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Ontogeny of worker body size distribution in bumble bee (*Bombus impatiens*) colonies

Margaret J. Couvillon^{1,2,*}, Jennifer M. Jandt^{1,*}, Nhi Duong³, and Anna Dornhaus¹

¹Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona, U.S.A.

²Laboratory of Apiculture and Social Insects, Department of Biological and Environmental Science, University of Sussex, Falmer, Brighton, U.K.

³Center for Insect Science, University of Arizona, Tucson, Arizona, U.S.A.

Abstract

1. Bumble bees exhibit worker size polymorphisms; highly related workers within a colony may vary up to 10-fold in body mass. As size variation is an important life history feature in bumble bees, the distribution of body sizes within the colony and how it fluctuates over the colony cycle were analysed.
2. Ten commercially purchased colonies of *Bombus impatiens* (Cresson) were reared in *ad libitum* conditions. The size of all workers present and newly emerging workers (callows) was recorded each week.
3. The average size of bumble bee workers did not change with colony age, but variation in body size tended to decrease over time. The average size of callows did not change with population size, but did tend to decrease with colony age. In all measures, there was considerable variation among colonies.
4. Colonies of *B. impatiens* usually produced workers with normally distributed body sizes throughout the colony life cycle. Unlike most polymorphic ants, there was no increase in worker body size with colony age or colony size. This provides the first, quantitative data on the ontogeny of bumble bee worker size distribution. The potential adaptive significance of this size variation is discussed.

Keywords

Bombus impatiens; bumble bees; polymorphism; size distribution; social insects

Introduction

In complex biological systems, individual components often interact to ensure the survival and the reproduction of the group (Maynard Smith & Szathmáry, 1995). These individual

components may be genetically identical, such as the cells that comprise a multi-cellular organism, or genetically dissimilar, as in the symbiotic relationship between eukaryotes and their mitochondria (Maynard Smith & Szathmáry, 1995). In complex systems, the whole may be greater than the sum of the parts, as different properties emerge from the interactions that would not be present at the individual level (Page & Mitchell, 1998; Sendova-Franks & Franks, 1999; Camazine *et al.*, 2001). Social insect colonies are considered complex biological systems (Bonabeau *et al.*, 1999; Fewell, 2003). Their within-colony genetic relatedness varies because colonies may be headed by either singly-mated or multiply-mated queens, which results in highly related or slightly related workers, respectively. The components that make up this system (i.e. members of a colony) may be broadly divided into the reproductive (queen/males) and non-reproductive (worker) castes (Hölldobler & Wilson, 2008), analogous to the reproductive and somatic cells within a multi-cellular organism.

Within the non-reproductive worker caste, there sometimes also exists further differentiation, analogous to the differentiated somatic cells of different tissue types. This differentiation within the non-reproductive worker caste may happen in several ways. In some insects, such as the honey bee (*Apis mellifera*), workers are similar in size and shape but differ in physiological attributes, like brain organisation, hormone levels and gene expression levels related to age (Robinson, 1987; Farris *et al.*, 2001; Ben-Shahar *et al.*, 2002; Whitfield *et al.*, 2003), all of which contribute to different behaviors (Lindauer, 1953; Seeley, 1982; Robinson *et al.*, 1994; Fjerdingstad & Crozier, 2006). In other insects, like some ants (Family: Formicidae), workers differ in more obvious morphological ways (Noirot & Pasteels, 1987; Hölldobler & Wilson, 1990; Stern & Foster, 1997), such as the dome-shaped head of some workers of the ant *Cephalotes* (Baroni Urbani, 1998; Powell, 2008) or the differently-sized workers within a colony of leaf cutter ants (Hölldobler & Wilson, 1990). All of the differentiations above contribute to variation in behaviours, which ultimately lead to division of labour (Beshers & Fewell, 2001) and to the functioning of the colony as a complex system. Morphological differences within the worker caste are especially interesting because they are produced by nutritional differences in the larval stage and fixed at eclosion, as most social insects are holometabolous (Couvillon & Dornhaus, 2009). Therefore, in order to examine morphological variation, it is imperative to study it at the colony level.

Bumble bee (*Bombus* spp.) workers, unlike other eusocial bees, exhibit a high degree of intra-colony morphological differentiation. That is, offspring of a singly-mated queen (in spite of a relatedness of 0.75) might differ by as much as 10-fold in their mass (Cumber, 1949; Plowright & Jay, 1968; Goulson, 2003). This size polymorphism is linked to bumble bee division of labour: smaller workers tend to feed and incubate brood and larger workers tend to forage, guard, and fan (Richards, 1946; Goulson *et al.*, 2002; Jandt & Dornhaus, 2009). Previous work has reported the distribution of bumble bee worker sizes for the species *Bombus terrestris* (Goulson *et al.*, 2002; Goulson, 2003). However, these descriptions were either derived from the distribution of all of the workers produced across the year, measured at the end of the colony cycle, or were collected on manipulated colonies at the end of the colony cycle (Couvillon & Dornhaus, 2010). Consequently, it is unknown

whether different-sized workers are actually present at the same time in the colony. Nor does this end-of-cycle measurement describe whether different-sized workers are produced as a cohort at the same time or merely accrue over time as a consequence of changes in nutrition across the colony cycle. In other words, does the worker body size distribution within a bumble bee colony change over time? Previous authors have suggested that worker body size does increase over time in some bumble bee species (*B. auricomus*, *B. griseocollis*, *B. fervidus*, and *B. perplexus*) (Sladen, 1912; Frison, 1927; Knee & Medler, 1965; Plowright & Jay, 1968); however, these data were anecdotal in nature and sometimes included examples to the contrary (*B. terricola* and *B. ternarius*) (Goulson, 2003). Clearly a detailed analysis of the size distribution over time is needed.

Here, the size distribution of workers of the bumble bee species *Bombus impatiens* was analysed. Between-colony variation, the effect of colony size, and the shape of the distribution were quantified. Importantly, these features were tracked over time and as population size (number of workers in the colony) changed. If the production of large workers is limited by the resources available to the colony, then under *ad libitum* feeding conditions, all workers produced should have a large body size, and body size may also increase as the population of workers increases.

Materials and methods

Study organism

Between September 2005 and August 2006, 10 queenright colonies of *B. impatiens* (Koppert Biological Systems, Romulus, Michigan) were obtained, and each was placed inside a wooden nest box with a Plexiglas cover to provide constant viewing of in-nest behaviour (see Jandt & Dornhaus, 2009 for details). Colonies were kept at room temperature and on a light:dark 8:16 h cycle. Although foraging behaviour has strong diurnal rhythms, brood care has weak or no diurnal rhythms (Yerushalmi *et al.*, 2006). Thus, larval feeding is performed around the clock and the effects of our laboratory light regime should neither have strongly influenced larval feeding behaviour in the nest nor our observations of the worker body size variation throughout colony development.

At the start of the experiment, colonies had 68 ± 27 workers. As the colonies used in our study were of commercial origin, the initial set of workers may have been comprised of mixed genetic ancestry. However, data collection began 2 weeks after colony set-up, a period after which bees that emerge are those raised by workers present. Bees had access to a foraging arena at all times, where they were provided with a sugary solution ('Bee-Happy', Koppert Biological Systems) and pollen *ad libitum*. When the colonies first arrived, all individuals were marked by gluing a numbered plastic tag ('Opalithplättchen') to the thorax. Afterwards, all newly emerging bees were marked and recorded on the date of eclosion. Thorax widths of workers, a standard measurement of body size in bumble bees (Goulson, 2003), were obtained *post mortem* using digital calipers to the nearest 0.01 mm. Any bee that died during the experiment was stored and measured as well. Rarely, we were unable to account for a bee, in which case it could not be included in the analysis; however, this was recorded and taken into account when estimating weekly population size. Large workers were readily distinguished from newly emerging gynes (i.e. virgin queens) as there

tends to be little overlap between the size of workers and gynes in this type of bumble bee (i.e. pollen-storers) (Goulson, 2003).

At week 1 of data collection, the queen's age was unknown; as such, data collection may have begun at slightly different times in the life-cycle for each colony. A bumble bee colony life-cycle includes a 'growth phase', when worker population size increases, and a 'senescent phase', when population size decreases (Cameron, 1989). Most colonies were early in their cycle, as all but three subsequently experienced a population size increase (Fig. 1).

These colonies were also used for other non-manipulative, observational studies. Therefore, the duration of time that data were collected on body size distribution varied between colonies. However, throughout these analyses reported here, no live worker bees were removed from the colonies. In some cases, the queen was removed once the colony began producing new reproductives, a signal that the colony was nearing the end of its annual cycle and entering into the senescent phase (Cameron, 1989). The point at which the colony became queenless is denoted in the figures.

Analyses

Statistics were done using Minitab (Student Version 14) and JMP (Version 7). Unless otherwise stated, averages are reported as mean $\pm$ standard error ($X \pm SE$). When analysing all 10 colonies separately, we used a Bonferroni corrected $\alpha = 0.005$ ($P = 0.05$, $k = 10$, $P/k = 0.005$) as our significance criterion. As each bee was individually marked and recorded daily, we knew which bees were alive and present in the colony each week. The degree to which the distribution of all adult worker body sizes per week was normal was analysed using a Shapiro–Wilk test.

Multiple linear regressions (MLR), with a block for colony-level effects, were used to determine whether average worker body size changed across colony age and/or different population sizes. The size of newly emerged bees (callows) per week was analysed separately. To understand within-colony patterns, linear regressions were calculated for each colony to determine whether average size of workers present in the colony and the size of newly emerging bees increased or decreased across colony age and/or different population sizes. To determine whether average size of all workers across colony age was better explained with a curvilinear fit, results from a second order regression were compared with those from the linear analyses. Furthermore, to determine whether there was a difference of worker body sizes present during the growth or senescent phase, we used linear regression to compare body size over time for each phase separately.

Finally, the standard deviation, degree of skewness, and degree of kurtosis were calculated to better understand the shape of the size distribution per week for each colony. The degree of skewness determines the presence and direction of a distribution's tail, while kurtosis determines how steeply the distribution curve is peaked. Multiple linear regressions, with a block for colony-level effects, were used to determine whether the shape of the distribution (standard deviation, skewness, and kurtosis) changed across colony age and/or different population sizes. Where there were significant interactions between colony level effects and

age or population size, separate linear regressions were calculated for each colony to identify within-colony patterns.

Results

Analyses of all bees in the colony

There were significant differences between colonies in average size of workers in the colony ($F_{9,892} = 12.80$, $P < 0.001$). Eight out of 10 colonies produced a normal worker body size distribution, as measured across the entire colony cycle (Table 1). This normality was maintained in 7 of the 10 colonies when worker sizes were examined weekly (Table 1). Colony populations were not manipulated, although steep declines in population size during the senescence phase of some colonies resulted in an overall significant population size decrease with colony age (see Fig. 1, black circles; MLR: $r^2 = 0.82$; colony differences: $F_{1,9} = 19.67$; $P < 0.001$; colony age: $F_{1,1} = 53.02$; $P < 0.001$; colony $\times$ age: $F_{1,9} = 8.34$; $P < 0.001$). As a result of the strong differences between colonies, the effects of population size were analysed separately from colony age.

Average body size of the workers present in the colony did not consistently increase or decrease with colony age (Fig. 1, grey circles; MLR: $r^2 = 0.11$; colony differences: $F_{1,9} = 45.75$; $P < 0.001$; colony age: $F_{1,1} = 1.96$; $P = 0.16$; colony $\times$ age: $F_{1,9} = 3.40$; $P < 0.001$), yet different trends were observed across colonies. One colony showed an increase in body size (F.06.03: $r^2 = 0.02$, $F_{1,736} = 15.92$, $P < 0.001$) and one showed a decrease in body size over time (F.05.02: $r^2 = 0.04$, $F_{1,344} = 15.17$, $P < 0.001$). There was no change in average worker body size over time in the other eight colonies ($P > 0.05$ for all other linear regressions). As there was no obvious monotonic trend, we repeated these analyses to determine if the data were more likely to follow a curvilinear fit (using quadratic regressions). In this case, there was again no significant relationship of worker body size with colony age in most (6 out of 10) colonies (F.05.02: $r^2 = 0.04$, $P = 0.001$; S.06.01: $r^2 = 0.10$, $P < 0.001$; F.06.02: $r^2 = 0.03$, $P < 0.001$; F.06.03: $r^2 = 0.02$, $P < 0.001$; $P > 0.03$ for all other colonies; see Fig. 1). In those colonies where the curvilinear fit was significant, the trend showed that body size tended to be lowest in the middle. As the curvilinear fit was no better at describing the data than the linear, we will focus on the linear regression in our discussion, as it is easier to interpret.

Changes in worker body size with colony age were also analysed separately during the growth and senescent phases of the colony cycle for each colony. The growth phase included the weeks until the peak in the population size (Fig. 1) and the senescent phase included the weeks after the peak in population size. There were no consistent trends across colonies with regards to the average body size during either phase (Table 2). In two colonies (S.06.01 and F.06.02), there was a decrease in average body size during the growth phase. In one colony, there was an increase in body size during the senescent phase (F.06.03), whereas in another there was a decrease (F.05.03). There were no significant trends in either phase in the other six colonies (Table 2).

Overall, average worker body size was lower with higher population size (Fig. 2; $r^2 = 0.89$; colony differences: $F_{1,9} = 45.41$; $P < 0.001$; population size: $F_{1,1} = 19.48$; $P < 0.001$; colony

× population size: $F_{1,9} = 16.98$; $P < 0.001$). However, when colonies were analysed individually, this was not consistent across colonies. There was a negative relationship between population size and average body size in three colonies (S.06.01: $r^2 = 0.92$, $F_{1,9} = 104.44$, $P < 0.001$; S.06.02: $r^2 = 0.69$, $F_{1,10} = 22.54$, $P = 0.001$; F.06.03: $r^2 = 0.97$, $F_{1,7} = 225.9$, $P < 0.001$), a positive relationship in one colony (F.05.03: $r^2 = 0.76$, $F_{1,9} = 28.65$, $P < 0.001$), and no relationship in the remaining six colonies (F.05.01: $r^2 = 0.11$, $F_{1,10} = 1.22$, $P = 0.30$; F.05.02: $r^2 = 0.13$, $F_{1,9} = 1.38$, $P = 0.27$; S.06.03: $r^2 = 0.38$, $F_{1,5} = 3.02$, $P = 0.14$; F.06.01: $r^2 = 0.25$, $F_{1,4} = 1.36$, $P = 0.31$; F.06.02: $r^2 = 0.68$, $F_{1,7} = 14.63$, $P = 0.006$; F.06.04: $r^2 = 0.20$, $F_{1,9} = 2.25$, $P = 0.17$).

Analyses of newly emerging bees (callows)

Overall, the size of callows produced within a colony decreased over time, although this pattern was not consistent across colonies (Fig. 3; MLR: $r^2 = 0.20$; colony differences: $F_{1,9} = 5.05$, $P < 0.001$; colony age: $F_{1,1} = 4.62$, $P = 0.03$; colony × age: $F_{1,9} = 2.14$, $P = 0.03$). There was a significant decrease in the average size of callows with colony age in only one colony when colonies were analysed separately (Fig. 3; S.06.01: $r^2 = 0.22$, $F_{1,41} = 9.34$, $P = 0.004$), but no significant change in callow size in the other eight colonies ($P > 0.02$ for all remaining colonies). Additionally, there was no evidence that population size affected the average callow size (Fig. 4; $r^2 = 0.35$; colony differences: $F_{1,9} = 1.30$, $P = 0.27$; population size: $F_{1,1} = 0.10$, $P = 0.75$; colony × age: $F_{1,9} = 0.43$, $P = 0.91$).

The shape of the worker size distribution

The degree of worker size polymorphism, measured here as the standard deviation of thorax sizes, varied across colonies and showed a general decrease with colony age (Fig. 5; MLR: $r^2 = 0.61$, colony differences: $F_{1,9} = 10.14$, $P < 0.001$; colony age: $F_{1,1} = 4.13$, $P = 0.05$; colony × age: $F_{1,9} = 0.91$, $P = 0.52$); however, this decrease was only significant in 4 out of the 10 colonies (F.05.02: $P = 0.006$; F.05.03: $P = 0.03$; S.06.02: $P < 0.001$; F.06.03: $P < 0.001$; for all other colonies: $P > 0.25$). On the other hand, the standard deviation increased with population size (Fig. 5; $r^2 = 0.72$; colony differences: $F_{1,9} = 14.96$, $P < 0.001$; population size: $F_{1,1} = 7.44$, $P = 0.008$; colony × population size: $F_{1,9} = 3.46$, $P = 0.001$). Again, however, when colonies were analysed separately, this positive relationship was only observed in two colonies (S.06.01: $P = 0.001$; F.06.03: $P = 0.003$); one colony exhibited a negative relationship (S.06.02: $P = 0.001$) and the other seven colonies showed no relationship using the Bonferroni-corrected $\alpha = 0.005$ (F.05.01: $P = 0.88$; F.05.02: $P = 0.04$; F.05.03: $P = 0.006$; S.06.03: $P = 0.06$; F.06.01: $P = 0.48$; F.06.02: $P = 0.02$; F.06.04: $P = 0.03$).

The degree of skewness also varied across colonies and became more negative over time (Figs 5 and 6; $r^2 = 0.83$; colony differences: $F_{1,9} = 28.49$, $P < 0.001$; colony age: $F_{1,1} = 7.45$, $P = 0.008$; colony × age: $F_{1,9} = 11.88$, $P < 0.001$), although this negative relationship was only observed in one colony when analysed separately (see Fig. 6: F.05.01: $P = 0.003$). In one colony there was a positive relationship (S.06.01: $P = 0.004$) and in the remaining eight colonies, there was no relationship (F.05.02: $P = 0.77$; F.05.03: $P = 0.15$; S.06.02: $P = 0.51$; S.06.03: $P = 0.08$; F.06.01: $P = 0.04$; F.06.02: $P = 0.43$; F.06.03: $P = 0.03$; F.06.04: $P = 0.03$). The distribution tended to be more positively skewed in larger population sizes (r^2

= 0.76; colony differences: $F_{1,9} = 16.14$, $P < 0.001$; population size: $F_{1,1} = 4.89$, $P = 0.03$; colony $\times$ population size: $F_{1,9} = 6.34$, $P < 0.001$), although this held true for only two colonies ($F_{06.01}$: $P = 0.002$; $F_{06.03}$: $P = 0.005$). One colony showed a negative relationship ($F_{06.04}$: $P = 0.001$) and the remaining seven colonies showed no relationship at all ($F_{05.01}$: $P = 0.03$; $F_{05.02}$: $P = 0.02$; $F_{05.03}$: $P = 0.19$; $S_{06.01}$: $P = 0.28$; $S_{06.02}$: $P = 0.69$; $S_{06.03}$: $P = 0.007$; $F_{06.02}$: $P = 0.74$).

Finally, there was no evidence to suggest that the degree of kurtosis changed over time ($r^2 = 0.35$; colony differences: $F_{1,9} = 1.97$, $P = 0.06$; colony age: $F_{1,1} = 1.56$, $P = 0.22$; colony $\times$ age: $F_{1,9} = 2.41$, $P = 0.02$) or as the population size increased ($r^2 = 0.46$; colony differences: $F_{1,9} = 2.23$, $P = 0.03$; population size: $F_{1,1} = 0.95$, $P = 0.33$; colony $\times$ population size: $F_{1,9} = 4.29$, $P < 0.001$).

Discussion

In the present study it was shown that the average size of worker bumble bees present in the colony, while different between colonies, does not consistently increase or decrease as the colony ages from its growth to its senescent phases. Overall, the worker sizes in a colony form a normal distribution and colonies exhibit significant variation in worker body size despite a standardised feeding regime. Although colony size was not manipulated to be independent from colony age, we found a statistical decrease in average worker size with increasing population size. However, this pattern was not consistent across colonies and different sizes of workers are produced throughout the colony cycle. Interestingly, the amount of variation among workers seemed to decrease with colony age, rather than increase, as is often the case for worker-polymorphic ant colonies. Similarly to ant colonies, however, variation may increase with population size. We conclude that worker size polymorphism in bumble bees is not necessarily a result of different-sized bees being produced at different stages in the colony life cycle, nor is food limitation a necessary factor in creating size polymorphism. This study is, to our knowledge, the first to describe this ontogeny of the worker body size distribution in a bumble bee colony.

Variation among workers in body size was maintained even though colonies were fed sugar and pollen *ad libitum*. This demonstrates that the maintenance of worker body size polymorphism is independent of variation in or limitation of available resources at the colony level, suggesting it may be adaptive. Colonies may be under selection to maintain worker polymorphism in *B. impatiens*. Alternatively, lack of effective organisational mechanisms to ensure equal care for all larvae may lead to worker polymorphism in bumble bees (Couvillon & Dornhaus, 2009).

It is also shown here that small colony size does not limit production of workers of a particular size; the average size of workers produced even decreased with colony population size in some colonies. This shows that production of small workers is not a result of an insufficient foraging force. Younger and larger colonies tended to show a higher degree of polymorphism among workers (higher standard deviation of average worker body size). These results are surprising as they run contrary to what is usually found in ants. Worker size polymorphism evolved many times within Formicidae (Davidson, 1978), and it has

been frequently found that the smaller-sized workers of a new colony give way to the larger, and sometimes more polymorphic, workers of a mature colony (Brian, 1952; Oster & Wilson, 1978; Herbers, 1980; Porter & Tschinkel, 1986). This was observed in ants of the genus *Pogonomyrmex badius*, for example, where not only do majors solely emerge late in the colony cycle, the average minor worker size also increases (Tschinkel, 1998). In contrast, no evidence was found that *B. impatiens* produce larger workers as the colonies grows or ages. Colonies may even contain more polymorphic workers when they are younger. In ants, there is a positive correlation between colony size and worker size in *Myrmecia* (Gray, 1971), *Pogonomyrmex* (Porter & Tschinkel, 1986), *Solenopsis* (Markin *et al.*, 1973; Tschinkel, 1988), and *Atta* (Wilson, 1983). Lastly, while worker size distribution in some ants might become more positively skewed over time (Wood & Tschinkel, 1981; Tschinkel, 1988; Wheeler, 1991), we found here that in bumble bees the distribution may become more negatively skewed as the colony ages.

Bumble bee colonies are annually founded by a queen that is typically singly-mated (Schmid-Hempel & Schmid-Hempel, 2000). The ultimate size that an adult worker reaches is not only determined by genetic factors, but by how much food is given to the developing larvae by adult nurses (Michener, 1974; Alford, 1975; Couvillon & Dornhaus, 2009). A normal colony cycle typically runs for several months, with the queen initially performing all colony tasks (nursing, laying, and foraging) until a small cohort of workers emerges (Sladen, 1912; Goulson, 2003). It is sometimes assumed that the first worker cohorts are significantly smaller than the bees produced later in the colony cycle, as resource abundance and population (i.e. available foragers) increase from spring to summer (Knee & Medler, 1965). Afterwards, the queen devotes herself to laying and the colony experiences a population explosion, where worker numbers will go from a few to possibly several hundred (Sladen, 1912; Cameron, 1989). Previous authors have suggested that worker body size increases over time in some bumble bee species (*B. auricomus*, *B. griseocollis*, *B. fervidus*, and *B. perplexus*) (Sladen, 1912; Frison, 1927; Knee & Medler, 1965; Plowright & Jay, 1968); however, these data were anecdotal in nature and sometimes included examples to the contrary (*B. terricola*, and *B. ternarius*) (Goulson, 2003).

How might bumble bees maintain body size polymorphism within individual colonies? Worker size polymorphism in bumble bees has been described previously (Knee & Medler, 1965; Plowright & Jay, 1968); however, most of this did not quantify size distributions. More recently, others have examined the size variation within (Goulson, 2003; Peat *et al.*, 2005b) and between (Peat *et al.*, 2005a) species, but this was less on the ontogeny of the distribution and more on the role of size polymorphism in the division of labour. Previous work has suggested that size variation in ants might be a result of limited food availability (Herbers, 1980; Rissing, 1987), as colonies, when resources are not limited, produce overall larger workers and fewer small ones. In contrast, we show here that in bumble bees, worker size variation is still present with unlimited resources. It is likely that the in-nest spatial organisation of bumble bee workers and larvae is the proximate mechanism maintaining variation in worker body size. This is because workers active as nurses tend to be found more in the centre of the nest, and larvae developing there receive more food, and thus develop into larger workers, whereas larvae in the periphery are fed less and develop into

smaller adults (Couvillon & Dornhaus, 2009; Jandt & Dornhaus, 2009). The spatial organisation of workers directly leads to variation in feeding rate over the nest surface and thus to worker size polymorphism (Couvillon & Dornhaus, 2009).

Why might bumble bees maintain a normal distribution of worker body sizes within individual colonies, instead of the highly skewed distribution usually seen in ants? Larger workers tend to be foragers and guards, and smaller workers tend to be nurses in bumble bee colonies (Jandt & Dornhaus, 2009), and one may expect that worker bees perform the tasks they are most suited for. Indeed, larger worker bees have been shown to be adept at foraging-related activities: they can bring back more nectar per time (Goulson *et al.*, 2002; Spaethe & Weidenmuller, 2002), are superior thermoregulators (Free & Butler, 1959; Heinrich, 1979), can see (Spaethe & Chittka, 2003) and smell (Spaethe *et al.*, 2007) better, and might learn better and remember longer (Worden *et al.*, 2005, although see Raine *et al.*, 2006; Raine & Chittka, 2008), all of which supports the hypothesis that size polymorphism is an adaptation for division of labour. There is, however, also evidence to suggest that larger bees are also better nurses, a task usually done by smaller workers (Cnaani & Hefetz, 1994), which is at variance with the hypothesis that size polymorphism is an adaptation for division of labour. However, if the large bees are simply higher-quality workers, then we would predict under *ad libitum* food conditions that colonies should rear disproportionately more large bees. In that case, we would expect to see a decrease in the variation of thorax widths when resources are not limited, because colonies are producing less small workers or an increase in the distribution skew, because colonies are rearing larger workers. We show here that worker size variation persists in *B. impatiens* even in the most resource-rich environments and the distribution skew is more likely to decrease, if it changes at all. Therefore, although the adaptive value of the production of bumble bee worker body size variation itself for division of labour remains unresolved, our results suggest that the maintenance of this variation may either be adaptive at the colony level for some yet undefined reason (Couvillon & Dornhaus, 2010) or it could simply be an epiphenomenon of unequal larval feeding within the nest. It is thus possible that the adaptive function of size polymorphism in bumble bees is quite different from that in ants.

Are there other adaptive explanations for size polymorphism that are independent of division of labour? It has been suggested that intra-colony variation might allow colonies to adapt more quickly to environmental conditions, as larger bees might forage better in cooler temperatures and smaller bees in warmer temperatures (Peat *et al.*, 2005a). However, although this has been applied to inter-specific variation in other organisms (Bergmann, 1847; Stillwell *et al.*, 2007), it remains unsupported in bumble bees (Couvillon *et al.*, 2010; Peat *et al.*, 2005a). Rather, perhaps size polymorphism in bumble bees might result from the trade-off of cheap, lower-quality workers and expensive, higher-quality workers. Future work should focus on cost-benefit ratio of different sized bees. Perhaps colonies with smaller bees actually consume less energy overall, although they are less efficient at collecting and processing that energy. Finally, workers actually perform a variety of tasks inside the nest in addition to feeding and warming the brood; however, these tasks are traditionally not included in division of labour analysis. It would be interesting to investigate

the efficiency of smaller workers performing these other tasks to give a better understanding of how and why bumble bees produce polymorphic workers.

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Fig. 1.

Average worker body size did not significantly change with colony age in *Bombus impatiens*, but larger colonies had on average smaller workers. Black filled circles represent the population size of workers in the colony; open circles represent the peak in colony population size; grey circles represent the average ($\pm$ SE) body size of all workers present in the colony each week. Regression lines are present when the relationship between average worker size and colony age was significant (Bonferroni's corrected $\alpha = 0.005$ was used). Vertical lines represent the point at which the queen was no longer present in the colony.

Fig. 2.

Average worker body size was overall negatively correlated with population size. However, this pattern was not particularly strong: different trends occurred across colonies. Regression lines are present when the relationship between average worker size and population size was significant for that colony (Bonferroni's corrected $\alpha = 0.005$ was used).

Fig. 3. Average body size of callows (newly emerging workers) decreased overall with colony age in *Bombus impatiens*. Black circles represent the population of all workers in the colony; grey circles represent the average ($\pm$ SE) callow body size each week. Regression lines are present when the relationship between average callow size and colony age was significant for that colony (Bonferroni's corrected $\alpha = 0.005$ was used). Vertical lines represent the point at which the queen was no longer present in the colony.

Fig. 4.

There was no relationship between average ($\pm$ SE) body size of callows and population size, either overall or when colonies were analysed separately. Each point represents the average callow size for one colony in 1 week.

Fig. 5.

The shape of the worker size distribution (in terms of standard deviation, skewness, and kurtosis) with colony age (left column) and with population (right column). Each symbol/colour combination represents a different colony.

Decrease Skew → --- → --- → **No Change** → --- → --- → **Increase Skew**

Fig. 6.

Thorax width frequency distribution across representative colonies with varying patterns of change in skew (decrease: F.05.01; no change: F.05.03; or increase: S.06.01 in skew).

Table 1

P-value results from Shapiro–Wilk tests of the null hypothesis that colonies produce a normal distribution of worker body sizes in the colony overall ('All'), and maintain a normal distribution of worker body sizes within the colony each week.

Colony	All	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10
F.05.01	0.251	0.183	0.247	0.260	0.221	0.321	0.405	0.511	0.548	1.00	1.00
F.05.02	0.827	0.152	0.210	0.339	0.365	0.696	0.968	0.871	0.989	0.920	0.920
F.05.03	0.049	0.698	0.231	0.110	0.209	0.454	0.488	0.462	0.028	0.062	0.885
S.06.01	0.043	0.575	0.324	0.169	0.029	0.026	0.012	0.121	0.168	0.333	0.305
S.06.02	0.0028	0.076	0.136	0.162	0.162	0.0004	0.006	0.004	0.006	0.013	0.013
S.06.03	0.0026	0.012	0.002	0.001	0.002	0.949	0.987	0.977	n/a	n/a	n/a
F.06.01	0.926	0.886	0.893	0.888	0.753	0.965	0.659	n/a	n/a	n/a	n/a
F.06.02	0.0003	0.703	0.096	0.0006	0.0001	0.0001	0.0003	0.0005	0.009	0.061	n/a
F.06.03	0.463	0.259	0.640	0.507	0.657	0.634	0.675	0.423	0.384	0.706	n/a
F.06.04	0.807	0.647	0.691	0.573	0.899	0.899	0.857	0.698	0.773	0.168	0.438

P-values represent the probability that the distribution of worker body sizes was not normal. All 10 colonies were analysed separately; therefore, Bonferroni's corrected $\alpha = 0.005$ was used as the significance criterion. Significant *P*-values are in bold. Seven of the 10 colonies demonstrated an overall normal body size distribution and maintained such a distribution through the colony cycle. 'N/A' represents weeks after colony death until week 10 for four colonies.

Table 2

Regression results for the relationship between worker body size and colony age (T_{age}) for each colony during their growth and senescence phases. Growth phase was defined as the time from week 1 until the peak in population size (represented in Fig. 1) and the senescence phase as the time that followed the peak in population size.

Colony	Growth phase			Senescence phase			
	N Wks	T_{age}	R^2	P	N Wks	T_{age}	P
F.05.01	3	-1.05	3.2%	0.300	8	0.96	2%
F.05.02	4	-0.91	0.6%	0.364	7	-2.39	2.9%
F.05.03	4	-0.10	0.0%	0.919	7	-3.01	5.2%
S.06.01	7	-3.61	6.0%	<0.001	4	2.07	7.3%
S.06.02	9	-0.92	0.3%	0.356	3	-0.00	0.0%
S.06.03	2	-0.61	0.4%	0.545	5	1.04	1.1%
F.06.01	2	-0.23	0.0%	0.822	4	0.12	0.0%
F.06.02	5	-4.09	3.8%	<0.001	4	2.33	1.7%
F.06.03	2	-0.38	0.1%	0.703	7	3.41	2.1%
F.06.04	3	-1.44	0.6%	0.151	8	-0.07	0.0%

N Wks represents the number of weeks included in the analyses. All 10 colonies were analysed separately; therefore, Bonferroni's corrected $\alpha = 0.005$ was used as the significance criterion. Significant regression results are in bold.