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# Elevated temperatures shape cognitive performance and foraging preferences in free-flying bumblebees

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Despite concerns about global warming on pollination services, its impact on pollinator cognition and foraging remains underexplored. We investigated how short-term heat exposure influences learning and foraging preferences of free-flying bumblebees. Bees foraging at 32°C or 24°C were presented with yellow and blue artificial flowers associated with sugar solution (rewarding) and an alternative—water (neutral) or quinine (aversive). During the initial trials with an aversive alternative to the positively conditioned stimulus (CS+), bees at 24°C learnt at a faster rate than at 32°C, but the overall proportion of bees that learnt was higher at 32°C. This indicates that elevated temperature may impair the time required for bees to learn to associate a sugar reward with a coloured stimulus. However, by the end of the trials, temperature had no negative effect on the overall proportion of bees that learnt to associate flower colour with sugar solution. Moreover, we discovered that bumblebees foraging in warmer conditions exhibited a preference for water already in their initial floral visits. The findings of this study suggest that rising environmental temperatures may affect the foraging behaviour and floral preference of insect pollinators, but on-field studies are required to assess if it could impact the ecosystem services they provide.

## 1. Introduction

Cognition enables animals to modify their behaviour based on acquired and processed information. Despite their small brain size, insects exhibit remarkably sophisticated cognitive abilities, demonstrating skills such as learning, memory, categorization

and even face recognition [1]. These capabilities are essential for executing key biological functions, including foraging and reproductive behaviours [2,3]. For obligate flower feeders, such as bees, the ability to identify the most rewarding flowers is critical for maximizing nutritional intake while minimizing energy expenditure. Consequently, the capacity to rapidly associate floral stimuli and rewards is often selected for as an adaptive trait [4]. Yet, the cognitive abilities of bees are likely to be affected by a range of environmental factors, including temperature fluctuations [5]. Indeed, as cognition is achieved by neuronal communication in the brain, it is highly dependent on physiology, well-known to be affected by temperature [6]. Potential learning and memory impairments resulting from exposure to high ambient temperatures are, to date, poorly understood, particularly in an ecologically relevant context [7,8]. To better understand how rising global temperatures may affect the cognition of insect pollinators in more realistic conditions, it is crucial to conduct studies that allow them to exhibit natural foraging behaviours in free flight.

Insect pollinators also possess a variety of mechanisms to cope with elevated temperatures. Among these, behavioural plasticity has garnered significant research attention, particularly in the context of behavioural thermoregulation [9]. One example of this plasticity is exhibited by honeybees that, instead of collecting nectar and pollen from flowers, collect water from sources such as lakes and ponds to cool themselves and their hives in warmer temperatures [10]. When foraging in higher temperatures, other bee species may, like honeybees, also increase their preference for foraging on water and indeed learn to associate sensory cues with water sources, but reports of this behaviour have never been published. As such, it remains unclear if bees adapt their preference for water depending on ecological conditions.

Here, we investigate whether ambient temperature affects associative learning and behavioural plasticity in free-flying buff-tailed bumblebee workers (*Bombus terrestris*) foraging at ambient temperatures of 24°C and 32°C. *Bombus terrestris* would frequently experience 24°C under natural foraging conditions, and this species exhibits optimal flight performance and foraging activity at ambient temperatures of 24–26°C [11,12]. Temperatures around 32°C are becoming increasingly common during the natural activity period of *B. terrestris* [13,14], although bees exposed to this temperature exhibit decreases in flight endurance [12] and cognitive performance (observed in constrained bumblebees after 2 h of exposure [15]). As brain temperature can exceed that of the body under elevated temperatures [16], cognitive performance is expected to be particularly vulnerable to heat. Among the underlying mechanisms, several studies have highlighted that the depolarization of insect neurons and glial cells—such as a drastic increase in extracellular $K^+$ concentration—may contribute to heat-induced cognitive impairments [17,18]. In this study, we specifically test the hypotheses that, in free-flying bumblebees, short-term exposure to elevated ambient temperatures (i) impairs associative learning and (ii) causes an increase in water foraging. In the first experiment, foraging bumblebees had a binary choice consisting of coloured flowers that provided sugar solution (the positively conditioned stimulus, CS+) or flowers with a different colour that provided water (initially considered a neutral conditioned stimulus, CS). When environmental conditions fluctuate (e.g. during heat waves), species frequently respond first through behavioural changes that, in certain situations, can be decisive for their survival [19,20]. Although water-cooling behaviour is poorly documented in bumblebees (but see [21]), a shift towards water foraging could help them cope with elevated temperatures. To determine if bees in the first experiment were exhibiting learning impairments at 32°C or whether they were instead exhibiting an increased preference for water, we then repeated the experiment but replaced the water with a negative conditioned stimulus (CS−), quinine.

# 2. Material and methods

## 2.1. Bumblebee rearing

Bumblebee workers (*B. terrestris*) from 12 commercial colonies (Koppert, Berkel en Rodenrijs, The Netherlands) were used in the experiments. Each colony contained approximately 100 workers and a single queen. As cognitive ability is not affected by colony size [22], and to minimize additional stress, the number of workers was not modified. Numbered identification tags (LPs Biodling, Säffle, Sweden; figure 1) were affixed to the thorax of all workers in the colony using superglue. Workers emerged during the experiment were also tagged. The bumblebees were provided with ad libitum access to sucrose solution within the colony (Koppert Naturpol Smart) and one pollen candy (a mixture of fresh-frozen organic pollen from Naturprodukter and Rawpowder Bipollen), which was replaced

weekly. All colonies were housed in dark, climate-controlled incubators. Throughout the experiment, the colony itself was maintained at a stable temperature of 26°C inside the incubator (50 ± 5% relative humidity), while workers exiting the colony were exposed to one of the two ambient temperature treatments described below.

## 2.2. Experimental set-up

The colonies in the incubators were connected to the experimental set-up via two plastic tubes (15 cm long, 15 mm diameter). These tubes led from the colony to a plastic tunnel (200 cm long × 30 cm wide × 30 cm high) that was connected to a wooden foraging arena (60 cm long × 60 cm wide × 40 cm high) through a sliding door (figure 1). The walls of both the tunnel and the foraging arena were lined with patterns to provide bumblebees with naturalistic visual feedback, to aid flight control [23,24]. Twelve artificial flowers were arranged in a 4 × 3 grid, spaced 10 cm apart within the foraging arena. Each artificial flower consisted of a laminated coloured paper circle (45 mm diameter) featuring a white cross to help bees localize a solution placed in a 0.2 ml microcentrifuge tube located at the flower centre [25]. The flowers were attached to a dark green plastic sheet on the arena floor using magnets at fixed locations, facilitating replacement and cleaning. This experimental set-up was replicated in two climate-controlled rooms, one set at a high ambient temperature (32 ± 1°C) and the other at an optimal temperature (24 ± 1°C). Both rooms were maintained at a relative humidity of 25 ± 10% with a 12 : 12 light–dark cycle from 07.00 to 19.00 Central European Time.

## 2.3. Temperature treatments

The experiments were carried out over two nine-week periods corresponding to two experiments: Experiment 1 took place from November 2022 to March 2023 with six colonies. Experiment 2 was conducted from February to April 2024 with an additional six colonies. Each nine-week period consisted of three experimental blocks, comprising 2 days of pre-training and 11 days of experiments. During each experimental block, two colonies were tested simultaneously, one in each experimental room. From day 1 to day 4 of the experiment itself, the temperature in one experimental room was 32°C, while the other was at 24°C. On the 5th experimental day of the first week, the bumblebees present in the tunnel and arena in each room were returned to their respective colonies and moved to the incubator in the other room, ensuring that each colony experienced both temperature treatments. Colonies were subsequently left undisturbed in their new incubator for 2 days (days 6 and 7) to recover from the stress of the transfer. At the beginning of the second week (day 8), the bees re-started the associative learning experiment, which continued until day 11, when the experiment ended for a given colony. In Experiment 1, a control experimental block was conducted with two colonies. In this experimental block, both rooms were kept at 24°C and the colonies were swapped between rooms after the first experimental week (as in the experiments described above). The purpose of this was to control for any effects related to the room, the process of moving the hives between rooms and the order in which the colonies experienced the temperature treatments—that is, we could test whether colonies exposed to 24°C during the second week, after experiencing 32°C in the first week, behaved similarly to colonies exposed to 24°C for both weeks. The order in which the colonies were exposed to the temperature conditions—starting at 24°C in the first week, followed by 32°C in the second week, the reverse order, or 24°C in both weeks—is referred to as the 'sequence' in the analyses.

## 2.4. Training

Each colony underwent a 2 days training phase at the same temperature they would experience during the first experimental week. During this phase, all bumblebees were given access to both the tunnel and the foraging arena, where a plate of pollen and a feeder providing ad libitum 50% w/w sugar–water solution were provided to encourage them to enter the foraging arena. To train the bees to feed from the artificial flowers, three flowers were placed in the tunnel and 12 flowers were placed in the foraging arena. Each flower contained 0.2 ml of a 50% w/w sugar–water solution and a 0.5 ml drop of pollen solution (a mixture of fresh-frozen organic pollen and water). The flowers used in this phase were half-blue half-yellow (to provide an equal opportunity to associate each colour with a reward, [26]), with a white cross in the middle (figure 1). To prevent the bumblebees from associating the foraging arena with food depletion, the flowers in the foraging arena were removed overnight, leaving

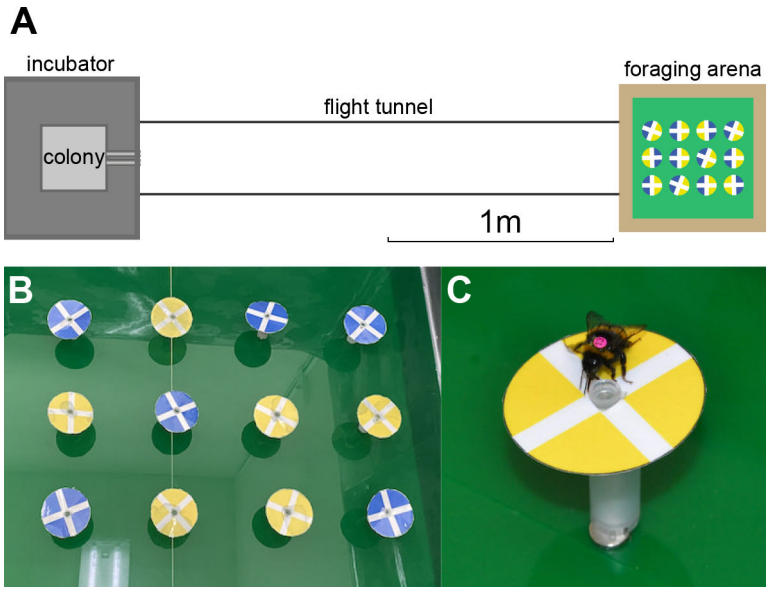


**Figure 1.** (A) The experimental set-up. A bumblebee colony was maintained at a constant temperature (28°C) in an incubator. The colony was connected via tubes to a flight tunnel and a foraging arena with artificial flowers. In the training phase, these flowers were half-blue and half-yellow. (B) In the learning sessions, the flowers were either blue or yellow and presented in a random arrangement. (C) Bees were able to access sugar solution, water or quinine from tubes inserted into the centre of the flowers.

only the pollen plate and sugar solution feeder. In the tunnel, six flowers with sugar solution and pollen solution remained. By the end of the 2nd day of training, most foragers were actively drinking from the flowers.

## 2.5. Associative learning and behavioural plasticity

Data were recorded during 1 h long 'learning sessions' held in the morning and afternoon, for 4 days. Throughout the experiment, bumblebees were allowed to freely enter and exit their colony. In the learning sessions, the artificial flowers from the training phase in the foraging arena were replaced with six blue flowers and six yellow flowers, each marked with a white cross indicating the centre of the flower (figure 1). Flowers of different colours were arranged pseudo-randomly in four different patterns that were changed after each learning session, to prevent bees from using spatial memory to learn the location of rewarding flowers. Prior to each learning session, the flowers were cleaned by wiping them with a water-dampened sponge, and were placed in a new configuration. Flowers were regularly replenished—typically every few foraging bouts—to prevent food depletion and to minimize the possibility that bees could visually detect a declining solution level. Access to the foraging arena was limited by a sliding door, such that only one individual was allowed in the arena during each foraging bout. This also prevented bees in the tunnel from observing the individual in the foraging arena, so we could exclude effects of social learning.

In Experiment 1, colonies one and two were presented with blue flowers containing 0.2 ml of a 50% w/w sugar–water solution (CS+) and yellow flowers containing water (CS). Conversely, colonies three to six were presented with yellow flowers that contained sugar solution (CS+) and blue flowers that contained water (CS). This design allowed us to control for potential biases in colour preference. The decision to use yellow as the CS+ in the control experimental block (colonies five and six) was based on observed colour biases during the first two experimental blocks, as detailed below.

In Experiment 2, all six colonies were presented with yellow flowers (CS+) containing 0.2 ml of a 50% w/w sugar–water solution and blue flowers (CS−) containing a 2% w/w quinine solution. During this experiment, only yellow flowers were rewarding because of the previously observed innate bias for blue flowers.

For the learning analyses, the value of each flower choice (defined here as a 'trial') was defined as follows: a value of 0 was assigned when a bumblebee either drank from or touched the unrewarded stimulus with its antennae (an incorrect choice), while a value of 1 was assigned for drinking from or touching the reward with antennae (a correct choice). Both drinking and antennal contact were scored as a positive response (1), since both behaviours contribute to taste perception and thus reinforcement

[27]. Indeed, antennal contact enables bees to detect the presence of solution within the artificial flower. Within the framework of this experiment, including antennal contact to code a 0 or a 1 is crucial. Indeed, when visiting the CS+ or the CS, bees regularly touched the solution with their antennae before proceeding to drink: if there was an antennal contact, they usually drank the solution (= 1). In contrast, when bees landed on the CS−, they often touched the solution with their antennae but rarely proceeded to drink after doing so. This behaviour indicates that the antennal contact is itself a meaningful choice, as it reflects the choice of the CS− (= 0) and the detection of quinine, but the bumblebees eventually do not drink it as it is aversive. A revisit of the same flower was considered as correct, as flowers were not emptied in a single visit or a foraging bout. During the whole experiment, an individual always received the same colour as CS+. We recorded all trials performed by an individual until they ceased foraging or flying for a duration of 3 min—after that time the worker was taken back to the flight tunnel. In total, we collected data from 265 individuals from 12 different colonies: 190 at 24°C (63 from Experiment 1 and 127 from Experiment 2) and 137 at 32°C (37 from Experiment 1 and 100 from Experiment 2), with some individuals participating in both temperature treatments.

## 2.6. Statistics

For the analyses, we used generalized linear mixed models (GLMM) with a binomial distribution, utilizing the lme4 package in R [28]. For each model, colony ID, bumblebee ID (nested within colony ID) and the experimental block and week (nested within experimental block) were included as random factors in the initial full model. We verified the assumptions of the binomial GLMM using the DHARMa package [29]. Simulated residuals showed no obvious deviations from uniformity, and tests indicated no significant overdispersion.

*Associative learning trends*. We evaluated the effect of ambient temperature on the associative learning ability of bumblebees during the first 10 trials. Ten trials are usually enough to detect a significant associative learning [30]. We developed two GLMMs, one for each experiment. Both models used flower choice as the response variable and included temperature treatment, sequence (a categorical variable with three levels), trial number and their interactions as fixed effects. Additionally, the model for Experiment 1 incorporated the rewarding colour as a fixed effect. To further investigate whether learning varied over longer periods (30 trials), we constructed two more GLMMs. These models also used flower choice as the response variable, with the same fixed effects as in the initial models. The first 10 visits represent the initial decision-making period, when bumblebees are first exposed to the task and begin to form associations. This phase is especially relevant for detecting early learning effects, treatment-driven differences in motivation or exploration in initial cue processing. In contrast, analysing the first 30 visits provides a broader view of performance over a longer timeframe, allowing us to assess whether treatment effects persist, intensify or diminish as the bumblebees gain more experience.

*Learning levels over the learning curve*. We further examined the differences in learning levels (i.e. the mean learning performance at a specific trial) at distinct stages of the learning process. First, we compared learning levels at trials 1, 10 and 30 using one GLMM per experiment (using either quinine or water as the alternative to sugar). These models include only bumblebees that completed the relevant number of trials, meaning that the sample size decreases with trial number (e.g. fewer bees reached trial 30 than trial 10). As we focused exclusively on data from the specified trials, the trial variable was treated as categorical. This analysis allowed us to directly compare the learning level achieved at these discrete intervals between the different conditions, irrespective of differences in learning rate (i.e. how fast the bees learn) or mean learning performance.

*Behavioural plasticity*. To determine if bumblebees adjusted their foraging preference in response to the availability of an alternative to sugar, we compared learning performance between Experiments 1 and 2 over 10 trials. We constructed a GLMM with flower choice as the response variable. The fixed effects included trial number, temperature treatment, experiment and the interaction between temperature and experiment.

We then assessed if the bumblebees that landed on the CS at elevated temperatures in Experiment 1 were drinking water or just touching it with their antennae. To do this, we selected all the trials (up to 30) where individuals chose the CS. A response value of 0 was assigned to bees that only touched the central tube with their antennae, a response value of 1 was assigned to bees that extended their proboscis into the central tube, indicating that they were drinking the water. We developed a GLMM with the response value (either 0 or 1) as the binary response variable, and included the temperature treatment, sequence, trial number, the rewarding colour and their interactions as fixed effects. This

**Table 1.** Generalized linear mixed models used in this study. Each model is listed with its corresponding research question and model specification. Fixed effects include trial, temperature and rewarding colour (when relevant). Random effects account for repeated measures at the level of individual bees (ID), colonies (Colony), experimental block and week.

| research question | model specification |
|---|---|
| does associative learning over 10 trials differ with water as CS? | Learning ~ Trial + Temperature + Rewarding Colour + (1 \| Colony/ID) |
| does associative learning over 10 trials differ with quinine as CS−? | Learning ~ Trial*Temperature + (1 \| ID) + (1 \| Experimental Block/Week) |
| does associative learning over 30 trials differ with water as CS? | Learning ~ Trial + Temperature + Rewarding Colour + (1 \| Colony/ID) |
| does associative learning over 30 trials differ with quinine as CS−? | Learning ~ Trial + Temperature + (1 \| ID) + (1 \| Experimental Block/Week) |
| are there differences at the 1st, 10th and 30th trial in Experiment 1? | Learning ~ Trial*Temperature + (1 \|ID) |
| are there differences at the 1st, 10th and 30th trial in Experiment 2? | Learning ~ Trial*Temperature + (1 \| ID) |
| does bumblebee drinking behaviour over 30 trials vary with temperature (Year 1)? | Drinking ~ Trial*Temperature + (1 \| ID) |

analysis allowed us to test if bees choosing the colour associated with water were actively using it as a resource.

For all analyses, we selected the final model from each full model, using a stepwise selection procedure by comparing the Akaike information criterion (AIC) values and choosing the model with the lowest AIC. In cases where AIC values were not significantly different, we chose the simplest model. All final models and the associated research question are summarized in table 1. All pairwise post hoc multiple comparisons were performed using the emmeans and multcomp packages in R, and *p*-values were adjusted using the Tukey method [31,32].

# 3. Results

## 3.1. Learning over 10 trials

In Experiment 1, we recorded 83 different individuals during the first 10 trials, with 69 of them performing all 10 trials. Learning was best explained by the model that included temperature, rewarding colour and trial as fixed factors, and bumblebee ID nested in colony ID as random factors (electronic supplementary material, table S1). The proportion of choices for the CS+ increased significantly over the trials ($\beta$ = 0.11, s.e. = 0.032, $p < 0.001$; figure 2A), and was significantly higher when blue was the colour associated with sugar ($\beta$ = −1.754, s.e. = 0.53, $p < 0.001$; figure 2A). Overall, there was no significant impact of temperature on the proportion of choices for the CS+ ($\beta$ = 0.48, s.e. = 0.444, $p$ = 0.28; figure 2A). The results indicate that, over 10 trials with water as the CS, bumblebees learnt to associate the CS+ with a reward, that temperature did not affect their ability to make this association and that they had a strong innate preference for the blue flowers.

In Experiment 2, we recorded 182 different individuals during the first 10 trials, with 107 of them performing all 10 trials. Learning was best explained by a model that included trial, temperature and their interaction as fixed factors, and bumblebee ID and week nested in the experimental block set as random factors (electronic supplementary material, table S2). The proportion of choices for the CS+ increased over the trials ($\beta$ = 0.102, s.e. = 0.032, $p$ = 0.002; figure 2B) and was, overall, higher at 32°C ($\beta$ = −1.609, s.e. = 0.316, $p < 0.001$; figure 2B). The significant interaction effect between temperature and trial suggests that the learning rate—that is, the rate at which the proportion of choices for CS+ increased over trials—was greater at 24°C than at 32°C ($\beta$ = 0.129, s.e. = 0.047, $p$ = 0.006; figure 2B). These results indicate that, over 10 trials with quinine as the CS−, bumblebees learnt to associate the CS+ with a reward, and that the likelihood of bees choosing the CS+ was higher at 32°C. However, the rate at which bees learnt to associate the CS+ with a reward was lower at 32°C.

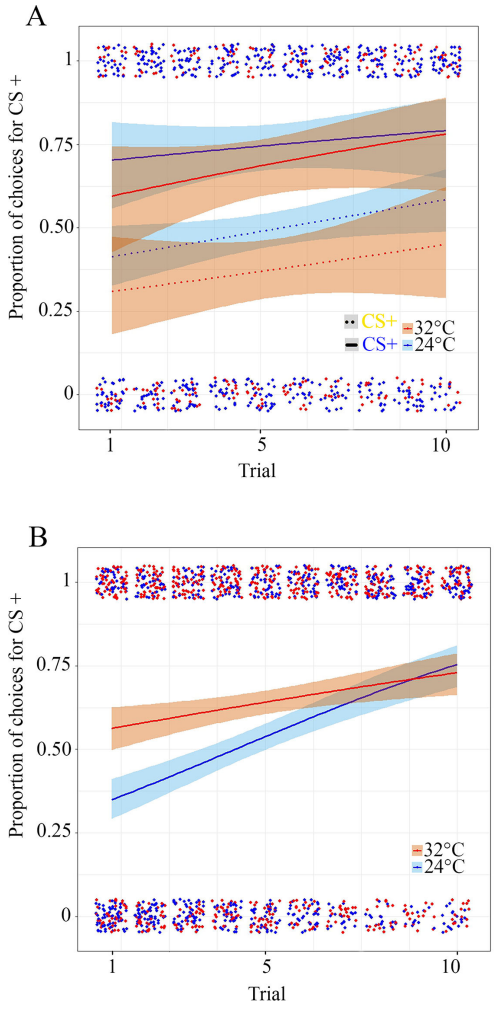


**Figure 2.** Proportion of correct choices in the first 10 trials. The points represent either the choice for the colour associated with the CS+ (response value = 1) or the choice associated with the CS− or the CS (response value = 0). The lines represent the learning curve with 95% CI predictions by temperature treatment group: 32°C (red) and 24°C (blue). (A) Experiment 1, when the water was associated with the CS. The solid lines represent the learning with blue as CS+, the dotted lines represent the learning with yellow as CS+ (763 observations from 83 bumblebees; 24°C, $n = 63$; 32°C, $n = 37$). (B) Experiment 2, when quinine was associated with the CS− (1402 observations from 182 bumblebees; 24°C, $n = 127$; 32°C, $n = 100$).

## 3.2. Learning over 30 trials

In Experiment 1, we recorded 83 different individuals during the first 30 trials, with 41 of them performing all 30 trials. Learning was best explained by a model that included temperature, rewarding colour, trial and the interaction between temperature and trial as fixed factors, and bumblebee ID nested in colony ID as random factors (electronic supplementary material, table S3). The proportion of choices for the CS+ increased significantly over trials ($\beta = 0.07$, s.e. = 0.014, $p < 0.001$; figure 3A) and was significantly higher with blue as CS+ ($\beta = -1.407$, s.e. = 0.318, $p < 0.001$; figure 3A). Overall, the proportion of choices for the CS+ was significantly higher at 24°C ($\beta = 0.739$, s.e. = 0.339, $p = 0.029$; figure 3A). The interaction between temperature and trial indicated that the learning rate was higher at 32°C, although this relationship was not significant ($\beta = -0.03$, s.e. = 0.016, $p = 0.065$; figure 3A). These results indicate that, over 30 trials with water as the CS, bumblebees learnt to associate the CS+ with a reward and that they had a strong innate preference for the blue flowers. At 24°C, a higher proportion of bees chose the CS+, but there was some indication that the learning rate was lower than at 32°C.

In Experiment 2, we recorded 182 different individuals during the first 30 trials, with 56 of them performing all 30 trials. Learning was best explained by a model that included fixed factors of trial and temperature, and random factors of bumblebee ID and the week nested in experimental block (electronic supplementary material, table S4). The proportion of choices for the CS+ increased significantly over the trials ($\beta = 0.108$, s.e. = 0.008, $p < 0.001$; figure 3B) and was, overall, significantly

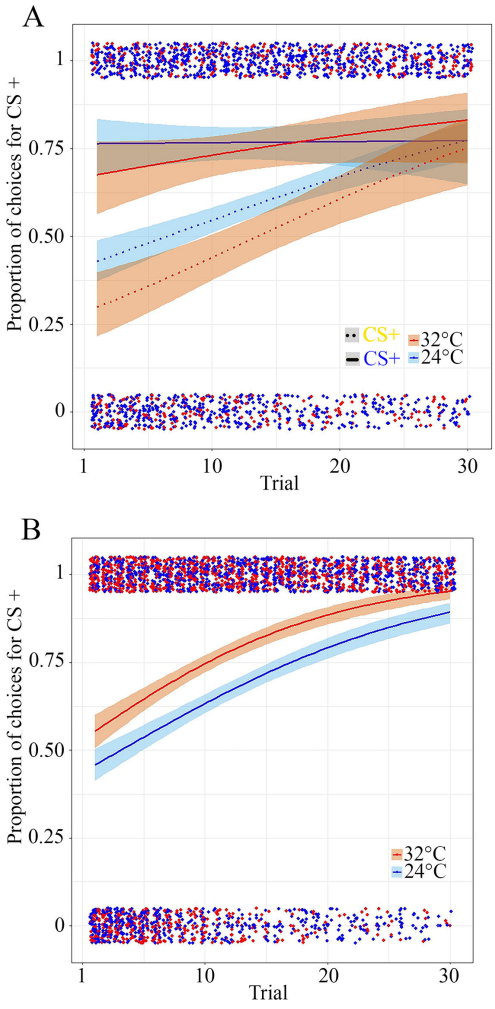


**Figure 3.** Proportion of correct choices in the first 30 trials. The points represent either the choice for the colour associated with the CS+ (response = 1) or the choice associated with the CS (water in Experiment 1) or the CS− (quinine in Experiment 2; response = 0). The lines represent the learning curve with 95% CI predictions by temperature treatment group: 32°C (red) and 24°C (blue). (A) Experiment 1, when the water was associated with the CS. The solid lines represent the learning with blue as CS+, the dotted lines represent the learning with yellow as CS+ (1781 observations from 83 bumblebees; 24°C, $n = 63$; 32°C, $n = 37$). (B) Experiment 2, when quinine was associated with the CS− (2892 observations from 182 bumblebees; 24°C, $n = 127$;, 32°C, $n = 100$).

higher at 32°C ($\beta = -1.168$, s.e. = 0.197, $p < 0.001$; figure 3B). These results indicate that, over 30 trials with quinine as the CS−, bumblebees learnt to associate the CS+ with a reward and that a higher proportion of bees choose the CS+ at 32°C.

## 3.3. Results of the comparison of the learning levels between the 1st, 10th and 30th trials

In Experiment 1, the final model included fixed factors of trial, temperature and their interaction, and random factor of bumblebee ID (electronic supplementary material, table S5). The results for both rewarding colours were combined for the analysis. Temperature had no significant impact on the proportion of choices for the CS+ at either trial 10 or trial 30, as indicated by the lack of significant interaction between learning and trial (respectively, $\beta = 0.308$ and 1.159, s.e. = 0.728 and 1.082, $z$-value = 0.423 and 1.071, $p = 0.673$ and 0.284; electronic supplementary material, figure S1). There was no significant difference in the innate choice of colour at trial 1 ($\beta = 0.457$, s.e. = 0.621, $z = 0.736$, $p = 0.462$; electronic supplementary material, figure S1).

In Experiment 2, the final model included fixed factors of trial, temperature and their interaction, and random factor of bumblebee ID (electronic supplementary material, table S6). Temperature had no significant impact on the proportion of choices for the CS+ at either trial 10 or trial 30, as indicated by the lack of significant interaction between learning and trial ($\beta = 0.479$ and −0.307, s.e. = 0.503 and 1.086, $z = 0.954$ and −0.283, $p = 0.34$ and 0.777, respectively; electronic supplementary material, figure

S1). However, a significant difference in the innate choices was observed at trial 1, where bumblebees at 32°C exhibited a significantly higher preference for the yellow colour compared with those at 24°C ($\beta = 1.267$, s.e. = 0.388, $z = 3.263$, $p = 0.001$; electronic supplementary material, figure S1).

### 3.4. Behavioural plasticity at elevated temperature

The results from Experiments 1 and 2 showed that the bees had different responses to temperature depending on whether the alternative to the reward was water or quinine—the proportion of bees choosing the CS+ in Experiment 1 was not affected by temperature, but was higher atn 32°C than at 24°C in Experiment 2. To investigate whether the bumblebees in Experiment 1 were displaying behavioural plasticity to prefer drinking water, we directly compared the results of Experiments 1 and 2. The final model included fixed factors of trial as well as temperature, experiment, their interaction and random factor of bumblebee ID. Over the first 10 trials at 24°C, there was no significant difference between the proportion of bees choosing the CS+ in each experiment ($\beta = -0.194$, s.e. = 0.131, $p = 0.452$). In contrast, at 32°C, the overall response to the CS+ was significantly higher when quinine rather than water was the alternative ($\beta = -1.14$, s.e. = 0.209, $p < 0.001$) (electronic supplementary material, figure S2). Thus, the proportion of bees that learn to associate a colour with sugar solution was not affected by the alternative at 24°C, but this proportion was significantly higher at 32°C when the alternative was aversive. This result suggests that some bumblebees in Experiment 1 were actively choosing to drink water. To further test this, we analysed whether bees that we recorded visiting the CS (i.e. the colour associated with water) during the first 30 trials were just touching the water with their antennae or whether they were drinking it. The choice to drink water was best explained by a model that included temperature, trial and their interaction as fixed factors and bumblebee ID as a random factor (electronic supplementary material, table S7). There was a significant impact of the interaction between trial and temperature: the proportion of bumblebees drinking water at 32°C increased significantly over trials while it decreased at 24°C ($\beta = -0.108$, s.e. = 0.038, $p = 0.005$; figure 4).

## 4. Discussion

In this study, we isolated the effects of ambient temperature on associative learning and behavioural plasticity in foraging workers by maintaining colonies at a constant temperature and utilizing artificial flowers. Rather than impairing overall learning as we hypothesized, short-term exposure to 32°C appeared to have no effect or to even improve it, depending on whether the alternative to the CS+ was water or quinine. Despite this, our findings indicate that exposure to elevated temperature may impair the time required for bees to learn to associate a sugar reward with a coloured stimulus, thus affecting the learning rate. We also discovered that some bumblebees developed a preference for flowers supplying water at the elevated temperature during the first few trials. This plasticity indicates that, in less than a few minutes, exposure to elevated temperatures is enough to trigger a behavioural response to heat, such as an increasing preference for water foraging.

In Experiment 1, across both the initial 10 and 30 trials, the proportion of bumblebees selecting flowers associated with sugar (CS+) was consistently higher in 24°C than in 32°C, although this difference was only statistically significant after 30 trials. Over 30 trials, the learning rate was faster at 32°C, and bumblebees eventually reached a similar proportion of CS+ choices by trial 30 at both temperatures (electronic supplementary material, figure S1, tables S5 and S6). In Experiment 2, the opposite trend was observed: bees foraging at 32°C consistently chose a higher proportion of CS+ flowers compared with those at 24°C. Nonetheless, the learning rate was faster at 24°C during the first 10 trials, and again, by trial 30, both groups achieved similar CS+ choice proportions (electronic supplementary material, figure S1). Thus, depending on the alternative available to the CS+, the effect of temperature on learning can vary. Providing an alternative that aids in coping with elevated temperatures, such as water, initially slowed foraging on the CS+ (electronic supplementary material, figure S2) but subsequently accelerated it, allowing bumblebees to reach learning thresholds within 30 trials that were comparable to those at 24°C. One explanation for this result is that elevated temperature exerts pressure to optimize learning and/or foraging on the rewarding flowers. In harsher conditions, animals must prioritize efficient foraging on rewarding options, leaving less room for exploration of unrewarding alternatives [33]. This trade-off between exploration and exploitation is frequently discussed in the context of food-depleted environments [34], but similar pressures may arise in other stressful conditions, such as elevated ambient temperatures. Our findings are consistent with

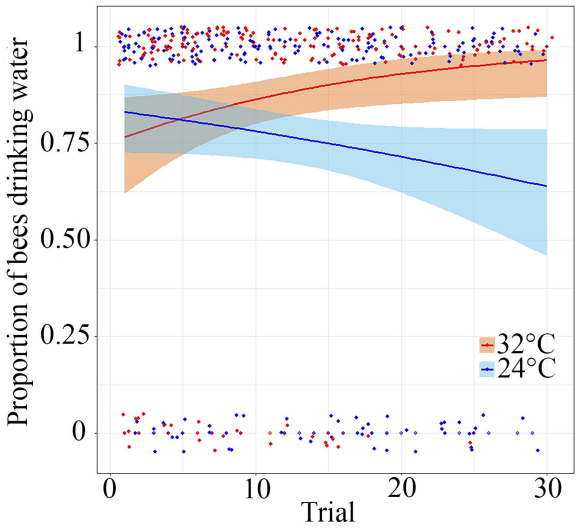


**Figure 4.** Proportion of bumblebees drinking water in the first 30 trials from Experiment 1. The points represent either a bumblebee actually drinking the water (response = 1) or a bumblebee touching the water with the antennae without drinking (response = 0). The lines represent the learning curve with 95% CI predictions by temperature treatment group: 32°C (red) and 24°C (blue) (354 observations from 26 bumblebees; 24°C, $n = 13$; 32°C, $n = 14$).

the hypothesis that, under low environmental pressures, animals should favour information gathering, while under more intense pressure, they should optimize their foraging strategy to maximize reward uptake [35]. Yet, it should be noted that these experiments were conducted sequentially rather than simultaneously (Experiment 1: November 2022–March 2023; Experiment 2: February–April 2024) and involved different sets of colonies. This temporal separation and the use of distinct colony groups could potentially contribute to some observed behavioural differences.

At 32°C in Experiment 1, we observed that some bumblebees, despite encountering both sugar- and water-associated flowers, continued to drink from the water-associated flowers. This behaviour was notably less common at 24°C. These findings suggest that bumblebees exposed to elevated temperatures exhibit behavioural plasticity that probably supports thermoregulation, as water absorption could aid in cooling. Importantly, this demonstrates a state-dependent valuation of resources [36]: the perceived value of the artificial flower's resource shifts with the environmental temperature. At 32°C, the low proportion of choices for the sugar-associated colour is unlikely to represent a mistake—as is evidenced by the higher proportion of choices for the CS+ at 32°C than at 24°C in Experiment 2—but rather a context-dependent decision reflecting the bumblebees' prioritization of water as a resource for thermal management. This plastic behavioural response is particularly advantageous in fluctuating environments, where different choices can maximize fitness depending on the prevailing conditions [20]. Unlike in honeybees [37], where water collection from water bodies is frequently observed, few studies have documented water foraging in bumblebees [21], and none have evaluated whether this behaviour serves as a buffering mechanism under warm conditions. Our results support the hypothesis that bumblebees use water foraging to mitigate the physiological effects of elevated temperatures and provide the first evidence that bumblebees may favour water over nectar as a floral resource. Behavioural adjustments often constitute the first line of response when environmental conditions change and, in some cases, can determine a species' ability to survive [19,20]. According to the principles of optimal foraging theory, foragers modify their diet to maximize energy intake [38]. Under stressful conditions, animals may also exhibit behavioural flexibility to mitigate physiological costs, such as overheating or oxidative stress. For example, pollinators may switch from collecting nectar to foraging for water, but behavioural shifts are not limited to resource choice; animals can also alter the timing or location of their foraging to avoid anthropogenic disturbances, such as humans or roads [39,40]. Despite the potential importance of such responses, studies examining how pollinators adjust their behaviour in response to environmental stressors remain scarce, yet this represents a promising avenue for research given its potential to buffer the impacts of multiple stressors.

We find no strong evidence to suggest that exposure to 32°C impairs the associative learning capabilities of bumblebees during 1–15 min foraging bouts. As such, our results are not consistent with previous work that has shown that exposure to elevated temperatures impairs insect cognition [15,41]. There are several possible explanations for this discrepancy. In other studies, the insects have been

unable to escape the ambient temperature either because they have been confined to tubes that restrict their behavioural repertoire [15] or because they were exposed to heat during their larval development [41]. In our experiments, bees could regulate the amount of time they were exposed to the temperature treatments when foraging by reducing their foraging time, the number of trips per worker and by increasing the worker turnover at 32°C [42]. This possibility is supported by data gathered with a similar set-up: using the same experimental set-up and hives, Gérard *et al.* [42] showed that, while differences in ambient temperature did not affect the duration of foraging trips for each bee, at 32°C the number of trips per worker decreased and the worker turnover increased [42]. Another possible explanation for the differences in the effect of temperature on learning observed here and in Gérard *et al*. [15] is that the brain temperature of bees in the different studies would not have been the same, although the studies were performed at the same two ambient temperatures. Sepúlveda-Rodríguez *et al.* [16] found that the brain temperatures of bumblebees resting in tubes for 2 h at 24°C and 32°C (as in Gérard *et al*. [15] and in the present study) were approximately 26°C and approximately 34°C, respectively. However, the brain temperatures of bumblebees that had flown in these temperatures for approximately 1 min—a situation that closely resembles that experienced by the bees in the current study—were approximately 34°C and approximately 38°C. Consequently, the discrepancies between the results of the present study and those of Gérard *et al.* [15] could be explained by variations in brain temperatures among the bees tested. Interestingly, learning performance was significantly higher at 32°C than at 24°C in Experiment 2. One possible explanation could be that these results are not related to the learning abilities *per se*, but rather to the ability to perceive the rewards. Indeed, higher temperatures increase the evaporation of sugar solution, thereby releasing more sugar volatiles and potentially enhancing the salience of the reward. However, this explanation appears unlikely, as previous studies have shown that bumblebees are unable to discriminate between sugar solution, water or quinine based on olfactory cues alone—except at very close range [43]. Further studies are therefore needed to determine whether 32°C optimizes neural processes related to learning and memory in *B. terrestris*, one of the rare bumblebee species living in warmer climates in Europe, including Mediterranean regions [44]. Our observations suggest that the duration and intensity of heat exposure is a critical factor to consider in future cognitive research. To make the experiments feasible in our laboratory, the bees were restricted to foraging flights over just a few metres. However, in nature, although some bees may concentrate their foraging flights to a small area around the nest [45], bumblebees typically travel much greater distances when foraging (e.g. several kilometres [46]). Experiments with longer travel distances would be necessary to determine if heat impairs bumblebee cognition under more naturalistic scenarios. Prolonged heat exposure can impact not only bumblebees but also flowers [47,48], particularly through the effects of drought [49]. The combined effects of heat and drought may alter flower phenotypes and the quality of floral rewards. However, the combined effects of climate change on plants and the potential impact of extended foraging time in high temperatures on pollinator cognition and foraging success remain unstudied, but this is a critical next step for future investigations.

**Ethics.** This work did not require ethical approval from a human subject or animal welfare committee.

**Data accessibility.** The data and the code related to this manuscript are provided in electronic supplementary material [50].

**Declaration of AI use.** We have not used AI-assisted technologies in creating this article.

**Authors' contributions.** M.G.: conceptualization, data curation, formal analysis, investigation, supervision, validation, visualization, writing—original draft; A.M.: formal analysis, investigation, writing—review and editing; C.S.: formal analysis, investigation, methodology, writing—review and editing; E.G.: formal analysis, investigation, methodology, writing—review and editing; J.T.: formal analysis, investigation, methodology, writing—review and editing; E.B.: conceptualization, funding acquisition, project administration, supervision, validation, visualization, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

**Conflict of interest declaration.** We declare we have no competing interests.

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