Overview

Many animals consume food from organisms in a single species, genus or family – a portion of the resources available to them in their environment [1,2]. This strategy – called dietary specialization – is a potentially perilous one. A dietary specialist takes on more risk than a generalist, whose diet contains more food species and so tends to be more stable over time as result of statistical averaging (the same concept explains why it is safer to invest in a diverse portfolio of stocks than just a few ones) [3,4]. Yet despite the benefits of generalizing, dietary specialists abound, particularly among insect herbivores [1]. Among bees, for example, many species will consume pollen from plants in only one genus or family; in North America, these pollen specialist bees make up approximately one quarter of the continent's total number of bee species [5–7]. Understanding why so many bees have adopted this dietary strategy is important for their conservation, for a foundational understanding of their biology, and for a more general understanding of why specialization is found across the tree of life [1,2,8].

One hypothesis for why bees specialize is that pollen specialization is the result of **evolutionary conflict between bees and plants**. Although bees and plants are mutualists, bees will sometimes exploit the pollination mutualism by harvesting large quantities of a plant's pollen while depositing little on other plants' stigmas. This could exert selection on the plant to evolve pollen defenses, and the bee counter-defenses; the bee might then come to specialize if the adaptations required to tolerate the pollen defenses entail losing the ability to utilize other types of pollen. Adaptation to plant defenses is likely central for explaining the prevalence of specialization seen in other taxa of insect herbivores [9–12], but this hypothesis is less intuitive for bees [10], which are mutualists of their host plants as well as herbivores. Yet there is strong evidence, first, that bees can exploit their relationship with plants and reduce plant fitness (e.g., by acting as pollen thieves, visitors that harvest pollen without pollinating) [13–15], and second, that plants defend themselves against this exploitation by arming their pollen with physical or chemical defenses (though other potential explanations for these defenses also exist) [16–18].

Research question

We investigated whether pollen defenses are important for explaining patterns of pollen specialization by bee species in southeastern Canada. We collected >2500 bees from eight genera of plants that host specialist bees as well as from 30 genera of control plants that do not. We use these data to ask 1) do generalist bees collect less pollen from the plants they visit if those plants are used by specialist bees? (note: we assume specialist bees only collect pollen from their host plants, though evidence suggests there are occasional exceptions to this [19–21]) and 2) Do generalist bees collect less pollen overall from plants that host specialist bees?

Methods

Plant and site selection

To investigate whether generalist bee species collect pollen from plants used by specialist bees, we sampled bees from populations of plants coming from eight different genera used by specialists. The populations occurred in eastern Ontario and southwestern Québec,

around Ottawa, ON, Canada. The plants were *Claytonia virginica*, *Salix* spp., *Vaccinium myrtilloides*, *Cornus sericea*, *Lysimachia ciliata*, *Pontederia cordata*, *Cucurbita pepo* and *Solidago canadensis*. Each of these plants is locally common and supports specialist bee species that occur in our study region [5]. Five of the plants were sampled in 2020 (*Cornus sericea*, *L. ciliata*, *P. cordata*, *Cucurbita pepo* and *S. canadensis*), and the other three in 2021 (*Claytonia virginica*, *Salix* spp., and *V. myrtilloides*).

For each of the plant species used by specialists, we selected three (or in one case, two) sites in our study region where the plant was abundant. Sites were a mix of deciduous hardwood forest and early-successional habitat (for *Claytonia virginica*, *Salix* spp., *Cornus sericea*, and *S. canadensis*), river and pond margins or banks (for *P. cordata* and *L. ciliata*), rocky outcrops (for *V. myrtilloides*), or agricultural fields (for *Curcurbita pepo*). The minimum distance between sites for a given species was 1.95 km, beyond the foraging distance of the bee species in our study region [22], ensuring that both bee and plant populations at different sites are independent.

At each site, we selected one to three nonhost plant species to use as control plants (Table 1), where a nonhost plant is defined as any plant species that does not support specialist bee species from our study region (see below). When selecting nonhost plants, we searched for plants in bloom with similar floral abundance to the host plant (when possible), and in close proximity to it (<1km), to ensure that the bee community available to visit the plants was similar between the different plant species. However, we violated this latter criterion on one occasion at a site with very few plants blooming. At this site, the maximum distance between plant patches was 1.6 km. However, in general, plant patches were close together: across sites, the median distance between plant patches was 200-m (in 2020; exact distances between plants were not measured in 2021).

When selecting nonhost plants, we did not restrict our choices to the native plant species blooming at a site, because non-native plants were typically among the more abundant plants at our study sites. There is not evidence that native bee species differentiate strongly between native and non-native plants (e.g., [23,24] though see [25]). Generally, there was at least one nonhost plant species that was native per site.

Our main criterion when picking nonhost plants was that they did not support any species of specialist bees that occur *inside* our study region; however, when possible we also picked nonhost plants that do not support specialist bees that occur outside of our study region. We violated this criterion with at least two nonhost plants: *Echium vulgare* and *Convolvulus arvensis*, both non-native plant species that host specialist bees in their native ranges on other continents [26,27]. In addition, three of our nonhost plants are part of families that host specialist bees: *Sororia sorbifolia* and *Prunus susquehanae* which are part of the Rosaceae family; and *Vicia cracca*, which is part of the Fabaceae family [5]. However, none of the three genera host any species of specialist bees. To ensure the inclusion of these plants did not affect our results we reran all analyses with these five plant species excluded.

Data collection

We sampled bees between June 4 and August 19 in 2020, and between April 13 and May 24 in 2021. We used hand nets to collect bees from our focal plants at each site, sampling from multiple flowering individuals of each plant species. Each plant species was sampled for a

total over two hours at each site, with sampling occurring over the course of two to four sampling days. We sampled during the peak hours of bee foraging activity, between 8:00 and 15:30, and we only sampled on days when bees were likely to be active, when it was sunny and air temperature was greater than 15°C. We only collected female bees of non-parasitic species, as neither male bees nor brood-parasitic bees collect pollen for their offspring. We sampled the same site on consecutive days whenever the weather permitted (the maximum number of days over which we spread out the two to four sampling days was nine).

At each site, two (or occasionally more) observers sampled bees during a total of twelve 10-minute sampling rounds on each of the focal plant species. Observers rotated between all focal plant species at a site on each day, unless bad weather cut short sampling. During each 10-minute sampling round, the observer set their timer and walked among a patch of the focal plant looking for bees contacting the reproductive parts of the plant species' flowers. When the observer saw a female non-parasitic bee, they netted the bee, and stopped their timer when the bee was securely in the net. All bees were then placed into individual clean vials, and stored in a cooler with icepacks. The observer then re-started their timer and continued to sample bees using these methods until ten minutes had passed.

Pollen removal

At the end of the field day, all bees except bumble bees (*Bombus* spp.) (queens in 2020 and both queens and workers in 2021), were returned to the laboratory at the University of Ottawa, and placed in a freezer, before being pinned and sampled for pollen. The bumble bees were sampled for pollen in the field, and then released.

For corbiculate bees (i.e., honey bees and bumble bees) with at least some visible pollen, we removed a sample of corbicular pollen from both legs using cleaned forceps. We placed the pollen sample onto a 1 mm^3 cube of fuchsin gel [28]. If no corbicular pollen was visible, we dabbed the fuchsin cube over the corbiculae. For bees with scopae (i.e., all non-corbiculate bee species we sampled), we took the 1 mm^3 cube of fuchsin gel and dabbed it over the scopae, making sure to sample both legs for non-megachilids. We then melted the cube of fuchsin gel onto a slide with either an alcohol lamp or hot plate. To minimize pollen contamination, we cleaned the lab bench and all tools with 70% EtOH between processing and removing pollen from each bee.

Pollen identification

We used a compound microscope at 200x magnification to identify the first 200 grains of pollen we encountered on each slide (or fewer than that if there were fewer than 200 grains of pollen on the slide). We identified pollen to the lowest taxonomic resolution possible, using a pollen reference library curated from our study sites. We identified pollen by starting in the middle of the slide and counting pollen grains in vertical transects to the left and right of our starting point, until we had identified 200 grains of pollen.

Bee identification

All bees were first identified to the genus level. To determine which bees were pollen specialists, we identified bees to the species level if they were from genera known to include

species of pollen-specialist bees, as listed by [5] (these genera were *Andrena*, *Megachile*, *Melissodes*, *Osmia*, *Perdita*, *Colletes*, *Calliopsis*, *Eucera*, and *Pseudopanurgus*). We considered all other bees to be pollen generalists. Bees were identified to the species level by JF (*Andrena*) and CA (all other genera) using the following keys [29–33].

Analyses: blinding the data

We conducted blind data analyses for all analyses [34]. In this type of analysis, a researcher blinds the data by randomizing it or by adding random noise; they then run the analysis on this blinded data, making the major analysis decisions before knowing what the results will be. Because researchers must wait to see the outcome of the analysis decisions, this method prevents the researcher from subconsciously making analysis decisions that favor the results they believe a priori are more likely or more publishable. In the manuscript for this paper, we will clearly identify any analysis decisions made on unblinded data as 'post-blind.' We blinded the data in different ways for the different analyses and describe how in each section below.

A script of the blinded analysis will be posted to GitHub (https://github.com/cmsmith91/specialist_bees), as will this document, before the data are unblinded.

Analyses: Do generalist bees collect less pollen from the plants they visit if those plants host specialist bees?

Before conducting our analyses, we first checked that specialist bees collected their host plant's pollen. We conducted this check on the real, unblinded data, as specialist pollen collection was not part of our research questions. We found that one specialist bee, *Eucera pruinosa*, rarely had any of its host plant's pollen on its scopae (median number of its host plant's pollen grains = 1, 2, and 7.5 for each of the three sites), despite being collected of off its host plant, *Cucurbita pepo*. This suggests it was foraging for nectar rather than pollen at the time of data collection, potentially because *Cucurbita pepo* was not producing pollen. Given that specialists on *Cucurbita pepo* were not collecting pollen from it, we think it is unlikely that generalists would be. We thus excluded bees visiting this plant from both analyses, along with bees sampled from other plants at the same sites.

We next examined whether generalist bees visiting plants used by specialist bees collect pollen from these plants, or whether they are instead visiting them for nectar only, or are collecting the plant's pollen with a mix of other pollen types (a potential strategy to ameliorate a pollen's unfavorable properties) [35]. Specifically, we tested if generalist bees collected less pollen from plants that host specialist bees than from nonhost plants. We used a generalized linear mixed model to answer this question. Our response variable was how much pollen each generalist bee removed from the plant species she was visiting when we collected her. We estimated this by counting the number of pollen grains belonging to that plant species in our sample of her pollen load (hereafter, we refer to this as the number of 'conspecific pollen grains'). Our predictor variable was plant type (host of specialist bees vs nonhost), and we included plant species and bee genus as random intercept effects. We also considered other model terms, and included

them if they improved the AIC value by at least two, which indicates that the term substantially improves model fit. The model terms we considered were year (as a fixed effect), and site (as a random intercept effect). (Note: we did not include a model term if adding it caused convergence problems). We modeled the error structure of the model with a beta-binomial distribution, which accounts for over-dispersion in our data, and treats the identity of each pollen grain (conspecific pollen versus heterospecific or no pollen) as a binomial process, with the probability of the binomial process being drawn from a beta distribution. For the binomial process, the number of trials was equal to 200, the maximum number of possible conspecific pollen grains in a pollen load (since we counted up to 200 pollen grains in each load). We used diagnostic plots from the R package *DHARMa* to check model assumptions [36]. We plan to use permutation tests to assess variable significance if the model fails to meet distributional assumptions once it is fit to the real (i.e., unblinded) data, because permutation tests are nonparametric.

In blinding the data for this analysis, we simulated data from a beta-binomial mixed model that was similar to the one used to analyze the data. In this model, the model intercept was equal to -0.05, the model coefficient for the effect of plant type equal to -0.6, and phi (a parameter that models overdispersion) was equal to 1.65. We included bee genus as a random effect, with its standard deviation equal to 0.5. The data were simulated with the function 'rBB' from the R package *PROreg* [37].

Analyses: Do generalist bees collect less pollen overall from plants that host specialist bees?

Next, we examined whether generalist bees remove less pollen in total from plants that host specialist bees than from nonhost plants. This differs from our first question because it considers how many generalist bees visit the plant and thus their visitation rate, in addition to how much pollen they collect from it during the visit. Our response variable was the total amount of pollen removed by generalist bees found visiting a plant at a site. We estimated this by taking all generalist bees visiting a plant and summing the fraction of the visited plant's pollen in their pollen load (this fraction was out of 200 possible grains, even if there were fewer grains than that in their load). If no generalist bees were collected from a plant, then we set this number equal to zero. Our predictor variable was plant type (host of specialist bees vs nonhost), with plant species as a random intercept effect. We also considered other model terms and included them if they improved the AIC_c value by at least two, which indicates that the term substantially improves model fit. The model terms we considered were year (as a fixed effect), and site (as a random effect). We used a gamma distribution to model the residual error structure (adding 0.001 to each value of the response variable, because the gamma distribution does not allow for values equaling zero). We used diagnostic plots from the R package *DHARMa* to check model assumptions [36]. We plan to use permutation tests to assess variable significance if the model fails to meet distributional assumptions once it is fit to the real (i.e., unblinded) data, because permutation tests are nonparametric.

In blinding the data for this analysis, we randomized plant type within sites.

Software used

All analyses were conducted in R [38]. We used the R package *glmmTMB* to fit the mixed models to the data [39], and the R package *MuMIn* to calculate AIC_c values [40].

Results

We collected 3013 individual bees total from generalist species, and 336 individuals from 15 species of pollen specialist bees. We collected pollen specialists from each of the 8 host plants that were the focus of our study, with specialists at both the genus and family levels. The pollen specialist bees we collected carried pure loads of their host plant's pollen, except for *Eucera pruinosa,* a specialist on the genus *Cucurbita* (Figure S1). We excluded bees visiting this plant from our analyses, along with bees sampled at the same site (see Analysis Methods). The sample size of generalist bees after this filtering step was 2837 generalist bees.

Do generalist bees collect pollen from plants used by specialists?

The following results are from analyses conducted on blinded data (see Methods).

For both models, we found that adding additional terms to the model failed to improve its fit as determined by AIC values (Table S1). We thus used the starting model structure for both analyses (see Analysis Methods).

Generalist bees visiting plants used by specialist bees carried less pollen from the plants they visited than did generalist bees visiting nonhost plants (plant type estimate ± SE = 0.61 ± 0.07, z-value = 9.18, p-value = 4.5e-20). The generalist bees visiting plants used by specialists carried an estimated 69 grains of the plant's pollen (out of the 200 maximum possible that we counted), whereas generalist bees visiting nonhost plants carried an estimated 98 grains of the plant's pollen, a 42.7% difference (Figure 1A).

By contrast, there was no difference between host plants and nonhost plants in the total amount of pollen that generalist bees removed in aggregate from the plant (plant type estimate ± SE = -0.24±0.38, z-value = -0.64, p-value = 0.52). (*Note*: we only report the following in the final manuscript if there is a significant effect of plant type). Generalist bees visiting plants used by specialist bees removed an estimated 0.21% fewer grains of pollen total than ones visiting plants not used by specialist bees (Figure 1B).

Two of the nonhost plants in our analyses host specialist bees in their native ranges on other continents, and three are part of families that host specialist bee species. We reran our analyses with these plants excluded and obtained qualitatively similar results: we found that individual bees from generalist species collected less pollen when they visited plants used by specialists than when visiting plants not used by specialists (plant type estimate ± SE = 0.62 ± 0.07, z-value = 9.37, p-value = 7.19e-21). And that there was no effect of plant type on the total

amount of pollen that generalist bees removed from plants at a site (plant type estimate ± SE = -0.05 ± 0.39, z-value = -0.12, p-value = 0.9).
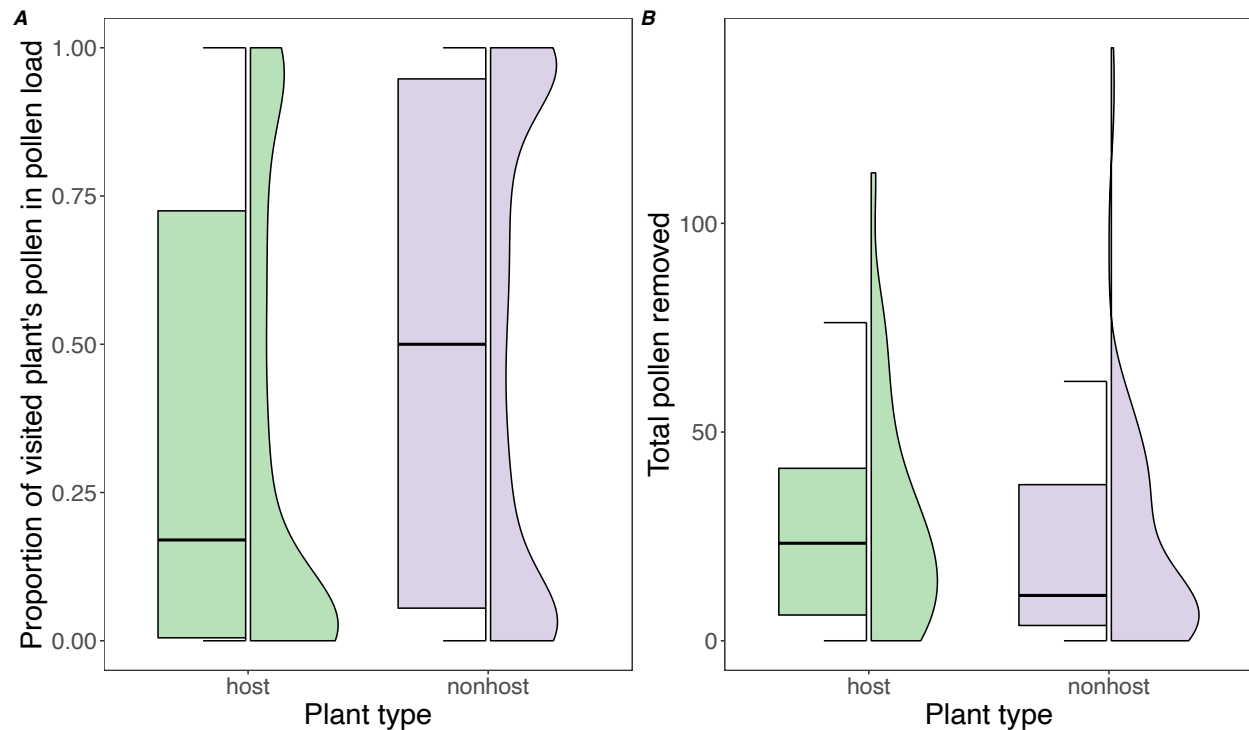

Figure 1. Box and violin plots comparing how much pollen generalist bees collect from plants used by specialists ('host' plants, green) and from plants not used by specialists ('nonhost' plants, purple). The plots show that when individual bees from generalist species visit plants used by specialist bees, they collect less pollen from the plant than when they visit other plants (A); However, generalist bees as a whole remove similar amounts of pollen in aggregate from plants used by specialists as from those not used by specialists (B). For the boxplots, the boxes encompass the first and third quartiles of the data and the thick black line is the median. The plot whiskers extend to 1.5 times the interquartile range.

Literature cited

1.   Forister ML, Novotny V, Panorska AK, Baje L, Basset Y, Butterill PT. 2015 The global distribution of diet breadth in insect herbivores. *Proc. Natl. Acad. Sci. USA* **112**, 442–447. (doi:10.1073/pnas.1423042112)

2.   Poulin R, Keeney DB. 2008 Host specificity under molecular and experimental scrutiny. *Trends Parasitol.* **24**, 24–28. (doi:10.1016/j.pt.2007.10.002)

3.   Batstone RT, Carscadden KA, Afkhami ME, Frederickson ME. 2018 Using niche breadth theory to explain generalization in mutualisms. *Ecology* **99**, 1039–1050. (doi:10.1002/ecy.2188)

4.   Schindler DE, Armstrong JB, Reed TE. 2015 The portfolio concept in ecology and evolution. *Front. Ecol. Environ.* **13**, 257–263. (doi:10.1890/140275)

5.   Fowler J, Droege S. 2020 Pollen specialist bees of the eastern United States. See https://jarrodfowler.com/specialist_bees.html.

6.   Fowler J. 2020 Pollen specialist bees of the central United States. See https://jarrodfowler.com/bees_pollen.html.

7.   Fowler J. 2020 Pollen specialist bees of the western United States. See https://jarrodfowler.com/pollen_specialist.html.

8.   Molina R, Horton TR. 2015 Mycorrhiza specificity: its role in the development and function of common mycelial metworks. In *Mycorrhizal Networks* (ed TR Horton), pp. 1–39. Dordrecht, the Netherlands: Springer Science + Business Media Dordrecht. (doi:10.1007/978-94-017-7395-9_1)

9.   Rothwell EM, Holeski LM. 2020 Phytochemical defences and performance of specialist and generalist herbivores: a meta-analysis. *Ecol. Entomol.* **45**, 396–405. (doi:10.1111/een.12809)

10.   Ehrlich PR, Raven PH. 1964 Butterflies and plants: a study in coevolution. *Evolution (N. Y).* **18**, 586. (doi:10.2307/2406212)

11.   Berenbaum M, Feeny P. 1981 Toxicity of angular furanocoumarins to swallowtail butterflies: escalation in a coevolutionary arms race? *Science (80-.).* **212**, 927–929. (doi:10.1126/science.212.4497.927)

12.   Becerra JX, Noge K, Venable DL. 2009 Macroevolutionary chemical escalation in an ancient plant-herbivore arms race. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 18062–18066. (doi:10.1073/pnas.0904456106)

13.   Parker AJ, Williams NM, Thomson JD. 2016 Specialist pollinators deplete pollen in the spring ephemeral wildflower *Claytonia virginica*. *Ecol. Evol.* **6**, 5169–5177. (doi:10.1002/ece3.2252)

14.   Hargreaves AL, Harder LD, Johnson SD. 2009 Consumptive emasculation: the ecological and evolutionary consequences of pollen theft. *Biol. Rev.* **84**, 259–276. (doi:10.1111/j.1469-185X.2008.00074.x)

15.   Koski MH, Ison JL, Padilla A, Pham AQ, Galloway LF. 2018 Linking pollinator efficiency to patterns of pollen limitation: small bees exploit the plant-pollinator mutualism. *Proc. R. Soc. B Biol. Sci.* **285**. (doi:10.1098/rspb.2018.0635)

16.   Rivest S, Forrest JRK. 2020 Defence compounds in pollen: why do they occur and how do

they affect the ecology and evolution of bees? *New Phytol.* **225**, 1053–1064. (doi:10.1111/nph.16230)

17. Irwin RE, Cook D, Richardson LL, Manson JS, Gardner DR. 2014 Secondary compounds in floral rewards of toxic rangeland plants: impacts on pollinators. *J. Agric. Food Chem.* **62**, 7335–7344. (doi:10.1021/jf500521w)

18. Stevenson PC. 2020 For antagonists and mutualists: the paradox of insect toxic secondary metabolites in nectar and pollen. *Phytochem. Rev.* **19**, 603–614. (doi:10.1007/s11101-019-09642-y)

19. Michener CD, Rettenmeyer CW. 1956 The ethology of *Andrena erythronii* with comparative data on other species (Hymenoptera, Andrenidae). *Univ. Kansas Sci. Bull.* **37**, 645–684.

20. Smith C, Weinman L, Gibbs J, Winfree R. 2019 Specialist foragers in forest bee communities are small, social or emerge early. *J. Anim. Ecol.* (doi:10.1111/1365-2656.13003)

21. Ritchie AD, Ruppel R, Jha S. 2016 Generalist Behavior Describes Pollen Foraging for Perceived Oligolectic and Polylectic Bees. *Environ. Entomol.* **45**, 909–919. (doi:10.1093/ee/nvw032)

22. Greenleaf SS, Williams NM, Winfree R, Kremen C. 2007 Bee foraging ranges and their relationship to body size. *Oecologia* **153**, 589–596. (doi:10.1007/s00442-007-0752-9)

23. Salisbury A, Armitage J, Bostock H, Perry J, Tatchell M, Thompson K. 2015 Enhancing gardens as habitats for flower-visiting aerial insects (pollinators): should we plant native or exotic species? *J. Appl. Ecol.* **52**, 1156–1164. (doi:10.1111/1365-2664.12499)

24. Williams NM, Cariveau D, Winfree R, Kremen C. 2011 Bees in disturbed habitats use, but do not prefer, alien plants. *Basic Appl. Ecol.* **12**, 332–341. (doi:10.1016/j.baae.2010.11.008)

25. Cahill CM, CaraDonna PJ, Forrest JRK. In press. Fitness consequences of non-native floral resource use for subalpine solitary bees: evidence of an ecological trap?

26. Trunz V, Lucchetti MA, Bénon D, Dorchin A, Desurmont GA, Kast C, Rasmann S, Glauser G, Praz CJ. 2020 To bee or not to bee: The 'raison d'être' of toxic secondary compounds in the pollen of Boraginaceae. *Funct. Ecol.* **34**, 1345–1357. (doi:10.1111/1365-2435.13581)

27. Fraberger RJ, Ayasse M. 2007 Mating behavior, male territoriality and chemical Communication in the European Spiral-Horned Bees, *Systropha planidens* and ,*S. curvicornis* (Hymenoptera: Halictidae). *J. Kansas Entomol. Soc.* **80**, 348–360. (doi:10.2317/0022-8567(2007)80[348:MBMTAC]2.0.CO;2)

28. Kearns CA, Inouye DW. 1993 *Techniques for Pollination Biologists*. 1st edn. Niwot, Colorado: University Press of Colorado.

29. Mitchell TB. 1960 Bees of the eastern United States. Volume I. *North Carolina Agric. Exp. Stn. Tech. Bull.* **141**, 1–538.

30. Ascher J, Pickering J. 2012 Discover Life bee species guide and world checklist (Hymenoptera:Apoidea;Anthophila). See http://www.discoverlife.org/mp/20q?guide=Apoidea_species.

31. Williams PH, Thorp RW, Richardson LL, Colla SR. 2014 *Bumble bees of North America: An identification guide*. Princeton, NJ: Princeton University Press.

32. Mitchell TB. 1962 Bees of the Eastern United States: volume II. *N. C. Agric. Exp. Stn. Tech. Bull.* **152**, 1–557.

33. Sheffield CS, Ratti C, Packer L, Griswold T. 2011 Leafcutter and mason bees of the genus *Megachile* Latreille (Hymenoptera: Megachilidae) in Canada and Alaska. *Can. J. Arthropod Identif.* **18**, 1–107. (doi:10.3752/cjai.2011.18)

34. MacCoun R, Perlmutter S. 2015 Blind analysis: hide results to seek the truth. *Nature* **526**, 187–189. (doi:10.1038/526187a)

35. Eckhardt M, Haider M, Dorn S, Müller A. 2014 Pollen mixing in pollen generalist solitary bees: a possible strategy to complement or mitigate unfavourable pollen properties? *J. Anim. Ecol.* **83**, 588–597. (doi:10.1111/1365-2656.12168)

36. Hartig F. 2021 DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version (0.4.4). https://cran.r-project.org/web/packages/DHARMa/index.html.

37. Najera-Zuloaga J, Lee D-J, Arostegui I. 2020 PROreg: patient reported outcomes regression analysis.

38. R Core Team. 2020 R: a language and environment for statistical computing.

39. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielson A, Skaug HJ, Maechler M, Bolker BM. 2017 glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* **9**, 378–400.

40. Bartón K. 2017 MuMIN: multi-model inference. R package version 1.40.0. , https://CRAN.R-project.org/package=MuMIn.

41. USDA, NRCS. 2018 *The PLANTS Database*. Greensboro, NC 27401-4901, USA: National Plant Data Team. See http://plants.usda.gov.

Table 1. List of focal plant species from which bees were collected (ordered alphabetically by family within each plant type). "Native status" refers to whether the species is thought to have been introduced to North America in the last 250 years, following [41].

Species	Family	Type	Native status
Solidago canadensis	Asteraceae	Specialist host	Native
Cornus sericea	Cornaceae	Specialist host	Native
Cucurbita pepo	Cucurbitaceae	Specialist host	Native
Vaccinium myrtilloides	Ericaceae	Specialist host	Native
Claytonia caroliniana	Montiaceae	Specialist host	Native
Pontederia cordata	Pontederiaceae	Specialist host	Native
Lysimachia ciliata	Primulaceae	Specialist host	Native
Salix sp.	Salicaceae	Specialist host	
Viburnum opulus var. americanum	Adoxaceae	Nonhost	Native
Viburnum lentago	Adoxaceae	Nonhost	Native
Rhus typhina	Anacardiaceae	Nonhost	Native
Daucus carota	Apiaceae	Nonhost	Introduced
Maianthemum canadense	Asparagaceae	Nonhost	Native
Impatiens capensis	Balsaminaceae	Nonhost	Native
Echium vulgare	Boraginaceae	Nonhost	Introduced
Myosotis scorpioides	Boraginaceae	Nonhost	Introduced
Erysimum cheiranthoides	Brassicaceae	Nonhost	Introduced
Alliaria petiolata	Brassicaceae	Nonhost	Introduced
Barbarea vulgaris	Brassicaceae	Nonhost	Introduced
Lonicera xylosteum	Caprifoliaceae	Nonhost	Introduced
Silene vulgaris	Caryophyllaceae	Nonhost	Introduced
Convolvulus arvensis	Convolvulaceae	Nonhost	Introduced
Sedum acre	Crassulaceae	Nonhost	Introduced
Euphorbia cyparissias	Euphorbiaceae	Nonhost	Introduced
Vicia cracca	Fabaceae	Nonhost	Introduced
Prunella vulgaris	Lamiaceae	Nonhost	Native
Origanum vulgare	Lamiaceae	Nonhost	Introduced
Glechoma hederacea	Lamiaceae	Nonhost	Introduced
Lythrum salicaria	Lythraceae	Nonhost	Introduced
Trillium grandiflorum	Melanthiaceae	Nonhost	Native
Anemone canadensis	Ranunculaceae	Nonhost	Native
Hepatica nobilis	Ranunculaceae	Nonhost	Native
Caltha palustris	Ranunculaceae	Nonhost	Native
Sorbaria sorbifolia	Rosaceae	Nonhost	Introduced
Prunus susquehanae	Rosaceae	Nonhost	Native
Acer campestre	Sapindaceae	Nonhost	Introduced
Saxifraga virginiensis	Saxifragaceae	Nonhost	Native
Verbascum thapsus	Scrophulariaceae	Nonhost	Introduced
Solanum dulcamara	Solanaceae	Nonhost	Introduced

Supporting tables and figures

Table S1. Results of AIC and AIC_c analyses. These were used to determine whether adding additional predictor variables to the model improved model fit, in comparison with the starting model (see main text for the variables included in the starting models). We used AIC values to compare the models of the 'proportion conspecific pollen' that individual bees carry, and we used AIC_c values (which adjust for small sample size) for the models of 'total pollen removed' by bees in aggregate. Additional predictor variables were only included if they reduced the starting model's AIC or AIC_c value by at least two.

| response variable	predictor variable added	AIC/AIC_c	df
proportion conspecific pollen	starting model	25477	5
proportion conspecific pollen	+ (1|site)	25479	6
proportion conspecific pollen	+ year	25479	6
proportion conspecific pollen	+ year + (1|site)	25481	7
total pollen removed	starting model	528	4
total pollen removed	+ (1|site)	530	5
total pollen removed	+ year	530	5
total pollen removed	+ year + (1|site)	533	6
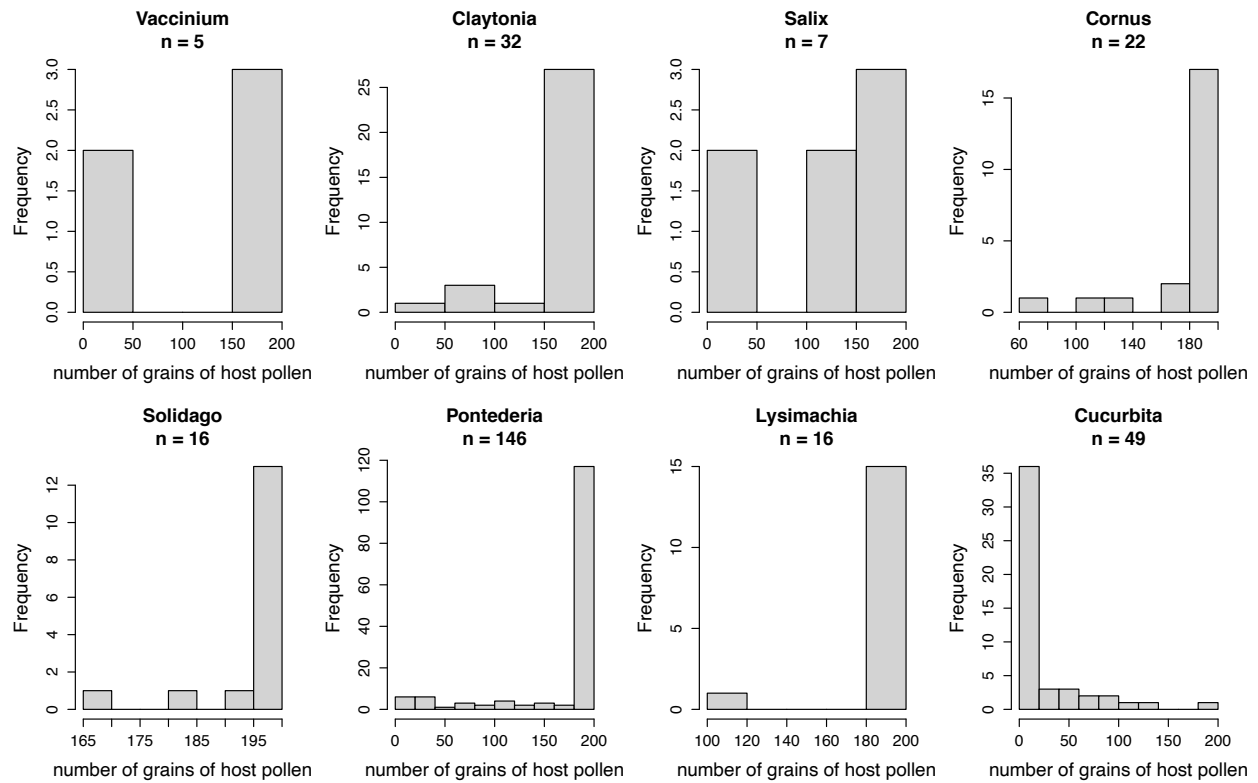
Figure S1. Histograms depicting the distribution of host pollen in the pollen loads of specialist bees visiting their host plants. The x-axis is the number of pollen grains an individual carried of its host plant out of a maximum possible of 200.