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Colony and individual life-history responses to temperature in a social insect pollinator

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Summary

1. Pollinating insects are of major ecological and commercial importance, yet they may be facing ecological disruption from a changing climate. Despite this threat, few studies have investigated the life-history responses of pollinators to experimentally controlled changes in temperature, which should be especially informative for species with complex life histories such as eusocial insects.

2. This study uses the key pollinator *Bombus terrestris*, a eusocial bumble bee with an annual colony cycle, to determine how temperature affects life-history traits at both individual and colony levels.

3. In two laboratory experiments, we reared *B. terrestris* colonies at either 20°C or 25°C, and measured differences in a set of life-history traits including colony longevity, queen longevity, worker longevity, production of workers, production of sexuals (queen and male production) and growth schedule, as well as effects on thermoregulatory behaviours.

4. Higher rearing temperature had a significant positive effect on colony longevity in one of the two experiments but no significant effects on queen or worker longevity. Higher rearing temperature significantly increased colony size but did not affect the timing of peak colony size. It was also associated with significantly higher queen production but had no effect on the production of workers or males or the timing of male production. Higher temperature colonies exhibited significantly more wing-fanning by workers and significantly less wax canopy construction. Hence an increase in rearing temperature of a few degrees increased colony longevity, colony size and queen production. However, individual longevity was not affected and so may have been buffered by changes in costly thermoregulatory behaviours.

5. We conclude that eusocial insects may show complex phenotypic responses to projected temperature increases under climate change, including effects on productivity and reproduction at the colony level. Such effects should be considered when predicting the impact of climate change on the provision of essential pollination services.

Key-words: pollination, climate change, bumble bees, colony cycle, phenology, thermoregulation

Introduction

The occurrence of global climate change (IPCC 2013) has created a pressing need to explore the role of temperature in the life history of animal and plant species. In extreme cases, large-scale perturbations in environmental temperature might be fatal in species that fail to adjust their life histories adaptively (Sinervo et al. 2010). It is especially important to consider the effect of temperature increases mediated by climate change in organisms that perform vital ecosystem services, such as pollinators (Brown & Paxton 2009; Vanbergen et al. 2013). Over a third of global food production is dependent on pollination (Kjøhl, Nielsen & Stenseth 2011), but many pollinators are in decline (Abrol 2012; Vanbergen et al. 2013). Several recent studies have explored the relationship between pollination and climate change by modelling or measuring changes in the phenologies of plants and their pollinators (Gordo & Sanz 2005; Memmott et al. 2007; Bartomeus et al. 2011; Gilman et al. 2012; Robbirt et al. 2014). These studies have led to the prediction that future climate trends could result in the disruption of current plant-pollinator interactions (Gordo & Sanz 2005; Memmott et al. 2007; Robbirt et al. 2014). One specific concern is that differential responses of plants and pollinators to temperature may cause a ‘mismatch' in their phenologies, resulting in

declines in plants, pollinators or both (Stenseth & Mysterud 2002; Visser & Both 2005; Miller-Rushing et al. 2010; Gilman et al. 2012).

However, while informative, these studies have not addressed the mechanisms underlying life-history responses of individual pollinator species, and so may overlook responses of relevant taxa to key variables such as temperature (Memmott et al. 2007; Visser et al. 2010; Bartomeus et al. 2011). Although the effects of temperature on insect life history have been studied experimentally in a number of cases (e.g. Calabi & Porter 1989; Sheehy 2002; Isitan, Gulel & Gunduz 2010), data are lacking on key pollinator species (but see Bosch, Kemp & Peterson 2000; Karlsson & Wiklund 2005). Notably, many of the most important pollinating insects are eusocial bees, e.g. honey bees, bumble bees and stingless bees (O'Toole 1993; Chapman & Bourke 2001), which have not been well studied in this context. Vogt (1986a) investigated the effects of rearing temperature on colony productivity and life history in the bumble bees *Bombus impatiens* and *B. affinis*. However, to our knowledge, no study has investigated the direct effects of temperature on life history variables in eusocial bees at both individual and colony levels. Such an approach is important because, in eusocial systems, the colony itself exhibits life-history characteristics such as growth, reproduction and decline (Wilson 1985; Starr 2006; Hou et al. 2010; Holland, Guidat & Bourke 2013). In turn, this means that environmental factors, such as temperature, could have effects on colonies different from those occurring in individuals. For example, a temperature change that reduces the longevity of individuals within a colony may not reduce the longevity of the colony as a whole.

Bumble bees (*Bombus* spp.) are ideal species in which to investigate the effect of temperature on individual and colony life history because they are key eusocial pollinators of both commercial crops and wild flowers (Goulson 2010). In temperate regions, bumble bees exhibit an annual colony cycle. Lone queens found colonies in spring by producing broods of

daughter workers. After a period of colony growth, queens produce new queens and males (sexuals), which leave the colony to mate with sexuals from other colonies. By the season's end, the old queen, workers and males have died, and the newly-mated queens have entered diapause to emerge post-hibernation as foundresses the following spring (Goulson 2010). However, variations on this life history exist in populations as a function of different climatic conditions. For example, in Mediterranean *B. terrestris* populations, queens aestivate during the warm dry summer rather than hibernating (Gurel, Gosterit & Eren 2008; Rasmont et al. 2008). Populations in the Mediterranean region, New Zealand and Tasmania also appear to have two colony cycles per year (bivoltinism; Donovan & Wier 1978; Buttermore 1997; Rasmont et al. 2005). In addition, there is evidence that bumble bee populations are already responding to temperature increases associated with climate change. Firstly, Bartomeus et al. (2011) documented trends for earlier (overwintering) queen and male emergence times in two North American bumble bee species in recent decades. Secondly, in the UK, there are growing records of winter-active *B. terrestris* queens and workers in recent years, with workers being observed up to two months later in the year than previously recorded (Robertson 1991; Edwards 2006; Farmer 2006; Goulson 2010; Stelzer et al. 2010; Stuart Roberts, personal communication).

During the current century, annual surface temperatures in Europe are predicted to increase with a 90-100% likelihood, with a disproportionate increase in extreme summer temperatures (IPCC 2013). For example, the annual average surface air temperature in 2080-2100 is predicted to be 1 to 5^{o}C higher than the 1986-2005 average over most of Europe (Collins et al. 2013; fig. 12.11). We therefore investigated the effect of increased temperature on the colony life history of the bumble bee *B. terrestris* (Linnaeus 1758), a common Palaearctic species. In two laboratory experiments, we manipulated the ambient rearing temperature of *B. terrestris* colonies and measured effects on colony longevity, individual queen and worker longevity and colony productivity. We also measured effects on workers' thermoregulatory

behaviour, i.e. wing-fanning (Heinrich 1979; Vogt 1986b; Weidenmuller, Kleineidam & Tautz 2002; Gardner, Foster & O'Donnell 2007) and extent of insulating wax canopy construction over the nest comb (Heinrich 1979). Winter activity of *B. terrestris* colonies in the UK could arise from either colonies persisting beyond the usual end of the colony cycle, or new colonies being founded in the autumn and winter, or both. Hence, in the second experiment, we used colonies of marked workers so that we could test for an increase in either colony longevity or individual workers' longevities as the result of increased temperature.

Materials & Methods

Colony culture and treatments

Colonies of *Bombus terrestris audax* were obtained from a commercial supplier (Biobest, Westerlo, Belgium) in August 2011 (Experiment 1) and February 2012 (Experiment 2). *B. t. audax* is the subspecies of *B. terrestris* found in the British Isles. Experiment 1 tested how temperature affects colony longevity, colony queen longevity (where 'colony queen' refers to the original foundress queen heading the mature colony), the production of sexuals and workers' thermoregulatory behaviour. To replicate Experiment 1 and test for effects on individual worker longevity and production of workers, Experiment 2 repeated Experiment 1 with marked workers. In both experiments, all colonies were queenright (i.e. contained a colony queen) and, at the start of the experiment, had not yet produced any adult sexuals or reached peak colony size (worker number). We selected colonies that were already partly developed for the experiments in order to test effects of temperature rises occurring in the second half of the growing season and specifically to test for effects on sexual production and colony longevity. Mean (range) colony size at the beginning of each experiment was 62 (41-94) workers in Experiment 1 (n = 24 colonies) and 62 (34-89) workers in Experiment 2 (n

= 20 colonies). Initial colony sizes were not significantly different across treatments (*t*-tests: Experiment 1, $t_{22} = 0.16$, $p = 0.873$; Experiment 2, $t_{18} = 0.17$, $p = 0.871$).

Each colony (including workers, queen and brood) was transferred to a wooden nest box (300 × 200 × 170 mm) with a clear plastic lid. Colonies were supplied with *ad libitum* sugar syrup nectar substitute, but a limited pollen supply (see *Supplementary material*). *Ad libitum* feeding with pollen was avoided to mimic field conditions, since in the field pollen supply is not necessarily maximised and hence it is likely that colonies would be unable to compensate for any detrimental life history effects of altered ambient temperature by increasing their food consumption; this design therefore allowed any effects of rearing temperature to be fully expressed. From the start of each experiment, colonies were kept in a constant environment (CE) room set at 20°C. In each experiment, colonies were randomly divided into two treatment groups (Experiment 1, 12 colonies per treatment; Experiment 2, 10 colonies per treatment), which were exposed to either 20°C or 25°C. The 20°C treatment was selected as representative of summer soil temperature in Southern England (e.g. http://www.bearsbythesea.co.uk/wxsoiltempseason.php). The 25°C treatment represents a 5°C increase, which is within the range of projected summer temperature increase in Europe over the 21st Century (Solomon et al. 2007; Collins et al. 2013). Although these temperatures are well within the habitable range of *B. terrestris*, given its wide Eurasian distribution, we predicted that they could still create large differences in the life history of this species, especially within a single population. The higher temperature was delivered by placing the nest-boxes housing the 25°C colonies on electric heatmats. For further details of the rearing and treatment of colonies, see *Supplementary material*. In both Experiments 1 and 2, realised in-nest temperatures were measured with a datalogger (EL-USB-2; Lascar Electronics, Salisbury, UK) placed inside the nest-box contacting the edge of the comb. Temperature in each colony was measured once (Experiment 1) or one to three times (Experiment 2) in a randomly determined order for a 45-hour session during the course of the experiment (see *Supplementary material*).

Colony, queen and worker longevity

In both experiments, colonies were monitored approximately daily (mode (range) = 1 (1-3) days between observations) to detect colony death, which was defined as the date when the number of living adult workers first reached zero. Colony longevity was defined as the time in days between the start of each experiment and the date on which colony death was reached (see *Supplementary material*). As a further measure of colony longevity, the time in days between the start of each experiment and the first census date (see *Colony productivity* below) at which colony size had declined to 10 or fewer workers was also recorded. This measure was used to account for the possibility that the presence of a small number of exceptionally long-lived workers would overestimate colony longevity by the first measure. Colonies were additionally monitored approximately every 2 days (mode (range) = 2 (1-3) days) for queen (foundress) death. Queen longevity was defined as the time in days between the start of the experiment and queen death.

In Experiment 2, individual worker longevity was measured by marking a subset of workers with individually-numbered tags (see *Supplementary material*). Workers were marked as callows (newly-eclosed adults), with 2-10 workers being marked per colony over days 1-15 of the experiment (cohort 1, i.e. workers eclosing relatively early in the colony cycle) and 0-8 workers being marked per colony over days 20-29 of the experiment (cohort 2, i.e. workers eclosing relatively late in the colony cycle). Approximately every day (mode (range) = 1 (1-3) days between observations), colonies were checked for marked workers, and each worker was recorded as either seen, not seen or dead. Individuals with detached discs were excluded from the analysis. As all marked workers were not seen every day, the date of death was estimated for each worker as the mean of the date last seen alive and the date first recorded dead (mean ± SD number of days between these two dates = 4.3 ± 7.3 days, n

= 160). Worker longevity was defined as the time in days between the date of worker eclosion and the estimated date of worker death.

Colony productivity

In Experiments 1 and 2, the number of adult workers in each colony was censused using a mechanical counter approximately once per week (Experiment 1, mean (range) = 6.9 (5-9); Experiment 2, mean (range) = 7.8 (5-13) days between observations). In Experiment 2 only, the production of workers was also estimated by recording daily all observed callow workers from day 1 to day 49 (with day 1 being the first day of the experiment). The recording of callow workers ceased at this time because almost all callows were males by this point. Because callow workers may sometimes have evaded detection, counts of callow workers may have been underestimates, although the magnitude of underestimation should not have systematically varied across treatments. In Experiment 1 only, each colony was checked for sexuals approximately every 2 days (mode (range) = 2 (1-3) days), and all sexual individuals detected were counted and removed. Removal of sexuals simulated the situation in wild nests, in which the sexuals usually depart within a few days of eclosion (Alford 1975). The first day on which adult males were detected within colonies was compared between treatments. In Experiment 2, sexuals were not removed but the number of new queens produced per colony was recorded.

Colony thermoregulation

The role of thermoregulatory behaviour in maintaining in-nest temperature was investigated by recording worker wing fanning (Experiment 1) and the extent of wax canopy constructed over the nest comb (Experiments 1 and 2). Extent of wax canopy was assessed on one day

(day 27) in Experiment 1 and on two days (days 15 and 27) in Experiment 2. See *Supplementary material* for further details.

Statistical analyses

In-nest temperature was compared between treatments in both experiments using ANCOVA or linear mixed models (LMMs). Colony and queen longevities were compared between treatments using *t*-tests (Experiment 1) or Cox's proportional hazards survival analysis (Experiment 2). Survival analysis was used in Experiment 2 to allow the inclusion of several colonies and queens for which only minimum values for longevity were known. Worker longevity (Experiment 2) was compared using an LMM. The number of workers per colony was compared between treatments using generalised linear mixed models (GLMMs; Experiments 1 and 2). For this analysis, separate models were used for the growth phase and the decline phase in each experiment, with the division between these phases being the mean time at which the number of workers per colony was largest (Experiment 1: day 31; Experiment 2: day 33). The timing of the peak number of workers (Experiments 1 and 2), and the number of workers produced (Experiment 2) per colony were compared between treatments using *t*-tests. The number of sexuals produced (Experiments 1 and 2) and the mean date of eclosion of the first male (Experiment 1) per colony were compared between treatments using *t*-tests or Wilcoxon signed rank tests. Thermoregulatory behaviour was compared between treatments using a GLMM for presence/absence of wing fanning (Experiment 2) and a Wilcoxon rank sum test for percentage cover of wax canopy (Experiments 1 and 2). Further details of the statistical analyses are provided in *Supplementary material*.

Results

Treatments

In Experiment 1, the recorded in-nest temperature of colonies was significantly higher in the 25°C treatment (ANCOVA, $F_{1,22} = 152.3$, $p < 0.001$) but was not significantly affected by day (ANCOVA, $F_{1,22} = 0.724$, $p = 0.404$) and there was no significant treatment-day interaction (ANCOVA, $F_{1,20} = 1.100$, $p = 0.173$; fig. S1). In Experiment 2, recorded in-nest temperature was again significantly higher in the 25°C treatment (LMM, n = 37 measurements from 20 colonies, $\chi^2 = 18.4$, d.f. = 1, $p < 0.001$), and there was neither a significant effect of day (LMM, $\chi^2 = 0.00$, d.f. = 1, $p = 0.958$) nor a significant treatment-day interaction (LMM, $\chi^2 = 0.46$, d.f. = 1, $p = 0.496$). These findings confirm that the 25°C treatment successfully elevated in-nest temperature relative to the 20°C treatment.

Colony, queen and worker longevity

In Experiment 1, there was no significant effect of treatment on colony longevity (means ± SE: 20°C treatment, colony longevity = 57.1 ± 1.7 days, n = 12 colonies; 25°C treatment, colony longevity = 61.4 ± 1.6 days, n = 12 colonies; *t*-test, $t_{22} = 1.87$, $p = 0.075$), although there was a trend for colony longevity to be greater in the 25°C treatment (95% confidence interval of mean increase from 20°C to 25°C = -0.5 – 9.1 days) and a significantly later decline to a colony size of 10 workers in the 25°C treatment (*t*-test, $t_{22} = 2.30$, $p = 0.031$). In Experiment 2, colony longevity was significantly higher in the 25°C treatment (means ± SE: 20°C treatment, colony longevity = 89.3 ± 4.0 days, n = 9 colonies; 25°C treatment, colony longevity = 103.2 ± 4.3 days, n = 5 colonies; Cox's PH, $z_1 = 2.70$, $p = 0.007$), but there was no effect of treatment on the time taken to decline to 10 workers (Cox's PH, $z_1 = 0.25$, $p = 0.800$). Hence, across both experiments, the higher temperature treatment had a significant but not completely consistent positive effect on colony longevity.

In Experiment 1, there was no significant effect of treatment on queen longevity (t-test, t_{22} = 0.43, p = 0.673, 95% confidence interval of mean increase from 20°C to 25°C = -7.0 – 10.7 days). In Experiment 2, there was also no significant effect of treatment on queen longevity (Cox's PH, z_1 = 1.48, p = 0.140). In Experiment 2, there was no significant effect of treatment on worker longevity (LMM, χ^2 =0.24, d.f. = 1, n = 160 workers from 20 colonies; p = 0.624; fig. 1). Therefore the higher temperature treatment had no effect on individual longevities of either colony queens or adult workers.

In Experiment 2, there was a significant effect of cohort on worker longevity (LMM, χ^2 = 32.93, d.f. = 1, p < 0.001), with workers from cohort 1 having greater longevity than workers in cohort 2 (fig. 1). There was no significant temperature-cohort interaction (LMM, χ^2 =0.62, d.f. = 1, p = 0.618). These results suggest that worker longevity is affected by relative eclosion date within the colony cycle, with earlier-eclosing workers living longer than later-eclosing workers.

Colony productivity

In Experiment 1, during the growth phase, the number of adult workers per colony (n = 24 colonies) was significantly higher in the 25°C treatment (GLMM, χ^2 = 15.03, d.f. = 1, p < 0.001) and was significantly increased by day, i.e. the passage of time (linear term: GLMM, χ^2 = 38.54, d.f. = 1, p < 0.001), but there was no significant treatment-day interaction (GLMM, χ^2 = 3.40, d.f. = 1, p = 0.065; fig. 2a). In the decline phase, the number of adult workers per colony was again significantly higher in the 25°C treatment (GLMM, χ^2 = 5.00, d.f. = 1, p = 0.025) and significantly decreased with day (GLMM, linear term: χ^2 = 65.5, d.f. = 1, p < 0.001, quadratic term: χ^2 = 407.8, p < 0.001), but there was no significant treatment-day (quadratic) interaction (GLMM, χ^2 = 2.83, d.f. = 1, p = 0.093; fig. 2a).

In Experiment 2, in the growth phase, although the mean number of adult workers was consistently higher in the 25°C treatment, the number of adult workers per colony (n = 20 colonies) was not significantly affected by treatment (GLMM, $\chi^2 = 2.46$, d.f. = 1, $p = 0.117$). There was a significant positive effect of day (GLMM, $\chi^2 = 14.60$, d.f. = 1, $p < 0.001$) and there was no significant treatment-day interaction (GLMM, $\chi^2 = 2.37$, d.f. = 1, $p = 0.124$; fig. 2b). In the decline phase, the number of adult workers was significantly higher in the 25°C treatment (GLMM, $\chi^2 = 62.30$, d.f. = 1, $p < 0.001$) and significantly decreased with day (GLMM, linear term: $\chi^2 = 52.36$, d.f. = 1, $p < 0.001$). There was also a significant treatment-day (quadratic) interaction (GLMM, $\chi^2 = 67.35$, d.f. = 1, $p < 0.001$), with the 25°C treatment being associated with a higher rate of decline in number of workers relative to the 20°C treatment (fig. 2b).

The week of peak worker number per colony did not differ significantly between treatments in either Experiment 1 (Welch's t-test, $t_{17} = 1.15$, $p = 0.267$; fig. 2a), or Experiment 2 (t-test, $t_{18} = 0.15$, $p = 0.884$; fig. 2b). Overall, therefore, across both experiments, the higher temperature treatment was associated with colonies attaining significantly greater size (adult worker numbers), except in the growth phase in Experiment 2, but did not affect the timing of peak worker numbers.

In Experiment 2, there was no significant effect of treatment on the total number of callow workers recorded per colony (t-test, $t_{18} = 1.68$, $p = 0.110$), although there was a trend for this number to be higher in the 25°C treatment (means ± SE: 20°C treatment = 37.0 ± 5.5 workers, n = 10; 25°C treatment = 51.1 ± 6.3 workers, n = 10).

In both Experiments 1 and 2, the total number of new queens produced per colony was significantly higher in the 25°C treatment (Wilcoxon signed rank tests, Experiment 1, W = 102.5, n = 24, p = 0.046; Experiment 2, W = 78, n = 20, p = 0.015; fig. 3). In Experiment 1, the total number of males produced per colony was not significantly affected by treatment (means ± SE: 20°C treatment = 15.6 ± 2.8 males, n = 12; 25°C treatment = 17.3 ± 3.9 males, n = 12; t-test, t_{22} = 0.13, p = 0.899). The mean date of eclosion of the first males was not significantly affected by treatment (t-test, t_{22} = 0.56, p = 0.577). In Experiment 2, the maximum number of males per colony recorded on any one census date was not significantly affected by treatment (t-test, t_{18} = 1.11, p = 0.28), although there was a trend for this number to be higher in the 25°C treatment (means ± SE: 20°C treatment = 28.5 ± 3.6 males, n = 10; 25°C treatment = 36.0 ± 4.5 males, n = 10). Therefore the higher temperature treatment significantly increased queen production but had no significant effect on male production or the timing of male production.

Colony thermoregulation

In Experiment 1, workers in colonies in the 25°C treatment had higher wing-fanning rates (fig. 4a) and colonies in this treatment were significantly more likely to exhibit wing-fanning compared with colonies in the 20°C treatment (GLMM, χ^2 = 40.37, d.f. = 1, p < 0.001; fig. 4b). The occurrence of wing-fanning was not significantly affected by day (GLMM, χ^2 = 0.22, d.f. = 1, p = 0.643) or treatment-day interaction (GLMM, d.f. = 1, χ^2 = 0, p = 1). By contrast, in both experiments, the percentage cover of wax canopy was significantly higher in the 20°C treatment (Wilcoxon rank sum test, Experiment 1: W = 6.5, n = 24, p < 0.001, fig. 4c; Experiment 2: day 15, W = 9.5, n = 20, p = 0.001; day 27, W = 12, n = 20, p = 0.002). Hence, overall, the higher temperature treatment was associated with significantly more wing-fanning by workers and a significantly lower extent of wax canopy construction.

Discussion

To investigate the effect of temperature increases on the life-history parameters of bumble bee pollinators, we conducted two experiments in which we reared *B. terrestris* colonies at two temperatures. We found a suite of responses (table 1). The higher temperature treatment was associated with significantly greater colony longevity in one experiment but not the other. However, it had no significant effect on queen or worker longevity. The higher temperature treatment significantly increased colony size (number of adult workers) but did not affect the timing of peak colony size. In both experiments, the higher temperature treatment significantly increased queen production but did not significantly affect worker or male production or the timing of male production. Finally, the higher-temperature treatment affected workers' thermoregulatory behaviour, leading to a significantly greater probability of wing-fanning and a significantly lower extent of wax canopy construction (table 1).

These findings have several implications. Firstly, they show that some colony life-history traits in bumble bees are phenotypically plastic with respect to temperature and hence that bumble bee colonies are likely to experience changes to their life histories following temperature rises due to climate change. In the short term, such changes, regardless of whether they were adaptive or not, could occur and persist via phenotypic plasticity alone. In the longer term, one would expect selection for adaptive responses to occur via natural selection acting on genetic variation for the relevant traits, with selection being based either on standing genetic variation, or on new mutations, or on genetic assimilation of standing variation coupled with phenotypic plasticity (West-Eberhard 2003).

Secondly, our findings show that higher temperature has a positive but probably weak effect on colony longevity in *B. terrestris*. No significant effect of temperature on colony longevity was found in Experiment 1. The significant positive effect found in Experiment 2 appeared due largely to the presence of a few, long-lived workers in the 25^{o}C treatment, since in this

experiment there was no significant effect of temperature on the time taken to decline to a colony size of 10 workers. The difference between the mean colony longevities in the two treatments was 4 days (Experiment 1) or 14 days (Experiment 2), with confidence intervals in Experiment 1 giving a 95% probability that the true increase was between -0.5 and 9.1 days, which is at most equivalent to two extra days of colony life per 1°C increase. Thus temperature increase is unlikely to have a marked effect on colony longevity in temperate bumble bees. For example, alone it seems insufficient to explain the existence of winter-active *B. t. audax* in southern Britain.

Thirdly, our findings show that, surprisingly, temperature differences did not affect queen or worker longevity. This suggests that the observed increases in colony longevity and adult worker number could have occurred because colonies at the higher temperature produced more workers, even though this effect was not significant (table 1). The lack of effects on individual longevity stand contrary to results of studies of other insects, including a study of worker fire ants, which have found that higher temperatures reduce individual longevity (e.g. Calabi & Porter 1989; Sheehy 2002; Isitan, Gulel & Gunduz 2010). The findings suggest that individuals within *Bombus* colonies could be robust with respect to longevity in response to increases in temperature associated with climate change. One possible reason for our findings, consistent with the large differences in the thermoregulatory behaviour of workers found between the treatments, is that colony-level thermoregulation buffers the effects of changes in ambient temperature. If so, the energetic and nutritive costs associated with workers changing their thermoregulatory activity between temperatures could not have been sufficient to alter longevity discernibly. In addition, the observed changes to thermoregulatory behaviours, and very probably changes to other thermoregulatory behaviours that were not measured (such as brood incubation), did not negate the differences in realised in-nest temperature between the treatments (mean differences between nests in the two treatments were approximately 8°C and 3°C degrees for Experiments 1 and 2, respectively; fig. S1).

This is in contrast with the finding from other studies that, within a wide range of ambient temperatures, brood temperature in bumble bee colonies is maintained at approximately 30°C (Heinrich 1979; Weidenmuller, Kleineidam & Tautz 2002; Gardner, Foster & O'Donnell 2007). However, unlike these previous studies, the present study measured in-nest temperature rather than brood temperature strictly speaking, which is less responsive to ambient temperature (e.g. Vogt 1986a). Nonetheless, in-nest temperature, as the temperature experienced by adult workers in the nest, would still be expected to have a potential influence on these workers.

We also found a strong effect of relative worker eclosion date on worker longevity, with workers eclosing relatively early in the colony cycle having significantly greater longevities than workers eclosing relatively late in the colony cycle. Similar patterns have been found in other bumble bee species and have been attributed to increased foraging mortality by late-emerging workers (Goldblatt & Fell 1987; O'Donnell, Reichardt & Foster 2000). However, since our colonies were not free-foraging, mortality of foragers stemming from external factors cannot explain this pattern in our study. Other possible reasons for the increased mortality in late-emerging workers could be the increased aggression between workers experienced by colonies late in the colony cycle (the competition phase; Van Der Blom 1986; Bloch & Hefetz 1999; Amsalem et al. 2009), or simply intrinsic differences in workers' longevities as a function of the time at which they are produced in the colony cycle. Similarly, in the honey bee *Apis mellifera*, worker longevity varies greatly according to stage of the perennial colony cycle, with 'summer', brood-rearing workers living less long than 'winter', non-brood-rearing workers (Smedal et al. 2009).

Fourthly, given that we found that the timings of peak colony size and first male eclosion did not differ according to temperature, our results suggest that colonies do not adjust the timing of colony events in the later part of the colony cycle in response to temperature. Vogt

(1986a) similarly concluded that a change in temperature did not affect the timing of events in the colony cycle. If colonies are dependent on an abundance of food at particular stages of development, adhering to a fixed developmental pattern is likely to prove maladaptive in the face of climate change, since an effect of climate change on the flowering times of plants (Gordo & Sanz 2005; Memmott et al. 2007; Bartomeus et al. 2011) could create a mismatch in bee-plant phenology (Miller-Rushing et al. 2010). Although the timing of queen emergence and colony establishment may also vary in response to temperature (Sparks & Collinson 2007; Bartomeus et al. 2011), our data suggest that such variation may result only in a 'frame shift' in the pacing of later colony development. Such relative inflexibility in the colony cycle could prove to be especially maladaptive if temperatures in different seasons change unevenly over time.

Finally, our findings of a significant positive effect of temperature on the production of queens suggest that colonies exposed to higher ambient temperatures in nature would produce more queens and so have higher reproductive success. No effect of higher temperature on queen production was found by Vogt (Vogt 1986a), although this may have been due to the small sample size in his study. Our finding is consistent with previous studies showing that the production of new queens depends on colony size in bumble bees (Owen, Rodd & Plowright 1980; Müller, Shykoff & Sutcliffe 1992; Bourke 1997; Lopez-Vaamonde et al. 2009), since colonies in the higher-temperature treatment also attained greater sizes. In unmanipulated colonies of *B. terrestris*, it is not unusual for a substantial proportion of colonies (c. 50%) not to produce queens (e.g. Lopez-Vaamonde et al. 2009); the lower-temperature treatment effectively reduced this proportion yet further (Fig. 3). Since an effect of temperature was found in queen, but not male, production, this may suggest that climate change in nature has the potential to influence population sex ratio, which could have knock-on effects on the viability of bumble bee populations.

In conclusion, the life-history responses of eusocial insects such as bumble bees to changing temperature are complex because effects on the colony and on individuals may be different. Our results show that individual-level life history may remain stable, perhaps because it is well protected by nest thermoregulation, but that temperature remains important because it can extend colony longevity and alter reproductive success at the colony level. With regard to the presence of winter-active *B. t. audax*, our findings suggest that temperature increases do not greatly extend the longevity of colonies, and hence that the increasing winter activity of this species in southern Britain is more likely to stem from colonies being founded in autumn and winter. Given the lack of experimental research in this area, it is not clear whether other social pollinators, including other bumble bee species, respond in the same way to temperature. Additional carefully controlled experimental studies, e.g. with variable temperature regimes, in combination with data from the field and modelling, should help construct a fuller understanding of how major social insect pollinators are likely to respond to climate change.

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Data accessibility

Data deposited in the Dryad Digital Repository:

http://dx.doi.org/10.5061/dryad.73k5g, (Holland & Bourke, 2015)

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Supporting information

Additional supporting information may be found in the online version of this article.

Supplementary material (including supplementary methods, supplementary results and supplementary references).

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Figure Legends:

Figure 1 Effect of temperature treatment and worker cohort on worker longevity (days between worker eclosion and death) in *Bombus terrestris* colonies (Experiment 2). 20°C C1: 20°C treatment, cohort 1 (n = 57 workers from 10 colonies); 25°C C1: 25°C treatment, cohort 1 (n = 66 workers from 10 colonies); 20°C C2: 20°C treatment, cohort 2 (n = 15 workers from 4 colonies); 25°C C2: 25°C treatment, cohort 2 (n = 22 workers from 3 colonies). Cohort

1 workers eclosed over days 1-15 and cohort 2 workers eclosed over days 20-30. Diamonds, thick horizontal lines, boxes and whiskers show the mean, median, interquartile range and range, respectively. ***, $p < 0.001$ (linear mixed model).

Figure 2 Effect of temperature treatment on mean weekly number of adult workers in *Bombus terrestris* colonies (a, Experiment 1; b, Experiment 2). Experiment 1: n = 12 colonies per treatment; Experiment 2: n = 10 colonies per treatment. 20 deg, black circles: 20°C treatment; 25 deg, white triangles: 25°C treatment. Error bars show ± 1 SE. Dotted lines show the division between growth and decline phases used in the statistical analysis. Difference between treatments within phases indicated by: NS, not significant, * $p < 0.05$, *** $p < 0.001$ (GLMM).

Figure 3 Effect of temperature treatment on number of new queens produced in *Bombus terrestris* colonies (a, Experiment 1; b, Experiment 2). Experiment 1: n = 12 colonies per treatment; Experiment 2: n = 10 colonies per treatment. Diamonds, thick horizontal lines, boxes and whiskers show the mean, median, interquartile range and range, respectively. *, $p < 0.05$ (Wilcoxon signed rank tests).

Figure 4 Effect of temperature treatment on thermoregulatory behaviour of workers in *Bombus terrestris* colonies (Experiment 1). n = 12 colonies per treatment. a: Mean worker wing-fanning rate (fanning events per worker per minute) for each filming session as a function of treatment and time. Black circles and white triangles show means for the 20°C and 25°C treatments, respectively. b: Proportion of filming sessions in which at least one wing-fanning event occurred as a function of temperature treatment. ***, $p < 0.001$ (generalised linear mixed model). c: Percentage of nest covered by a wax canopy as a

function of treatment, assessed on day 27 of the experiment. Diamonds, thick horizontal lines, boxes and whiskers show the mean, median, interquartile range and range, respectively. ***, $p < 0.001$ (Wilcoxon rank sum test).

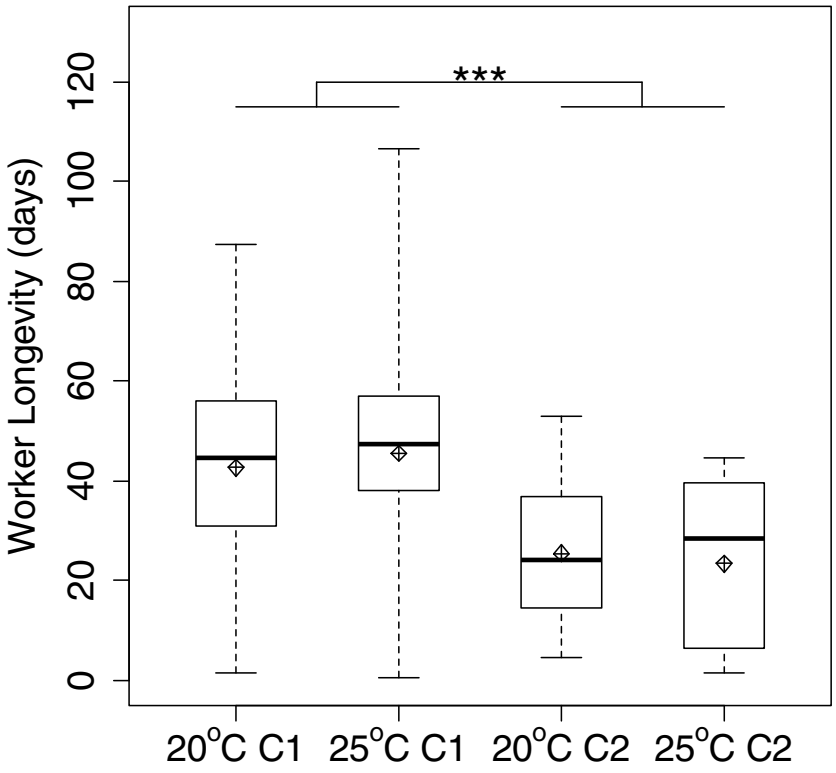

Figure 1

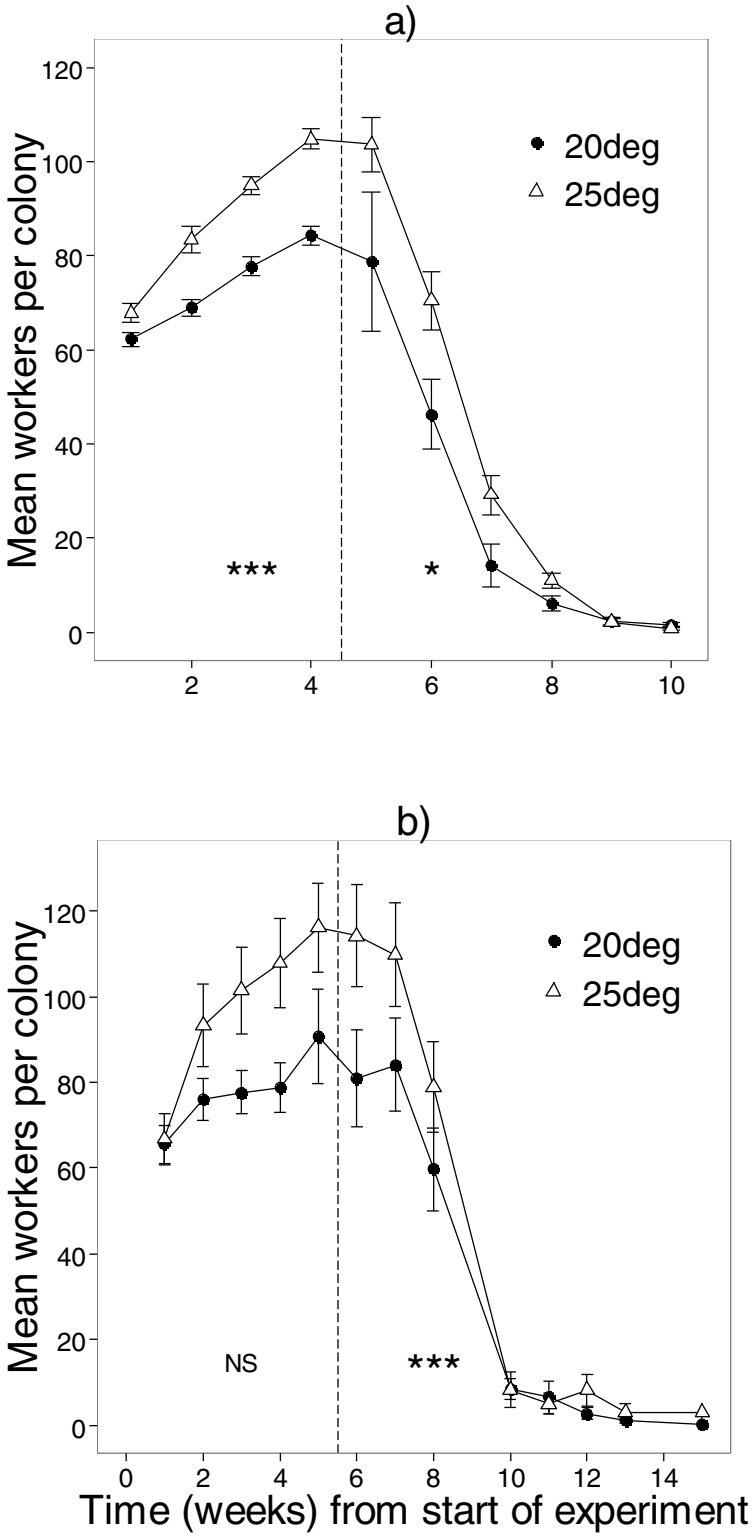

Figure 2

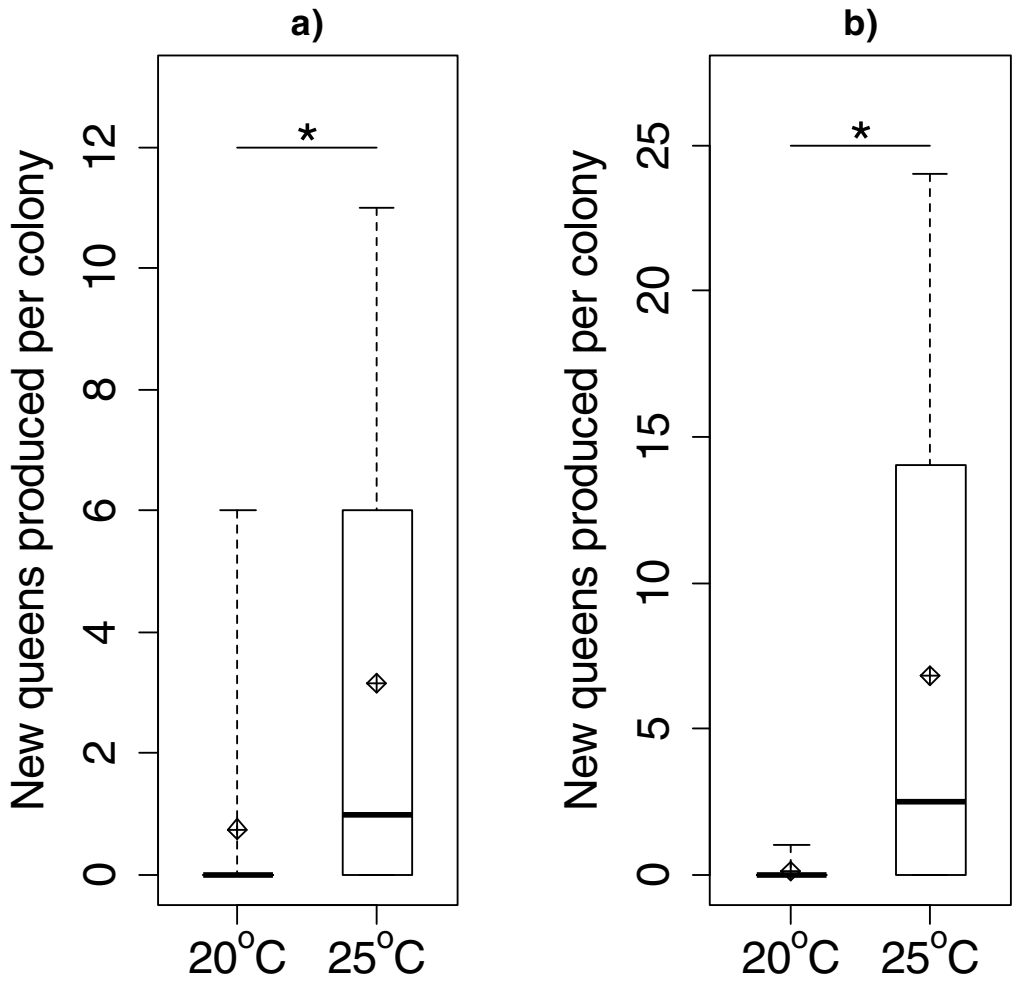

Figure 3

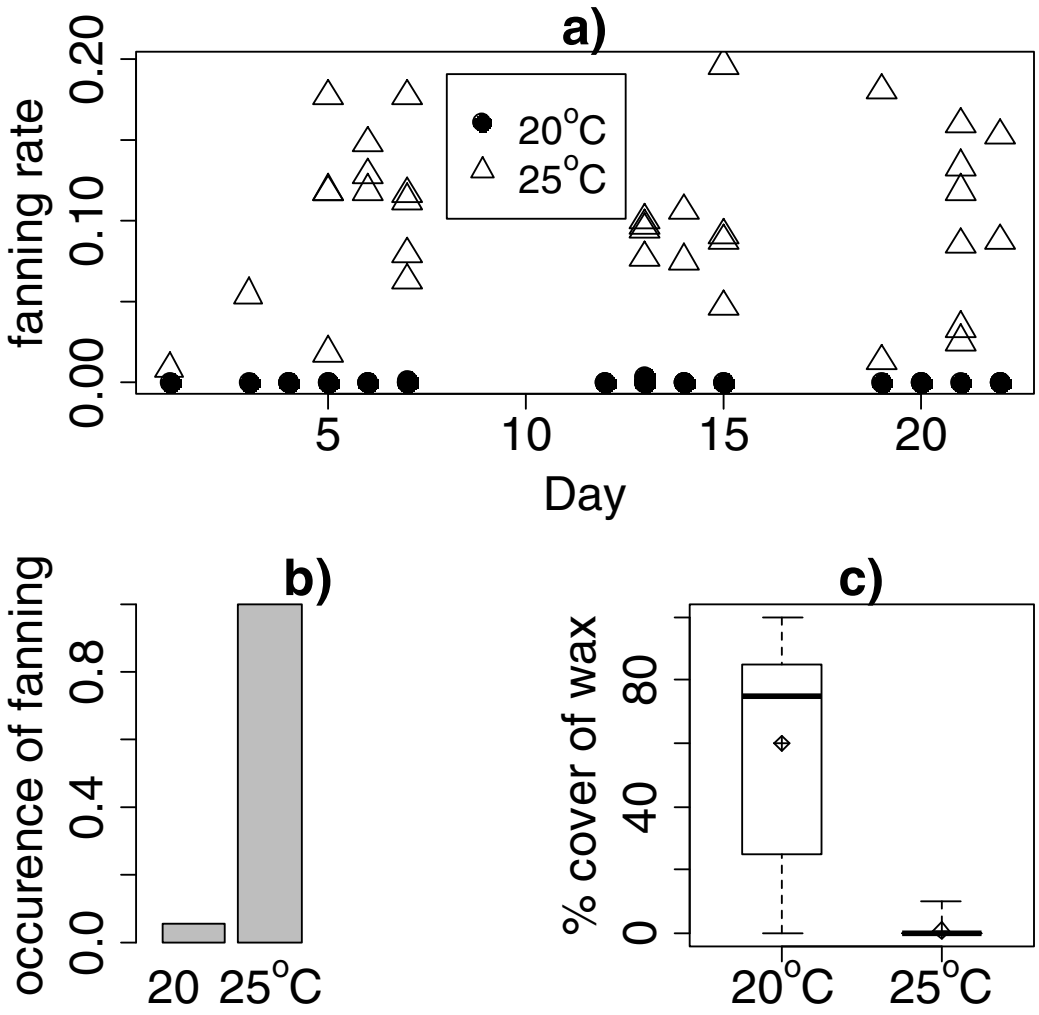

Figure 4

Table 1 Summary of main effects of temperature treatment (25°C v. 20°C) on life-history and behavioural metrics in *Bombus terrestris* colonies. +, significantly positive effect; -, significantly negative effect; NS, no significant effect; n/a, not applicable (effect not tested). See *Results* for details.

Life-history or behavioural metric	**Effect of higher rearing temperature**	
	Experiment 1	**Experiment 2**
Colony longevity	NS	**+**
Time to reach 10 workers	**+**	NS
Queen longevity	NS	NS
Worker longevity	n/a	NS
Number of adult workers		
Growth phase	**+**	NS
Decline phase	**+**	**+**
Timing of peak colony size	NS	NS
Number of workers produced	n/a	NS
Number of queens produced	**+**	**+**
Number of males produced	NS	NS
Date of eclosion of first male	NS	n/a
Probability of wing fanning	**+**	n/a
Extent of wax canopy cover	**-**	**-**