* so time spent foraging is likely negligible), the power output of each of the six bees would be 33.3 mW, which agrees with the previous estimate by Livesey *et al.* (2019).

## Conclusion

The generation and retention of heat within bumblebee nests is highly important in determining success and fecundity. Bumblebees have evolved mechanisms both individually and socially to control the generation and retention of heat in their nests. This study establishes the paradigm of incubation effort totalling heat loss and isolates the first of these mechanisms with respect to a bumblebee colony, namely radiative heat transfer. Modelling shows that the maximum values for radiative heat loss may account for up to a third of the incubation effort of a microcolony. While small, this shows that heat loss via radiation may be non-trivial, however, nesting substrate conductivity could influence the rate of radiative heat loss.

The microcolony system has some limits in its comparability with wild bumblebee nests. In a wild bumblebee nest, insulating material may be gathered in the nest cavity, trapping a layer of warmer air and material around the brood. This would reduce the immediate temperature difference between the brood and the surrounding nest, thus reducing the direct rate of radiative heat loss from the brood to the substrate. Further investigation into the mechanisms of heat loss from bumblebee colonies will allow a more complete picture of the energy budget of bumblebee colonies.

## Chapter 3: The Significance of Insulation and its Consequences for Colony Fecundity in Bumblebees *Bombus terrestris*

### Abstract

Declines in bumblebees and other pollinating insects continue to cause concern due to their important roles in pollination of crop plants and wild flower communities. Field trials have highlighted how colonies perform differently in urban environments compared to agricultural environments, the availability of flowers and floral nectar could be a key factor in determining bee abundance. However, the energetic and time costs of incubation are affected by both the climatic conditions and the thermal efficiency of nesting substrates. A complete understanding of the efficiency of heat transfer in bumblebee nests is required to understand the density and availability of nectar sugars required to support successful bumblebee colonies.

This study investigates whether heat loss by convection and conduction is relevant to the thermal budget of a colony and whether it could affect colony fecundity. To test how insulation influenced the thermal efficiency of small nests, small groups of orphaned worker bumblebees in softwood boxes were provisioned with different types of insulation material. The effect of the different types of insulation on thermal efficiency within the colonies was quantified by the average brood temperature and the associated carbohydrate (sugar syrup) consumption.

Electrical heating apparatus was used to quantify the efficiency of different types of insulating materials in microcolony boxes. Developmental and demographic models were used to predict the effect of temperature on growth rate in bumblebee colonies and how this could affect reproduction.

Insulation treatments increased average brood temperature by less than a degree, whilst modelling indicated that reduction in average brood temperature by a degree could result in a 10 % decrease in the number of new queens produced by the colony. These findings demonstrate how the thermal properties of a substrate could reduce colony energy demand by limiting heat loss, and how shortfalls in incubation could negatively affect reproductive success.

Both changes in land use and the influence of changing climate patterns on nectar availability from wild flower species could therefore alter the viability of bumblebee colonies due to both the incubation status of the colony and the availability of sugar from the surrounding landscape. Relatively small shortfalls in incubation appear to potentially have large impacts on queen production. The findings reported here suggest that this demographic amplification could be highly consequential when studying the effects of landscape attributes on the performance and population viability in nesting bumblebees.

## Introduction

### Shortfalls in Incubation

Incubating worker bumblebees generate approximately 35 mW of heat energy each (Livesey et al., 2019), so as the number of worker bees in the colony grows, it is plausible to suggest that the total amount of heat energy the colony is capable of generating also increases. In order for the colony to grow at an optimum rate, the brood must be maintained close to the optimum developmental temperature, which is dependent on the rate that nectar sugars, which fuel incubation, can be gathered from the surrounding environment. In order for a colony to generate sufficient heat throughout the season, the landscape surrounding the colony must provide resources, both spaciouly and temporally, that supply nectar sugars at a sufficient rate.

A bumblebee colony could experience incubatory shortfalls for a number of reasons, here incubatory shortfall is defined as the brood temperature dropping below optimum for an extended period of time.

Three plausible scenarios in which a colony could enter incubatory shortfall are:

#### **Acute Power Deficit**

In this scenario, a drop in the ambient temperature of nest substrate means that the power required to heat the brood to an optimum temperature is more than the total incubatory power of the workers available. For example, an acute temperature drop could be the result of a late spring frost. Even if forage resources may be rich and nectar stores full, the power output of the colony is limited by the number of workers that have been produced. Consequently, further drops in ambient temperature cannot be mitigated as there are no more worker bees that can be recruited to the task. This results in a temporary drop in brood temperature until ambient temperatures warm.

#### **Weather-Driven Acute Starvation**

Although bumblebees are generally considered to be more robust than other nectavores, foraging can still be disrupted by the weather. The number of foraging trips are reduced during rainfall (Peat and Goulson, 2005) and in general bumblebees tend to forage less in inclement weather (Free and Butler, 1959). When weather conditions inhibit foraging for more than a day or so it is likely that the colony will begin to enter starvation. Under these conditions, the behaviour of bees changes. Under starvation, bumblebees will cease incubation tasks and also show a weaker response to nest invading parasites (Carter and Dill, 1991). Providing the landscape has suitable nectar resources, foraging will resume once the weather improves. As



nectar is returned to the colony incubation can resume. Incubatory shortfalls such as these may cause large temperature deficits, but they are likely to occur over relatively short timespans.

### **Landscape-Driven Chronic Power Deficit**

An incubatory shortfall can occur when a landscape has poor forage resources for all or part of the growing season. As resources in the landscape become sparser more bees will start to forage in order to maintain a sufficient rate of nectar supply to avoid starvation. If resources are poor enough then the incoming nectar will be insufficient to maintain optimal incubation effort. At this point the number of incubating bees is reduced because some must switch to foraging tasks. This results in the thermogenic power output of the remaining incubating workers decreasing below the incubation demand, so the colony enters in an incubatory shortfall. While actively foraging (i.e during the daytime), maintaining an optimum thermogenic output becomes energetically unaffordable due to the scarcity of nectar resources in the landscape. This type of sustained incubation shortfall could cause delays in larval development, potentially slowing growth to such a point that the colony does not produce any reproductives before the end of the colony season.

### **Convective and Conductive Heat Loss**

Convective and conductive heat loss share a key difference to radiative heat loss, convection and conduction rely on the transfer of energy between colliding particles whereas radiant heat energy is transferred in the electromagnetic spectrum, requiring no physical contact between particles. In any substance above absolute zero, molecules vibrate at a level determined by their kinetic energy. When materials of differing temperatures come into proximity, collisions occur between these molecules. Collisions between molecules on this scale are elastic, meaning that the total kinetic energy of the system is conserved. In effect, as a hotter molecule, which moves faster, collides with a cooler, slower molecule, kinetic energy is transferred to the cooler molecule, causing it to vibrate faster.

Convection refers to instances where heat energy is transferred via a fluid, either liquid or gas. Convection can be separated into two categories: forced and natural. 'Forced convection' is where a fluid is actively driven across a surface and heat is exchanged at the boundary layer between fluid and the surface (Jaluria, 1980). For example forced convection occurs in the cooling system of a car where air is forced through the panels of a radiator by the movement of the car through the air. Bumblebees have been observed to use fanning behaviours to cool

their brood in response to high temperatures (Weidenmüller, Kleineidam and Tautz, 2002), in this case forced convection is used to regulate high brood temperatures.

'Free (or natural) convection' relies on the establishment of buoyancy driven circulation patterns (Jaluria, 1980). This is where heat warms a fluid at the interface between the two materials and as the fluid warms it become less dense. The surrounding cooler, denser fluid then pushes the warmer molecules up as a rising column. Global convective circulation patterns known as 'Hadley cells' drive weather patterns and winds. In bumblebee nests, free convection will establish with the heat generated by incubation driving circulation within the nest cavity.

### Importance of Insulation, Convection and Conduction in Bumblebee Nests

The importance of heat loss by these factors is likely to vary because nest site selection varies between bumblebee species. For example, of the U.K *Bombus* species, *B. terrestris*, *B. lapidarius* and *B. ruderatus* tend to prefer underground nest sites while *B. hortorum*, *B. sylvarum* and *B. muscorum* will nest on the soil surface (Kells and Goulson, 2003). Nest site choice almost certainly has significant implications for the relative magnitudes of conductive and convective heat loss from the colony. This general division between subterranean and surface nesters raises several key questions about the differences in thermal efficiency of nesting between the two environments.

Ground-nesting species have a tendency to seek out and occupy the old nests of small rodents, which are often already furnished with insulating materials. Subterranean nesting presents several benefits. Nest temperature will be buffered to some degree from large changes in climate, as the ground will act as a 'thermal store', resisting external temperature changes. Amongst ground-nesting species, there are more specific preferences relating to the nature of the nest site; *B. terrestris* and *B. lapidarius* tend to favour a long entrance tunnel to their nests while species like *B. hortorum* require only a few inches (Free and Butler, 1959). Differences in nest architecture, such as tunnel length, will have implications for convective heat loss and ventilation within the nest and hence, will influence the incubation effort required and demand for nectar sugars of the colony.

Surface and tree-nesting may be more suitable in warmer climates, where risk of exposure to extreme low temperature is small. A colony of *B. pullatus* observed in the Costa Rican rainforest was found to nest in a banana tree approximately 5.5 m above the forest floor. The nest itself was constructed from banana leaves held together by a mixture of soil and wax, with an opening approximately 10 cm across and an estimated volume of one or two litres. The nest was too well ventilated to allow the use of ether in collecting and counting the colony

and its brood (Janzen, 1971). This degree of ventilation would allow for a significant amount of convective heat loss. In warmer climates thermal efficiency may be a much weaker factor in nest site selection. Average temperature in these regions is around 25 °C, meaning that that colonies only face a small temperature deficit from optimum and therefore, are likely to be far less limited by incubation demands than more temperate species.

The arctic species *B. polaris* is found on tundra meadows within the arctic circle, where summer air temperatures can drop below 5 °C at night (Richards, 1973). Despite this, *B. polaris* constructs nests on the soil surface, therefore forgoing any insulative benefit of ground-nesting. There may be several reasons why *B. polaris* opts to surface-nest in such a challenging thermal environment. One reason why surface nesting may be preferable is that the nest will receive some degree of solar heating, which may be particularly valuable in the arctic where spring and summer daylight hours are particularly long. Subterranean nesting may be unfeasible for *B. polaris* as the soils of tundra meadows are saturated in water from the melting permafrost on which they sit (Brown *et al.*, 1980). *B. polaris* has been observed to form nests from organic matter, such as mosses and lichens, that can be suspended above the brood by a wax canopy produced by the bees. These nest construction behaviours may be more heavily relied upon in areas where soil nesting is unsuitable. The use of lichens and mosses as insulation likely provides a feasible alternative to soil-nesting, with regards to minimising heat loss through forced convection. Evidently, *B. polaris* has evolved to manipulate both the generation and management of heat within its nests. The nesting behaviours of *B. polaris* in such a cool environment demonstrates the significance of insulation in reducing heat loss from the nest, allowing the colony to grow and reproduce.

A behaviour that appears common amongst almost all bumblebee species is their ability to form canopies of wax over their brood. These canopies be constructed from wax and other organic matter. Heinrich (1979) reports that a colony of *B. occidentalis* tore apart a piece of filter paper and mixed the fibres with wax to build a canopy over the brood. Colonies reared at higher temperatures (25 °C) produce smaller areas of wax canopy covering compared to colonies reared at cooler temperatures (20 °C). Colonies reared at higher temperatures were also found to exhibit fanning behaviours with increased frequency (Holland and Bourke, 2015). The formation of a wax canopy over the brood probably reduces convective heat loss by trapping warm air close to the brood, which will also reduce the rate of direct convective heat loss at the boundary layer as the difference in temperature between the brood and air will be smaller.

Bumblebees have developed physical and behavioural adaptations that allow them to generate and retain heat within their nests. The maintenance of suitable nest temperature is implicit in

the growth and survival of bumblebee colonies. Different species have developed a range of life history traits to maximise heat retention within their nests. These traits will have high adaptive significance if maintenance of nest temperature has a strong effect on colony fecundity. However, the functional significance is not yet well understood.

## Aims and Objectives of the Study

The purpose of this study was to determine the ecological significance of convective and conductive heat loss in colonies of bumblebees by:

- A)** Determining the relative importance of conductive and convective heat loss in bumblebee microcolonies, using two different types of insulation.
- B)** To quantify the impact of insulation on the thermal efficiency of bumblebee microcolony boxes.
- C)** To use developmental and demographic models to quantify the adaptive significance of nest insulation by estimating impact on colony growth and fecundity.

## Methods

### Provenance and Bee Husbandry

Throughout the experiment, queenright colonies of *Bombus terrestris audax* were obtained from a commercial supplier (Koppert B.V., Rodenrijs, Netherlands). Colonies were housed in a controlled temperature room (CTR) (25 °C +/- 2° C, humidity approximately 40 %) to establish, prior to exposure to cooler temperatures of approximately 21 °C. Throughout the experiment bees were fed *ad libitum* with sugar syrup (Attraker; Koppert B.V., Rodenrijs, Netherlands).

### Microcolonies

In total, 42 microcolonies were established, each containing 6 worker bees. Microcolony boxes were constructed from soft wood and had dimensions 160x160x45 mm. Each box was divided into two equal halves with a small hole allowing the bees access between the two sides and a clear plastic lid. One side was a brooding compartment containing an 8 g pollen ball made from powdered pollen mixed with sugar syrup. In order to record brood temperature each pollen ball had a K-type thermocouple (RS Components, Corby, UK) embedded in the middle. The pollen ball was formed from a dough made from ground pollen mixed with a small amount of sugar syrup. The other side of the box had three 2 mL centrifuge tubes drilled with small holes and filled with sugar syrup for feeding.

### Treatment Design

In order to study the effects of insulation on microcolony brood temperatures and syrup consumption, microcolonies were established with three different insulation treatments. The two insulation treatments were applied separately so that the relative individual effect on brood temperature compared to the uninsulated treatment was clear. This is important as it separates conductive heat loss to the substrate from convective heat loss within the nesting cavity. This allows a comparison of the adaptive significance of nest site selection and nest insulation behaviours as two separate behaviours, influencing the thermal efficiency of bumblebee nests.

#### Treatment One: Uninsulated

The standard rearing set up for microcolonies of bumblebees in the lab was considered as an 'uninsulated' treatment. This consists of a softwood microcolony box with a Perspex lid. Additionally a square of corrugated cardboard and a slightly larger square of felt are placed on top of the Perspex. This is both to help retain heat and protect from light disturbance.

### Treatment Two: Insulation by Cotton Wool

To reduce the magnitude of convective heat loss, cotton wool was selected as an insulating material. Commercial colonies are normally supplied with a layer of cotton wool, which the bees often use to form a canopy over their brood. After the bees had established some brood, the colony lids were removed and microcolonies were supplied with a 1.5 g ball of cotton wool that had been previously removed from a commercial colony box. These boxes were also covered with one piece of corrugated cardboard and one piece of felt.

### Treatment Three: Insulation by Polystyrene

To reduce magnitude of heat lost through conduction, the microcolony boxes were placed on top of a sheet of polystyrene 60 mm thick. This insulated the bottom of the microcolony box and reduced conduction of heat to the shelving that the microcolonies are kept on. An additional sheet of felt was placed over the lid of boxes in this treatment to reduce heat loss from the top of the box. This treatment provides insulation for the bees that they cannot modify and so only tests the effect of an insulating substrate.

### Establishment of microcolonies and exposure to temperature stress

Microcolonies were housed in an underfloor heated, controlled temperature room (25 °C +/- 2 °C) and left to establish for 3-5 days. After this time, brooding was confirmed by visual examination for the presence of brood cells and incubation was confirmed by measurements of brood temperature. Once brooding had been established the microcolony was supplied with insulation material, which depended on the treatment group it had been assigned to. In order to account for any differences between commercial colonies, microcolonies derived from each commercial colony were divided equally between the three treatment groups. Microcolonies were randomly assigned treatments within each cohort.

To expose the microcolonies to an increased incubation deficit, the heating system was switched off and the room left to cool to room temperature. The heating was switched off at 15:20 on the 23<sup>rd</sup> of May and between the 27<sup>th</sup> of May and the 7<sup>th</sup> of June the room temperature was maintained between 21.2 °C and 22.2 °C.

In order to measure the effect of insulation on average brood temperature, the brood temperatures of the microcolonies were measured two to three times each day. In order to measure differences in sugar demand between treatments, feeders were filled with sugar syrup and weighed into and out of each box. Three 2 mL centrifuge tubes were drilled with 1 mm holes and inserted through the side of each microcolony, these acted as syrup feeders. Feeders were changed regularly, at least every 48 h, and the microcolonies did not consume

enough syrup to empty the feeders during this time. So syrup was available *ad libitum*. Both average brood temperature and total syrup consumption were used to compare the effect of each treatment.

## Theoretical Modelling

Models can be used to better understand how social insect colonies function. A demographic model can show how population structures change in response to different perturbations. In order to show how changes in development rate, due to rearing temperature, could influence colony demographics, both a developmental and demographic model were combined.

## Models of Temperature Dependant Insect Development

Temperature is a key factor in determining the developmental rate of insects. Several different types of model have been proposed that describe the relationship between rearing temperature and development time in insects. The simplest of these models accounts for both the accumulated time and temperature above the developmental threshold temperature. This degree-day concept is based on the accumulation of enough time-temperature units in order to complete each developmental stage. Although, relatively simple degree-day models have been used to good effect to predict the emergence times of insect pests (Ahmad and Ali, 1995). These models are linear and so do not account for non-linear changes in development rate at the extremes of temperature.

Other models have been proposed that account for non-linear response in development rate, at the extremes of the developmental temperature range. These are called curve-linear models and are especially relevant when modelling the growth rates of social insects, as they provide a better estimate of how small reductions in temperature near the optimum development temperature could affect development rate (Lamb, 1992). This is particularly relevant for social insects such as bumblebees, where nest temperatures can be held near-optimum for the majority of the season and is less dependent on ambient temperatures. For this reason, a curve linear model was selected as most appropriate.

In order to estimate the growth rate of bumblebee colonies an existing model was parameterised using estimates of the minimum and maximum development temperatures for bumblebees. A model of insect development proposed by (Briere *et al.*, 1999) was selected. It uses the minimum and maximum temperature thresholds for development and an empirical constant to produce a curve linear relationship between rate of development and temperature.

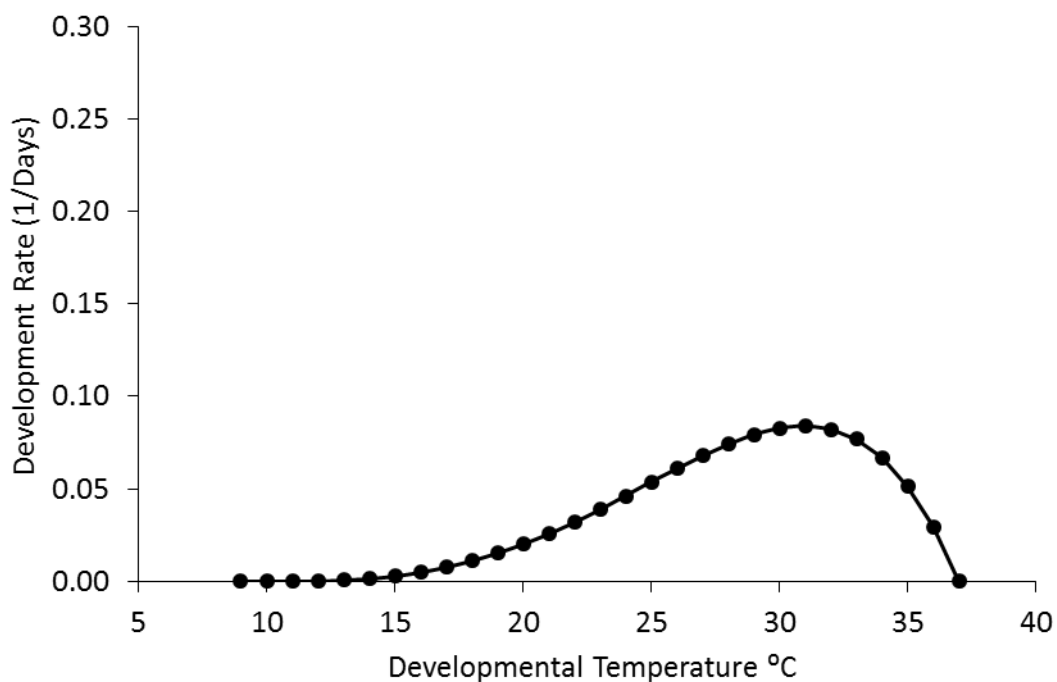
In order to produce a plausible developmental curve for bumblebee larvae, the Briere model, in the form presented by (Shi *et al.*, 2016) was used so that:

$$r = aT(T - T_1)(T - T_2)^{1/2}$$

**Eq. 7.**

Where  $r$  is developmental rate at a given temperature  $T$ ;  $a$  is an empirical constant ( $2.7 \times 10^{-4}$ );  $T_1$  is the minimum development temperature (11 °C) and  $T_2$  is the maximum developmental temperature (37 °C). This yields the developmental curve presented in **Fig. 9**.

### Demographic Model



**Fig. 9. Briere model curve of bumblebee brood development rate with respect to temperature.** Model is parameterised with hypothetical values. Axis scaled to allow comparison to **Fig. 15**.

In order to assess the effect of changes in colony development rate on overall colony fecundity (queen and male production), a demographic model of a colony of *B. terricola* was used (Cresswell, 2017). This is a matrix population model that predicts changes in the demographic structure of the colony overtime. In the original publication, the model is used to compare the predicted effects of two stressors, neonicotinoid pesticide (imidacloprid) and predation of worker bees by Crab spiders, on colony demographics. In order to do this, the model contains several parameters that can be adjusted to reflect the impact of different stressors on colony



dynamics. For example, the fecundity of the queen can be changed to reflect experimentally observed effects of a stressor.

In this study, the model is used in conjunction with a hypothetical developmental curve to estimate how nest temperature could change the number of new queens and males produced by a colony. In order to do this the fecundity detriment was calculated at one degree temperature intervals between 11 °C and 35 °C. An assumption made in this model is that reductions in colony fecundity over the course of a colony season are comparable to a reduction in the fecundity of the queen. This is based on the idea that, in a colony maintained below optimum developmental temperature over the length of the colony season, the effect on the colony demographic as a result of reduction in peak colony size, due to increased developmental time for each successive brood, will manifest itself in the same way that direct stressors inhibit the queens fecundity, hence reducing the amount of brood she lays. This approach allows the consideration of percentage reductions in developmental rate as detriments to colony fecundity and convert this to a loss in potential queen and male production.

The temperature dependant fecundity detriment was calculated using the following equation, so that:

$$Fecundity\ Detriment = 100 \times \left( 1 - \frac{r_T}{r_{max}} \right)$$

**Eq. 8.**

Where  $r_T$  is the rate of development at a given temperature and  $r_{max}$  is the maximum modelled rate of development.

Estimates of the difference in development rate between insulated and uninsulated boxes based on their average temperature were inputted into this model, to demonstrate the adaptive significance of insulation.

## Quantifying Thermal Efficiency using Electrical Simulation

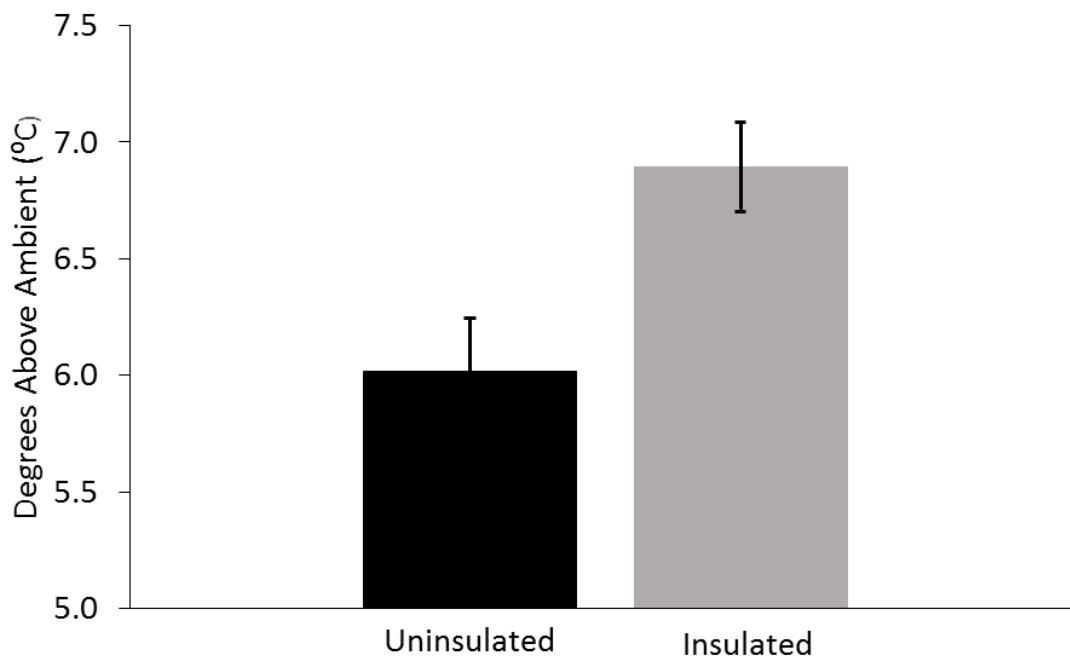
To quantify how each insulation treatment affected the power required to heat a microcolony, electrical heating apparatus was used to generate power/temperature curves for each treatment. As in the radiative heat loss experiments, a 22  $\Omega$  resistor was embedded into the

surface of each pollen ball and connected to a variable voltage power supply. When the current is switched on, the resistor generates a known amount of heat energy depending on the voltage across it. The effect of insulation on pollen ball temperature was tested across a range of bee-realistic power outputs, by varying the voltage across the resistor. In order to make comparisons with experimental microcolonies, 8 g pollen balls with K-type thermocouples, embedded as described previously, were used. The three treatments were the same as in the initial insulation experiment with bumblebee microcolonies. Cotton wool was carefully removed from the microcolonies and used for the electrical simulation as the bees had added their own wax and shaped a canopy from the cotton wool that had been supplied. In order to generate similar equilibrium temperatures to those that were observed in the initial bumblebee microcolony experiment, a range of power outputs between 40-220 mW were tested. This represents a power output of approximately 1-7 bees according to the per worker incubation power estimate of 35 mW (Livesey *et al.*, 2019). In order to quantify the difference in thermal efficiency at one realistic power output, the effect of insulation on the brood lump equilibrium temperature at a power output of 160 mW was tested separately. Preliminary work had shown that this was approximately the power required to maintain a box at 6.5 °C above ambient temperature.

## Results

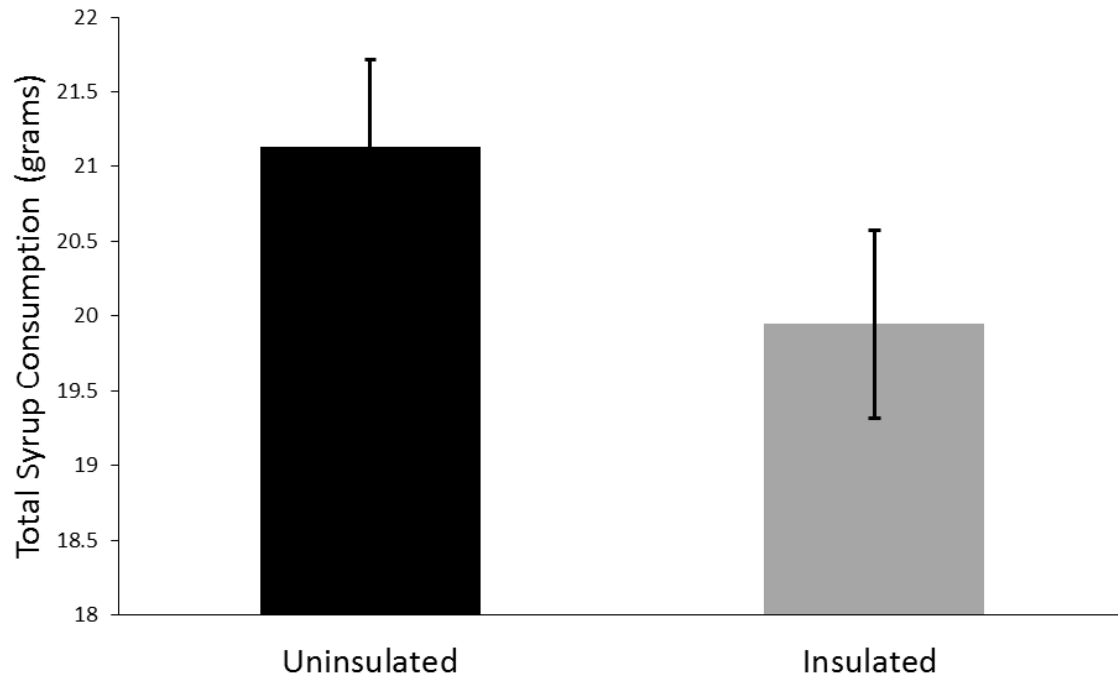
### Microcolonies

When the cotton wool and polystyrene insulation treatments were pooled, a significantly higher average brood temperature was found in the insulated treatment (mean = 6.9, S.D = 0.87), when compared to the uninsulated treatment (mean = 6.0, S.D = 0.73) (independent samples t-test,  $t_{30} = 2.8$ ,  $P < 0.05$ ). There was no detectable difference in mean brood temperature between the two insulation treatments.



**Fig. 10. Average brood temperature (degrees above ambient) for insulated (grey, N=22) and uninsulated (black, N=10) boxes. Error bars show one standard error.**

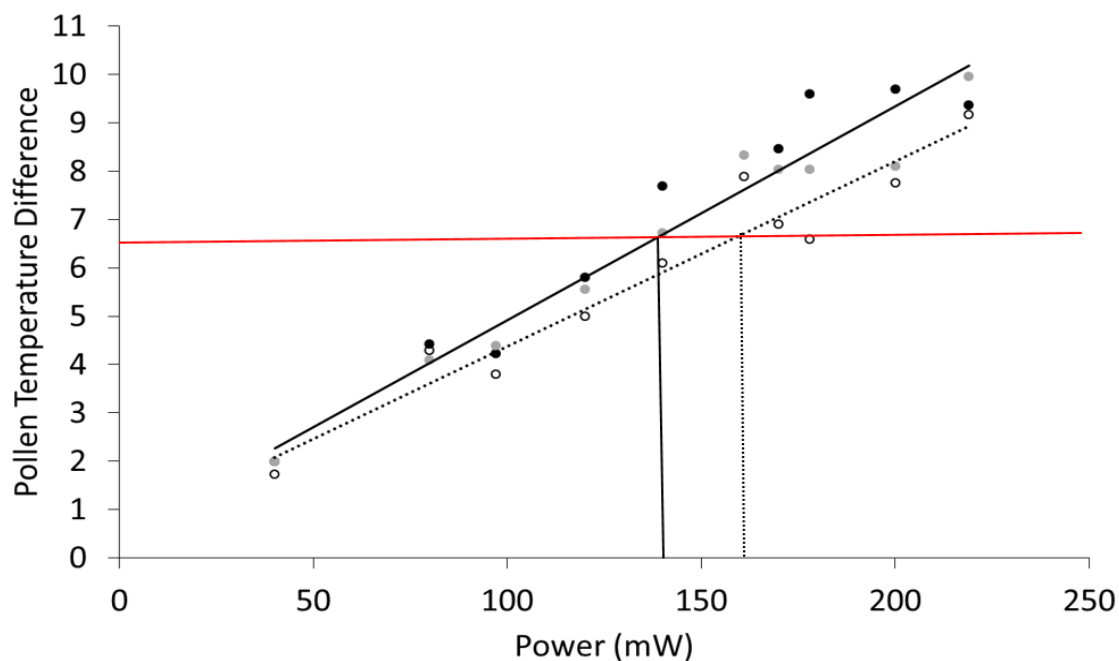
Average total syrup consumption per microcolony over 250 h was not significantly different between uninsulated (mean= 21.1, S.D = 1.83) and insulated boxes (mean = 19.9, S.D = 2.94) (independent samples t-test,  $P > 0.05$ ,  $t = 1.17_{30}$ ).



**Fig. 11. Average total syrup consumption in microcolonies.** Uninsulated control box (black, N=10) and Insulated boxes (grey, N=22). Error bars show standard error.

## Electrical Simulations

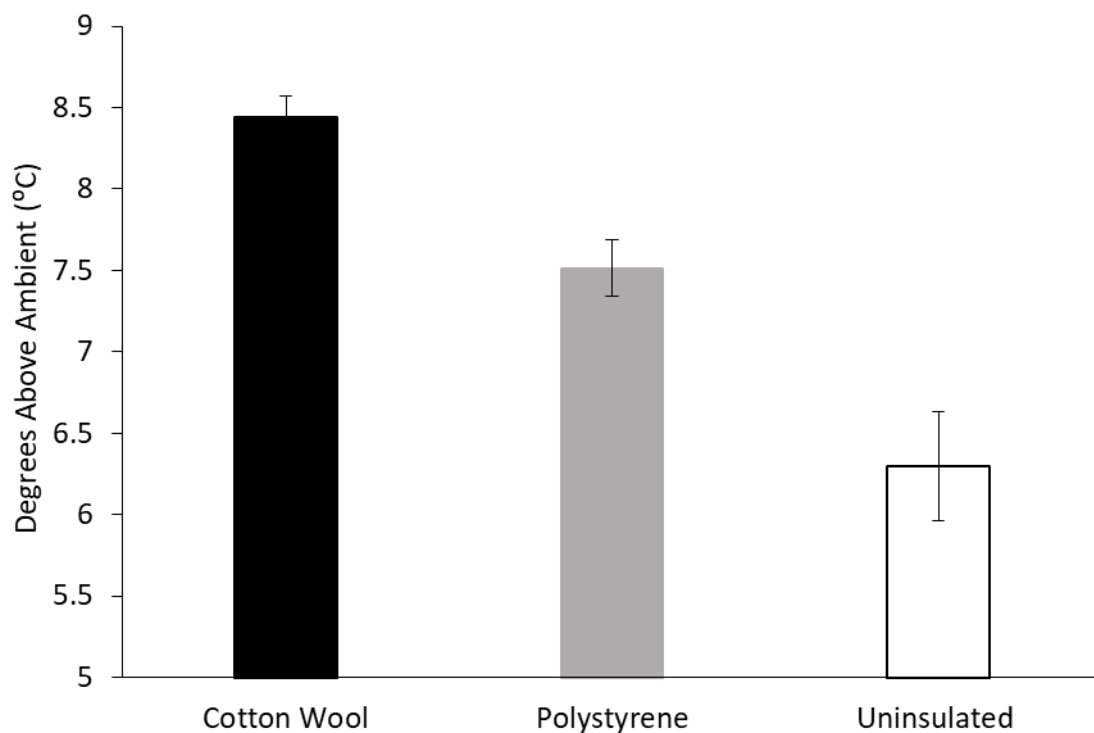
In the electrical simulation of brood incubation, brood lump temperature increased proportionally with power (ANCOVA,  $F_{1,29} = 332$ ,  $P < 0.01$ ) and the heating effect did not depend on insulation (ANCOVA,  $F_{1,29} = 0.06$ ,  $P > 0.05$ ). Brood temperature was set to 6.5 °C, as this was approximately the average temperature above ambient achieved by microcolonies in our bee experiment, regardless of treatment. This yields the following power demands: insulated colonies require a power input of 155 mW while uninsulated require an input of 136 mW, a difference of 19 mW.



**Fig. 12. Equilibrium temperature of microcolony boxes heated with a 22  $\Omega$  resistor at a range of power outputs.** Black (cotton wool) and grey (polystyrene) points show the two insulation treatments, open points show uninsulated treatments. Trend lines are fitted to the uninsulated boxes (dashed) and to the insulated boxes (solid), together as one data set. The red line indicates 6.5 °C pollen lump temperature, the average observed across all boxes during the bee experiments. The vertical solid and dashed lines shows the estimated power required to generate 6.5 °C of pollen ball heating in insulated and uninsulated boxes respectively.

In order to test for small differences in thermal efficiency between insulation treatments, heating was repeated multiple times at the 160 mW power level. Cotton wool and polystyrene insulation treatments sustained higher equilibrium temperatures than the uninsulated boxes and there was no significant difference in equilibrium temperature between cotton wool and polystyrene insulation (one-way ANOVA,  $F_{2,12} = 16.1$ ,  $P < 0.05$ ).

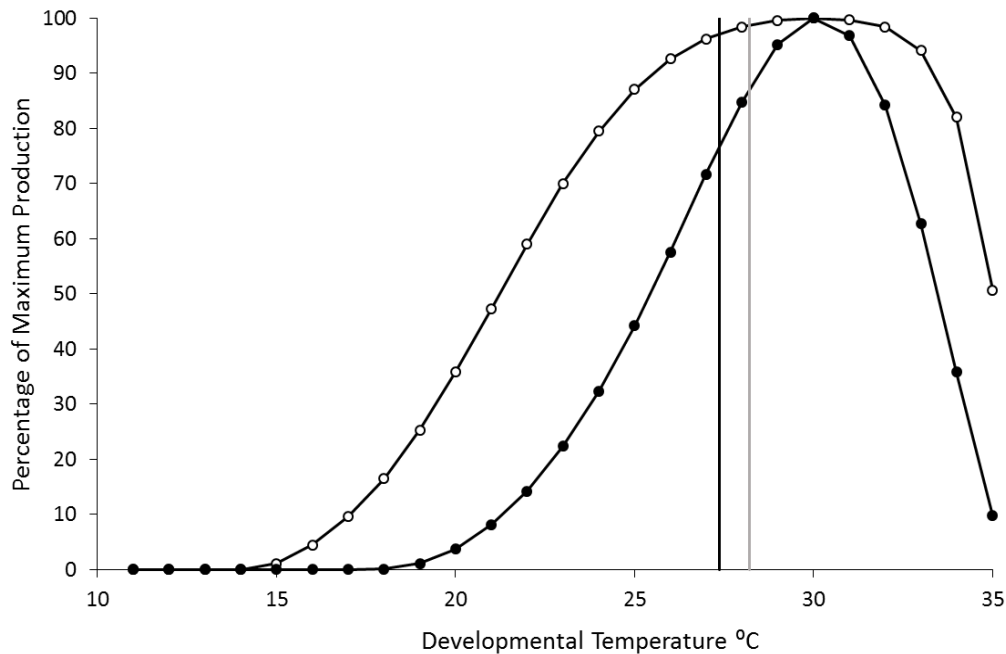
*A priori* contrasts indicate that the mean temperature above ambient for cotton wool (mean = 8.4, S.D = 0.29) and polystyrene (mean = 7.5, S.D 0.38) were significantly higher than the mean temperature above ambient for uninsulated boxes (mean = 6.3, S.D = 0.92) ( $t_{4,6} = 3.96$   $P < 0.05$ ). Additionally, the average temperature of cotton wool boxes was significantly higher than polystyrene boxes ( $t_{7,39} = 4.32$ ,  $P < 0.05$ ).



**Fig. 13. Equilibrium temperatures of pollen balls supplied with 160 mW of power.** Cotton wool (Black, N=5). Polystyrene (Grey, N=5) and Uninsulated (White, N=5). The cotton wool was removed from a microcolony used in the insulation experiment, it weighed 2.2 g and was approximately 2 cm thick when laid over the pollen ball. Both the cotton wool and the uninsulated boxes had a piece of cardboard and a piece of felt on top. A 60 mm thick square of polystyrene was placed underneath a microcolony box with a piece of cardboard and two layers of felt on top of the box. Each treatment was set up in the same way as it had been in the original microcolony experiment.

## Modelled Effect of Temperature on Rate of Colony Growth and Reproduction

Combination of developmental and demographic models shows divergence between queen and male production with changes in developmental temperature.

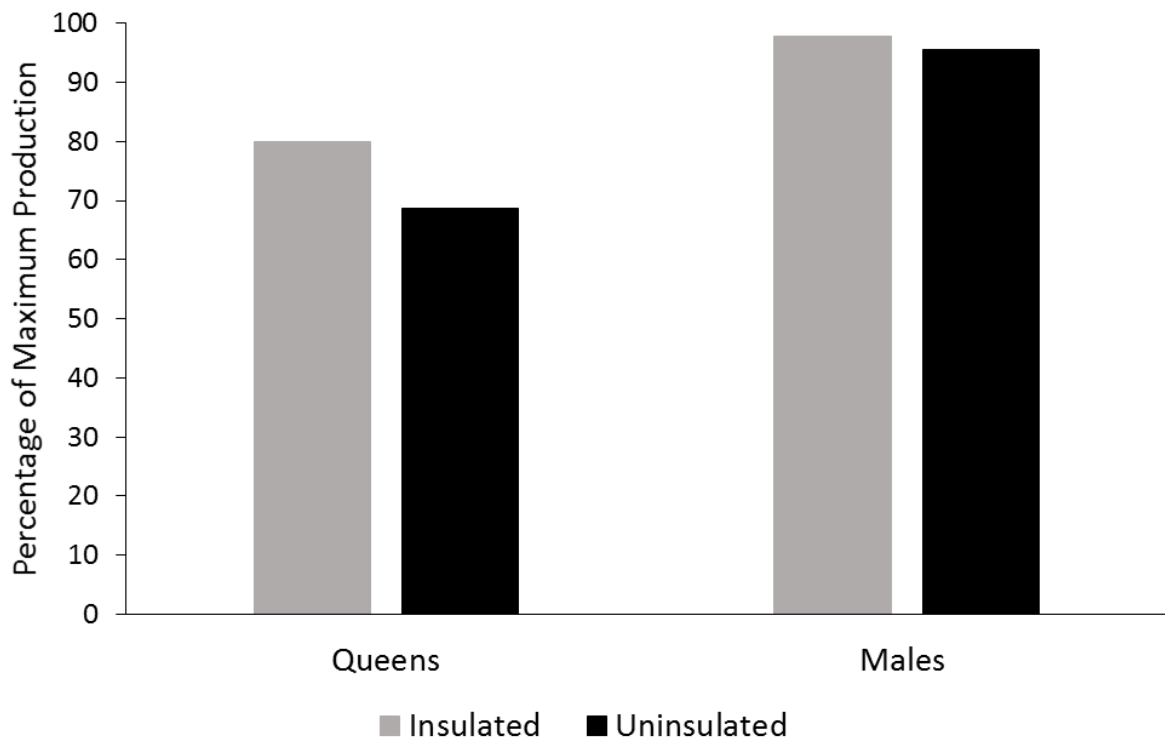


**Fig. 14. Modelled queen and male production in colonies of *B. terricola* at a range of developmental temperatures.** Males are represented by open points, queens are represented by closed points. The black and grey vertical lines represent the average brood temperatures of uninsulated and insulated colonies respectively.

Between 22 °C and 30 °C, the relationship between brood temperature and developmental rate is nearly linear; in this range each degree below optimum reduces queen production by approximately 10 %.

The average brood temperature for insulated microcolonies was 28.2 °C and for uninsulated colonies, was 27.4 °C. As an example, the developmental model shows that developmental rate in insulated microcolonies would be 6 % higher relative to optimum.

When the differential eclosion rate due to insulation was applied to the demographic model the elevated developmental rate of insulated colonies was projected to result in queen production being 11.4 % higher than uninsulated boxes, relative to a colony under optimum conditions.



**Fig. 15. Modelled reproductive output of insulated and uninsulated colonies.** Reproductive output is presented as a percentage of the maximum modelled reproductive output of a colony with no fecundity detriment.



## Discussion

At a thermal equilibrium, the incubation effort of a colony will be equal to the rate of heat loss by convection, conduction and radiation. The experiments in Chapter One show that the magnitude of radiative heat loss in microcolonies of bumble bees is relatively small and experimentally undetectable. Therefore, the majority of heat loss must be through convection and conduction. This study confirms that the magnitude of convective and conductive heat loss could have a significant effect on the thermal equilibrium of a nest and that insulation reduces the rate of heat loss, raising equilibrium temperature.

The results presented here suggest that there are two key physical factors that influence the ability of a bumble bee colony to maintain an optimum brood temperature:

### The Thermal Challenge

The thermal challenge is the difference between the ambient temperature of the environment and the optimum development temperature of the bees. The thermal challenge experienced by bees varies significantly. For example, in the arctic, ambient temperature may drop to near freezing overnight, exposing colonies to a thermal challenge of near 30 °C, whereas tropical species may only experience a thermal challenge of 5-6 °C.

### The Thermal Efficiency

The thermal efficiency is a reflection of the energy required to maintain a given equilibrium temperature in a nest or nesting substrate. For example, the uninsulated microcolony boxes used in the experiments require 160 mW of heating power to sustain a brood temperature approximately six degrees above ambient.

The interaction of these two factors determines the incubation effort required to maintain an optimum brood temperature.

### Insulation Increases Average Brood Temperature in Microcolonies.

Assuming an optimum brood temperature of 31 °C, microcolonies were exposed to a thermal challenge of 10 °C below optimum. Experimental microcolonies were unable to maintain optimum brood temperature with their given insulation and thermogenic capacity (**Fig. 10**). Previous work had shown that under a reduced thermal challenge of approximately 6 °C, microcolonies of six bees are able to sustain a brood temperature of near 31 °C (Livesey *et al.*, 2019). The microcolony boxes used by Livesey *et al.* were of the same construction as the uninsulated boxes used in this study, which demonstrates that under reduced thermal challenge, the rate of conductive and convective heat loss can be matched by six incubating

bees, allowing them to achieve a near optimum brood temperature. When exposed to the lower ambient temperature and hence larger thermal challenge of this experiment, the incubating effort of six bees establishes a lower average equilibrium temperature of around 27 °C. In both this study and in previous work by Livesey *et al.* microcolonies maintained their brood approximately 6 °C above the ambient temperature. However, due to the lower ambient temperature of 21 °C in this experiment microcolonies experienced an incubatory shortfall, failing to maintain their brood at an optimum temperature.

The results from this experiment demonstrate that the addition of insulating materials increases the thermal efficiency of the microcolony boxes. Insulated microcolonies established and maintained higher average brood temperature than the uninsulated microcolonies. The addition of insulation to the microcolonies allows a higher equilibrium temperature to be reached where the rate of heat loss is equal to the rate that heat can be generated by the incubating bees. While exposed to a 10 °C thermal challenge, none of the microcolonies were able to sustain their brood temperatures near optimum. No difference in syrup consumption was detected between insulated and uninsulated microcolonies (**Fig. 11**), suggesting that the microcolonies ability to sustain an optimum brood temperature was being limited by the thermal efficiency of the nest and the maximum incubatory power available to them. This is most similar to the 'Acute Power Deficit' scenario, the key difference being that microcolonies were exposed to relatively low ambient temperatures for an extended period of time. This suggests that nest site selection by queens could have strong adaptive significance as the thermal efficiency of the nest site is particularly important during the early stages of colony development when incubator power output could be limited, due to the number of worker bees.

### Modelling Suggests that Nest Insulation Boosts Colony Fecundity

Average brood temperature has a strong effect on the larval development time in insects. Over the course of the colony season, small differences in average brood temperature could result in large decreases in both queen and male production of a colony. Modelling in *B. terricola* shows that the insulation treatment could have increased developmental rate of larval bumblebees by 6 %. The increase in colony growth could increase queen production by 11.4 % relative to optimum (**Fig. 15**). Male production is not significantly effected as at 26 °C, 90 % of maximum male production is achieved and for each additional degree up to the optimum temperature only small increases in male production are predicted (**Fig. 14**). This model is based on data from colonies of *B. terricola*, which is a ground nesting species, and therefore, there may be differences in the response in other species. However, this is a first estimation of the potential impact of incubation shortfall on colony fecundity.

The production of queens and males does not follow the same trajectory; male production begins to increase at lower average brood temperatures (**Fig. 14.**). A colony that is struggling to sustain a brood temperature near optimum may have an overall reduced output of reproductives, with a sex ratio strongly favouring males. It has been previously observed that some bumblebee colonies fail to produce any queens and instead produce only males, possibly due to nutritional state (Pelletier and McNeil, 2003). The allocation of workers to foraging tasks in order to avoid starvation may then limit growth due to shortfalls in incubation as well as by protein requirement for larval development. Another driver of the bias towards male production is the relative energetic cost of rearing males versus queens; on average the dry weight of queens is twice as much as males (Duchateau and Velthuis, 1988), so equal nutritional investment leads to more numerous males.

The response in queen and male production to brood temperature demonstrates how populations could be limited by shortfalls in incubation as a result of reductions in floral resources. Shortfalls caused by reduced nectar availability, termed landscape-driven chronic power deficit, could be a strong driver of bumblebee declines in agricultural landscapes. Semi-natural grasslands support pollinators (butterflies and bumblebees) at higher densities than neighbouring agricultural areas, however these semi-natural areas may act as pollinator sources for agricultural areas (Öckinger and Smith, 2007). Bumblebee colonies placed in agricultural landscapes fail to maintain any nectar stores for the majority of the growth period, they also fail to produce any gynes (Samuelson *et al.*, 2018). It may be the case that colonies that enter prolonged incubation shortfall fail to keep up with reproductive timings and so produce few or no queens before the end of the colony season.

The demographic model (**Fig. 14**) demonstrates the strength of the relationship between queen production and brood temperature in *B. terricola*. If this applies to other species, such as *B. terrestris*, then the strength of this effect may have been overlooked in field trials measuring bumblebee colony fecundity under stress conditions and in different environments. For example, in a field trial testing the impacts of thiacloprid on colony fecundity in flower-rich and flower-poor environments, colonies located in flower-rich areas did not have increased fecundity and queen production was low in all treatments (Ellis *et al.*, 2017). This lack of queen production may have been due in part to climatic conditions at the field sites, but temperature was not considered when locating colonies within the environment and as has been shown here, a difference of even one or two degrees in brood temperature can have significant impacts on queen production. The strength of the effect of rearing temperature on fecundity is important to consider when locating colonies for study in the field. Increases in the thermal efficiency of nests due to insulation could have large effects on colony fecundity and so nesting behaviours may have high adaptive significance.

## Nest Thermal Efficiency could Impact Carbohydrate Demand

Electrical simulation of nest heating investigated the potential differences in power demand required to maintain a fixed temperature between insulated and uninsulated treatments. In one experiment, empty microcolonies boxes with each of the three treatments were left to reach a thermal equilibrium at power outputs between 40 and 220 mW (**Fig. 12**). No significant difference in trend was observed between the three insulation treatments. However, in a subsequent experiment, microcolony boxes were heated at the 160 mW power level. Multiple replicates (n=5) showed that the cotton wool and polystyrene insulation treatments established significantly warmer equilibrium temperatures (**Fig. 13**). This suggests that insulation treatments do increase thermal efficiency however, the effect of insulation of equilibrium temperature is not detectable at lower heating power.

In order to demonstrate how differences in nest thermal efficiency may influence foraging demand, the trend lines for each treatment from the thermal efficiency trend experiment (**Fig. 12**) were used to estimate changes in carbohydrate demand and foraging. The two insulated treatments (cotton wool and polystyrene) were considered as one group, with the uninsulated boxes making a separate group. Trend lines were fitted to each group and used to estimate the difference in incubation power required to sustain a brood temperature 6.5 °C above ambient.

The difference in estimated difference in heating power requirement between the uninsulated and insulated boxes was approximately 19 mW. Over the course of 24 h, a power output of 19 mW is equivalent to 1642 J of energy. The energy content of sugar is approximately 16600 J g<sup>-1</sup> (Weast and Astle, 1983). Therefore an estimate of the additional mass of sugar required to maintain a brood lump 6.5 °C above ambient temperature in uninsulated colonies was 99 mg day<sup>-1</sup>. Had the microcolonies been foraging for their nectar instead of it being provided *ad libitum*, this extra demand in sugar would have resulted in additional foraging activity. Foraging reward data from two different plants species can be used to examine how availability of floral resources can affect the foraging effort required to maintain sufficient brood incubation.

*Delphinium nelsonii* is a variety of larkspur that bumblebees are known to forage on. *D. nelsonii* provides an estimated carbohydrate reward per trip of 110 mg (Hodges, 1985), and a rate of 10.6 µl min<sup>-1</sup>. *Monarda fistulosa* (wild bergamont) is also a known nectar source for bumblebees, however it yields lower amounts of carbohydrate per trip, 67 mg at a reward rate 2 µl min<sup>-1</sup> (Cruden, Hermanutz and Shuttleworth, 1984). Considering *Delphinium* as a 'rich' environment and *Monarda* as a 'poor' environment, an additional carbohydrate requirement of 99 mg would result in 0.9 additional foraging trips in a rich environment and 1.5 additional trips in a poor one. Considering the capacity of an average workers honey crop to be 100 µl

(Heinrich, 1979b), it would take 50 min to fill a honey crop when foraging on *Monarda* and only 10 min when foraging on *Delphinium*. The additional time cost in a rich environment is approximately 10 min, however due to the low reward rate of *Monarda*, the additional time cost in a poor environment could be 75 min. *Monarda* is colloquially known as bee balm and is generally considered an attractive species for foraging bumblebees, and therefore, *Delphinium* may be an unusually rich forage resource. Nonetheless, the difference in both reward rate and total sugar yield between these two plant species illustrates how forage resources can impact the colony dynamics, specifically how a poor environment requires more hours of labour to meet the sugar demand of a colony. The increased demand for foraging could result in shortfalls in incubation, negatively affecting colony growth and reproduction.

The effect of landscape richness on colony growth and reproduction has been demonstrated in field trials. Where richness is defined by the rate at which sugar can be gathered from it, both the type of plants and their density within the landscape contribute to the richness of the landscape. In a study conducted by Westphal *et al.* (2006) to simulate rich and poor environments, colonies were placed in either close proximity (500 m) or further away (2900 m) from a mass flowering nectar resource. Foraging trip duration was recorded. Colonies located further from the floral resource had foraging durations 24 % longer and gained considerably less weight compared to colonies located in closer proximity to resources (Westphal, Steffan-Dewenter and Tscharrntke, 2006). It is clear then that in a poor environment, more foragers would need to be mobilised in order to acquire sufficient nectar resources required to fuel an increased incubation demand. In turn, this could lead to incubation shortfall in the colony if too few workers are left to provide sufficient incubation of the brood.

Differences in thermal efficiency between nesting substrates impact the sugar demand of the colony. The impact of increased sugar demand on the colony can be amplified depending on the richness of the landscape. Allocation of workers to meet increased foraging demand could push the colony into incubation shortfall which has significant negative implications for colony growth and fecundity.

### Nesting Behaviours could have High Adaptive Significance

The two insulation treatments tested were designed to limit heat loss in different ways. The polystyrene treatment limited heat loss through conduction from the microcolony box, while the cotton wool treatment limited convective heat loss from the brood to the air within the box. These conditions represented the potential effects of two behaviours exhibited by bees: the first being nest site selection, determined by the queen at the beginning of the colony season and the second being insulation behaviours, where bees construct nest insulation from both

organic matter and secreted wax. These results show that both of these behaviours can significantly increase the thermal efficiency of a nest and that this could result in large differences in fecundity, suggesting that they could be strongly adaptive. Bumblebees exhibit a wide range of nesting preferences, generally separated into ground and cavity nesters. Preferences for nest site must depend on both the physical environment and climate.

Soils can have several properties that make them desirable as a nesting substrate. Nesting in soils buffers the diurnal temperature fluctuations and the degree of buffering increases with depth (Parton and Logan, 1981). Soil temperature measurements from short grass prairies in Colorado, USA show that in July, soil surface temperatures peaked above 40 °C, but at 10 cm depth, peak temperatures were near 30 °C. Here soil nesting would protect from high summer temperatures that may be lethal to bumblebee broods and it is likely that the soil provides some protection from late spring frosts. Although soil nesting may seem ideal, it is not always feasible. For example, *B. polaris*, which establishes colonies within the arctic circle, is a surface nesting species. The tundra meadows *B. polaris* feeds on grow on top of permafrost, and summer melting makes these soils waterlogged meaning that subterranean nesting is unfeasible. Instead *B. polaris* forms surface nests, well-insulated with mosses and lichens (Pape, 1983). The construction of a well-insulated nest may be a behaviour with high adaptive significance; bumblebees can learn behaviours such as the manipulation of a piece of string from a small minority of innovators within their colonies (Alem *et al.*, 2016), which could be highly relevant in the development of nest building behaviours within a colony.

Not all bumblebees nest exclusively underground. *Bombus hypnorum* is an invasive species to the UK (Goulson and Williams, 2001) and has been rapidly expanding its distribution, as a tree nesting species it forgoes the benefits of soil nesting. Although surface and tree nest species do not benefit from the thermal stability of the soil they may benefit from warmer summer surface temperatures and radiant heat incident upon them directly from the sun. Surface nesting species most likely experience temperature fluctuations of greater magnitude and rate than those underground. Surface nesting species may then trade off thermal stability in the nest for the energetic benefits of solar heat or other benefits such as protection from flooding and predation by large mammals. A nest of *Bombus pullatus* observed by Janzen (1971) in the Costa-Rican rainforest had several structural traits that may reflect the thermal challenge of that environment. The nest had notably large openings that should render it a poorly insulating structure, however the colony appeared to be in a strong state. Average temperatures in the area are around 25 °C and so the colony is exposed to a very small thermal challenge. In these warmer areas inhabited by bumblebees thermal efficiency may be a weaker driver of nest site selection than factors such as flooding and predation risk.

## Conclusion

These findings demonstrate how physical differences in bumblebee nest structure may increase the thermal efficiency of the nest. This allows a colony to maintain a higher average brood temperature while sugar demand remains the same. Developmental models have demonstrated the potential effect of incubation shortfalls on bumblebee colony fecundity, notably how queen production is particularly sensitive to chronic shortfalls in incubation. This demonstrates the potential for a mechanistic link between shortfalls in incubation and reductions in colony fecundity. Findings from modelling show that a one degree drop in brood temperature can have a severe impact on colony reproduction. This indicates that it is plausible that shortfalls in incubation, caused by sugar scarcity, could result in declining numbers of bumblebees, particularly in agricultural areas where sugar resources provided by mass flowering crops can be more transient. Further work is required to determine the sugar density requirements of successful colonies in a field setting. However, findings shown here demonstrate the potential adaptive significance of nesting behaviours in bumblebees and how heat loss from a nest can be reduced by the addition of insulation to increase thermal efficiency.

## Chapter 4: Thesis Conclusion

The ecological and economic significance of pollinating insects is clear due to pollination services they provide to both natural and agricultural ecosystems (Gallai *et al.*, 2009) . Declines in insects have been associated with a range of anthropogenic drivers including reductions in floral resources (Goulson *et al.*, 2015), however, clear mechanistic links have yet to be established. Temperature is a key factor determining rates of growth and development in insects (Wigglesworth, 1939). Bumblebees maintain their brood well above ambient temperatures by generating heat within the nest (Schultze-Motel, 1991), this is a metabolically demanding process. Nectar sugars from the surrounding environment fuel the process of incubation within the bumblebee colony and shortfalls in the supply of nectar to a colony cause worker bees to stop incubating and the brood temperature to fall (Carter and Dill, 1991).

Since the maintenance of brood temperature within bumblebee colonies is so important for their growth and development, it is important to understand how the transfer of heat within bumblebee nests could influence their demand for nectar sugars. Energy in the form of heat is transferred by three mechanisms: convection, conduction and radiation. In order to investigate how the different mechanisms of heat loss influenced the ability of worker bees to maintain brood temperature, a series of experiments were conducted.

No difference in average brood temperature or sugar consumption was detected in microcolonies with either a 'good' or 'bad' radiant barrier treatment, suggesting the magnitude of radiant heat loss may be small. Further work used observations of brood cell emissivity to estimate the maximum possible radiant heat loss from the brood of microcolonies used in the experiment. A model of the Stefan-Boltzmann equation demonstrated that the maximum possible radiant heat loss in experimental models was approximately 70 mW, however, this assumed that the surrounding substrate acted as a perfect absorber of radiant heat. The conclusion from these findings was that radiative heat loss from bumblebee brood structures makes up a small, but not insignificant proportion of the total heat loss from a colony.

Experiments demonstrated that insulation, both around the outside and within the brood compartment, increased average brood temperature. Cotton wool was placed within the nesting compartment, this tested the bumblebee's ability to modify insulation material in order to reduce heat loss from the nest, while in a separate treatment polystyrene blocks were placed beneath the microcolony boxes. These two insulation treatments were compared to a control treatment, consisting of a standard microcolony box with no additional insulation. Average brood temperature in the insulated boxes was nearly a degree higher, while there



was no detectable difference between the total syrup consumption in insulated and uninsulated boxes. This demonstrates how the addition of insulation both external and internal increases the thermal efficiency of microcolonies, allowing them to sustain a higher average brood temperature. This suggests that both nest site selection and nest insulation behaviours could be in important traits effecting the thermal efficiency of the colony.

Combination of a developmental and demographic model demonstrated the influence of shortfalls in incubation on colony fecundity. This highlighted to the sensitivity in queen production to relatively small drops in average brood temperature over the course of the colony season. This suggests that both nest site selection and nest insulation behaviours could have high adaptive significance. Since workers stop incubating under starvation, this is also evidence for a mechanistic link between reductions on floral resource availability and reduction in bumblebee colony fecundity.

The findings presented here demonstrate the importance of nesting behaviours with regards to bumblebee colony fecundity and suggest that they could have strong adaptive significance. Nectar resource scarcity in a landscape has the potential to limit reproductive success, bumblebee colonies that are poorly insulated will be more susceptible to this. Intensification of agriculture has the potential to reduce wildflower abundance in the United Kingdom and it is plausible to suggest that the changes in nectar availability could be partially responsible for the declines in bees and pollinating insects. To better understand the how reductions in nectar resources could influence bumblebee populations further work is required to fully understand the nectar requirements of successful bumblebee colonies.

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