



## RESEARCH ARTICLE

# Short-term exposure to heatwave-like temperatures affects learning and memory in bumblebees

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## Abstract

Global warming has been identified as a key driver of bee declines around the world. While it is clear that elevated temperatures during the spring and summer months—the principal activity period of many bee species—is a factor in this decline, exactly how temperature affects bee survival is unknown. In vertebrates, there is clear evidence that elevated ambient temperatures impair cognition but whether and how heat affects the cognitive abilities of invertebrates remains unclear. Cognitive skills in bees are essential for their survival as, to supply the hive with nutrition, workers must be able to learn and remember the location of the most rewarding floral resources. Here, we investigate whether temperature-related cognitive impairments could be a driver of bee declines by exploring the effect of short-term increases in ambient temperature on learning and memory. We found that, in comparison to bees that were tested at 25°C (a temperature that they would typically experience in summer), bees that were exposed to 32°C (a temperature that they will be increasingly exposed to during heatwave events) were significantly worse at forming an association between a coloured light and a sucrose reward and that their capacity to remember this association after just 1 h was abolished. This study provides novel experimental evidence that even just a few hours of exposure to heatwave-like temperatures can severely impair the cognitive performance of insects. Such temperature-induced cognitive deficits could play an important role in explaining recent and future bee population declines.

## KEYWORDS

behavior, *Bombus terrestris*, cognition, global warming, proboscis extension reflex, stimulus

## 1 | INTRODUCTION

Wild bees are responsible for pollinating more than 75% of plants globally and their pollination services are estimated to be worth several billions of dollars (IPBES, 2016; Potts et al., 2016). However, it is now widely accepted that wild bee species are declining across

several continents (Dicks et al., 2021) and that these, as well as future declines, are a consequence of climate change (Duchenne et al., 2020; Gérard et al., 2020; Soroye et al., 2020). Indeed, in a recent meta-analysis assessing the relative importance of different drivers of bee declines, climate change—and elevated temperatures, in particular—has been evaluated as “important” or “very important” on

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every continent (Dicks et al., 2021). Rising spring and summer temperatures and the increased frequency of heatwaves (IPCC, 2021; Perkins-Kirkpatrick & Lewis, 2020) have been postulated to have the largest effect on bees because they coincide with the most active period in their life cycle (Duchenne et al., 2020, 2020), although exactly what these effects are and the underlying mechanisms remain unknown (Dicks et al., 2021). A better understanding of how temperature affects different aspects of bee ecology is essential for developing effective methods for limiting bee and other insect pollinator declines (Diaz et al., 2019; Dicks et al., 2021; Soroye et al., 2020).

High ambient temperatures are known to affect cognition in several vertebrate species but their effect on invertebrates that, unlike vertebrates, cannot tightly regulate their brain temperature, remains mostly unknown (Soravia et al., 2021). When experiencing high ambient temperatures, mice, rats, and zebra finches have impaired associative learning and memory abilities (Danner et al., 2021; Erfani et al., 2019), and similar effects have been observed in fruit flies (Zhang et al., 2008). Heat-induced impairment of cognitive abilities could have negative consequences for fitness, as it would affect an animal's ability to learn novel cues and to make appropriate decisions and behavioural adjustments in response to fluctuating conditions within its habitat (Coomes et al., 2019; Rozan et al., 2007). For example, reduced spatial memory caused by elevated ambient temperatures in vertebrates can affect foraging and mating success (Branch et al., 2019; Lee et al., 2015). To date, however, the relationship between elevated temperature and cognitive abilities in most taxa, particularly in invertebrates, remains to be empirically tested.

Wild bees are central place foragers—they must locate floral resources, fly to them efficiently, learn which flowers are the most profitable and remember their location, return to their nest and then remember and return to the most profitable flowers again—behaviours that require cognitive capacities such as learning, memory, and navigation (Giurfa, 2013). Among social species, impairments to these capabilities will have severe consequences on the fitness of the colony, as workers will not be able to efficiently identify and navigate between productive floral resources, limiting their capacity to provide nutrition for the colony. While there is an increasing number of studies on the effect of increasing temperatures on bee ecology (Gérard et al., 2021; Maebe et al., 2021; Soroye

et al., 2020), the potential impacts of higher temperatures on their cognition have not been studied.

Here, we begin to investigate whether temperature-related cognitive impairments could be a driver of current bee declines by testing the hypothesis that bumblebees exhibit reduced learning and memory capacity when exposed to short term elevated temperatures (see Table 1 for the duration of each phase). We simulated temperatures that might be experienced by a bee on a foraging trip (i.e., between 66 and 82 min on average, and up to 209 min according to Westphal et al., 2006) during a Northern Hemisphere summer heatwave. Heatwaves can be defined as the period when “at least three consecutive days are above the 90<sup>th</sup> percentile of  $T_{max}$  for each calendar day” (Faye et al., 2021; Perkins & Alexander, 2013; Perkins-Kirkpatrick & Lewis, 2020). Based on this definition, a temperature of 32°C can represent a heatwave in many parts of the world, from North America to East Asia or Temperate Europe (Perkins-Kirkpatrick & Lewis, 2020). We therefore tested the cognitive capabilities of buff-tailed bumblebee workers (*Bombus terrestris*) exposed to ambient temperatures of either 25 or 32°C. At ambient temperatures of 25°C, *B. terrestris* workers have a high foraging activity and optimal flight performance (Kenna et al., 2021; Kwon & Saeed, 2003). At 32°C, *B. terrestris* will forage, although activity decreases and they have reduced flight performance (Kenna et al., 2021; Kwon & Saeed, 2003). Moreover, other bumblebee species have been observed foraging at this temperature, which is becoming increasingly common throughout their distribution ranges (e.g. Couvillon et al., 2010; Peat et al., 2005). By mid-century in the US, for example, 32°C is expected to be reached on an additional 20–30 days during the year (Vose et al., 2017). Thus, while 32°C lies at the upper limit of temperatures at which bumblebees forage, it is a temperature that many species in the Northern Hemisphere will increasingly experience during the colony's peak activity period.

To investigate the effect of temperature on the associative learning and memory capabilities of bumblebees, we used the free-moving proboscis extension response (FMPER) method in a differential conditioning task (Muth et al., 2018). Bees rely strongly on visual cues to locate and differentiate between floral resources (Avargès-Weber & Giurfa, 2013; Frasnelli et al., 2018), so we used differential visual learning tests to assess the effect of the different temperature treatments on bumblebee learning and memory. Blue light (wavelength peak: 460 nm) associated with 50% sucrose solution was the

	Starvation phase (2 h)	Learning phase (~1 h30)	One-hour break	Test phase (10 min)
Group 1, 25°C (n = 50)	25°C, without access to water	25°C	25°C	25°C
Group 2, 32°C (n = 50)	32°C, without access to water	32°C	32°C	32°C
Group 3, 32°C (n = 25)	25°C, with access to water	32°C	32°C	32°C
Group 4, 32°C (n = 25)	32°C, with access to water	32°C	32°C	32°C

TABLE 1 Different conditions during the four phases of the FMPER experiment

positively conditioned stimulus (CS+), yellow light (wavelength peak: 520 nm) associated with 2% quinine solution was the negatively conditioned stimulus (CS-), and white light (with a broad spectrum encompassing that of both the blue and yellow lights) was used as a neutral stimulus. The choice of light colour was based on the innate colour preferences of bees and the stimuli used fell well within the spectral sensitivity range of *B. terrestris* (Gumbert, 2000; Skorupski et al., 2007).

## 2 | MATERIALS AND METHODS

### 2.1 | General

The experiments were conducted in the Department of Zoology at Stockholm University (Sweden) during the spring and summer of 2021. We used workers from seven colonies of *Bombus terrestris audax* (Koppert), which were kept in the dark in a climate-controlled room (60% humidity, 25°C). The hives were provided *ad libitum* with sucrose water (Koppert Naturpol smart sucrose solution) and pollen candy, consisting of a mixture of fresh-frozen organic pollen (Naturprodukter, Rawpowder Bipollen). Only workers were selected for the experiments because they are primarily responsible for foraging and gathering resources for the colony during most parts of bumblebee life cycle.

We performed the learning and memory tests using the free moving proboscis extension reflex protocol (Muth et al., 2018) applied to four treatment groups as described below, with a total of 150 workers tested (Table 1). The experiment included four phases—a 2 h starvation phase, a learning phase (lasting 1.5 h for each batch of 10 individuals), a 1 h rest phase, and a test phase (details below).

### 2.2 | Starvation phase

Individual workers from different colonies were captured under red light and placed directly into 50 ml transparent Falcon tubes that were covered entirely by aluminum foil except at the ends. The tapered end of the tube had a 6 mm diameter hole to allow the bumblebees to extend its proboscis and the other end had a 25 mm diameter hole covered with metallic mesh. The tubes were placed in the dark for 2 h—the starvation phase—which gave the bees time to acclimatise to the tube and encouraged them to interact during the learning phase of the experiment. The starvation phase was carried out in two adjacent experimental rooms—one in which the air temperature was set at 25°C and another where it was set to 32°C. In each room, the humidity was maintained constant at 60%.

### 2.3 | Learning phase

After the starvation phase, the bees experienced the learning phase, during which they were trained to associate coloured lights with

either a positive or a negative stimulus. As with the starvation phase, individuals in the learning phase were placed in one of the two experimental rooms set at either 25 or 32°C.

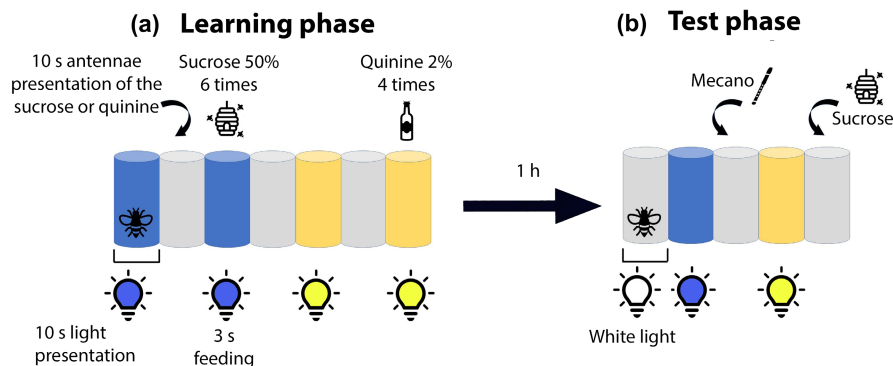
During the learning phase (Figure 1a), the stimuli (blue or yellow light) were presented for 10 s, then the bees were presented either with sucrose solution (in blue light, CS+) or quinine (in yellow light, CS-) by touching the solution to the antennae from a plastic stick (that was cleaned with alcohol between each presentation) presented from the 6mm hole for 3 s (Figure S1). The bee was then able to drink the sucrose or the quinine solution. White light (neutral) was presented for 10 s between each stimulus presentation to help the bee distinguish between conditioned light colours (blue and yellow). The bees' responses to the conditioned stimuli were quantified using binary data: we attributed a '1' if the bee extended its proboscis and '0' if it did not. During this learning phase, experiments were repeated six times for CS+ and four times for CS-, with an automated pseudo-random presentation. The CS+ stimulus was presented more times than the CS- to maintain motivation, as pilot experiments suggested that presenting quinine six times drastically reduced motivation. To control for the effect of room on the responses, we first conducted learning tests at 25°C in each room. These tests showed that the experimental room had no effect on the observed behaviour (Figure S2;  $p = .696$ ). After the learning phase, the bees had 1 h rest phase where they were placed once again in the dark in one of the two experimental rooms, before experiencing the test phase.

### 2.4 | Test phase

During the test phase (Figure 1b), the following sequence of five different stimuli were presented for 10 s to each bee. (1) White light was presented as a neutral stimulus to assess the ability of bees to distinguish between conditioned stimuli and unconditioned stimuli, (2) the positive conditioned stimulus CS+ (blue light) was presented without any sucrose reward or quinine, (3) a stick without any solution on it was presented to the antennae as a mechanosensory stimulus in white light (neutral stimulus), to check if the bees were responding to the movement of the stick alone, or to the contact between the stick and the antennae. We then (4) presented the negative conditioned stimulus CS- (yellow light) without any sucrose reward or quinine and, finally, (5) presented the stick with a sucrose solution, in white light, as a positive control to evaluate if the bee was still motivated by the sucrose solution. As in the learning phase, the responses were quantified using binary data (proboscis extension or not).

### 2.5 | Treatment groups

Individual bumblebees were placed into one of four treatment groups. Group 1 ( $n = 50$ ) experienced the whole experiment at 25°C (i.e., starvation phase, learning phase, 1 h break and test phase). Group 2 ( $n = 50$ ) experienced the whole experiment at 32°C. Unlike these first two groups, groups 3 and 4 had access to water (ad



**FIGURE 1** Experimental protocol. (a) Learning phase. Each colour represents the lights used as positive (CS+, sucrose) and negative (CS-, quinine) conditioned stimuli that were presented for a duration of 10 s. Sucrose and 2% quinine solutions were presented using a plastic stick. The CS+ and CS- stimuli were presented pseudo-randomly with 6 and 4 presentations, respectively, to maintain motivation. (b) Test phase. During this phase, the last stage (white light with sucrose) was used as a control for bumblebee's ability to extend their proboscis only to sucrose solution. Every grey cylinder represents a stage conducted in white light

libitum, in a cotton wool inside the tube) during the starvation phase, to ensure that the potential differences observed in the learning and test phases at 32°C were not due to dehydration experienced during the starvation phase. Group 3 ( $n = 25$ ) experienced the starvation phase at 25°C then the learning and test phases at 32°C and group 4 ( $n = 25$ ) experienced the whole experiment at 32°C. Differences between groups 3 and 4 also allowed us to test if temperature differences during the starvation phase had an impact on learning and memory.

As some bumblebees were dying during the starvation phase at 32°C, we also assessed if the body size of bees influenced their survival. Body size was measured as the inter-tegular distance (ITD; distance between the two insertion points of the wing), which is a reliable proxy for bumblebee body size (Cane, 1987). Indeed, a positive relationship between body size and starvation resistance often exists in insects (e.g., Gergs & Jager, 2013; Lehmann et al., 2006), thus synergistic effects between starvation and heat stress may have occurred, being lethal for some individuals.

## 2.6 | Statistical analyses

We used Generalised Linear Models (using the lmer4 package in R; R Core Team, 2020) with a binomial distribution to analyse the effects of the different stimuli on the responses. To assess if ambient temperature affected the learning capacity of bumblebees, we built a model that fitted proboscis extension (coded as a binary variable) as a response variable, temperature during the learning phase, trial number, the type of stimulus (blue colour alone, blue colour associated with sucrose, yellow colour alone or yellow colour associated with quinine) and the interaction between temperature and the type of stimulus as fixed effects. We did not include colony ID as random factor because it was explaining less than 0.001% of the variance that remained in the residuals. To assess the impact of water availability and temperature during the starvation phase on learning, we built a model that fitted proboscis extension as a response variable,

water availability, temperature during the starvation phase and their interaction as fixed effects, and colony ID as a random effect. To assess the impact of temperature and the type of stimulus (i.e., white light, CS+, mechano-stimulus, CS- and sucrose) on associative memory, we built a model that fitted proboscis extension during the test phase as a response variable, temperature during the test phase and the type of stimuli as fixed effects. We did not include colony ID as random factor because it was explaining less than 0.001% of the variance that remained in the residuals. In this model, we only included individuals that learned to extend their proboscis with the CS+ during the learning phase, to test the hypothesis that even when bees at 32°C learned to associate the blue colour with the reward, they performed worse at the memory test. Individuals that learned have been defined as individuals extending their proboscis to the blue colour during the sixth trial of the learning phase. As for the positive control (i.e., sucrose), 100% of the individuals extended their proboscis during the test phase, we used the method of bias-reduced logistic regression (using the brglm2 package in R; R Core Team, 2020) in the generalized linear model as it is adapted for models with a binomial distribution which include a level of a predictive variable that have a probability of 1. Indeed, it returns estimates that are always finite, even when the maximum likelihood estimates are infinite because of the probability of 1 (Kosmidis & Firth, 2021). We also built the same model including all individuals, whether they learned or not (results including all individuals for the memory test are described in Supporting Information S1; Figure S3). To assess the impact of water availability and temperature during the starvation phase on the test phase, we built a model that fitted proboscis extension as a response variable, water availability, temperature during the starvation phase, their interaction and the interaction between stimulus (i.e. blue colour with sugar, blue colour alone, yellow colour with quinine and yellow colour alone) and water availability as fixed effects, and colony ID as a random effect. Finally, to assess if there was a relationship between body size (measured as the ITD) and survival during the starvation phase at 32°C, we built a model with state (dead or alive) fitted as response variable, body size fitted as a fixed

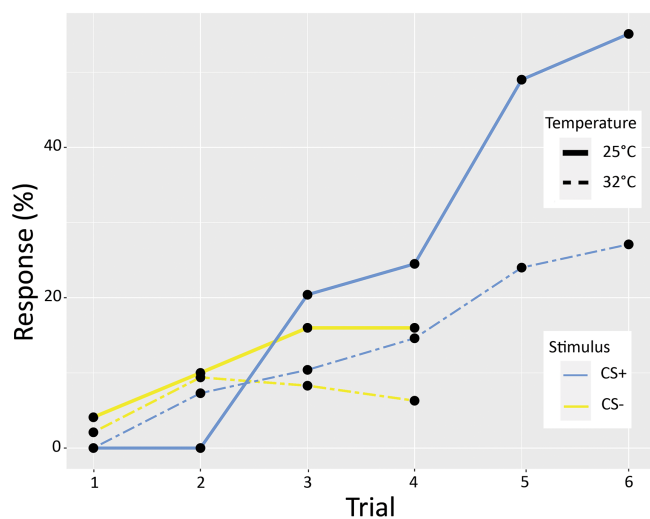
effect, and colony ID fitted as a random effect. The results of this analysis are described in Supporting Information S2 (Figure S4).

### 3 | RESULTS

First, we found that the proportion of bumblebees extending their proboscis in response to the CS+ was neither impacted by water available during the starvation phase ( $p = .30$ ), nor by temperature experienced during this time ( $p = .83$ ; Table S1 for details of the model output).

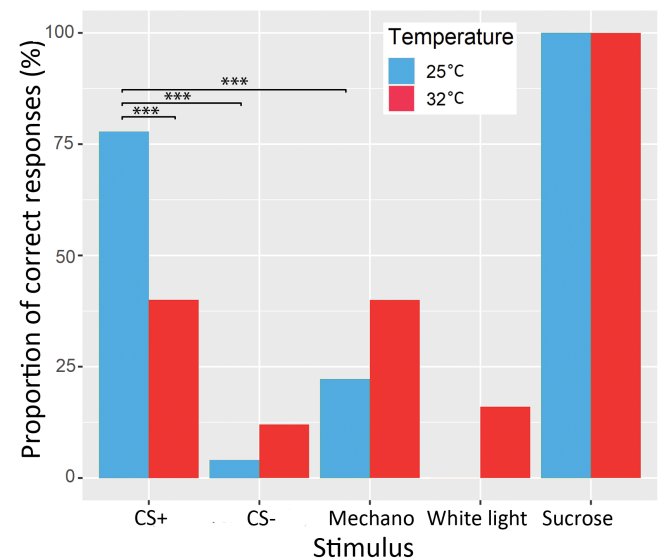
During the learning phase, bumblebees extended their proboscis significantly more often when presented with the blue light (CS+) than when presented with the yellow light (CS-;  $p < .001$ ). The number of bumblebees extending their proboscis when presented with the CS+ increased significantly across trials at both 25 and 32°C ( $p < .001$ ; Figure 2), although the proportion of correct responses was significantly higher at 25°C than at 32°C ( $p = .001$ ; Figure 2; Table S2 for details of the model output). By the sixth trial, when presented with the CS+, 55.1% of the bumblebees at 25°C extended their proboscis, while this proportion was halved at 32°C, with only 27.1% responding (Figure 2). While the number of bumblebees extending their proboscis when presented with the CS- increased significantly across trials at both 25 and 32°C ( $p < .001$ ; Figure 2), the proportion of proboscis extension to the CS- was not significantly different between the two temperature treatments ( $p = .84$ , pairwise post hoc multiple comparison; Table S3 for the pairwise comparison).

For the test phase, which occurred 1 h after the learning phase, we analysed only bees that had learned to associate the blue light



**FIGURE 2** The effect of temperature on a differential conditioning task in the learning phase. The proportion of bumblebees extending their proboscis across trials in response to the conditioned stimulus (blue light) and the unconditioned stimulus (yellow light).  $N = 50$  for 25°C and  $N = 100$  for 32°C

with a sucrose reward (i.e., individuals that extended their proboscis to the CS+ during the sixth trial of the learning phase, see Section 2). The results revealed that, as for the learning phase, bumblebees that experienced both the learning and test phases at 25°C performed better than bumblebees that experienced these phases at 32°C ( $p < .001$ ; Figure 3), with bumblebees at 32°C being less likely to extend their proboscis when presented with the CS+ (Figure 3; Table S4 for details of the model output). Indeed, when presented with the CS+, 77.8% of bumblebees at 25°C extended their proboscis, while only 40% responded at 32°C (Figure 3). We also found that the type of stimulus presented had a significant impact on proboscis extension ( $p < .001$ ; Figure 3). Using a post hoc multicomparison test, we once again found that bumblebees that experienced 25°C during the learning and test phases extended their proboscis significantly more often when presented with the CS+ than when presented with the CS- ( $p < .001$ ), the white light ( $p = .002$ ) or the mechanosensory stimulus ( $p < .001$ ). However, for bees that experienced 32°C during the learning and test phases, there were no significant differences between the proportion that extended their proboscis to the CS+, the CS- ( $p = .08$ ), the white light ( $p = .25$ ) or the mechanosensory stimulus ( $p = .999$ ). Nonetheless, these bees extended their proboscis significantly more in response to the positive control (i.e., sucrose) than to the CS+ ( $p = .02$ ) or any other stimulus ( $p < .03$  for all three other stimuli). All bees extended their proboscis when presented with sucrose solution, and temperature treatment had no effect ( $p = .158$ ), suggesting that the differences we observed in the learning responses between temperature treatments were not caused by differences in sucrose sensitivity. Finally, proboscis



**FIGURE 3** Proportion of bumblebees extending their proboscis during the test phase at the different temperatures and in response to different stimuli 1 h after the learning phase. Only bees that learned to associate the CS+ with the sucrose reward at the sixth trial were included. For each stimulus,  $n = 31$  for 25°C and  $n = 21$  for 32°C. The CS+ and CS- stimuli test the ability of the bees to remember the conditioned association they learned 1 h prior (\*\*\*) indicates  $p < .001$

extension in response to the CS+ or the CS− was not affected by water availability during starvation ( $p = .48$ ), temperature during starvation ( $p = .68$ ) or their interaction ( $p = .99$ ).

## 4 | DISCUSSION

While the impact of global warming on animals is becoming the focus of an increasing body of research, very little is known about how temperature affects cognition, particularly in invertebrates (Soravia et al., 2021). Here, we provide the first evidence that elevated ambient temperatures severely impact the learning and memory abilities of an important insect pollinator group, bumblebees. While temperature had no significant effect on the proportion of bees that extended their proboscis in response to a neutral stimulus (yellow light) that was associated with an aversive stimulus (quinine) during the learning phase, the rate at which bumblebees learned to associate a neutral visual stimulus (blue light) with a positive reward (sucrose solution) at an ambient temperature of 32°C was significantly lower than for those that performed the same task at 25°C. For bees in 32°C that did learn to extend their proboscis in response to the blue light at the end of the learning phase, their ability to remember this association after just 1 h was significantly impaired in comparison to those kept at 25°C. Indeed, at 32°C, bumblebees did not extend their proboscis significantly more to the CS+ than to the CS− or the neutral mechanosensory stimulus, suggesting that all memory of the positive reward was lost. Our findings are consistent with studies in humans, mice, goldfish, nematodes and fruit flies, which also indicate that heat-stress impairs learning and memory (reviewed in Soravia et al., 2021).

The cognitive impairment observed in this study, in natural circumstances, would likely affect the ability of workers to discriminate between flowers that provide good nectar resources (in this case, represented by the blue light) and those that provide less favourable resources (in this case, the yellow light). Such impairments may also affect the ability of bumblebees to remember landmarks, making it more difficult for foragers to find their way back their colony or to return to rewarding floral resources. Taken together with the recent finding that *Bombus terrestris* has a reduced flight performance at temperatures above 25°C (Kenna et al., 2021), our results suggest that even relatively short periods of elevated temperatures are likely to have a disastrous impact on the ability of bumblebee workers to efficiently supply their colonies with nutrition. In addition, our results suggest that, after a short exposure time, higher temperatures would impair bumblebees' ability to remember plants that provide no reward or are even aversive.

How does temperature disrupt bumblebee learning and memory? The answer to this question is not clear as the effect of temperature on neuronal function is poorly understood. Below lethal limits, temperature has been shown to affect motor control by changing the signalling rates in central pattern generators (reviewed in Robertson & Money, 2012). However, while a disruption to the motor control of the proboscis extension reflex could potentially explain the findings

of this study, it is highly unlikely, as temperature treatment did not affect the bees' ability to extend their proboscis in response to sucrose solution in the test phase. Instead, we hypothesise that elevated temperature causes a disruption to neuronal signalling in the periphery of the visual system and/or in the brain regions that are important for visual learning and memory, such as the mushroom bodies and the central complex (Plath et al., 2017). Increases in ambient temperature would increase the amount of kinetic energy in the nervous system, which would affect the rates of the chemical reactions and protein conformational changes that underlie proper neuronal function (Huey & Kingsolver, 1989; Robertson & Money, 2012). Such effects would be exacerbated in insects such as bees because they lack the ability to tightly regulate their brain temperature as vertebrates can.

While 32°C is close to the upper limit of the temperatures at which bumblebees forage (Heinrich, 2004), it is not beyond what they can experience during the peak of their annual activity period. Under normal conditions, bumblebees may avoid foraging when ambient temperatures are high due to the increased metabolic cost and the risk of overheating (Heinrich, 2004), rather than to evade the detrimental neural effects specifically. For example, in an ambient air temperature of 35°C, the thoracic temperature of a flying bumblebee approaches 45°C, which is close to their lethal limit (Heinrich, 2004). However, with rising spring and summer temperatures, as well as the increasing intensity and duration of heatwaves, bumblebees will need to trade-off feeding the colony against foraging in suboptimal physiological conditions, likely forcing them to forage more often in unfavourably warm conditions (although future studies on the relationship between bumblebee activity and heatwaves are necessary to test this). During our experiments, we noticed that some bees died during the starvation phase at 32°C. These bees had smaller body sizes than the survivors, suggesting that heatwave-like events may have an additional survival cost. The combination of an increased cost of foraging—due to increased metabolic costs and inefficiency caused by poor cognitive function—with a survival cost on the smallest foragers would, even over just a short period of time, make heatwaves particularly stressful for bumblebee colonies (Botias et al., 2021).

Our finding that just a few hours of exposure to 32°C led to significant cognitive impairments becomes even more concerning considering the origin of our study species: *B. terrestris* evolved in the warm climate of the Mediterranean, which is particularly rare among bumblebees (Rasmont et al., 2015), but should make it robust to warmer temperatures. The fact that we observe an impact in cognitive abilities already at 32°C is surprising, not only because this species will certainly be increasingly exposed to such temperatures throughout their activity period but also because it suggests that many other bumblebee species that are adapted to cooler climates may be even more affected by both seasonal and short-term increases in temperature. For example, we expect that the cognitive capacity of species with a more temperate or arctic origin may be more negatively impacted by high ambient temperatures. Bumblebees that are forced to forage in particularly warm



conditions (something that may occur during extended heatwaves) may succumb to a state called “heat stupor”, and eventually death (Martinet et al., 2015). For example, the maximum critical temperature of bumblebees (i.e.,  $CT_{max}$ , the temperature which leads to muscular spasms before an unresponsive state) is reached between 40 and 53°C depending on the duration of the stress, the species, and the method applied (Hamblin et al., 2017; Martinet et al., 2015; Oyen & Dillon, 2018).

The impact of high ambient temperature could even be accentuated by potential effects of stressful temperatures experienced during development. While it is already known that elevated developmental temperature can impact bee morphology (Gérard et al., 2018; Guiraud et al., 2021), its effects on cognition remain unclear. In honeybees, exposure to temperatures just outside the optimum for larval development can affect the learning and memory capacity of adults (Jones et al., 2005; Tautz et al., 2003), suggesting that exposure to temperatures that cause heat-stress in honeybee colonies may also have quite detrimental effects on the cognition of adults. When the internal ambient temperature of a colony reaches 32°C, *B. terrestris* workers begin to fan the brood, indicating heat-stress (Vogt, 1986; Weidenmüller et al., 2002). At this temperature, *B. terrestris* workers also decrease their foraging activity by 70% compared to 25°C (Kwon & Saeed, 2003). The timing of the heat stress event could indeed be critical and may permanently affect cognition if it occurs in the early stages of development (Buchanan et al., 2013). The effects of exposure to high ambient temperatures even for just a portion of development may ultimately lead to changes in behaviour. For example, *B. terrestris* workers that were exposed to 33°C during just a portion of their development displayed more maladaptive responses to basic stimuli, such as sucrose solution or light, than those that experienced a constant temperature of 26°C throughout their development (Perl et al., 2022). The ability of workers to forage efficiently is central to the survival of bumblebee colonies but the relationship between exposure to elevated temperature (either ambient or during development) and foraging behaviour remains poorly explored for now (e.g. Richman et al., 2020). Due to increasing average temperatures across the globe, as well as the frequency of punctual stressful climatic phenomena, there is an urgent need to investigate how high temperature, cognition and fitness interact in insects, particularly those that provide essential ecosystem services, such as pollinators. Our results encourage further research to focus on this topic, particularly in a context of the current global pollinator decline.

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## AUTHOR CONTRIBUTIONS

Emily Baird conceived the ideas. Maxence Gérard, Emily Baird, Bérénice Cariou, and Anahit Amiri contributed to the design of the methodology; Anahit Amiri collected the data; Anahit Amiri and Maxence Gérard analysed the data; Maxence Gérard led the writing of the manuscript with critical input from Emily Baird. All authors contributed critically to the drafts and gave final approval for publication.

## DATA AVAILABILITY STATEMENT

Data available from Dryad Digital Repository: (Gérard et al., 2022). <https://doi.org/10.5061/dryad.w3r2280t0>

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