Dispatch

# Pollination: influencing bee behaviour with caffeine

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**Summary**
Plant secondary metabolites found in floral nectar can affect the behaviour of pollinating insects, but how these changes benefit plants directly is little understood. An experimental study with bumblebees shows that recalling a caffeine-enhanced odour memory can increase flower visitation.

Although we have known for about half a century that nectar is more than just alimentary sugars in a watery solution [1], it is only in recent years that more attention is given to the rich mixtures of nectar compounds and the role they play in plant-insect interactions. Plant metabolites found in nectar mainly serve as volatile chemical signals, nutrients for pollinators, deterrents for unwanted flower visitors and herbivorous insects, or defence chemicals that protect the flower's components from attacks by microbes and pathogens [2, 3]. Several metabolites can interact with neurons binding to neurotransmitter receptors and thus might directly elicit some of the behavioural effects observed in pollinating insects [4]. Caffeine, a bitter-tasting purine alkaloid, is one of these neuroactive compounds. It is synthesised in the leaves and fruit of several species (for example in plants of the genus *Citrus, Tilia* and *Coffea* [3, 5], Figure 1A), where it serves as a toxic defense against herbivores. However, it is also found in the nectar of their flowers, which is paradoxical as the nectar is produced specifically for consumption by visiting pollinators [4]. Whilst much remains to be understood about the action of caffeine-containing nectar on the physiology and behaviour of pollinating insects, it has been previously shown that at low concentrations caffeine has the potential to increase the foraging and cognitive performance of bees [6, 7]. Therefore, Arnold and colleagues [8] turned to caffeine when setting out on a quest for a solution to a real-world problem in crop pollination.

Crop and fruit plants are bred for traits that are primarily selected for the needs of humans, but this can potentially render the plant's rewards less tasteful or preferable for bees. Even though crops are grown in high densities over a large area, thus providing a massively abundant food source, bees may still forage from co-flowering wild plants. The traditional solution since ancient times is to supplement crop sites with managed bees, by moving honeybee hives between fields. These days bumblebee colonies are available for that, supplied to farmers by commercial breeders in easily transported boxes. Yet at times this might not be enough to provide the necessary levels of pollination to farmed plants, even when both managed and wild pollinators are integrated into the agricultural landscape through new diversified farming systems, with wildflower margins and corridors. Managed field margins provide refuges and diversified food sources attracting a range of pollinators to crop fields, but modelling suggests that increasing field margins around monocultures shifts pollinator visits away from the crop, even when at lower densities [9]. The effect on plant diversity is pronounced, suggesting that not only quantity but also quality counts when bees assess floral rewards. Differences in the flight ranges also impact the pollinator's decision which flowers to visit. Some insects, such as bumblebees, are able to fly over longer distances due to their larger body size [10], yet within a bumblebee colony foragers differ substantially in their size which affects how they learn about flowers deciding which ones to explore and exploit during their foraging trips [11].

What then if one could somehow increase a bee's interest in the crop before releasing it into the field? More so, given that commercially bred bumblebees are naïve and have never visited a flower before. Arnold's team hypothesised that priming naïve bumblebees with caffeine-laced sucrose and the crop's odour prior to foraging might generate an olfactory memory that would be strong enough to guide the bee's choices in an environment in which a crop competes with other flowers. To test this idea, they created an experimental foraging arena in which bees encountered robotic flowers that all looked the same and offered the same reward but differed in their odour [8]. Bees that were initially primed with caffeine-laced sucrose solution and a strawberry crop odour inside their nest recognised the memorised odour and searched for food more often on the strawberry-scented flowers, although these offered only caffeine-free sucrose. Bees in control colonies that were not primed, chose randomly amongst the flowers. The effect was transient, as caffeine-primed bees soon learned that both odours in the foraging arena signalled the presence of a reward. Whilst further work should assess the robustness of this intervention for novel applications, this study clearly demonstrates the effect priming can have on flower visitation, without the need to continuously provide caffeine-containing reward. It is a prime example of the directions that translational pollination research can take.

In the present study, bees could only track floral scents in an artificial olfactory landscape. However, in reality, flowers display colours and patterns that guide bees when they search for flowers, detect and recognise them, and eventually land on them to extract the reward. These visual cues can often dominate when it comes to decision-making, and may be indispensable for the tight control of flight and landing movements. Moreover, it has been shown that bees can also rely on other cues in flowers, less perceptible to the human observer, such as temperature and electrostatic patterns [12, 13], humidity [14], and petal texture [15], and are capable of distinguishing and learning combinations of various cues [16]. Flowers are complex, multisensory structures [17] (Figure 1B, C), and how caffeine and other neuroactive compounds affect the mechanisms of multimodal learning and memory formation is an open question.

How much and how often do bees have to ingest caffeine to become more active or strengthen their memories? Because it is deterring or even toxic at high concentrations, similar to some of the other plant secondary metabolites found in nectar, it is a matter of careful dosing. When caffeine concentrations in sugar solutions are low, it is sufficiently masked by the sweetness of the nectar's sugar content, so that honeybees readily imbibe it and show the resulting memory effects when the reward is paired with an odour (Figure 1). It only takes a few brief exposures to the odour in the presence of a caffeine-laced sucrose reward to form a much more robust olfactory memory than without caffeine [6]. Caffeine interacts with various cellular targets in the bee's body, nervous system and brain [4, 18], and, in addition to pre-ingestive taste perception, physiological tolerance thresholds in key pollinators are likely to exert selective pressures on the distribution of caffeine and other costly phytochemicals in the flower and in the rest of the plant's tissues [3].

Earlier studies reporting the effects of caffeine on bee behaviour continuously exposed bees to it. This scenario could be useful for when bees have already worked out preferential routes that take them to dense patches of the caffeine-providing trees and shrubs in full bloom, as would be the case in orchards and farms at the peak of their flowering period. But in situations where flower densities are low, such as in wild communities or when an individual plant is early or late in flowering, then caffeine-providing flowers are more likely to compete for pollinators with other plants. Strongly fluctuating rewards as well as sparse flower distributions can push bees towards exploring other flowers and deciding to visit more than one type of flower to obtain enough rewards. In such case, a dominant long-term memory for the odour of the caffeine-rewarding flower could still prove very beneficial, influencing the rate and frequency with which bees discover multimodal displays of flowers and evaluate other rewards and helping the caffeine-investing plant to secure profitable shares in pollinator visits.

As well as increasing the visits made by a pollinator, it might be that there are other behavioural changes that make it worthwhile for the plant to spike their nectar with neuroactive compounds, despite the potentially detrimental impact on its taste [3, 6]. An interesting observation made by Arnold and colleagues [8] is that the caffeine-dosed bees tended to spend more time on the strawberry-scented flowers during their initial visits. The lures containing the odour were the same in all the artificial flowers, with equal opportunity to discover the reward. Yet the expectation of reward evoked by the recalled memory seemed to have motivated the bees to remain on the artificial flowers for longer. A more detailed dissection of the behavioural interactions with the flower could help to reveal further positive effects for the plant, elucidating how such increased time on the flower might facilitate the transfer of pollen and enhance the chance of successful pollen deposition.

Diving deeper into the intricate complexities of floral displays and spiked nectar cocktails allows us to pursue new questions about learning mechanisms, the nature of foraging decision-making, and behavioural control in pollinating insects. It continues to be an adventurous journey, full of surprises, about how cleverly plants hone in on these mechanisms and utilise them to their advantage.

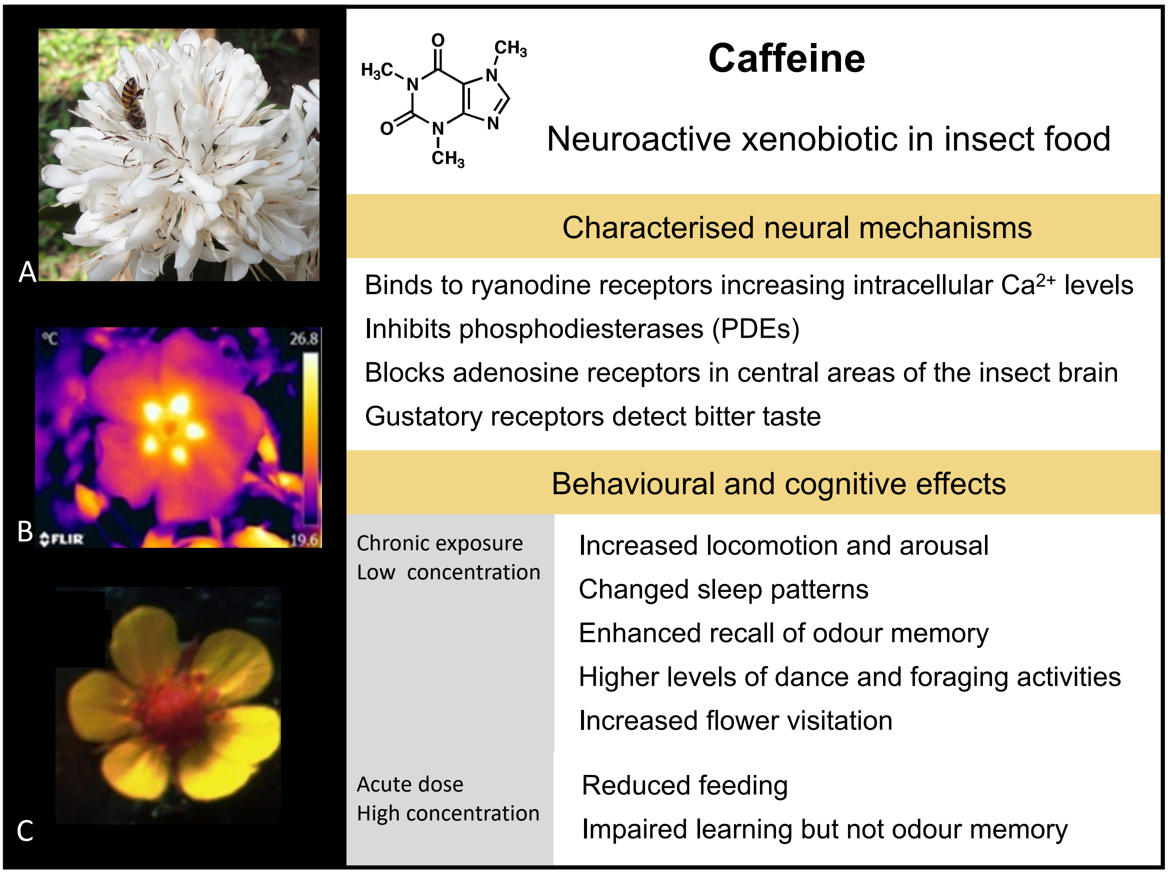


**Figure 1**: (A) The Eastern honeybee, *Apis cerana*, foraging on coffee flowers in the Sahyadri mountain range (Kerala, India, photo by N. Hempel de Ibarra). *Caffea* species typically contain only caffeine as a secondary metabolite in its nectar [6]. It needs to be studied further how different low concentrations of ingested caffeine affect memory recall for uni- and

multimodal sensory information present in floral displays, such as temperature or colour patterns (B, C). (B) Thermal pattern image of *Cistus veguinii* [19], (C) Multispectral image of *Fragaria vesca*'s visual display, shown in "bee colours" (RGB-coded bee receptor excitations [20]). Main panel: Currently known behavioural and neural effects of caffeine when ingested by insects [3, 4]. The evidence base is still scarce, and future research should include more non-model species to understand specific adaptations in different pollinating insects. Inset: Structural formula of caffeine found in low concentrations in the nectar of coffee, citrus, linden plants.

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