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The impact of experimental warming on bumblebees: Higher temperatures induce behavioral changes in *Bombus terrestris* queens

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With 5 figures

Abstract: Bumblebees (*Bombus terrestris*) are important pollinators in most temperate and boreal ecosystems, but these regions are increasingly affected by heatwaves that are a symptom of anthropogenic climate change. Bumblebee queens may be particularly vulnerable to elevated temperatures during their solitary period following overwintering. Using an experimental system with numerous replicates, we investigated the oviposition behavior of overwintered spring queens, the resulting colony characteristics, and the mating behavior of newly emerged fall queens at three different temperatures: optimal (27 °C; C27), moderately high (32 °C; C32), and high (35 °C; C35). Spring queens exposed to higher temperatures exhibited unusual behavior, with spring queens in the C35 group more frequently digging up and destroying egg cells, ultimately leading to colony failure. Compared to C27, spring queens in the C32 treatment displayed more frequent oviposition, a higher colony formation rate, a higher number of offspring per colony, a shorter colony development period, and lower body sizes in their adult offspring. These results indicate that elevated temperatures lead to behavioral changes in spring queens and affect the characteristics of resulting colonies. Fall queens exhibited mating avoidance behavior at higher temperatures, resulting in a lower mating rate. Our results demonstrate that bumblebee queens respond to thermal conditions that can lead to colony failure when thermal limits are exceeded. In nature, behavioral changes induced in bumblebee queens exposed to persistent heatwaves during the solitary period may be seriously deleterious to bumblebee populations in many regions.

Keywords: Bumblebee; elevated temperature; oviposition behavior; mating behavior; pollinator; climate change

1 Introduction

Bumblebee species in the genus *Bombus* Latreille, 1802 distributed globally across climate zones from subarctic to tropics (Maebe et al. 2021b) are crucial pollinators in many natural and agricultural ecosystems (Velthuis & van Doorn 2006). The diversity and abundance of bumblebee is, however, declining worldwide due to human activity (Marshall et al. 2018). Loss of habitat and application of chemicals have local impacts on *Bombus* viability (Minnameyer et al. 2021). Moreover, anthropogenic climate change is predicted to accelerate future decline of bumblebee populations (Vasiliev & Greenwood 2021). In particular, elevated temperatures and unseasonal weather extremes such as heatwaves can directly impact bumblebee populations (Vasiliev & Greenwood 2021). Globally, heatwaves are becoming longer and more frequent, irrespective of season and region (Kaiser et al. 2023; Thompson et al. 2023). For example, in 2021 an unprecedented heatwave in the Pacific Northwest peaked at 49.6 °C at 50 ° latitude (White et al. 2023). Similar climate extremes including high temperatures are expected to have profound impacts on the natural environment (Easterling et al. 2000) including insect populations (John et al. 2024). Although bumblebees display some resilience to climate change through plastic and adaptive responses associated with their morphology, physiology, behavior, phenology, and genetics (Maebe et al. 2021b), anthropogenic climate change has already been implicated in distributional

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changes and a decrease in the biodiversity of bumblebees (Soroye et al. 2020; White & Dillon 2023).

Bumblebees in temperate, subtropical and tropical areas have responded to elevated temperatures in several ways (Martinet et al. 2021a). For example, body size and flight ability of bumblebees decreases with rising temperatures (Kenna et al. 2021; Gérard et al. 2023), reducing foraging activity and pollen collection (Gérard et al. 2022b), while elevated temperatures during the hibernation period can decrease the viability of fertilized queens (Yoon et al. 2010). The annual life cycle of most bumblebee species depends on oviposition by fertilized queens that have survived the winter by hibernating. Nest temperature is critical and when they are present, bumblebee workers maintain a temperature of approximately 27–29 °C through wing fanning (Vogt 1986; Westhus et al. 2013). However, during the period directly after queen hibernation when there are no workers, oviposition by fertilized queens will be severely affected by excessive heat. While the effects of heat exposure on workers, brood, males, and whole colony responses have been relatively well studied (reviewed by White & Dillon 2023), the relationship between elevated temperatures and oviposition behavior of fertilized queens remains unclear. Little is known about the plasticity of queen response.

Bombus terrestris is the most widespread bumblebee species, with a range extending from warm to cool climates (Martinet et al. 2021a). It is widely used as a pollinator for crops and other flowering plants in greenhouses and fields (Velthuis & van Doorn 2006) and has been successfully reared in indoor systems for this purpose (Velthuis & van Doorn 2006). Based on their maintenance of nest temperatures through wing fanning (Vogt 1986; Westhus et al. 2013) and increased wing fanning at nest temperatures of 30 to 32 °C (Grad & Gradisek 2018), we hypothesized that 27 °C is the optimal ambient temperature for bumblebees (Yoon et al. 2005), 32 °C is their upper temperature limit, and 35 °C is the temperature threshold that exceeds tolerance limits. Additionally, temperatures of 32 and 33 °C are considered thermal stress conditions for bumblebees (Gérard et al. 2022a, b, 2023; Perl et al. 2022), which they can encounter while foraging (Couvillon et al. 2010), in their underground nests (Grad & Gradisek 2018), and during summer (Rasmont et al. 2015). Previous studies have shown the effects of elevated temperatures during colony development on morphological traits, foraging parameters, and colony characteristics of bumblebees (Gérard et al. 2022a, b, 2023), so we documented behavioral changes and their subsequent impacts on spring and fall queens under high temperatures of 32 °C and 35 °C.

We investigated oviposition behavior of bumblebee queens exposed to elevated nest temperatures and the effect on subsequent colony formation. We also sought evidence for a temperature threshold for successful oviposition and mating for bumblebee queens. To do this we observed oviposition behavior of spring *Bombus terrestris* queens following overwintering, subsequent colony development, and the mating behavior of newly emerged fall queens under experimental conditions.

2 Materials and Methods

2.1 Bumblebees

Bombus terrestris bumblebee queens (17th generation of indoor rearing since 2014) were obtained from the National Academy of Agricultural Science in Wanju, Republic of Korea, and housed in an indoor rearing system following a previously reported method (Yoon et al. 2005). The bumblebees were kept at 27 °C and 65% relative humidity in continuous darkness prior to experimentation. Bumblebee queens were placed in ventilated wooden nest boxes (9.5 × 15.0 × 10.5 cm) to initiate oviposition. After the emergence of 5–10 workers, the oviposition plates were transferred to ventilated plastic nest boxes (15.5 × 16.5 × 10.5 cm) until 40–50 workers had emerged. Nests were then moved to larger boxes (22.0 × 28.0 × 14.0 cm) for further colony development. The bumblebees had *ad libitum* access to pollen bread (1:1 ratio (w/v) pollen and 40% sucrose solution) and 40% sucrose solution. For mating, >10 queens 5–6 days after emergence, and 20–30 males 7–8 days after emergence, were harvested from different colonies to avoid inbreeding (Maebe et al. 2021b). They were distributed in seven wooden mating boxes with steel mesh sides (60 × 50 × 65 cm) and nutrients at 23 °C with a 14L:10D photoperiod under 1,000 lx lights. Queens that mated singly with males were then separated and placed in wooden boxes under the same conditions for one week before hibernation.

2.2 Hibernation

Mated queens were weighed and then stored in ventilated tubes at 2.5 °C and a relative humidity of 70% under constant darkness for 12 weeks, resulting in hibernation (Yoon et al. 2010). After hibernation, the queens were weighed and placed in wooden flight boxes with steel mesh sides (60.0 × 50.0 × 65.0 cm) at 23–25 °C for three days to induce flight orientation. These queens, of the 18th generation, were provided *ad libitum* 40% sucrose solution and pollen bread and subsequently reared under various temperature treatments.

2.3 Temperature treatments

Three temperature treatments, 27 °C, 32 °C, and 35 °C, were conducted either in an insectary or in temperature- and humidity-controlled incubators at Dong-A University from January 2022 to December 2023. Experimental colonies were divided into three treatment groups and various treatment subgroups (Fig. S1). Queens that emerged from hibernation were reared at 27 °C (optimal temperature; OT) in incubators, 32 °C (moderately high temperature; MHT) in an insectary, or 35 °C (high temperature; HT) in incubators under a relative humidity of 65%. Thirty overwintered queens were used for each treatment and temperature transposition treatments. Treatment subgroups were held at constant temperatures of 27 °C, 32 °C, or 35 °C (C27, C32, and C35, respectively), throughout the queen's life cycle thus: Transposition of the queen from 27 °C to 32 °C after the first oviposition event (O) (27T32O); transposition of the queen from 27 °C to 32 °C after the emergence of ten workers (W) (27T32W); transposition of the queen from 32 °C to 35 °C after O (32T35O); transposition of the queen from 32 °C to 35 °C after W (32T35W); transposition of the queen after O from 35 °C to 32 °C (35T32O); and transposition of the queen at the expected W period (due to the absence of workers) from 35 °C to 32 °C (35T32ΔW). In addition, twelve overwintered queens were exposed to 38 °C in incubators under a relative humidity of 65%. Oviposition by overwintered queens at 38 °C was observed (C38). All data were collected under the four temperatures for at least one generation.

2.4 Oviposition of fertilized queens after hibernation and colony development

The oviposition rate, period, and early oviposition behavior of spring queens under the temperature treatments were observed for 30 days after the treatment began. The size of the egg cells and the number of eggs per egg cell were recorded for 40 days after the temperature treatment began using a microscope (Nikon SMZ800N, Nikon, Tokyo, Japan) and associated software (*i*-Solution Lite, IMT *i*-Solution Inc., Burnaby, Canada). The degree of dispersion of the egg cells was calculated as the proportion of egg cells that were positioned at least one egg cell width from others, relative to the total number of egg cells formed. To measure the thickness of the egg cells and the number of eggs per egg cell, the upper part of an egg cell was partially opened and then covered again after observation. Thickness of each egg cell was measured at four points. The brood care behavior of the queens under the C27, C32, and C35 treatments was recorded using a camera (Canon EOS 700D, Canon, Tokyo, Japan).

To monitor colony development, the day of emergence for the adult offspring, the number of newly emerged queens, workers, and males per colony, the colony formation rate, and the lifespan of the spring queens were recorded. Colony formation was considered successful once >50 workers, including at least one male and a queen, were present (Yoon et al. 2005). The body weight of newly emerged queens, males, and workers 1–2 days after emergence was also recorded using an electronic scale (Mettler AE260 Delta Range, Mettler Toledo, Columbus, Ohio, USA) and body length and width of the workers measured using digital calipers (Cocraft, Insjön, Sweden). For weighing, bumblebees were secured with a transparent plastic tube cap without anesthesia. Body length and width of workers were measured after ten seconds of anesthesia with CO_2.

2.5 Mating of newly emerged queens

For mating experiments, queens 5–6 days after emergence and males 7–8 days after emergence were harvested from different colonies. They were placed in wooden mating boxes with steel mesh sides (small 46 × 46 × 47 cm or large 60 × 50 × 65 cm) and provided with pollen bread and a 40% sucrose solution under standard lighting conditions (1,000 lx, 14L:10D). The large mating boxes were used for mating involving >10 queens and 20–30 males. The cumulative mating rate, mating duration, and the time of mating occurrence for newly emerged queens and males from C27, C32, or 32T35W was observed for 120 min. During mating, the queens and males, while in separate incubators with the same conditions, were moved to ventilated wooden nesting boxes (9.5 × 15.0 × 10.5 cm) to estimate mating duration. The mating experiments using newly emerged queens and males from C27 or C32 were recorded at 23 °C, 27 °C, 32 °C, or 35 °C. Mating experiments using newly emerged queens and males from 32T35W were conducted at 32 °C or 35 °C. Mating experiments were conducted at 23 °C in a mating room and at 27 °C, 32 °C, or 35 °C in incubators. The mating avoidance behavior of the newly emerged queens was recorded using a camera (Canon EOS 700D).

2.6 Statistical analysis

Statistical difference among different groups were tested using one-way analyses of variance (ANOVAs) followed by Tukey's multiple comparisons. Statistically significant differences in the rate of colony formation among different treatment groups were determined by Chi-square tests. Linear regression analysis was used to assess the relationship between temperature and oviposition (i.e., the number of oviposition events, number of egg cells, dispersion of egg cells, and digging of egg cells). Additionally, a two-way ANOVA was used to determine the relationship between temperature and exposure duration on the thickness of egg cells.

3 Results

3.1 Oviposition of spring queens under elevated temperatures

The oviposition rate and oviposition period for the spring queens under the different temperature treatments were examined for 30 days after treatment began (Fig. 1). Spring queens in the C38 group were used solely as a negative control for extremely high temperatures. Spring queens were observed to oviposit under the C27, C32, and C35 treatments, with longer preoviposition periods at higher temperatures (Fig. 1A). Spring queens exhibited more oviposition events in the 35T32O and C32 treatments ($F = 2.840$; df = 6, 52; $P = 0.018$; Fig. 1B), indicating a shorter period between each oviposition event. The number of egg cells followed a similar pattern to the number of oviposition events but was sig-

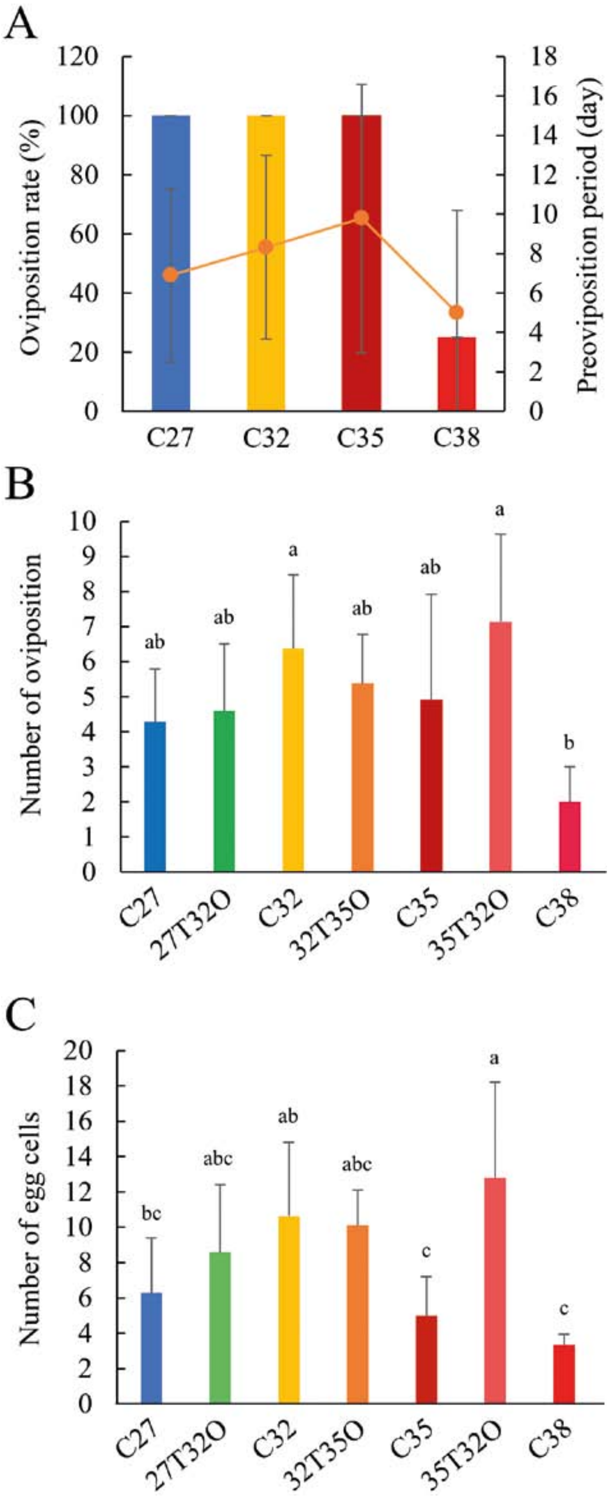

Fig. 1. Oviposition measurements for spring queens under different temperature conditions, measured for 30 days following treatment initiation: (A) the oviposition rate ($P = 0.0001$) and preoviposition period ($n = 11$ for C27, 22 for C32, 19 for C35, and 3 for C38; $P = 0.374$), (B) the number of oviposition events ($n = 7$ for C27, 5 for 27T32O, 16 for C32, 8 for 32T35O, 13 for C35, 7 for 35T32O, and 3 for C38; $P = 0.018$), and (C) the number of egg cells ($n = 3–16$; $P = 0.0001$). Different letters indicate significant differences (Tukey's multiple comparisons at $P = 0.05$ level).

nificantly lower in the C35 and C38 treatments ($P = 0.0001$; Fig. 1C). Linear regression analysis revealed the relationship between higher temperatures and the number of oviposition events (Regression model ANOVA; $F_{(2, 35)} = 8.628$; $P = 0.001$; $R^2 = 0.3302$; Fig. S2A) or the number of egg cells ($F_{(2, 29)} = 7.006$; $P = 0.003$; $R^2 = 0.3258$; Fig. S2B).

The spring queens distributed egg cells on the oviposition plate more widely under higher temperatures (Fig. 2A), and the degree of dispersion for the egg cells was higher in the C35 and C38 groups ($P = 0.0001$; Fig. 2B). There was also a positive relationship between the temperature and size (width, length, and thickness) of egg cells ($P = 0.0001$; Fig. 2C and S3). At higher temperatures, the egg cells became dark brown in color, and their thickness increased with rising temperatures ($F_{5,367} = 105.959$; $P = 0.0001$; $\eta^2 = 0.591$) and prolonged exposure ($F_{2,367} = 38.566$; $P = 0.0001$; $\eta^2 = 0.174$), indicating that temperature is the more influential factor (Fig. 2C). Additionally, the number of eggs within each egg cell decreased as the temperature increased ($F = 11.690$; df = 14, 108; $P = 0.0001$; Fig. 2D).

We observed brood care behavior of the spring queens (Fig. S4). C27 spring queens directly cared for the eggs in the egg cells, while C32 and C35 queens engaged in wing fanning to decrease the nest temperature (Video 1). C35 spring queens consistently exhibited repetitive digging of the egg cells (Fig. S4) and ovicidal behavior (Fig. S4 and Video 2), leading to hatching failure. Ovicidal behavior and larval discarding by 32T35O spring queens were also observed. Higher temperatures were significantly correlated with the dispersion of egg cells (Regression model ANOVA; $F_{(2, 37)} = 58.163$; $P = 0.0001$; $R^2 = 0.7583$; Fig. S5A) and the digging of egg cells ($F_{(2, 38)} = 55.395$; $P = 0.0001$; $R^2 = 0.7446$; Fig. S5B).

3.2 Colony development of spring queens under elevated temperatures

In terms of colony development, the time until the emergence of new workers ($P = 0.0001$), queens ($P = 0.141$), and males ($P = 0.020$) was slightly shorter in the C32 treatment but showed no significant difference between the C32 and C27 treatments (Fig. S6A–C). By contrast at C35 only a few workers emerged very late, with no males or queens observed. Although there was no significant difference between the C27 and C32 treatments, the number of newly emerged queens per colony was higher in the C27 treatment ($P = 0.040$), while the number of newly emerged workers was higher in the C32 treatment ($F = 14.270$, df = 7, 33, $P = 0.0001$; Fig. 3A–C). We also examined the rate of colony formation for the spring queens (Chi-square test: $x^2 = 26.415$; df = 7; $P = 0.0001$; Fig. 3D). Spring queens in 27T32W and C32 treatments successfully formed colonies with more than 50 workers, at least one male, and a queen. Spring queens in the 32T35O, C35, and 35T32ΔW treatments did not establish colonies. The time for colony formation was shorter for C32 than C27 treatments, while the 32T35W and 35T32O

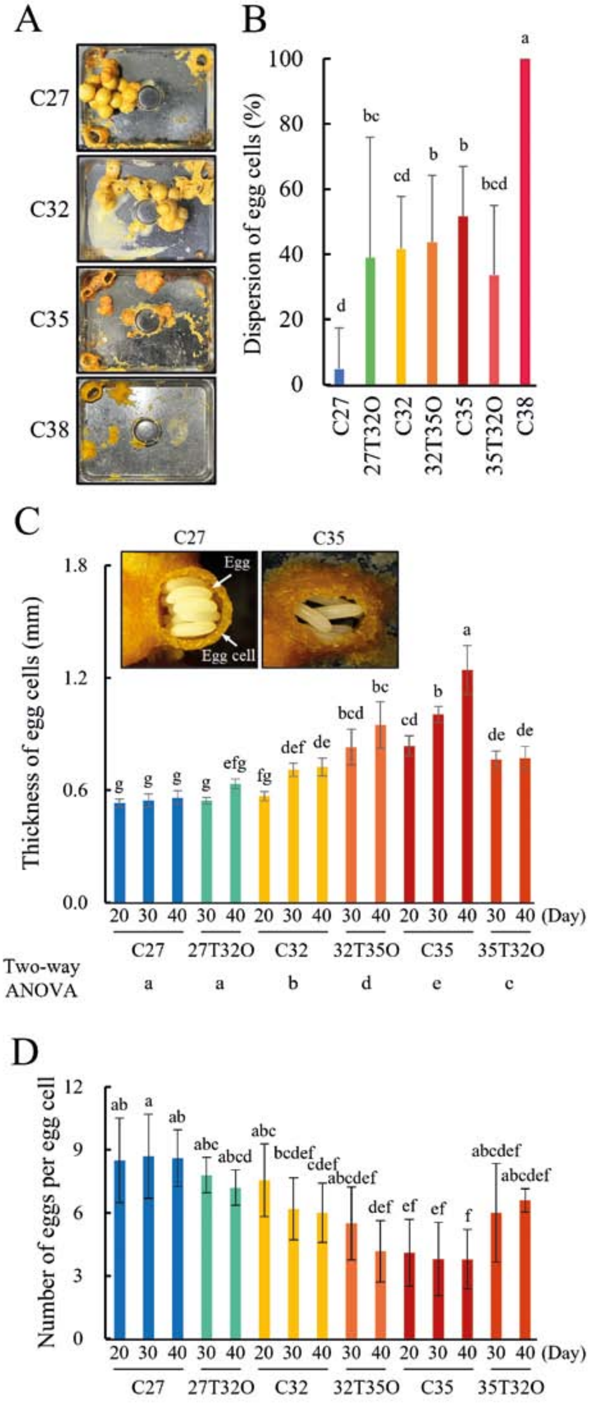

Fig. 2. Egg cells of spring queens under different temperature conditions, observed for 40 days following treatment initiation: (A) representative images of the egg cells, (B) dispersion of the egg cells (n = 3–16; P = 0.0001), (C) the thickness of the egg cells (n = 3–9; P = 0.0001), and (D) the number of eggs per egg cell (n = 4–14; P = 0.0001). In (C), the representative image depicts the number and distribution of eggs within the egg cell as well as the thickness of the egg cell. In (B–D), different letters indicate significant differences among the treatments (Tukey's multiple comparisons at P = 0.005 level). In (C), different letters below the X-axis of the figure indicate significant differences (two-way ANOVA test followed by posthoc test).

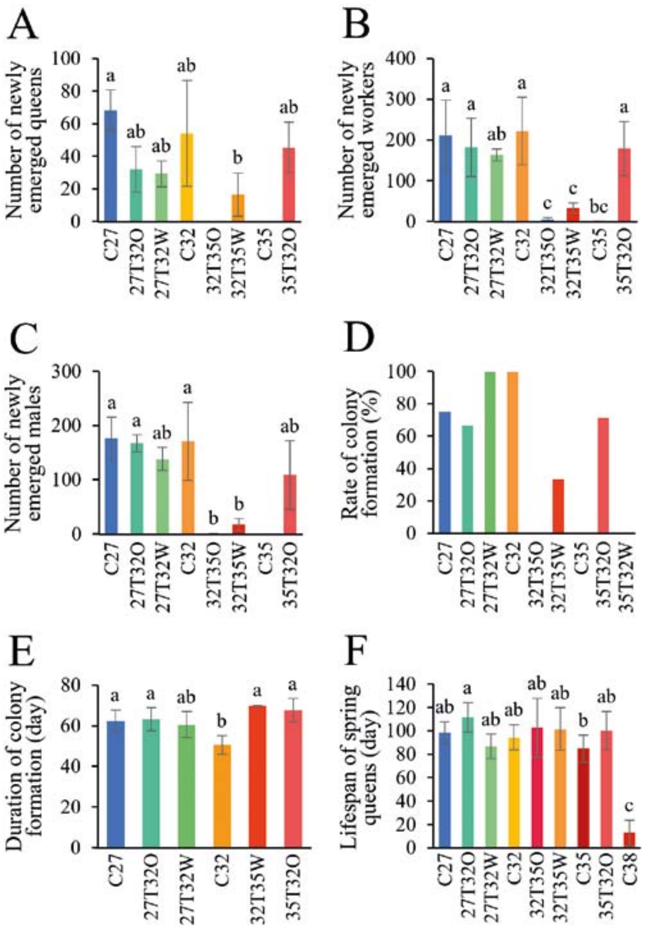

Fig. 3. Colony development under different temperature conditions: the (A) number of newly emerged queens per colony (n = 3–7; P = 0.040), (B) number of newly emerged workers per colony (n = 2–8; P = 0.0001), (C) number of newly emerged males per colony (n = 2–7; P = 0.0001), (D) rate of colony formation (n = 3–8; P = 0.0001), (E) Duration for colony formation (n = 1–8; P = 0.0001), and (F) lifespan of spring queens (n = 3–12; P = 0.0001). In (A–E), the low number of colonies (n = 1 or 2) was attributed to the failure of colony development under high-temperature conditions. Different letters indicate significant differences among the treatments (Tukey's multiple comparisons at P = 0.005 level).

spring queens displayed a slight delay (F = 8.590; df = 5, 18; P = 0.0001; Fig. 3E). The lifespan of spring queens was lower under higher temperatures (Fig. 3F). Overall, these results indicate that the C32 spring queens exhibited enhanced colony formation and shorter colony development periods. Moreover, it is important to note that, in the presence of workers, the spring queens demonstrated resistance to higher temperatures.

We found that the body weight of newly emerged fall queens, males, and workers decreased in the higher temperature treatments (Fig. 4A–C). Workers exhibited the most significant decrease in their body weight (F = 4.660; df = 7, 33; P = 0.001), length, and width, followed by queens (F = 16.710; df = 5, 923; P = 0.0001; Fig. 4A–C and S7).

Additionally, among newly emerged bees the average body mass of males relative to queens decreased as temperatures increased (1:2.18, 1:2.14, and 1:1.78 for C27, C32, and 32T35W, respectively) (Fig. S8). These results indicate that elevated temperatures result in decreases in the body sizes of adult offspring.

3.3 Mating behavior of fall queens under elevated temperatures

The cumulative mating rate, mating duration, and the time of mating occurrence were observed for newly emerged fall queens and males from the C27 group under mating temperatures of 23 °C, 27 °C, and 32 °C (Fig. 5A). Under higher temperatures, the mating rate decreased (P = 0.024), with shorter mating durations (P = 0.0001). Mating was generally concentrated within a 30-minute period under all temperatures. The mating of newly emerged queens and males from the C32 or 32T35W treatment were similar to C27, with a dramatic decrease in mating rates at 35 °C (P = 0.001 and P = 0.005, respectively; Fig. 5B, C). Notably, fall queens under 35 °C mating conditions exhibited a higher frequency of mating avoidance behavior (Video 3).

4 Discussion

Bombus terrestris has a wide natural geographic range, extending from Europe to North Africa, where it displays intraspecific variation in thermal tolerance that might reflect adaptation to local climates and/or phenotypic plasticity (Maebe et al. 2021a). Rapid anthropogenic climate warming is imposing new demands on bee populations and while elevated temperatures are known to effect colony production, body size, and foraging behavior (Kenna et al. 2021; Gérard et al. 2022b, 2023; Naumchik & Youngsteadt 2023), the extent of phenotypic plasticity is unclear. In particular, the influence of climate change on colony establishment and viability in terms of the oviposition behavior of spring queens and mating behavior of newly emerged fall queens. In captive *B. terrestris* under experimental conditions we found that overwintered spring queens and newly emerged fall queens were sensitive to elevated temperatures. Resulting behavioral changes may contribute to current and projected declines in bumblebee populations (Ghisbain et al. 2024).

Colony establishment and population development depends on oviposition and brood care by solitary spring queens. We discovered that spring queen oviposition behavior exhibited plasticity with regard to nest temperature; higher temperatures increased oviposition and resulting egg cells that was accompanied by wider dispersion of egg cells on the oviposition plate, more space between eggs within the egg cell, and increased thickness of the egg cells. The positive relationship between temperature and spring queen response indicates potential to mitigate thermal stressors.

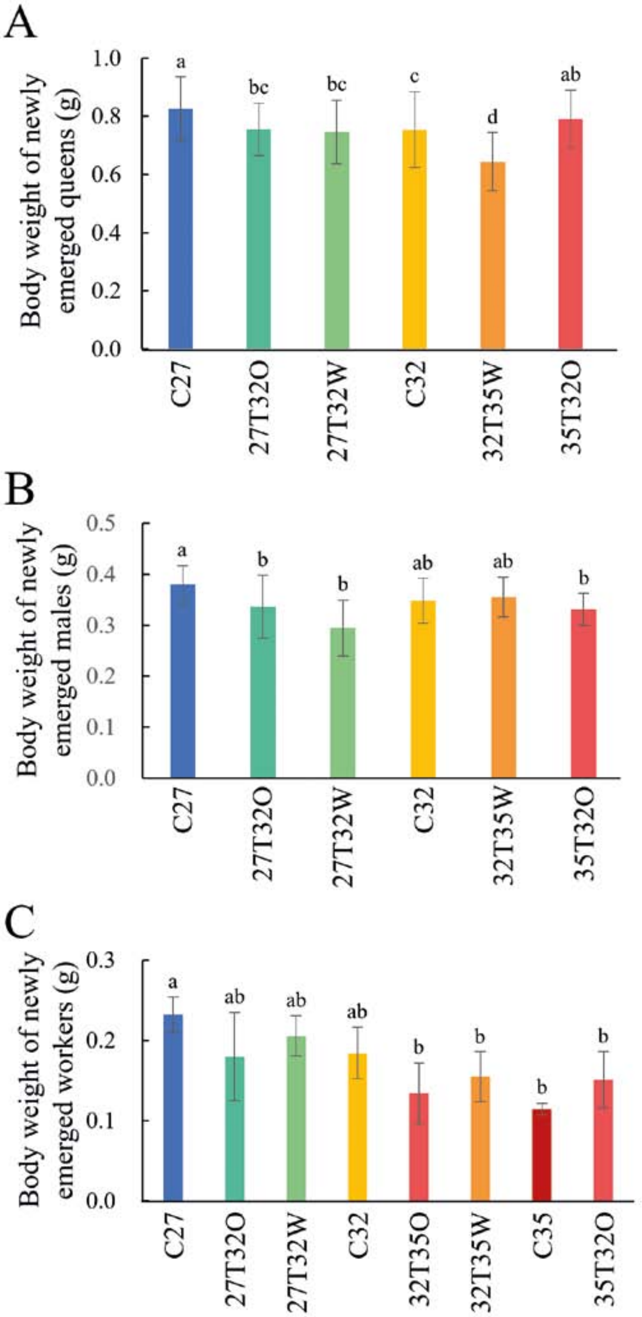

Fig. 4. Body weights of newly emerged queens, males, and workers under different temperature conditions: the (A) body weight of newly emerged queens (fall queens) (n = 114 for C27, 103 for 27T32O, 76 for 27T32W, 423 for C32, 5 for 32T35W, and 182 for 35T32O; P = 0.0001), (B) body weight of newly emerged males (n = 20 for C27, 30 for 27T32O, 7 for 27T32W, 42 for C32, 5 for 32T35W, and 26 for 35T32O, 10 replications; P = 0.001), (C) body weight of newly emerged workers (n = 5–10 for C27 and 27T32O, 3 for 27T32W, 8 for C32, and 7 for 32T35O and 32T35W, 4 replications; P = 0.001; In C35, only one worker emerged from each of the two colonies, thus n = 2). Different letters indicate significant differences among the treatments (Tukey's multiple comparisons at P = 0.005).

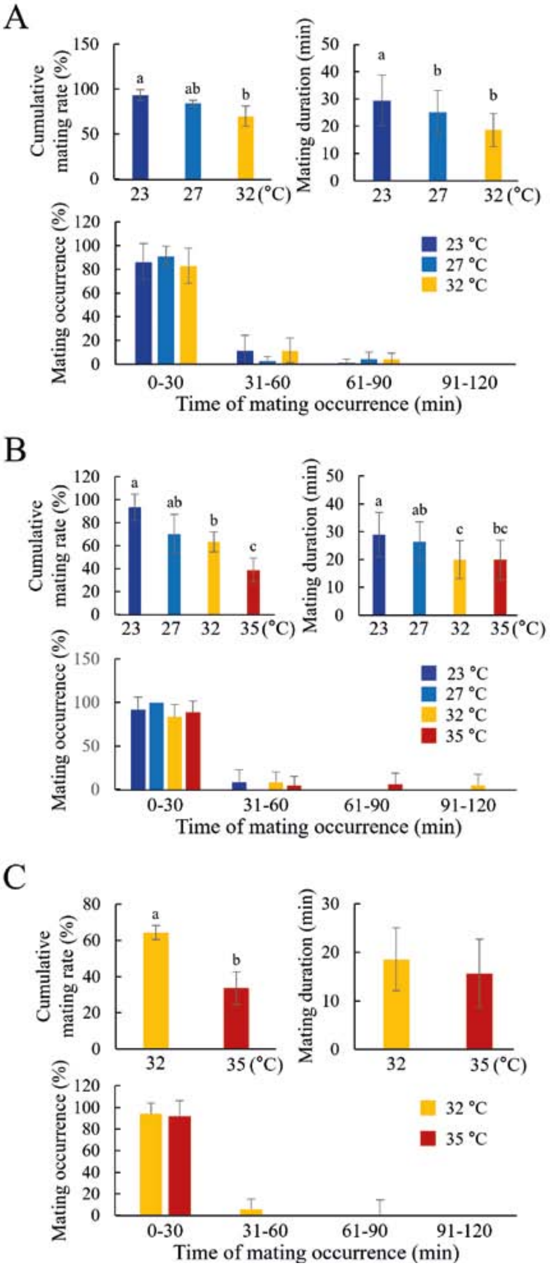

Fig. 5. Mating behavior of fall queens at different temperatures. (A) Mating behavior of newly emerged fall queens and males from the C27 treatment: the cumulative mating rate (*n* = 10–30, triplicates; *P* = 0.024; left), mating duration (*n* = 32–44; *P* = 0.0001; middle), and the time of mating occurrence (*n* = 32–44; right). Data were recorded at temperatures of 23 °C, 27 °C, and 32 °C. (B) Mating behavior of newly emerged fall queens and males from the C32 treatment: the cumulative mating rate (*n* = 10–30, triplicates; *P* = 0.001; left), mating duration (*n* = 12–37; *P* = 0.0001; middle), and time of mating occurrence (*n* = 12–37; right). Data were recorded at temperatures of 23 °C, 27 °C, 32 °C, and 35 °C. (C) Mating behavior of newly emerged fall queens and males from the 32T35W treatment: the cumulative mating rate (*n* = 5, triplicates; *P* = 0.005; left), mating duration (*n* = 14–19; *P* = 0.224; middle), and the time of mating occurrence (*n* = 14–19; right). Data were recorded at temperatures of 32 °C and 35 °C. Different letters indicate significant differences among the treatments (Tukey's multiple comparisons at *P* = 0.005 level).

This is consistent with plasticity exhibited by bumblebees in maintaining nest temperatures (Maebe et al. 2021b). Thermal stress induces behavioral changes in social insects (Perez & Aron 2020) and cycles of oviposition – ovicide in bumblebees are sensitive to external conditions such as temperature (Carnell et al. 2020). Consistent with this, we found that spring queens established colonies most rapidly at 32 °C, but when kept warmer at 35 °C they exhibited abnormal behavior including brood neglect, damaging egg cells, and ovicide, leading to recruitment failure. For *Bombus terrestris* spring queens 38 °C represents an extremely high temperature resulting in oviposition failure and shortened lifespan (Kelemen et al. 2019).

Queens kept at the coolest experimental conditions (27 °C) had a longer period between oviposition and emergence of adult offspring, with fewer workers and males compared to the 32 °C treatment (Yoon et al. 2005). Treatments involving higher temperatures (32T35O, C35, and 35T32ΔW) produced few workers, no males or queens, and ultimately failed to establish a colony. Collectively, the spring queens kept at 32 °C were more likely to establish a successful colony most rapidly, and in the presence of workers the spring queen demonstrated resistance to elevated temperatures. Although colonies established faster at 32 °C than 27 °C they produced adult offspring with lower body weights. Decreased body size of workers emerged from higher temperatures can have a negative impact on pollen collection and pollination (Kenna et al. 2021; Gérard et al. 2022b, 2023), and the disproportionately small size of males may negatively affect their mating interactions and reproductive fitness.

Bumblebees normally maintain a nest temperature of 27–29 °C through wing fanning (Vogt 1986; Westhus et al. 2013), and we found that spring queens and newly emerged workers at 32 °C exhibited this behavior. However, spring queens at 35 °C failed to regulate nest temperature despite wing fanning, as also reported in *B. impatiens* (Bretzlaff et al. 2024). In addition to greater chances of colony failure, elevated temperatures during development results in impaired behavioral response (Perl et al. 2022). Based on these results and previous findings that the appropriate nest temperature for oviposition and colony formation in spring queens appears to be below 32 °C. Reduced body size of adult offspring at this temperature could be attributed to physiological responses adopted to cope with thermal stress (Naumchik & Youngsteadt 2023). This suggests a trade-off between colony size and body size (Castillo et al. 2015), representing a potential strategy by which bumblebees respond to thermal stress. Elevated temperatures during development induced allometric changes in bumblebee morphological traits (Gérard et al. 2023), and workers reared at higher temperatures exhibit a higher visiting rate but shorter visiting time at flowers when foraging (Gérard et al. 2022b). This could reflect functional complementarity in the adaptive response to high temperatures.

The mating rate of newly emerged queens and males was significantly reduced at higher temperatures, with shorter mating, and more frequent mating avoidance, particularly at 35 °C. Given that climate change can affect bumblebee phenology (Gérard et al. 2020), our results suggest that earlier emergence of adult offspring could lead to a mismatch between the seasonal climate conditions and the ecological and physiological traits of bumblebees during their annual lifecycle. It also suggests that a longer and warmer fall period may contribute to the decline of bumblebee populations due to the mating avoidance behavior of newly emerged fall queens. Furthermore, heat shock (40 °C) negatively affects sperm viability in bumblebees (Martinet et al. 2021b). Therefore, higher temperature may affect the fertility of newly emerged fall males, including the number and viability of their sperm. In the present study, shorter mating durations at higher temperatures may negatively affect the quantity of sperm reaching the queen's oviduct and this could further imperil bumblebee population viability.

Within the calibration tolerances of our insectary and incubators our data indicate that elevated nest temperatures induce behavioral changes in bumblebee queens, suggesting the potential impact of climate warming on bumblebees. *Bombus terrestris* queens generally nest underground (Maebe et al. 2021b) so insulation provided by soil and vegetation cover may mitigate the impact of heatwaves, but nest temperatures can still increase, particularly in areas with sparse vegetation and direct sunlight, and we note that nests can be close to, or protrude through, the soil surface (Fig. S9). Therefore, bumblebees are likely to experience elevated nest temperatures in at least some natural settings that are similar to the experimental temperatures in this study. *Bombus* in regions experiencing novel temperature extremes including Europe and North America (Kaiser et al. 2023; White et al. 2023) may be most vulnerable.

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