Warm flowers, happy pollinators

We tend to regard insects as 'cold blooded' and plants as passively adopting the temperature around them. Yet, a bumblebee's body temperature can be above 40°C, and needs to be at least 30°C for its flight motor to run smoothly. Flowers have invented a number of tricks to cater to this need: they warm themselves (and thus the nectar they offer to the bees) to several degrees above ambient temperature. The bees, in turn, use surprising strategies to identify warmer flowers.

Heather Whitney[1] and Lars Chittka[2]

[1]Cambridge University & [2]Queen Mary, University of London

Title image. Bees looking for floral warmth? Bumblebee (*Bombus terrestris*) foragers exploring Van Gogh's Sunflowers (copy painted by Julian Walker).

The café in the botanic gardens at Cambridge is a wonderful place to sit on a cold, bright February day. From there you can watch the emerging spring flowers, early bumblebees and posturing birds from the warmth, and a hot cup of tea sets you up well to face the brisk winds outside. However, the emerging spring flowers and their pollinators manage to cope in the spring chill without the warmth we so crave at this time of year. Or do they? We now know that flowers have a huge variety of ways of increasing their temperature; as well as this being of developmental advantage to the flower it could also act as an additional incentive for pollinator visits, much as the hot cup of tea is an additional incentive to visit the warmth of the café.

Floral warmth

The ability to create a warm flower may well have arisen early in the evolution of the angiosperms. Members of several basal angiosperm families such as the Nymphaeaceae and Magnoliaceae have the ability to actively produce heat in their flowers. The mechanism used is the alternative respiratory pathway, mediated by an enzyme – alternative oxidase (AOX) – that occurs inside the mitochondrial membrane. While this pathway occurs in all plants (its usual role is thought to be the production of reactive oxygen compounds that are important for defence against microbes), most plant species appear not to take advantage of its ability to produce heat. This capacity can be considerable, heating tissues by over 35°C above ambient temperature in the case of *Philodendron selloum*, an arum lily! In several species a lower but more constant temperature is preferred and respiratory heat production is regulated to achieve fairly constant flower temperatures in widely variable environmental temperatures (see references in Seymour et al, 2003).

However, the flowers that actively produce heat are relatively few. Other flowers rely on a more 'passive' method: they make the most of the ambient sunlight by converting it into warmth. Many of the flowers that show the most effective adaptations to increase floral warmth are Arctic or Alpine species, where low temperatures can result in reduced seed production. In fact, flower temperature can be a greater factor than pollinator visits in the amount of seed produced. Therefore a higher floral temperature is beneficial: it ensures protection of the flower during periods of cold, stabilises floral development, helps with pollen germination, ovule development, and may help pollen tube growth (Kevan 1989). Features thought to increase floral temperature include flower shape, size and angle to the sun.

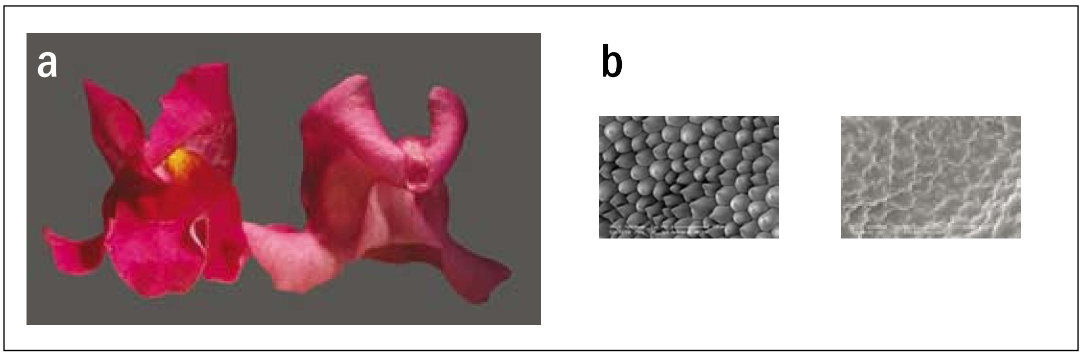
One particularly effective feature is that of heliotropism, where the flower consistently follows the sun. Although a range of flowers (for example sunflowers) have this feature, it is particularly prevalent in flowers that occur in the Arctic, and could be seen as a particularly 'arctic' adaptation, as it allows the flower to fully utilise the midnight sun that occurs in the far north. For the flowers of the arctic poppy *Papaver radicatum* this adaptation can increase the flower temperature by up to 6 degrees above ambient for 50% of their lives, which increases the number of days these flowers can actively grow by an extra 25% (Kevan 1989). When the sun-tracking ability of the flower was artificially prevented, both the quantity and quality of the seeds was reduced (Kevan 1989).

The arctic poppy has another adaptation that may help it in the harsh arctic conditions. The petal epidermal cells are 'reversed papillate'. This means that the inner tangential epidermal cell walls have papillae (cells with a pronounced conical shape) pointing into the internal cells of the petal. It is thought that these structures help to focus the light into these internal cells, warming the petal and making the most of the heating ability of the light. These 'reversed papillate' cells also occur in early spring flowers such as *Crocus* where increasing the floral temperature is important (McKee and Richards, 1998).

Conical cells on the petal epidermis are very widespread – it has been estimated that 80% of angiosperm flowers have them (see references in Dyer et al, 2007). They occur in a couple of different forms, the reversed papillate form of *Papaver* and *Crocus*, and the external conical form where the cells project out from the petal surface (Figure 1). However, the extent to which these cells can influence the temperature of the flower has only recently been investigated.

In 1994 Noda and colleagues from the John Innes Centre, Norwich, identified a snapdragon (*Antirrhinum majus*) mutant that lacked conical cells. It was possible to

Figure 1. (a) Wild type snapdragon (*Antirrhinum majus*) flowers have a deeply saturated purple colour (left) while *mixta* mutant snapdragons are pink (right). Both these flowers produce exactly the same floral pigments. It turns out that the only direct effect of the *mixta* mutation is a change of shape in the epidermal cells, with indirect effects on both floral colour and temperature. (b) Scanning electron microscope images of *Antirrhinum* petal surfaces: conical shaped cells of the wild type (left); flat cells of a *mixta* mutant petal (right). The conical cells act as lenses to focus the light into the pigment-containing vacuoles.



identify them due to the fact that the mutant flowers were significantly paler than those of the wild-type plants (Figure 1a). While the wild-type, magenta, *Antirrhinum* flowers have conical petal epidermal cells, those of this mutant, which appears paler-pink in comparison, were found to be flat (Figure 1). The pigments produced in both the flower types are exactly the same; thus it is the shape of the cells that leads to the difference in the colour we perceive.

The mutation was found to occur in the *mixta* gene, which encodes a transcription factor. Transcription factors are required to activate specific genes, for example for developmental processes during the growth of a cell. The MIXTA transcription factor is required for the activation of directional cell expansion (Noda et al, 1994). In virtually all cases, conical cells are only found on the petals of flowers, not the leaves, and this restricted expression pattern has led to the suggestion that conical cells are produced to enhance the attractiveness of the flower to potential pollinators. The fact that the presence of these cells affects the colour of flowers added weight to this suggestion – but could this be the only function for conical cells?

To study the effect conical cells had on pollinators, field experiments were carried out on snapdragon flowers of both wild-type and *mixta* flowers. Using these two flower sets, which were genetically identical except for the mutation in the *mixta* gene, meant that the effect of a single trait on pollinator behaviour, in this case that of conical cells, could be observed. Both lots of flowers had had their anthers removed (emasculated), to prevent self-pollination. It was found that, under these conditions, the *mixta* flowers produced much less seed than the wild-type. This was not the case when the flowers were pollinated by hand.

So both sets of flowers were equally capable of producing seed if pollinated, and the only reason for the reduced seed set in the *mixta* flowers was due to a reduced visitation rate by pollinators (Glover and Martin 1998). Indeed, this was later observed in greater detail – fewer bumblebees were observed visiting the *mixta* flowers when they were planted in randomly arranged plots with wild-type flowers (Comba et al, 2000).

So conical cells do enhance the attractiveness of the flower to pollinators – but is this only due to the effect on colour? Work done with flower-naïve *Bombus terrestris* bumblebees has shown that while bumblebees can tell the two flower types apart by colour, the bees do not show any innate preference for either colour, nor does the greater contrast with green of the darker colour make it any easier for the bees to find the flower (Dyer et al, 2007). What else about the effect of conical cells on the flowers could be attracting the pollinators, and leading them to choose conical-celled flowers over those with flat cells? Comba and colleagues also found that the snapdragon flowers with conical cells were significantly warmer than the flat-celled flowers (Comba et al, 2000).

The snapdragon *mixta* mutant was isolated due to the difference in colour it showed from the wild-type snapdragon flowers. The effects of colour and temperature can be closely linked in flowers. Conical cells are thought to act as lenses, focusing light into the epidermal cell vacuoles that contain floral pigments such as anthocyanins (see references in Dyer et al, 2007). It is easy to imagine how this 'focusing' effect of the conical cells could affect both temperature and colour. The colour that animals perceive in an object is a function of the light that the object reflects – which is dependent on the incident light minus the light the object's surface absorbs. At the same time, of course, absorbed light is also converted into heat. In addition, temperature may influence the production of pigments that result in floral colour. In several species that have a variety of colours, a temperature difference between different colours has been found.

However, there does not appear to be a simple and consistent pattern in terms of which flower colours are associated with more warmth. In the arctic poppy *P. radicatum*, where two forms – white and yellow – exist, the yellow flowers were found to be significantly warmer, which is what one might expect. White surfaces bounce back (do not absorb) much of the incident light, whereas yellow flowers absorb some light and might convert the light energy to heat. In *Crocus*, however, where three colours were studied, the purple and white flowers were found to be warmer than the yellow flowers (McKee and Richards, 1998). In general, one might predict that a darker flower would be warmer, but the yellow crocuses deviate from the prediction that they should be warmer than the white flowers. Clearly, more data are needed so that we understand whether there is a general pattern linking flower colour and temperature, in such a way that pollinators might actually be able to anticipate the temperature of a flower from its colour, without first having to probe it.

Thus, pigmentation can have an influence over temperature, but the reverse may also be true. Flower pigmentation is controlled by a range of internal and external factors, and one of these factors is temperature. In petunia, a period of moderately-low temperature increases anthocyanin production and so enhances flower pigmentation (Shvarts et al, 1997). As a darker flower would be warmer, this 'universal stress response' of anthocyanin production (plants produce anthocyanins in response to many stressful environmental triggers) in this case may have the additional advantage of helping to ameliorate the situation.

Figure 2. Thermographic image of a bumblebee on a flower taken with an infrared camera. Brighter colours indicate higher temperature. Photo by B. Bujok, M. Kleinhenz, J, Tautz (Beegroup Würzburg), with permission.

Responses of pollinators

Bees have spectacular thermoregulation abilities and can raise their body temperature to 30°C above ambient temperature to near 40°C (Figure 2). Indeed, they need such high temperatures to be able to fly. They do this by shivering their flight muscles, but this takes a lot of energy (Heinrich and Esch, 1994). Hence, a good strategy would be to obtain some of the heat from external sources. Warmer flowers are more attractive to pollinators. It has previously been observed that insects will bask on warm, sunlit leaves or flowers. In Arctic flowers, warmer flowers were found to attract more pollinators due to the use by the insects of the flower as a heating site during low temperatures (Kevan, 1989).

Heat-producing flowers reward insects with a direct energy reward. This was shown by some elegant research by Roger Seymour and colleagues who studied *Cyclocephala colasi* beetles, which are the main pollinators of the lily *Philodendron solimoesense* in French Guiana (Seymour et al, 2003). The flower produces heat so that, at night, the floral chamber is 3.4-5.0 degrees warmer than the ambient temperature. Since the beetles have to produce heat to keep their body temperatures high enough for activity, beetles that remain in a warm flower during the night use less than half the energy they would have used if they had stayed out in the open.

Flowers that warm themselves by more passive means can still use temperature as a heat reward. In *Oncocyclus* irises, the very dark floral colour of the flower contributes to the floral microclimate such that the flower is 2.5 degrees warmer than the ambient night temperature. The pollinators of this flower, male solitary bees, shelter in the flowers and are able to use this additional warmth to start foraging earlier than those kept at a lower temperature (Sapir et al, 2006).

These observations show that insects sometimes use warm flowers as shelters – but can they also 'forage for heat' by picking warmer flowers in their typically brief visits during nectar collection? Social bees are the intellectuals of the insect world, with outstanding learning abilities. Can they learn to pick the warmer flowers, and learn the cues that identify them from a distance?

As discussed, in snapdragons a single gene difference leads to a reduction in floral temperature but also to subtle colour change (Comba et al, 2000). In recent laboratory experiments, the *Antirrhinum mixta*/wild-type flower system was mimicked, using artificial flowers (Figure 3a) that were similar to those displayed by the wild-type and *mixta* flowers (pink and purple; Figure 1a). Equal amounts of 'nectar' were supplied on each artificial flower, but while the pink flowers remained at room temperature, the purple flowers were 8 degrees warmer. The bees were able to distinguish the two temperatures, and preferred to visit the warmer (purple) flowers (Figure 3b). This means that bees can use floral colour as a cue to both distinguish between flowers of different temperatures and then to discriminate against the cooler flowers (Dyer et al, 2006).

These experiments show how bumblebees cleverly reduce their own investment in making heat by seeking out flowers with warmer nectar – they are essentially collecting warmth, because warmer nectar constitutes a direct metabolic reward. This discrimination against cooler flowers could be the reason why the *mixta* mutant flowers were avoided by pollinators in the field trials: having sampled both the cooler flat celled flowers and the warmer conical flowers the bees actively chose to visit only the warmer flowers, which they could distinguish using flower colour as a cue. These results suggest that warmth is

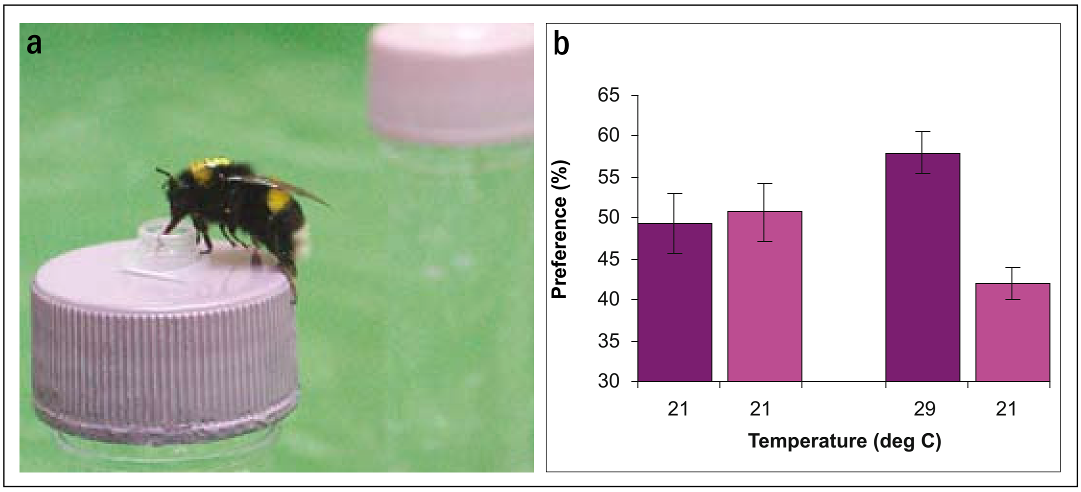


Figure 3. (a) A bumblebee worker imbibing sucrose solution from an artificial flower in a laboratory flight arena. Artificial flowers were made from plastic tubes with painted lids, and a small nectar reservoir glued onto the top. Water inside the tubes allowed to stabilise the temperature of the flowers (Photo by A G Dyer); (b) Bees preferred the warmer purple flowers over the cooler pink flowers (right pair of columns) – but this preference was not apparent when the flowers were equal in temperature (left two columns). This shows that the preference for purple was not innate, but had to be learnt in conjunction with the temperature difference.

a significant factor in both the attraction and retention of a pollinator to a flower species, and could therefore be important in the maintenance of floral features in the economy of nature.

Scent and nectar production

Warmer flowers may also increase the attractiveness of a flower from a distance by enhancing the evaporation of floral scents. In the case of the flowers heated actively by the alternative respiratory pathway, it has been found that the temperature was important in attracting pollinators by olfaction. Work by Angioy et al (2004) on the thermogenic flowers of dead horse arum (*Helicodiceros muscivorus*) showed that the effect of heat on oligosulphide volatiles was important in attracting blow flies, the pollinators of this plant. As heat is also an important oviposition cue for blow flies, temperature itself may again be an important cue in this plant-pollinator interaction, as well as influencing other cues such as volatiles. As discussed below, warmer flowers usually produce more nectar of greater quality, so an ability to use temperature as a cue would be an advantage to potential pollinators.

Nectar production is a temperature dependent biological process, and investigations have found that at low temperatures nectar secretion in most species decreases (Corbet, 1979). This could mean that if a flower is warmer it could constitute a better source of nectar, which would act as an additional incentive for pollinators to choose warmer flowers. However, nectar production is hugely variable, even within the same plant individual, and is subject to many environmental factors. Thus, warmth as an indicator of nectar production might not necessarily be a particularly reliable cue, and choosing warmer flowers for their metabolic reward of heat alone would provide sufficient motivation for a chilly pollinator.

Conclusions

Flowers have a wide array of ways to attract and reward pollinators. The complex factors linking temperature as both a reward and a cue, as well as its influence on other rewards and cues is gradually being understood. However, it is good to know as you observe the activity of bumblebees from the warmth of a café, that as well as getting food from their mutualist partners, the bees are also getting warmth. A warm flower and a hot sweet drink – what more could any chilly pollinator ask for? What the bees appear to be doing is indeed a bit like us drinking a hot drink on a cold day. If you need to warm up, you can produce your own heat, at the expense of some of your energy reserves - or you can consume a warm drink, and save on investing your own energy. A fascinating observation is that bees do not just prefer the warmer drinks – they also learn to predict the flower temperature from the flower colour.

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Heather Whitney is a Postdoctoral Researcher at the Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA.

Lars Chittka is Professor of Sensory and Behavioural Ecology at the School of Biological & Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS. Email: l.chittka@qmul.ac.uk