BRIEF COMMUNICATIONS

Bees associate warmth with floral colour

Pollinators may be seeking more than just food as a reward when they choose one flower over another.

Floral colour signals are used by pollinators as predictors of nutritional rewards, such as nectar[1–3]. But as insect pollinators often need to invest energy to maintain their body temperature[4] above the ambient temperature, floral heat might also be perceived as a reward. Here we show that bumblebees (*Bombus terrestris*) prefer to visit warmer flowers and that they can learn to use colour to predict floral temperature before landing. In what could be a widespread floral adaptation, plants may modulate their temperature to encourage pollinators to visit.

Some beetles spend extended periods (about 24 hours) inside specialized thermogenic flowers, even in the absence of a nutritional reward[5], and basking insects will take advantage of floral suntraps[6]. Visits to flowers by pollinating insects in order to imbibe carbohydrates in nectar are typically much briefer. But it is possible that endothermic pollinators might also seek a metabolic reward in the form of heat, given that the temperature of floral nectar is the same as the flower containing it. Differences in floral temperature occur widely between and within plant species[6–9] and, if these variations can influence the preference of pollinators, pollinators may forage adaptively by paying attention to temperature when choosing between flowers[10].

To test whether warmer nectar is preferred by pollinators, we connected a bumblebee nest box to a flight arena where sucrose solution (20% by volume) was available from two identical feeders, one at room temperature (18.5 °C ± 0.3 s.d.) and the other at 18.5 °C, 22.5 °C, 27 °C or 29.5 °C (for details, see supplementary information). There was a significant increase in bees' preference for the warmer feeder (Pearson's $R = 0.9870$, $P = 0.012$), and this preference was significant when the temperature difference was 4 °C or more (Fig. 1a). In this case, bees were using spatial positioning to identify the warmer feeder.

To test whether bees can learn to use flower colour to identify warmer flowers, we exposed them to coloured artificial flowers (four purple 'flowers' at 28.8 ± 0.2 °C and four pink 'flowers' at 20.8 ± 0.1 °C), which were positioned randomly and which each presented 20 µl sucrose solution (40% by volume) (see supplementary information).

Choice frequency for bees landing on the warmer purple flowers was 58.0% (± 2.6 s.d.; $\chi^2 = 25.6$, d.f. = 1, $P < 0.001$; $n = 10$ bees) (Fig. 1b). This was significantly higher than the choice frequency in a control group, for which there was no temperature difference between the purple and pink flowers, and indicates that the bees did not simply prefer purple flowers (Fig. 1b; mean, 49.4% ± 3.0 s.d.; independent samples *t*-test, $t = 9.54$, d.f. = 18, $P < 0.001$; $n = 10$ bees per treatment). When the pink flowers were warmer than the purple ones, the pink colour was preferred (Fig. 1b; mean, 61.6% ± 3.8 s.d.; $\chi^2 = 53.8$, d.f. = 1, $P < 0.001$; $n = 10$ bees).

We conclude that the bees preferred to land on the warmer flowers, even though the similarly coloured alternative contained the same nutritional reward. In another control experiment in which flowers varied in temperature but not colour, discrimination fell to chance levels (50.8% ± 3.1 s.d.; $\chi^2 = 0.3$, d.f. = 1; $P = 0.64$, NS; $n = 10$ bees), indicating that the bees must use colour as a cue, rather than directly gauging temperature by remote perception.

Our findings indicate that floral temperature can serve as an additional reward for pollinator insects when nutritional rewards are also available. They may also have implications for the evolution of specific floral structures and for the connection between floral sensory cues, floral temperature and pollinator behaviour[9].

Hot spot: a thermographic image of a bumblebee taken with an infrared camera — brightness indicates higher temperature.

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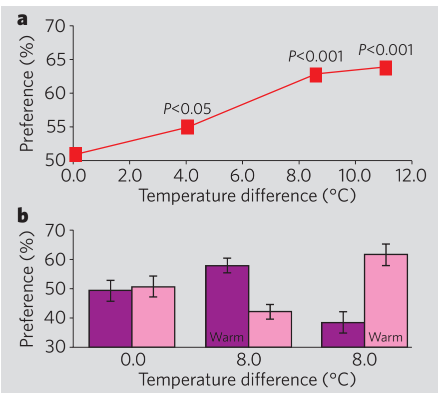

Figure 1 | Temperature preferences and flower-colour use by bees. a, Bee preference for sucrose solution at different temperatures above room temperature (18.5 °C); *P* values for χ^2; d.f. = 1. **b,** Bees learn to associate colour with temperature: when purple flowers are warmer than pink flowers by 8 °C, bees prefer the warmer purple flowers (central column pair); when pink flowers are warmer, these are chosen more often (right column pair). When there is no temperature difference, bees show no preference for either colour (left column pair). For each bee 100 choices were evaluated, and ten bees were used per group.

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Supplementary information

Methods

The bee flight arena was a box with a wooden frame (dimensions: L=110cm, W=70cm, H100 cm), with wire mesh on the 3 of the four sides, and UV transparent Plexiglas screens in the top and front panels. The arena floor was painted green (Humbrol No. 2; UK). Natural illumination in the arena was simulated using six Activa 172 Professional 36W fluorescent tubes (Sylvania, Germany). Since normal 50Hz strip lights will be perceived as flickering by insects' eyes, the lights were fitted with special ballasts (Philips HF-B 236 TLD), which convert the frequency to 4.3 KHz. Laboratory and feeder[1]. Artificial flower (and nectar) temperatures were measured with a Hanna (Cluj-Napoca, Romania) HI 8757 portable Microprocessor K-type thermocouple.

Experiment on temperature preference

Gravity feeders of the type used by von Frisch[2], p19. were used. These feeders minimise evaporation, since the sucrose solution is almost fully enclosed by a glass dome (contents 50ml); only in the tiny grooves in the base plate (from which the bees feed) is there contact between air and sucrose solution. Thus, even over an 8h period, concentration changed only by 0.3% (n=5), when the starting concentration was 20% (vol), the air temperature 18.5^{o}, and the feeder temperature was 29.5^{o}, the highest temperature difference between feeder and environment that was tested (see below). Concentrations were determined with an Atago HSR500 pocket refractometer (Tokyo, Japan).

Feeders were placed directly onto Techne DB-2A heating blocks (Cambridge, UK); each block was insulated around its circumference with 7 mm polystyrene foam (level with the base plate of the feeder) so that the sucrose solution was heated, but there was minimal influence on the landing platform. The feeders were placed 30cm apart on the arena floor at 1m distance from the nest entrance.

Feeders were heated to temperatures from 18.5ºC to 29.5ºC; these temperatures are within the range of floral temperatures reported in natural conditions[3]. Positions were switched between experiments to eliminate the possibility that bees could use previous experience to solve the task in each new test. Foragers collected sucrose for at least 6 hours before a test. Feeders were cleaned with ethanol before every hour during training and before testing. During a test, bee choices were counted for 1h. This procedure was repeated four times for each temperature.

Experiment with coloured flowers

Artificial flowers were built from Sterilin tubes (Bibby Sterilin Ltd., Stones, Staffordshire, UK: Ø=26 mm; height 8 cm), and we glued the lids of 0.5ml Eppendorf (Hamburg, Germany) tubes onto the top to hold the sucrose. The lids of the Sterelin tubes were painted either purple or pink. Purple artificial flowers were filled with warm water (28.8ºC ± 0.2ºC); pink flower tubes contained cool water (20.8ºC ± 0.1ºC) to stabilise temperature of the sucrose reward. The tempered reward droplets were placed into the lids of the Eppendorf tubes.

Purple artificial flowers were painted with a mixture of Humbrol (Hull, UK) paints No. 65, 68 and 200 (in a mixture of 1:2:7 vol.), whereas for pink flowers, we used the same paints in a ratio of 2:1:7). These colours were chosen to closely match those of wild type snapdragon (*Antirrhinum majus*) flowers (purple) and the pink MIXTA mutants which have a modified epidermis structure, resulting in a subtly different colour and floral temperature[4-6]. Reflectance spectra were measured with an Ocean Optics S2000 spectrophotometer (Dunedin, Fla., USA) and colour loci plotted in bee colour space (methods see ref.1; Figure S1). The colour distance between stimuli was 0.044 hexagon units, which means that the colours are potentially difficult, but not impossible, to distinguish for bees[7]. To confirm that this particular pair of colours could be discriminated, individual bees were trained with purple artificial flowers containing 20µl of 40% (vol.) sucrose solution, while visits to pink flowers carried a penalty: they contained 20µl of 0.012% quinine hemisulfate salt solution[7]. Discrimination was initially 50% and gradually rose to 76% ± 9.7% (n=5 bees; $\chi^2 = 25.7$, df=1; $p < 0.001$) by the time each bee had visited 60 flowers, so bees were clearly able to distinguish the two colours.

a) b)

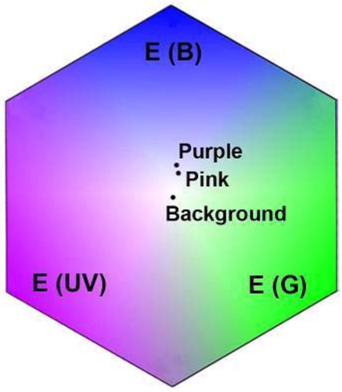

Figure S1. Artificial flowers, and the colour loci of the two types (purple and pink) in the bee colour space. A) Bombus terrestris *worker imbibing sucrose solution from an artificial flower.* ***B)*** *Colour loci of artificial flowers in the colour hexagon, where angular position from the centre denotes hue, so that colours lying upwards from the centre will be perceived by bees as bee-blue (i.e. stimulating predominantly the bees' blue receptor), whereas those that lie in the lower left corner will be perceived as bee-UV (stimulating predominantly the bees' UV receptor). Colour loci in the centre specify uncoloured objects (e.g. white). Distance between colour loci indicates the extent to which they appear as similar to bees: colours close together will be more difficult to discriminate than those far apart.*

Artificial flowers were brought to temperature (purple: 28.8ºC ± 0.2ºC; pink: 20.8ºC ± 0.1ºC) immediately before tests started. The temperature difference between flowers did not change substantially during tests: temperature decay was 0.7ºC ± 0.4ºC (n=3) for the warmer flowers over a 5 min period, while that of the cooler flowers did not change to a measurable extent. Foraging bouts typically lasted < 4 min; if bees occasionally foraged for longer periods, choices after 4 min were not counted. Bees were trained individually for 100 visits.

Flowers contained 40% sucrose concentration. Any difference in preference for higher temperature could not have been caused by differences in viscosity. This is because ingestion rates for bumblebees imbibing sucrose solution are unaffected by concentrations from 10%-40% (and corresponding viscosities of 1<cp<7)[8]. Viscosity of sucrose solution changes from cp=6.2 at 20.8ºC to cp=4.6 at 28.8ºC for 40% [9]

sucrose solution (and from cp=2.1 at 18.5ºC to cp=1.5 at 29ºC for the 20% sucrose solution[9] used in the temperature preference experiment). This means that all the viscosity values fall into the range where ingestion rates are independent of viscosity[8].

We also wished to make sure that the concentration of the sucrose solution droplets in the flower colour learning experiment did not change over the duration of a foraging bout (maximally 4 minutes). Over a 5 minute period, there was a slight increase in concentration over this period, but this increase was minimal: 0.22% (± s.d.=0.14%; n=10) in the cooler flowers and 0.27% (± s.d.=0.12%; n=10) in the warmer flowers.

Individual bees collected sucrose until they returned to the colony; between foraging bouts, artificial flowers were replaced with new ones, to eliminate the possibility of bees using scent marks[10-11]. Testing continued for 100 visits and landings on the respective stimuli were scored as the dependent variable. Data from the 10 bees per group were pooled after Chi-squared goodness-of-fit tests showed no statistical heterogeneity within groups of bees.

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