Peer Community In Ecology

The importance of understanding bee nutrition

Ignasi Bartomeus based on peer reviews by *Cristina Botías* and 1 anonymous reviewer

Clément Tourbez, Irène Semay, Apolline Michel, Denis Michez, Pascal Gerbaux, Antoine Gekière, Maryse Vanderplanck (2023) Heather pollen is not necessarily a healthy diet for bumble bees. Zenodo, ver. 3, peer-reviewed and recommended by Peer Community in Ecology. https://doi.org/10.5281/zenodo.8192036

Submitted: 11 April 2023, Recommended: 07 August 2023

Cite this recommendation as:
Bartomeus, I. (2023) The importance of understanding bee nutrition. *Peer Community in Ecology*, 100534. 10.24072/pci.ecology.100534

Published: 07 August 2023

Contrasting with the great alarm on bee declines, it is astonishing how little basic biology we know about bees, including on abundant and widespread species that are becoming model species. Plant-pollinator relationships are one of the cornerstones of bee ecology, and researchers are increasingly documenting bees' diets. However, we rarely know which effects feeding on different flowers has on bees' health. This paper (Tourbez et al. 2023) uses an elegant experimental setting to test the effect of heather pollen on bumblebees' (*Bombus terrestris*) reproductive success. This is a timely question as heather is frequently used by bumblebees, and its nectar has been reported to reduce parasite infections. In fact, it has been suggested that bumblebees can medicate themselves when infected (Richardson et al. 2014), and the pollen of some Asteraceae has been shown to help them fight parasites (Gekière et al. 2022). The starting hypothesis is that heather pollen contains flavonoids that might have a similar effect. Unfortunately, Tourbez and collaborators do not support this hypothesis, showing a negative effect of heather pollen, in particular its flavonoids, in bumblebees offspring, and an increase in parasite loads when fed on flavonoids. This is important because it challenges the idea that many pollen and nectar chemical compounds might have a medicinal use, and force us to critically analyze the effect of chemical compounds in each particular case. The results open several questions, such as why bumblebees collect heather pollen, or in which concentrations or pollen mixes it is deleterious. A limitation of the study is that it uses micro-colonies, and extrapolating this to real-world conditions is always complex. Understanding bee declines require a holistic approach starting with bee physiology and scaling up to multi-species population dynamics.

References:

Gekière, A., Semay, I., Gérard, M., Michez, D., Gerbaux, P., & Vanderplanck, M. 2022. Poison or Potion: Effects of Sunflower Phenolamides on Bumble Bees and Their Gut Parasite. Biology, 11(4), 545. https://doi.org/10.3390/biology11040545

Richardson, L.L., Adler, L.S., Leonard, A.S., Andicoechea, J., Regan, K.H., Anthony, W.E., Manson, J.S., & Irwin, R.E. 2015. Secondary metabolites in floral nectar reduce parasite infections in bumblebees. Proceedings of the Royal Society of London B: Biological Sciences 282 (1803), 20142471. https://doi.org/10.1098/rspb.2014.2471

Tourbez, C., Semay, I., Michel, A., Michez, D., Gerbaux, P., Gekière A. & Vanderplanck, M. 2023. Heather pollen is not necessarily a healthy diet for bumble bees. Zenodo, ver 3, reviewed and recommended by PCI Ecology. https://doi.org/10.5281/zenodo.8192036

Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.5281/zenodo.8116028
Version of the preprint: 2

Authors' reply, 28 July 2023

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Decision by Ignasi Bartomeus, posted 17 July 2023, validated 17 July 2023

Paper accepted pending minor edits

Thanks for implementing the required changes. The paper now is very clear and provides reproducible evidence of how heather pollen affects bumble bees. I have a couple of very minor requests before final acceptance.

First, I missed in the text if Bombus terrestris naturally forages on heather (and in which proportions if known). This is very relevant for interpreting the results, and can be briefly stated in the introduction, or maybe in discussion. Also, the Cox models ignore random effects, right? Just state this, and justify why this is ok in methods.

Second, there are a few grammatical issues. e.g. line 144: "parasiteS ... fed WITH ... WERE parasite free"; line 356 needs a space in emergenceresulting.

Best,
Ignasi

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.04.06.535809
Version of the preprint: 1

Authors' reply, 06 July 2023

Download author's reply
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Decision by Ignasi Bartomeus, posted 08 May 2023, validated 08 May 2023

Minor revision to clarify methodology

This is a very nice experimental test to assess the effect of diet in different aspects of bumblebees fitness. Given the current environmental challenges, understanding the physiological mechanisms by which floral resources (both pollen and nectar) affect bumblebees capacity to reproduce and withstand parasites is timely.

The manuscript is clear and the experiments robust. Both reviewers apreciated the manuscript and have some minor comments to enhance it. As you will see, Cristina Botías has interesting caveats on some experimental aspects. I think an honest discusion on the limitations or potential blind spots of the experimental dessign will strengthen the manuscript.

I would also recommend focusing the results around effect sizes, rather than on p-values. There are several ways to do so. First, estimates (for cathegorical factors those are usually mean and SE differences between factors) can be provided in a table in the appendix. This is important specially for researchers doing further meta-analysis. Extracting those from figures is painful. Second, this same estimates can be addedd when relevant in text, for example, instead of stating a significant increase in parameter X, you can mention which is the raw of percentual mean increase in this parameter. I think this will reinforce some of your results, as highlighting the effect sizes (e.g. a 20% increase in parameter X) will help the readers.

I am looking forward to read the revised version.

Best,

Ignasi

Reviewed by anonymous reviewer 1, 20 April 2023

In the manuscript "Role of pollen flavonoids in the heather- bumble bee-parasite interactions", the authors tested the effect of heather pollen in microcolonies success, with and without the presence of a pathogen whose effect has been shown to diminish with heather nectar. I find the manuscript to be easy to read, the experimental design is appropriately controlled and coherent with the goals and hypotheses and the results appear to be correctly interpreted. The figures are appropriate and in line with the text. Both methods and discussion are the appropriate length and depth. Both data and scripts for analyses are freely available and uploaded.

In general, I find the paper timely and of interest to bumblebee conservation or rearing, and ready for publication in a peer reviewed journal should the authors decide so. I would recommend some minor changes, like a more appealing title (maybe stating the main results as heather pollen being detrimental to bumblebee colony development) and checking some sentences for English language (e.g., "Evidence is…" should better be "There is evidence that…"). Also the introduction would profit from the inclusion of a bit more literature about the different effects from flavonoids that have been found so far (every time it was mentioned that some results are "controversial", it would be nice to have a brief summary of the contradicting results found so far).

Reviewed by Cristina Botías, 05 May 2023

The paper entitled "Role of flavonoids in the heather-bumble bee-parasite interactions" by Tourbez et al. is an interesting study that highlights the need to consider both nectar and pollen when addressing medicinal effects of a plant towards pollinators. Overall, the questions asked in this study are interesting, important and topical, and in general the methods are adequate, and the results clearly presented. However, in my opinion,

some parts of the paper need clarification by the authors. I have a few suggestions that I hope can be useful to improve the manuscript.

Line 112: The use of willow pollen as "control" needs further explanation by the authors. Given that bumblebees normally collect pollen from diverse floral resources, wouldn't have been more appropriate to use a polyfloral pollen blend as the control pollen? Also, since willow pollen contains flavonoids, and that these are heat-sensitive and soluble in organic solvents, did the authors consider performing any treatment to decrease the concentration of flavonoids in the control pollen?

It would also be very useful to have a comparative table of flavonoids present in heather and willow pollen. Is the value supplied in Appendix A the concentration of flavonoids measured in the willow pollen? Please clarify this issue.

Line 120: The commercial bumblebee colonies very often carry pathogens, so bumblebees may have been infected by some parasite/s before starting the experiment. According to the information provided in the methods, the purchased colonies were not checked for the presence of potential pathogens before starting the experiment. Unless the supplier provided a guarantee that they were parasite-free colonies, the possible influence of other pathogens in the results can not be discarded, and this should be mentioned in the discussion.

Lines 138-141: Honeybee collected pollen can also carry some pathogens. As mentioned above, if there is no guarantee that pollen pellets were parasite-free, this should be mentioned in the paper.

Line 146: As far as I understand from the table in Appendix A, the control willow pollen, and the heather pollen was spiked with a similar amount of ethanol than the flavonoids-supplemented willow pollen. This information would be useful here, just a sentence mentioning it, to help repeatability of the assay.

Lines 150-160: I found the identification of Crithidia bombi by microscope problematic. At least four different trypanosomatids (with similar mophology) have been detected in Bombus terrestris (Bartolomé et al., 2021. Environ Microbiol. 2021 Jan;23(1):478-483). Unless the source of C. bombi pool was analysed by a PCR at some stage, it can't be proved that the trypanosomatid inoculated and observed under the microscope was C. bombi. If the authors kept the faeces samples or the inoculated dead bumblebees, I would recommend using molecular tools to corroborate the identity of the pathogens inoculated. If doing this additional analysis is not feasible, the possible misidentification of the trypanosomatid used in this study should be mentioned somehow in the discussion.

Line 158: To help reproducibility of the experiment, it would be useful to describe the method used to supply the 10uL inoculate to the bees.

Lines 337-341: In the paper it is not mentioned if the males were analysed for pathogen presence, but here in the discussion, it is assumed that they were infected just by developing in microcolonies with inoculated workers. I find the hypotheses described here plausible and interesting, but it should also be considered that maybe not all males, or even neither of them, got infected, and thus, the differences found in fat body content between experimentally inoculated workers and non-inoculated males.