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INSIDE JEB

Grey-backed mining bees home in on willow signature scent

A grey-backed mining bee (*Andrena vaga*) collecting pollen from a male willow catkin. Photo credit: Hannah Burger.

Selecting a family meal that keeps all your picky diners happy can be a trial, especially if they all have different preferences. But when it comes to fussy offspring, grey-backed mining bees (*Andrena vaga*) have it relatively easy. Hannah Burger, from the University of Konstanz, Germany, explains that these finicky larvae only thrive on willow pollen, so the industrious adults are strongly attracted to the scent of willow catkins. But what makes willow fragrances stand out from the bouquets of other blooms for these loyal insects? Burger and her colleagues Giovanni Galizia (University of Konstanz), Manfred Ayasse (Ulm University, Germany) and Stefan Dötterl (Paris Lodron University of Salzburg, Austria) embarked on an ambitious series of experiments to discover which willow scent components grey-backed mining bees home in on when locating their larvae's favoured dining preference.

'I asked local bee experts to help me find nest aggregations', says Burger, who collected bees from the Botanical Garden of the University of Ulm, Germany, and near Konstanz. 'I just picked them up; the sting of this species cannot penetrate skin', she says, explaining that she and her colleagues needed to collect the bees in early spring before they began foraging, to record their natural preferences. In addition, she gathered samples of male catkins from seven willow species, including grey willow (*Salix cinerea*) and white willow (*S. alba*), when the catkins were fresh, to capture their fragrance. Back in the lab, Burger, Melanie Marquardt and Katharina Babucke (both from Ulm University) passed each aroma cocktail through a gas chromatograph, to separate the individual components from the mixture, before passing them on to be sniffed by a bee's antenna, to discover which components the bees reacted most strongly to.

Identifying 37 individual scent components, the team discovered that the bees' antennae reacted strongly to the spicy, nutty odour of 1,4-dimethoxybenzene, which comprises 74% of willow scent. However, 1,4-dimethoxybenzene also turns up in other flower fragrances that the bees ignore, so it is unlikely to be fragrance that guides them to pollen packed willow catkins. It was only when the team recorded the response of the bee's antenna to one minute component of the overall mixture – 4-oxoisophorone, which has a woody odour – that they realised they had discovered the key scent that helps grey-backed mining bees locate willow catkins. Even though 4-oxoisophorone only comprises 1.3% of the willows' scent cocktail, the nerve signal produced by the compound in the antenna was much stronger than the team expected.

Burger and Kim Heuel (University of Konstanz) then checked how the bees' brains reacted to the scents of different willows, revealing several groups of nerves in the insects' brains that recognised willow scents specifically. And when the team recorded which clusters of nerves in the bees' brains responded to 4-oxoisophorone as the odour became stronger, they found one group that was able to detect the woody odour at concentrations a thousand times more dilute than other key odours in the scent cocktail, allowing them to pick up the trail before they were aware of the other scent components.

Finally, Marquardt and Babucke checked whether the bees depend on their eyes or their antennae to track down flowering willows, and it turned out that the bees mainly prefer to follow their sense of smell, but they also need to see the catkins to get really interested. Even though 4-oxoisophorone is one of the most dilute components in willow fragrance, it's the key that keeps worker bees coming back to fill up on willow pollen for their fussy larvae.

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Burger, H., Marquardt, M., Babucke, K., Heuel, K. C., Ayasse, M., Dötterl, S. and Galizia, C. G. (2021). Neural and behavioural responses of the pollen-specialist bee *Andrena vaga* to *Salix* odours. *J. Exp. Biol.* **224**, jeb242166.

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