

Pollen foraging mediates exposure to dichotomous stressor syndromes in honey bees

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Abstract

Recent declines in the health of honey bee colonies used for crop pollination pose a considerable threat to global food security. Foraging by honey bee workers represents the primary route of exposure to a plethora of toxins and pathogens known to affect bee health, but it remains unclear how foraging preferences impact colony-level patterns of stressor exposure. Resolving this knowledge gap is crucial for enhancing the health of honey bees and the agricultural systems that rely on them for pollination. To address this, we carried out a national-scale experiment encompassing 456 Canadian honey bee colonies to first characterize pollen foraging preferences in relation to major crops and then explore how foraging behavior influences patterns of stressor exposure. We used a metagenetic approach to quantify honey bee dietary breadth and found that bees display distinct foraging preferences that vary substantially relative to crop type and proximity, and the breadth of foraging interactions can be used to predict the abundance and diversity of stressors a colony is exposed to. Foraging on diverse plant communities was associated with increased exposure to pathogens, while the opposite was associated with increased exposure to xenobiotics. Our work provides the first large-scale empirical evidence that pollen foraging behavior plays an influential role in determining exposure to dichotomous stressor syndromes in honey bees.

Keywords: honey bees, pathogens, neonicotinoids, crop pollination, metabarcoding

Significance Statement

Insect-mediated pollination is an important ecological process that is crucial for food production. Managed honey bee colonies are one of the most important insect pollinators, but their health has been under threat from a variety of stressors. Bee workers are primarily exposed to stressors while foraging and understanding how bee foraging preferences are related to exposure risk could provide pivotal information to improve management efforts. Here, we studied honey bee foraging preferences in relation to prominent Canadian crops and across a gradient of modified environments. We found that honey bees show distinct, measurable foraging preferences and that dietary diversity is a strong predictor of the stressors that colonies are exposed to.

Competing Interest: The authors declare no competing interests.

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Introduction

The western honey bee, *Apis mellifera*, is the most frequent floral visitor of crops worldwide (1, 2), playing a vital role in the productivity of agricultural systems (3). Recent declines in the health of managed honey bee colonies, seen most prominently as an increase in annual colony losses (4), present a considerable threat to global food security. No singular entity has emerged as a primary driver of colony losses (5, 6), but rather, poor health as a result of exposure to a multitude of stressors (7) has been suggested as a possible cause. These stressors include pathogens (8, 9), pests (10), xenobiotics (11–13), and poor-quality diets (14–16). Pin-pointing individual stressors that induce colony loss has been difficult (17, 18), and attempts to model the specific mechanisms by which stressor exposure induces colony mortality have failed to produce meaningful management strategies (19, 20). Some work has suggested that pests and pathogens could play the largest role (21), but more broadly, declines in bee health have been attributed to the entangled effects of multistressor exposure (22), the complexities of which are only beginning to be explored (7). While these general stressors have been known for some time, it is not immediately obvious how foraging behavior influences patterns of exposure.

Foraging bees must travel outside of the colony to collect pollen and nectar, and through this process, they may be exposed to xenobiotics, pests, and pathogens. Pollen is a primary food source, providing access to important nutrients for foraging bees and the colonies they support (23–26). Colony-level foraging decisions generally occur in advance of mass pollination events (27), e.g. foraging scouts may locate ideal food sources and communicate this to the rest of the colony via a waggle dance, and are often driven by cost-benefit ratios that favor high-quality resources in close proximity to the hive (28). Both the abundance and diversity of floral resources within a landscape shape these patterns of interaction; complex environments support shorter foraging distances (29, 30), but can also promote the rapid discovery and abandonment of food sources (27). Contrastingly, homogenous agroecosystems support a wider geographic foraging range (31) and a decrease in the frequency of waggle dances (29), indicating a reduction in the discovery of new food sources and thus the richness of plant-bee interactions. Despite its obvious importance to stressor exposure, we know little about how the foraging preferences of worker bees mediate colony-level exposure to stressors. Resolving this knowledge gap is crucial for mitigating threats to honey bee health and increasing the resilience of agricultural systems that depend on honey bees for pollination.

Previous work has demonstrated that honey bees can exhibit measurable foraging preferences in relation to crop species (32–35), but this type of work has long been inhibited by the logistically constrained task of quantifying plant-pollinator interactions. Recent advancements in methodological development (36–40) have provided a new avenue for exploring plant-pollinator interactions and present an opportunity to resolve knowledge gaps about foraging behavior and its influence on stressor exposure. Here, we set out to quantify honey bee foraging preferences in relation to prominent Canadian crops and explore how patterns of plant interaction drive exposure to the pathogens and xenobiotics nested within anthropogenically modified environments. Applying a metagenetic approach to characterize honey bee dietary breadth (36), we asked: do honey bees have distinct foraging preferences when presented with common Canadian crops? does the structure of a landscape impact foraging behavior? and finally does foraging behavior mediate exposure to stressors?

Results

Experimental overview

We used an established experimental design (13) to characterize honey bee foraging preferences and explore how foraging behavior mediates exposure to the stressors nested within Canadian landscapes (Fig. 1). At the start of the beekeeping season, we randomly assigned colonies to apiaries near (i.e. in or directly adjacent) or far (>1,500 m) from the following 8 common Canadian crops: cranberry, lowbush blueberry, highbush blueberry, apple, commodity canola, hybrid seed-production canola, corn, and soybean. We sampled pollen from these colonies at 2 time points during the experiment (see Materials and methods) and subjected these samples to multilocus pollen metabarcoding to identify the source and relative abundance of different plant taxa in each sample following established methods which have been demonstrated to provide results comparable to microscopic mellissopalynology (36). Overall, our pollen metabarcoding libraries were deep sequenced to an average depth of 11,210,193 ($\pm 2,298,792$) barcode reads and identified over 480 plant genera, including the 5 associated with our focal crops (*Vaccinium*, *Malus*, *Brassica*, *Zea*, and *Glycine*). We additionally tested the pollen, nectar, and nurse bees from each colony for chemical residues, and the nurse bees for pests and pathogens (7). Dietary values presented below are the multilocus average relative abundance of reads associated with the genera for each relevant focal crop. Statistical significance was determined using a threshold of $P < 0.05$.

Honey bees display strong foraging preferences

The 456 honey bee colonies used in our experiments displayed strong foraging preferences—quantified by pollen metabarcoding—in relation to common Canadian crops. Only 4 of our 12 experiments had a “proximity-dependent” effect wherein focal crop pollen dietary abundance (the relative dietary proportion comprised of the genera associated with the focal crop being studied) differed significantly between colonies placed near and far from the focal crop (Tables S1 and S2). In both of our cranberry experiments, as well as 2 of our blueberry experiments, we detected a statistically significant difference in the relative dietary abundance of *Vaccinium* pollen between our near and far groups. When a honey bee colony was placed directly adjacent to cranberry crops, cranberry pollen comprised an average of 28.5% ($\pm 21.8\%$ SD) of their diet, and when placed far away from cranberry crops, we detected no cranberry pollen in their diets. This indicates that cranberry pollen is a floral resource honey bees may forage on only when it is convenient, e.g. spatially close and abundant (Fig. 2). Blueberry crops were not a highly accessed floral resource overall. Even when colonies were placed directly adjacent to the crop, blueberry pollen only comprised an average of 3.2% ($\pm 3.6\%$) of their diet. This indicates that blueberry pollen may not be a favorable dietary component, but honey bees will forage on it to some degree if it is easily accessible (Fig. 2). Across all canola experiments, distance to the focal crop (near or far) had no discernible impact on foraging preferences, and canola comprised a substantial ($47.9\% \pm 21.6\%$) portion of each colonies’ diet regardless of the relative proximity of the crop (Fig. 2). Similarly, apple foraging behavior did not differ between near and far groups, but only comprised a small dietary proportion ($5.9\% \pm 5.8\%$), indicating that it was not as highly favored as other crops like canola. Both corn pollen and soybean pollen were rarely, if ever, detected in pollen samples from these experiments (Fig. 2). Notably, our pollen analysis does not indicate the frequency of nectar feeding,

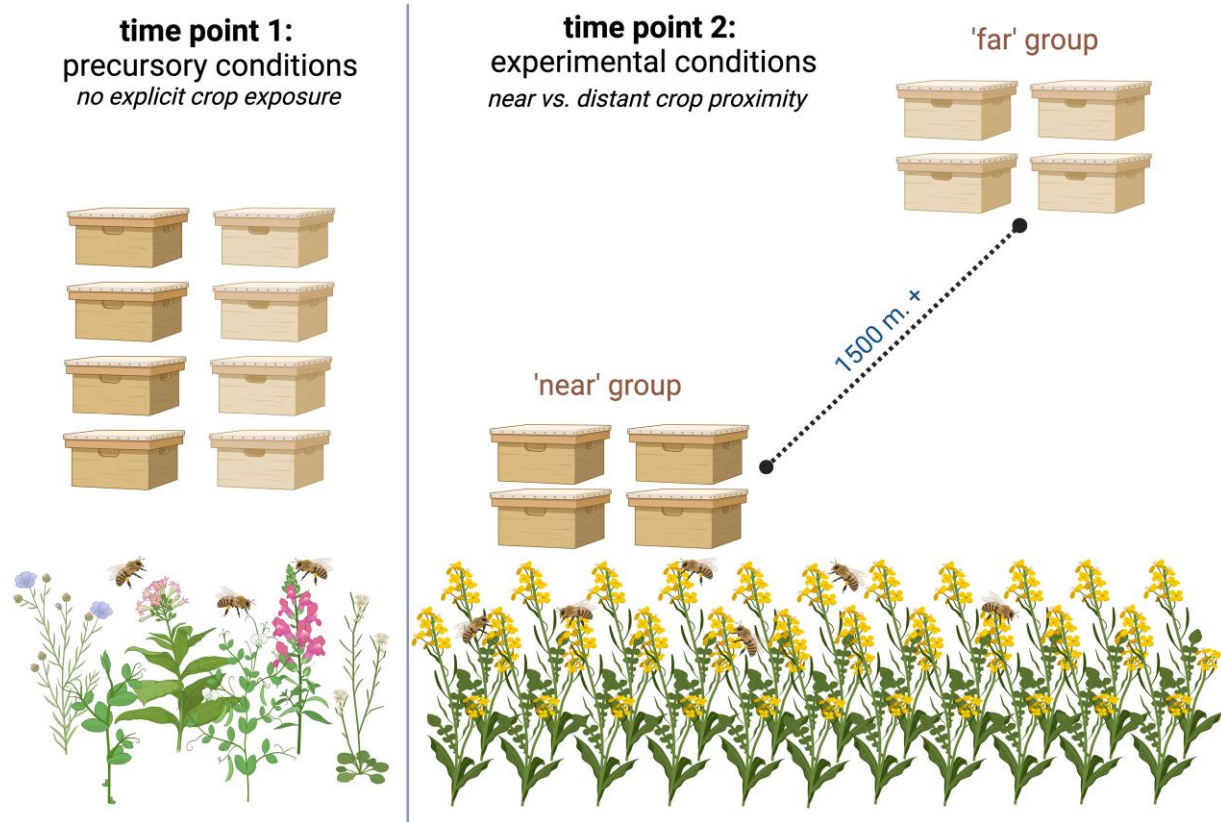


Fig. 1. Experimental design. At the start of the season, colonies were randomly assigned to apiaries that were either “near” or “far” from focal crops. Colonies were sampled after randomization prior to crop exposure (time point 1) and during periods of maximum exposure (time point 2). Image created with [BioRender.com](https://www.biorender.com).

and thus, we cannot make any conclusions about this type of foraging interaction.

Landscape composition predicts dietary diversity

We next explored how the relative abundance of land cover types at each site influenced dietary diversity—a quantification of the variety and abundance of plant genera within each mixed pollen sample (Fig. S1). We estimated dietary diversity via Shannon Weaver’s index of species diversity and used it as an indicator of the breadth of foraging interactions; higher diversity values were strongly correlated with a low predominant dietary component and a high number of unique plant genera (Fig. S1). Agricultural land cover, urban land cover, and grassland cover were all significantly associated with dietary diversity (Table 1). Agricultural land was negatively correlated with dietary diversity (Fig. 3a), while urban land was positively associated with dietary diversity (Fig. 3b). Grassland was negatively associated with dietary diversity; however, this relationship was driven by a low number of observations in a single province (Fig. 3c). Forest land cover displayed no detectable association with dietary diversity (Fig. 3d).

Dietary diversity predicts stressor exposure

Our stressor dataset was heavily skewed by low or no observations, and thus, we selected a subset of variables with sufficient observations (>25 detections) to undergo mixed-effects logistic modeling, using experiment (time, location) as our random effect. Of the 18 stressors deemed suitable for logistic analysis, 8 were significantly associated with dietary diversity, assessed via log-

odds estimates and P-values (Table 2). Log-odds ratios indicated that as dietary diversity decreased, we were significantly more likely to detect 4 xenobiotics: coumaphos, picoxystrobin, clothianidin, and thiamethoxam (Fig. 4; Table 2). In contrast, as dietary diversity increased, we were significantly more likely to detect *Nosema* spores, European Foulbrood, as well as 2 xenobiotics: flupyradifurone and pyrimethanil (Fig. 4; Table 2). Five stressor variables had both a sufficient number of observations and variation in those observations to undergo general linear mixed-effects modeling: *Nosema* spore abundance, Varroa mite abundance, summed total viral loads, thiamethoxam LD₅₀ risk quotients (RQs), and clothianidin RQs. LD₅₀ RQs quantify the relative risk of adverse effects of exposure given the observed concentration, estimated dietary intake, and the known lethal dose (see Materials and methods for a description of these calculations). Due to the nonnormal distribution of stressor observations, we opted to use quasi-Poisson mixed-effects models to better account for the high number of low or no detections. Both neonicotinoid LD₅₀ RQs were significantly negatively associated with dietary diversity (Table 3); the highest observations of both thiamethoxam and clothianidin were seen in colonies with low dietary diversity (Fig. 5a and b). In contrast, total viral loads were significantly positively associated with dietary diversity (Table 3); colonies with high dietary diversity tended to have the greatest total pathogen loads (Fig. 5c). To determine whether the association between dietary diversity and quantitative stressor abundance was confounded by the influence of landscape composition, we ran the same quasi-Poisson mixed-effects models, using relevant land cover parameters as the model input.

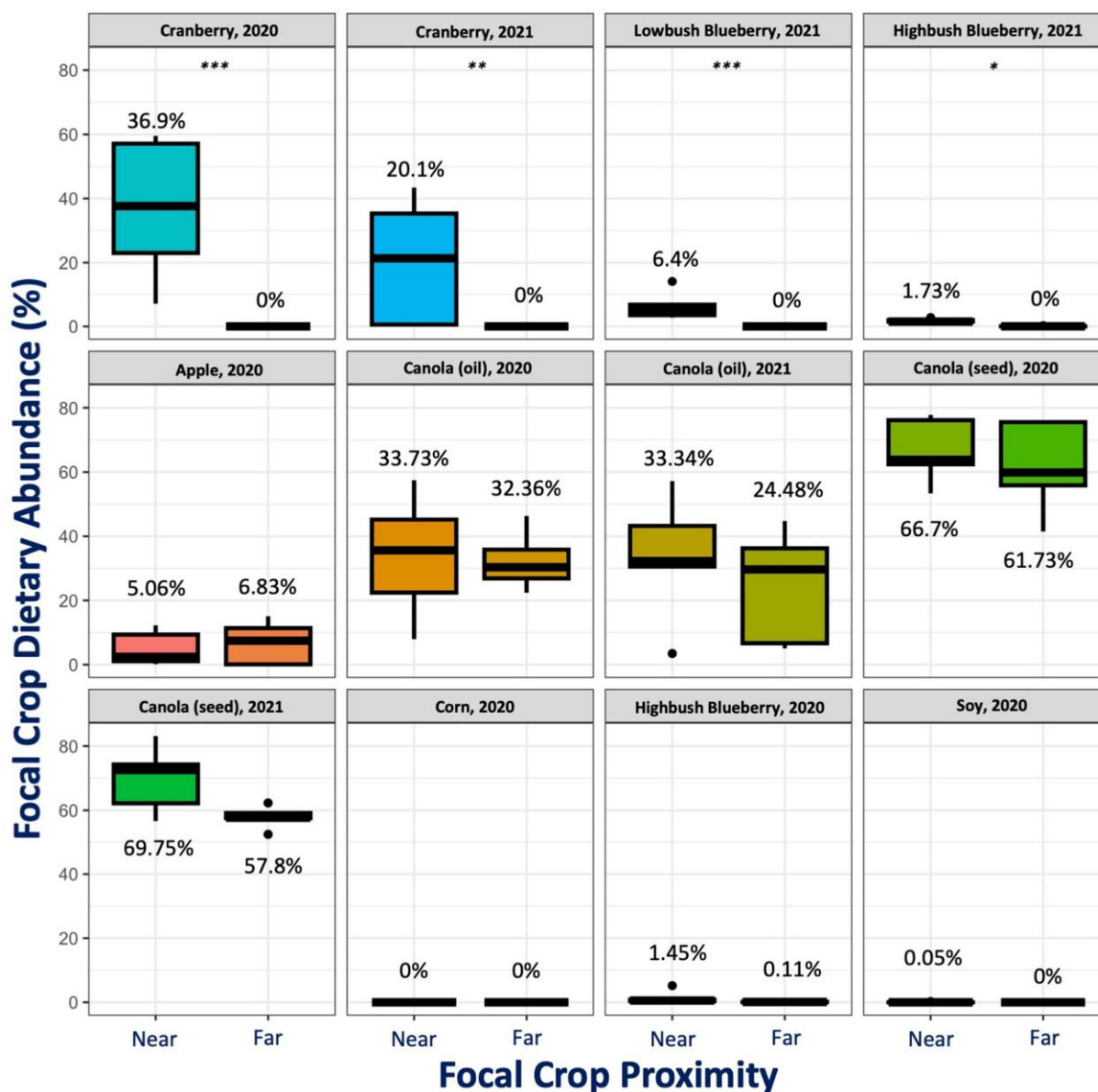


Fig. 2. Bees display distinct foraging patterns. The boxplots show differences in focal crop pollen abundance between near and far sites. Focal crop dietary abundance was estimated as the multilocus average relative proportion of each pollen sample comprised of the genera associated with the crop of interest. The asterisks indicate statistically significant differences ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$), calculated using Kruskal–Wallis nonparametric analysis.

Table 1. Landscape composition is significantly associated with dietary diversity ($P < 0.05$), analyzed via Pearson's product moment correlation tests.

Predictor	Response	t	r	P-value
Agriculture	Dietary diversity	-3.076	-0.279	0.003
Urban	Dietary diversity	3.421	0.307	<0.001
Grassland	Dietary diversity	-2.745	-0.251	0.007
Forest	Dietary diversity	0.953	0.089	0.343

Output of product moment correlation tests exploring association between landscape composition and dietary diversity. Agricultural land cover was negatively associated with dietary diversity, urban land cover was positively associated with dietary diversity, and grassland cover was negatively associated with dietary diversity (grassland observations were limited to a single province); t is the test statistic, r is the Pearson correlation coefficient, and P -value indicates the significance of the relationship.

Landscape composition was a weak predictor of stressor abundance (Fig. 5d–f), suggesting that our dietary diversity models are not substantially confounded by the known influence of landscape composition.

Discussion

The honey bee colonies used in our experiments exhibited distinct foraging preferences; canola was a highly sought-after floral resource, while cranberry foraging appeared to be driven by convenience, and other crops (blueberry, corn, and soybean) were clearly not a dominant dietary source of pollen. There was a strong preference for both canola production systems in our

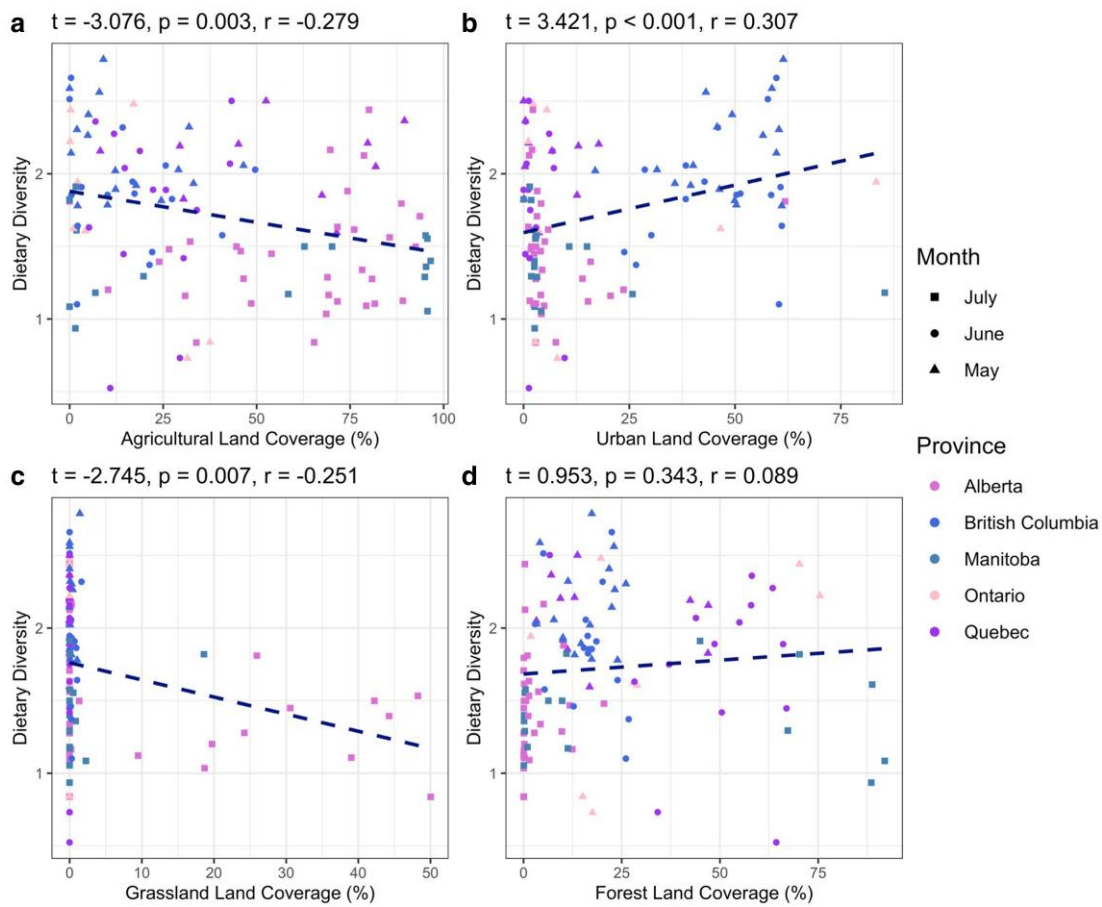


Fig. 3. Landscape composition is significantly associated ($P < 0.05$) with dietary diversity. Pearson's product moment correlation tests assessed raw association between landscape types and dietary diversity. Land coverage (%) values indicate the relative abundance of a land-type within a 1,500 m radius of the study site. Agricultural land cover was negatively associated with dietary diversity, urban land cover was positively associated with dietary diversity, and grassland cover was negatively associated with dietary diversity (grassland observations were limited to a single province); t is the test statistic, r is the Pearson correlation coefficient, and P -value indicates the significance of association. The dashed lines indicate the direction of association.

experiments, especially the seed production variety; even colonies placed $>1,500$ m from flowering canola had diets dominated by *Brassica* pollen. This is in sharp contrast to the lack of interest in other crop genera—even when placed directly adjacent to blueberry plants during anthesis, pollen stored by honey bees rarely contained any *Vaccinium* pollen, implying that they may be actively foraging outside of their expected range to access preferred floral resources. One crop in particular was only notably foraged on by “near” groups: cranberry, indicating a moderate preference that is potentially driven by convenience. The strong foraging preference for canola seen in our experiments coincides with that seen by Rollin et al. (34), as does the indifference to blueberry species seen by Girard et al. (35). Interestingly, our experiment documented no pollen foraging of corn by honey bees, in direct contrast to the results seen by Danner et al. (33), but coinciding with the results seen by Tsvetkov et al. (12).

To some degree, foraging patterns may be explained by pollen dispersal mechanisms—corn is anemophilous, relying on wind as the predominant vector for pollen dispersal (41, 42). Anemophilous (wind-dispersed) species are unlikely to comprise a substantial proportion of any honey bees' diet due to their small size and lack of pollenkitt, an adhesive compound found on the outer exine layer that aids in the dissemination of entomophilous (insect-dispersed) pollen (43, 44). Soybean may not rely on

anemophily for dispersal of pollen, but some work has suggested it predominantly self-pollinates (45), and thus may not invest resources into the attraction of insect pollinators. Dispersal mechanisms provide a simple explanation for some foraging patterns but fail to account for the variable behavior seen in our cranberry and blueberry experiments. This lack of interest in blueberry pollen has been seen previously (35), wherein the authors propose that it may be the result of a poor nutrient profile. More broadly, blueberry is thought to require buzz pollination, i.e. sonication of flowers to promote anther dehiscence (46), which honey bees are not known to do (47, 48). Thus, low dietary abundance could be a result of insufficient sonication abilities, coupled with low overall pollen production rates. Alternatively, if nutritional demands really are driving foraging patterns, the consistent treatment of canola (*Brassica*) as a high-value and highly accessed floral resource could imply that it has special dietary value. Experimental work has suggested that diets comprised of *Brassica* pollen can reduce early mortality of worker bees (49), though its protein/lipid ratio is relatively low (50), contradicting the hypothesis that preferences are driven by protein content (51). Evidently, more work is needed to understand the specific mechanisms that determine foraging value and the related preference for canola pollen.

Across all 12 experiments and 114 sites, our work has provided robust evidence that within Canadian landscapes, diets of lower

Table 2. Dietary diversity is associated with the detection of 8 stressors of interest, analyzed via mixed-effects binomial logistic regressions.

Response	Estimate	SE	P-value
Xenobiotics			
Boscalid	1.426	0.829	0.086
Pyraclostrobin	0.867	0.664	0.192
Fluopyram	0.171	0.819	0.834
Pyrimethanil	2.708	0.989	0.006
Clothianidin	-1.956	0.732	0.008
Chlorantraniliprole	-1.093	0.657	0.096
Thiamethoxam	-2.325	0.767	0.003
Flupyradifurone	2.596	1.238	0.036
Coumaphos	-1.730	0.783	0.027
Picoxystrobin	-7.325	2.568	0.004
Pests and pathogens			
Nosema spores	2.064	0.863	0.016
Black queen cell virus	-2.680	1.880	0.154
Sacbrood virus	-0.057	0.731	0.938
Varroa destructor virus	-0.114	0.567	0.841
European foulbrood	1.441	0.678	0.034
Varroa mites	0.738	0.503	0.142
Deformed wing virus	-0.383	0.552	0.488
Israeli acute paralysis virus	0.549	1.027	0.593

Output of mixed-effects binomial logistic regressions testing for association between dietary diversity and the presence or absence outcome of a stressor response. Estimate is the log-odds of detection as dietary diversity increases, SE is the standard error of that estimate, and P-values indicate the statistical significance of the relationship.

diversity are associated with greater exposure to xenobiotics (Fig. 4), specifically the neonicotinoids thiamethoxam and clothianidin (Fig. 5a and b), while diets of higher diversity are associated with greater exposure to pathogens (Figs. 4 and 5c). These findings demonstrate that pollen foraging behavior and related patterns of plant interaction drive exposure to dichotomous stressor syndromes, characterized by diets of low diversity and increased xenobiotic exposure, or diets of high diversity and increased pathogen exposure. Landscape composition is evidently influential in determining these patterns of interaction (Fig. 3), and while the relationship between landscape composition and dietary diversity was significant, the associated correlation coefficients were weak (Table 1). Further, landscape composition alone was a weak predictor of stressor abundance (Fig. 5d–f), demonstrating the key role that foraging preferences play in mediating exposure. Pollen foraging preferences providing linkage between the structure of a landscape and exposure to the stressors nested within that landscapes are intuitive, and yet, our work is the first to empirically document this on a large scale.

Our findings suggest that patterns of stressor exposure occur on a dichotomous scale, and the degree to which any given colony experiences one of these contrasting stressor syndromes is mediated by patterns of plant interaction. Foraging on singular pollen sources within agricultural contexts led to increased xenobiotic, and specifically neonicotinoid, exposure—highlighting the risks associated with crop pollination and its impact on colony health. Though our work is one of the first to provide direct evidence of this linkage, the risks associated with crop pollination have been known for some time. Crop monocultures are routinely treated with xenobiotics (52–54), such as the neonicotinoid thiamethoxam (Figs. 4 and 5a), which is known to impair flight ability (55), alter motor function (56), and cause oxidative damage (57). Similar deleterious effects have been documented after exposure to clothianidin (58–60), the detection of which was again associated with low dietary diversity (Figs. 4 and 5b). Though it remains hotly debated how detrimental neonicotinoid

Table 3. Dietary diversity is associated with quantitative stressor levels, analyzed via quasi-Poisson mixed-effects linear regressions.

Predictor	Response	Estimate	SE	t-value	P-value
Diet predicting stressor abundance					
Dietary diversity	Nosema spores	0.321	0.207	1.549	0.124
Dietary diversity	Mites	0.266	0.342	0.778	0.438
Dietary diversity	Viral load	0.846	0.308	2.751	0.007
Dietary diversity	Thiamethoxam	-1.768	0.404	4.377	< 0.001
Dietary diversity	Clothianidin	-1.147	0.442	-2.593	0.011
Landscape predicting stressor abundance					
Urban land	Viral load	-0.009	0.008	-1.086	0.280
Agricultural land	Thiamethoxam	0.018	0.008	2.413	0.018
Agricultural land	Clothianidin	-0.002	0.007	-0.376	0.707

Output of quasi-Poisson mixed-effects linear regressions testing for association between the response (stressor abundance) and the predictor (dietary diversity, or landscape composition), controlling for experiment (location, time) as a random effect. Estimates are quasi-Poisson log-odds coefficients; P-values indicate the statistical significance of the relationship.

exposure may be to honey bee health, recent work has demonstrated the degree of impact may be genotype dependent (61), suggesting that some colonies may be more susceptible than others. While crop pollination remains necessary, developing strategies to reduce colony reliance on singular pollen sources could present a pathway to reduce the degree of xenobiotic exposure. Agroecosystems populated by crop monocultures provide little diversity of pollen sources to foraging bees and thus could be the root cause of this linkage. It remains unclear if low dietary diversity is associated with nutritional stress, but if this hypothesis holds true, honey bee colonies foraging on crop pollen may be more likely to experience the synergistic effects of multistressor exposure via the compounded exposure to xenobiotics and a poor-quality diet.

Increasing the diversity of floral resources available to foraging bees may reduce singular reliance on crop pollen, and any associated crop-specific xenobiotics, but this strategy comes with its own set of risks. Our work suggests that diets with a diverse mix of pollen sources are associated with increased pathogen exposure (Figs. 4 and 5c), representing an alternative but potentially equally stressful scenario for managed honey bee colonies. This foraging behavior was promoted by urban land cover (Fig. 3b), coinciding with previous findings that suggest that cities promote high foraging diversity, likely as a result of high beta plant diversity and fine-grain heterogeneity of floral resources (62). Interestingly, other work has provided evidence that cities, heavily comprised of impermeable surfaces (63–65), can concentrate invertebrates into small densely populated green spaces, facilitating the development of pathogen transmission hubs (66). This is likely attributable to the overlap in resource access patterns when the availability of floral resources is limited; interspecific interactions are a primary source of microorganism transmission (67–70). Disease spill over from honey bees has led to the spread of pathogens among other bee taxa (71), primarily via overlap in plant interactions (67, 72), and while honey bees may have been the catalyst for the initial introduction of many microorganisms, transmission is multilateral. Our work provides linkage between these findings, suggesting that cities promoting diverse foraging

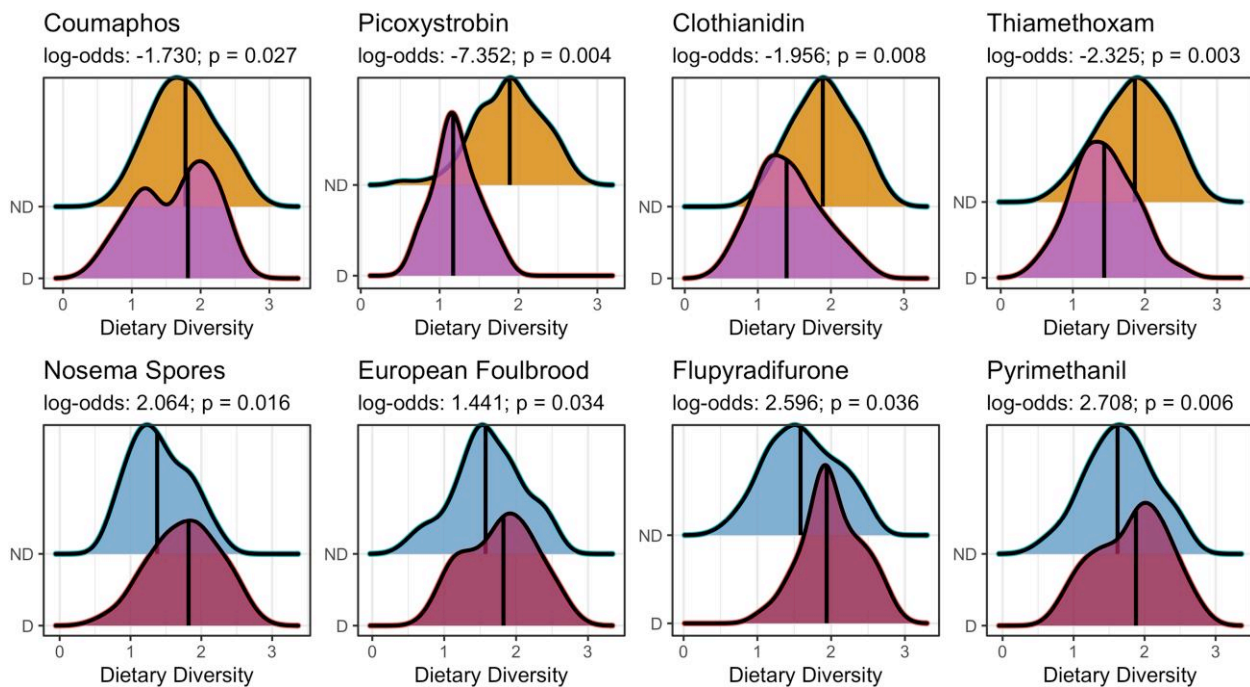


Fig. 4. Dietary diversity is a strong predictor of stressor exposure. Ridge plots display significant association ($P < 0.05$) between dietary diversity and the detection outcome of 8 stressors, analyzed via mixed-effects binomial logistic regressions. Log-odds ratios indicate a change in the probability of detection as dietary diversity increases; negative values indicate a reduction in the likelihood of exposure; positive values indicate an increase in the likelihood of exposure.

behavior and increased pathogen exposure are an intertwined phenomenon. It is possible that pollinator gardens and other pockets of biodiversity within urban developments could act as poisoned oases (73), providing diverse diets at the expense of exposure to pathogens.

Here, we present the first large-scale evidence that pollen foraging preferences in modified environments mediate exposure to dichotomous stressor syndromes. Colonies foraging at agricultural sites on a small number of pollen sources experience a greater risk of xenobiotic exposure, while colonies foraging at urban sites on diverse pollen sources experience a greater risk of pathogen exposure. The dichotomous nature of stressor exposure found in our 456 honey bee colonies suggests that modified landscapes, regardless of the nature of those modifications, present a broad risk to honey bee health. This implies that despite our best efforts, no anthropogenically modified environment is wholly “safe” for honey bee colonies, and supporting pollinators may require a radical change in our urban and agricultural land management strategies. What remains largely unknown is how dietary diversity (e.g. nutrition) impacts colony health—integrated with our findings here, this key question could provide crucial information to improve our efforts to combat colony loss and maintain healthy, robust colonies to pollinate our crops and increase the resilience of our food production systems.

Materials and methods

Experimental design

We used an established experimental design (7, 13) to explore how crop proximity impacted the frequency of plant interaction in 8 Canadian crops: cranberry (*Vaccinium*), highbush blueberry (*Vaccinium*), lowbush blueberry (*Vaccinium*), commodity canola (*Brassica*), hybrid seed-production canola (*Brassica*), apple (*Malus*), soybean (*Glycine*), and corn (*Zea*). Crops of particular interest

(both canola production systems, highbush blueberry, cranberry) were replicated across a second year, totaling 12 spatially and/or temporally separate experiments. Each experiment followed the same general design (Fig. 1); at the beginning of the pollination period for each crop, we selected 40 honey bee colonies at a neutral apiary and sampled under “t1” precursory conditions (described below) to confirm that no experimental group differed substantially in their foraging behavior or stressor load preceding the start of each experiment. We selected colonies of equal size and visually inspected them to ensure that they were healthy with no obvious signs of disease prior to the start of the experiments. We then randomly assigned each of the colonies to 1 of 10 experimental sites. Five of those sites were in or directly adjacent to the focal crop of interest (“near” crop proximity), and the remaining 5 were placed a minimum of 1,500 m away from the focal crop (“far” crop proximity), with all sites being at least 1,500 m away from another experimental site. After moving the colonies to sites, we performed “t2” sampling (experimental conditions) 2–4 weeks after placement, coinciding with the timing of peak exposure from the relevant focal crops. For all crops except corn, this period coincided with peak bloom, while for corn, this period coincided with seeding the crop. We do not provide exact GPS coordinates for apiaries to protect the identity of the beekeepers and farmers involved. The approximate location (municipality, province) of each site is provided in [Supplementary file \(S2\)](#).

Sampling

At each time point (t1 precursory conditions and t2 experimental conditions), we sampled several colony matrices to quantify biotic and abiotic stressors and the composition of stored pollen (bee bread). We sampled 4 g of bee bread (or ~100 hive cells) from each of 4 colonies at a site by harvesting freshly processed bee bread, identifiable by the color and light degree of compaction. We then pooled all 4 samples and divided the resulting 16 g

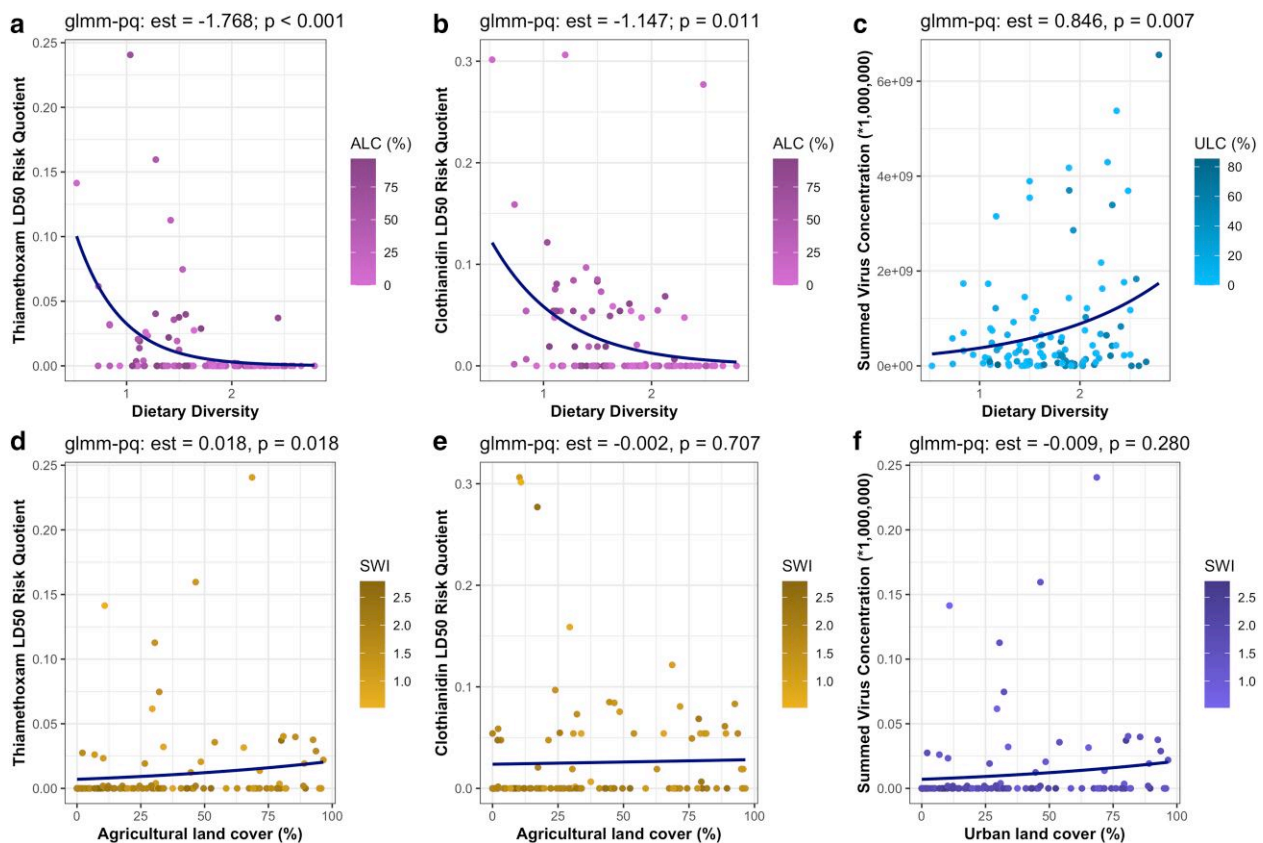


Fig. 5. Dietary diversity is a significant predictor of stressor abundance. Quasi-Poisson mixed-effects models ($glmm-pq$) tested for association between the response (stressor abundance), and the predictor (dietary diversity, or landscape composition), controlling for experiment (location, time) as a random effect. Estimates are quasi-Poisson log-odds coefficients; P -values indicate the statistical significance of the relationship. Dietary diversity (a–c) was a significant predictor ($P < 0.05$) of stressor exposure, while relevant landscape parameters (d–f) were weak predictors of stressor exposure. ALC (agricultural land coverage) and ULC (urban land coverage) are landscape gradients values that indicate the relative abundance of a land-type within a 1,500 m radius of the study site. SWI (Shannon’s index) are dietary gradients that indicate the relative diversity of a pollen sample.

sample into 2 portions: 10 g to undergo pollen metabarcoding (described below) and 6 g to undergo xenobiotic analysis (described below). We next sampled nectar from each colony at each site; we collected 3 mL from the comb, using a 1-cc syringe with the needle removed, and transferred it to a 5 mL centrifuge tube. Nectar was used for xenobiotic analysis (described below). We then sampled nurse bees for pathogen and xenobiotic analysis. We randomly sampled 200 bees from each site (~50 per colony), which were placed on dry ice and stored in a -80°C freezer. We quantified *Varroa destructor* mite abundance using an alcohol wash method (74) and standardized *Varroa* mite counts for each colony to represent the number of mites per 100 bees, which was then averaged across the 4 colonies at each site and time point.

Pathogen quantification

We quantified a number of pathogens in the collected samples of hive bees using established methods (75, 76) performed by the National Bee Diagnostic Centre (Beaverlodge, Alberta). This included qPCR quantification of acute bee paralysis virus, black queen cell virus, chronic bee paralysis virus, deformed wing virus, Israeli acute paralysis virus, Lake Sinai virus, Kashmir bee virus, sacbrood virus, and *Varroa destructor* virus-1. The causative agent of European foulbrood (*Melissococcus plutonius*) was detected using qPCR20 with a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, USA). *Nosema* spores were quantified using microscopy (76). Each sample was first homogenized in 12 mL of GITC (guanidinium thiocyanate) buffer. We then isolated RNA using a 200 μL aliquot and the

NucleoSpinRNA kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) and used 800 ng to synthesize cDNA at 46°C for 20 min using the iScript cDNA synthesis kit (Bio-Rad laboratories, Hercules, USA). The resulting 20 μL of dDNA was diluted with 60 μL of nuclease-free sterile water, and 3 μL was used for qPCR analysis. Our quantitative PCR analysis of each sample to determine the presence and abundance of pathogens of interest used SSoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, USA) and the previously published primers described by Borba et al. (76). Triplicate assay amplifications used ~30 ng of cDNA, and a CFX384 Touch Real-Time Detection System (Bio-Rad Laboratories, Hercules, USA). Standard curves were generated from plasmids that contained the target sequences with diluted copy numbers ranging from 10^2 to 10^7 . PCR program specifications were as follows: initial denaturation for 3 min at 95°C , followed by 10 s at 95°C and 20 s at 60°C for 40 cycles. We confirmed the accuracy of these approaches via melt-curve analysis (65 – 95°C with increments of 0.5°C at 2 s/step); real-time qPCR data were analyzed with the CFX Manager Software (Bio-Rad Laboratories, Hercules, USA).

Xenobiotic quantification

Multiresidue quantification of 239 xenobiotics was carried out on samples of pollen and nectar following standard methods (77, 78) by the Agriculture and Food Laboratory (Guelph, Ontario), using a modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction. For each sample that underwent analysis, a 2 g subsample was extracted into 1% acetic acid (in acetonitrile,

anhydrous sodium acetate, and magnesium sulfate), and the resulting supernatant was then evaporated and diluted in methanol with ammonium acetate (0.1 M). Extracts were analyzed via LC/ESI-MS/MS (liquid chromatography and electrospray ionization tandem mass spectrometry) using an established approach (77). To compare the relative risk of xenobiotic exposure, we calculated the RQ of each chemical (79, 80) based on its relative abundance and LD₅₀ (median lethal dose that kills 50% of bees in test cages), as well as the approximate weight of pollen and nectar that an invasive honey bee consumes per day, where:

$$RQ = \frac{(\text{residue in nectar } (\mu\text{g kg}^{-1}) \times 140 \times 10^{-6} \text{ kg} \times \text{bee}^{-1}) + \text{residue in pollen } (\mu\text{g kg}^{-1}) \times 9.6 \times 10^{-6} \text{ kg} \times \text{bee}^{-1}}{\text{acute oral LD}_{50} (\mu\text{g bee}^{-1})}$$

Pollen metabarcoding

We followed an established protocol for pollen metabarcoding (36). Briefly, we extracted DNA from pollen samples using the NucleoMag DNA Food Kit (Macherey-Nagel, Düren, Germany) by combining 10 g of bee bread with 20 mL of lysis buffer: 70% autoclaved filtered water (Millipore Sigma, Burlington MA, USA), 20% 10× STE (100 mM NaCl, 10 mM Tris, 25 mM EDTA), and 10% diluted SDS (10% sodium dodecyl sulfate). We then sealed each conical tube, inverted them 10 times, and then homogenized the suspension by shaking samples for 10 min in an orbital shaker (G25 Incubator Shaker, New Brunswick Scientific, Edison, NJ, USA) at 25 °C and 375 rpm. Immediately after removing the samples from the orbital shaker, we pipetted 3 mL of the homogenized sample into a 7 mL cylindrical tube containing 10 small (1.4 mm) and 2 large (2.8 mm) ceramic beads and bead beat it (Bead Mill 24, Fisherbrand, Ottawa, ON, Canada) for 4 × 30 s cycles, at a speed of 6 m/s. We then transferred 550 μL of the homogenized suspension to a 1.5 mL Eppendorf tube. We warmed CF lysis buffer for 10 min in a 65 °C water bath, then added 550 μL of the warmed buffer and 10 μL of proteinase K (from the NucleoMag DNA Food kit) to the homogenized sample, and vortexed (Mini Vortex Mixer, VWR, Mississauga, ON, Canada) the sealed tube for 30 s. We then incubated the sample at 65 °C for 30 min in a block heater (Isotemp 145D, 250 V, Fisherbrand, Ottawa, ON, Canada), inverting every 10 min. After incubation, we added 20 μL of RNase A (New England Biolabs, Ipswich, MA, USA) and allowed the sample to incubate at room temperature (20 °C) for an additional 30 min. After incubation, we centrifuged the sample for 20 min at 14,000 rpm (Centrifuge 5810 R, 15 amps, Eppendorf, Hamburg, Germany), transferred 400 μL of the upper liquid layer to the binding plate, added 25 μL of NucleoMag B-Beads and 600 μL of binding buffer CB (both from the NucleoMag DNA Food kit), and then ran an extraction program on the KingFisher Flex extraction robot (ThermoScientific, Waltham, MA, USA). Each of the 5 deep well plates used to complete the extraction program contained either 600 μL of CMW buffer (wash 1), 600 μL of CQW buffer (wash 2.1), 600 μL of 80% EtOH (wash 2.2), or 100 μL of buffer CE (elution). After the extraction program was complete, we transferred 80 μL of the eluted sample to a fresh 1.5 mL Eppendorf tube and froze it at −20 °C until we began DNA amplification.

We carried out 3 PCR programs—first amplifying the DNA barcode locus of interest, then extending the length of the amplified sequence, and finally indexing the samples with unique combinations of forward and reverse primers. We used validated primers (36) to amplify loci from 2 barcoding regions, ITS2 and rbcL (Table 4). We used 96 well plates containing 84 pollen samples, 6 negative controls, and 6 positive controls (Banana, *Musa* sp.). Each reaction included 11 μL of water, 12.5 μL of 2× Taq Pol Mix

An LD₅₀ was used if it was derived using workers from any subspecies of *A. mellifera*, followed the Organization for Economic Co-operation and Development guidelines for acute oral or diet toxicity tests on honey bees, and provided per bee or per body weight dosages. If tests using a high purity of the active ingredient were unavailable, studies on formulations or a combination of xenobiotics were used. Xenobiotic concentrations that fell below the limits of quantification or detection were assigned a concentration equal to that limit.

(New England Biolabs, Ipswich, MA, USA), 0.5 μL of each relevant forward and reverse primer (1 μM), and 0.5 μL of sample DNA (~36.2 ng) into each well. PCR cycling conditions were (Eppendorf Mastercycler, Ep Gradient, Hamberg, Germany): initial denaturation (94 °C, 10 min, 1 cycle), followed by 40 cycles of denaturation (94 °C, 30 s), annealing (54 °C, 40 s), and extension (72 °C, 1 min), and then a final extension cycle (72 °C, 10 min). The product of this first PCR (PCR1) was used as the template for a second PCR (PCR2; Table 4) and the same chemistry as described above (with 0.5 μL of PCR1 product instead of sample DNA). Cycling conditions were the same for PCR1 and PCR2, with the exception of a higher annealing temperature (56 °C instead of 54 °C). After each respective PCR program, we used gel electrophoresis to confirm sufficient amplification of each sample and identify any potential contamination using the negative controls. Following PCR2, we prepared samples for Illumina Sequencing by performing a third PCR (PCR3) program that tagged each sample with a unique combination of forward and reverse primers; PCR3 program specifications follow that described above, with the exception of a higher annealing temperature of 60 °C. We then normalized the resulting PCR3 product using a SequelPrep Normalization kit (Invitrogen, Burlington, ON, Canada) and shipped the normalized libraries on dry ice for Illumina Sequencing (Illumina MiSeq PE250) at Genome Quebec. Each library was pair-end sequenced in its own lane. On average, each ITS2 library generated 11,970,673 (±2,893,265) reads, and each rbcL library generated 10,449,713 (±1,366,310) reads.

Pollen metabarcoding data processing was completed in Python (v. 3.10.7) and R (v. 4.2.1; R Core Team), using the *dada2* (81) (v. 1.16.0) and *purrr* (82) (v. 0.3.4) packages. We first paired forward and reverse reads, trimmed primer sequences, and grouped identical sequences as ASVs (amplicon sequence variants). We then built a database that linked species to sequences associated with each primer using the MetaCurator method (83). We used this database to parse through returned sequence data and identify the species associated with each unique grouped sequence, setting a precursory condition of >0.95 similarity. After identifying the plant species associated with each sequence, we consolidated classifications at the genera level and filtered data to control for sequence mistagging (84). Sequence patterns within negative control samples were used to remove detections with a high likelihood of representing mistag-associated false detections (84).

Landscape composition

We used georeferenced raster data from Agriculture and Agri-Food Canada's 2020 and 2021 Annual Crop Inventory to

Table 4. PCR primer specifications.

Primer	Sequence
PCR1	
rbcL1 (F)	AGACCTWTTTGAAGAAGGTTTCWGT
rbcL1 (R)	TCGCATGTACCTGCAGTAGC
ITS2 (F)	ATCGGATACTTGGTGTGAAT
ITS2 (R)	TCCTCCGCTTATTGATATGC
PCR2	
rbcL1 (F)	CAGCGTCAGATGTGTATAAGAGACAG AGACCTWTTTGAAGAAGGTTTCWGT
rbcL1 (R)	GCTCGGAGATGTGTATAAGAGACAG TCGCATGTACCTGCAGTAGC
ITS2 (F)	CAGCGTCAGATGTGTATAAGAGACAG ATCGGATACTTGGTGTGAAT
ITS2 (R)	GCTCGGAGATGTGTATAAGAGACAG TCCTCCGCTTATTGATATGC

(F) indicates a forward primer and (R) indicates the relevant reverse primer. Bolded values are changes to each respective primer between PCR1 and PCR2 programs.

determine the composition of land cover around sites. The Annual Crop Inventory delineates land cover that was present during the crop growing season for a region each year. Using QGIS (version 3.26.0), we converted the raster data to vector data with polygon features, clipped the land cover data to a 1,500 m buffer radius around each apiary location, and calculated the planimetric area of each land cover type. The resulting land-type classifications were grouped based on their description. Urban landcover was characterized as the combined total of urban and developed land types. Grassland landcover was a singular group characterized by the grassland landcover type. Forest landcover was characterized as the combined total of coniferous, broadleaf, and mixed-wood land types. Agricultural landcover was characterized as the combined total of all land classifications related to agriculture, including: vegetables, blueberry, cranberry, berry, corn, potatoes, fallow, vineyards, orchards, crops, barley, rapeseed, flaxseed, oats, soybeans, spring wheat, winter wheat, beans, peas, buckwheat, hemp, rye, sugar beets, faba beans, lentils, mustard, triticale, sunflower, millet, and ginseng.

Data analysis

All statistical analyses were completed in R (v. 4.2.2, 2022-10-31, R Core Team). To control for sampling effort (i.e. differences in the number of metagenetic sequence hits per sample and its effect on the number of species detected), we filtered each sample to the lowest sequence hit observation for each primer using the “rarefy” function included in the *vegan* package (85) (v. 2.6-4). After filtering the data, we converted observations into relative frequencies and consolidated the 2 primers’ estimates to generate multilocus averages, which are more robust than using single locus estimates (36). We used histograms and residual plots to evaluate the parametricity of response variables and selected subsequent tests based on the distribution. For the focal crop experiments, we used the genus of interests’ multilocus average relative pollen abundance as our response variable and performed non-parametric repeated measures analysis using a Friedman rank sum test (for within subjects effects across time) and a Kruskal–Wallis rank sum test (for between subjects effects). We treated time as the repeated measure and experimental group (“near” vs. “far” crop exposure) as a fixed effect, and when the interaction was statistically significant ($P < 0.05$), we performed post hoc analysis using Dunn’s test (86) (*dunnstest* package, v. 1.3.5). We calculated Shannon’s diversity indices using the *vegan* package, which were used as the dietary variable for subsequent analyses. For the landscape analysis, we used linear models (each site was unique, with little or no experiment-level clustering of landscape parameters), and for stressor analysis we used mixed-effects models. Many of the stressor response variables had insufficient

observations (<25 detections) for either quantitative or logistic modeling and thus were excluded from analysis. All response variables that met that minimum requirement underwent logistic modeling (10 xenobiotics, 8 pests and pathogens), and a smaller subset that featured both sufficient detection observations and a substantial range of values (e.g. high and low observations) underwent additional quantitative modeling (*Nosema* spores, mites, total summed viral load, thiamethoxam LD₅₀ RQ, clothianidin LD₅₀ RQ). To account for the influence of time and location, we used experiment (e.g. location × time) as a random effect in all of our models and analyzed the relationship between variables of interest via mixed-effects linear regressions. For our logistic regressions, we used the “glmer” function from the *lme4* package (87) (v. 1.1-31), and for our quasi-Poisson regressions, we used the “glmmPQL” function included in the *mass* package (88) (v. 7.3-58.3). We plotted results figures using the *ggplot2* package (89) (v. 4.2.0) with the *ggribes* extension (v. 0.5.4).

Supplementary Material

Supplementary material is available at PNAS Nexus online.

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Author Contributions

A.Z., L.J.F., E.G.-N., P.G., R.W.C., S.E.H., S.F.P., M.M.G., M.B., and I.M.C. designed the research. S.B.W. and S.F.K. analyzed the data. S.B.W. wrote the manuscript. All authors performed the research and revised the manuscript.

Preprints

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Data Availability

All data underlying the findings are provided in Supplementary file (S2). All code used for analysis has been deposited on GitHub (sbwizenberg/BeeCSI—PNAS-Nexus). We do not provide exact GPS coordinates for apiaries to protect the identity of the

beekeepers and farmers involved. The approximate location (municipality, province) of each site is provided in [Supplementary file \(S2\)](#).

References

- Hung K-LJ, Kingston JM, Albrecht M, Holway DA, Kohn JR. 2018. The worldwide importance of honey bees as pollinators in natural habitats. *Proc Biol Sci.* 285:20172140.
- Le Conte Y, Navajas M. 2008. Influencia de los cambios climáticos en las poblaciones de abejas y sus enfermedades: -EN- climate change: impact on honey bee populations and diseases -FR- changements climatiques : impact sur les populations d'abeilles et leurs maladies -ES. *Rev Sci Tech OIE.* 27:485–510.
- Ricketts TH, Regetz J, Steffan-Dewenter I, Cunningham SA, Kremen C, Bogdanski A, et al. 2008. Landscape effects on crop pollination services: are there general patterns? *Ecol Lett.* 11: 499–515.
- Smith KM, Loh EH, Rostal MK, Zambrana-Torrel CM, Mendiola L, Daszak P. 2013. Pathogens, pests, and economics: drivers of honey bee colony declines and losses. *EcoHealth.* 10:434–445.
- Aebi A, Neumann P. 2011. Endosymbionts and honey bee colony losses? *Trends Ecol Evol.* 26:494.
- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. 2010. Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol.* 25:345–353.
- French SK, Pepinelli M, Conflitti IM, Jamieson A, Higo H, Common J, et al. 2024. Honey bee stressor networks are complex and dependent on crop and region. *Curr Biol.* 34(9):1893–1903.e3.
- Cepero A, Martín-Hernández R, Bartolomé C, Gómez-Moracho T, Barrios L, Bernal J, et al. 2015. Passive laboratory surveillance in Spain: pathogens as risk factors for honey bee colony collapse. *J Apic Res.* 54:525–531.
- Cornman RS, Tarpy DR, Chen Y, Jeffreys L, Lopez D, Pettis JS, et al. 2012. Pathogen webs in collapsing honey bee colonies. *PLoS One.* 7:e43562.
- Le Conte Y, Ellis M, Ritter W. 2010. *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? *Apidologie.* 41:353–363.
- Fisher A, Rangel J. 2018. Exposure to pesticides during development negatively affects honey bee (*Apis mellifera*) drone sperm viability. *PLoS One.* 13:e0208630.
- Das A, Sau S, Pandit MK, Saha K. 2018. A review on: importance of pollinators in fruit and vegetable production and their collateral jeopardy from agrochemicals. *J Entomol Zool Stud.* 6:1586–1591.
- Tsvetkov N, Samson-Robert O, Sood K, Patel HS, Malena DA, Gajiwala PH, et al. 2017. Chronic exposure to neonicotinoids reduces honey bee health near corn crops. *Science (1979).* 356: 1395–1397.
- Dolezal AG, Toth AL. 2018. Feedbacks between nutrition and disease in honey bee health. *Curr Opin Insect Sci.* 26:114–119.
- Tosi S, Nieh JC, Sgolastra F, Cabbri R, Medrzycki P. 2017. Neonicotinoid pesticides and nutritional stress synergistically reduce survival in honey bees. *Proc Biol Sci.* 284:20171711.
- Huang Z. 2012. Pollen nutrition affects honey bee stress resistance. *Terr Arthropod Rev.* 5:175–189.
- Grozinger CM, Zayed A. 2020. Improving bee health through genomics. *Nat Rev Genet.* 21:277–291.
- Watson K, Stallins JA. 2016. Honey bees and colony collapse disorder: a pluralistic reframing. *Geogr Compass.* 10:222–236.
- Magal P, Webb GF, Wu Y. 2019. An environmental model of honey bee colony collapse due to pesticide contamination. *Bull Math Biol.* 81:4908–4931.
- Myerscough MR, Khoury DS, Ronzani S, Barron AB. 2015. *Why do hives die? Using mathematics to solve the problem of honey bee colony collapse.* Springer Singapore.
- Genersch E. 2010. Honey bee pathology: current threats to honey bees and beekeeping. *Appl Microbiol Biotechnol.* 87:87–97.
- Neov B, Shumkova R, Palova N, Hristov P. 2021. The health crisis in managed honey bees (*Apis mellifera*). Which factors are involved in this phenomenon? *Biologia (Bratisl).* 76:2173–2180.
- Bonoan RE, Gonzalez J, Starks PT. 2020. The perils of forcing a generalist to be a specialist: lack of dietary essential amino acids impacts honey bee pollen foraging and colony growth. *J Apic Res.* 59:95–103.
- Hendriksma HP, Toth AL, Shafir S. 2019. Individual and colony level foraging decisions of bumble bees and honey bees in relation to balancing of nutrient needs. *Front Ecol Evol.* 7:177.
- Hendriksma HP, Shafir S. 2016. Honey bee foragers balance colony nutritional deficiencies. *Behav Ecol Sociobiol.* 70:509–517.
- Keller I, Fluri P, Imdorf A. 2005. Pollen nutrition and colony development in honey bees: part 1. *Bee World.* 86:3–10.
- Tereshko V, Lee T. 2002. How information-mapping patterns determine foraging behaviour of a honey bee colony. *Open Syst Inf Dyn.* 9:181–193.
- Schmickl T, Thenius R, Crailsheim K. 2005. Simulating swarm intelligence in honey bees: foraging in differently fluctuating environments. In: *Proceedings of the 7th Annual Conference on Genetic and Evolutionary Computation*; Washington, DC. New York, (NY): Association for Computing Machinery. p. 273–274.
- Steffan-Dewenter I, Kuhn A. 2003. Honeybee foraging in differentially structured landscapes. *Proc R Soc Lond B Biol Sci.* 270:569–575.
- Beekman M, Ratnieks FLW. 2000. Long-range foraging by the honey-bee, *Apis mellifera* L. *Funct Ecol.* 14:490–496.
- Danner N, Molitor AM, Schiele S, Härtel S, Steffan-Dewenter I. 2016. Season and landscape composition affect pollen foraging distances and habitat use of honey bees. *Ecol Appl.* 26:1920–1929.
- Bänsch S, Tschamtko T, Ratnieks FLW, Härtel S, Westphal C. 2020. Foraging of honey bees in agricultural landscapes with changing patterns of flower resources. *Agric Ecosyst Environ.* 291:106792.
- Danner N, Härtel S, Steffan-Dewenter I. 2014. Maize pollen foraging by honey bees in relation to crop area and landscape context. *Basic Appl Ecol.* 15:677–684.
- Rollin O, Bretagnolle V, Decourtye A, Aptel J, Michel N, Vaissière BE, et al. 2013. Differences of floral resource use between honey bees and wild bees in an intensive farming system. *Agric Ecosyst Environ.* 179:78–86.
- Girard M, Chagnon M, Fournier V. 2012. Pollen diversity collected by honey bees in the vicinity of *Vaccinium* spp. crops and its importance for colony development. This article is part of a Special Issue entitled “Pollination biology research in Canada: perspectives on a mutualism at different scales”. *Botany.* 90: 545–555.
- Wizenberg SB, Newburn LR, Pepinelli M, Conflitti IM, Richardson RT, Hoover SER, et al. 2023. Validating a multi-locus metabarcoding approach for characterizing mixed-pollen samples. *Plant Methods.* 19:120.
- Wizenberg SB, Newburn LR, Richardson RT, Pepinelli M, Conflitti IM, Moubony M, et al. 2023. Environmental metagenetics unveil novel plant-pollinator interactions. *Ecol Evol.* 13:e10645.
- Bell KL, Fowler J, Burgess KS, Dobbs EK, Gruenewald D, Lawley B, et al. 2017. Applying pollen DNA metabarcoding to the study of plant-pollinator interactions. *Appl Plant Sci.* 5:1600124.
- Richardson RT, Lin C-H, Quijia JO, Riusech NS, Goodell K, Johnson RM. 2015. Rank-based characterization of pollen assemblages

- collected by honey bees using a multi-locus metabarcoding approach. *Appl Plant Sci.* 3:1500043.
- 40 Richardson RT, Lin C-H, Sponsler DB, Quijia JO, Goodell K, Johnson RM. 2015. Application of ITS2 metabarcoding to determine the provenance of pollen collected by honey bees in an agroecosystem. *Appl Plant Sci.* 3:1400066.
 - 41 Tang M, Gu W, Ma Q, Li YJ, Zhong C, Li S, et al. 2019. Water adsorption and hygroscopic growth of six anemophilous pollen species: the effect of temperature. *Atmos Chem Phys.* 19:2247–2258.
 - 42 Hall JA, Walter GH. 2011. Does pollen aerodynamics correlate with pollination vector? Pollen settling velocity as a test for wind versus insect pollination among cycads (Gymnospermae: Cycadaceae: Zamiaceae). *Biol J Linn Soc Lond.* 104:75–92.
 - 43 Lu X, Ye X, Liu J. 2022. Morphological differences between anemophilous and entomophilous pollen. *Microscopy Res Techn.* 85: 1056–1064.
 - 44 Pacini E, Franchi GG. 2020. Pollen biodiversity—why are pollen grains different despite having the same function? A review. *Bot J Linn Soc.* 193:141–164.
 - 45 Li M, Sha A, Zhou X, Yang P. 2012. Comparative proteomic analyses reveal the changes of metabolic features in soybean (*Glycine max*) pistils upon pollination. *Sex Plant Reprod.* 25:281–291.
 - 46 Cooley H, Vallejo-Marín M. 2021. Buzz-pollinated crops: a global review and meta-analysis of the effects of supplemental bee pollination in tomato. *J Econ Entomol.* 114:505–519.
 - 47 Rosi-Denadai CA, Araújo PCS, Campos LADO, Cosme L, Guedes RNC. 2020. Buzz-pollination in Neotropical bees: genus-dependent frequencies and lack of optimal frequency for pollen release. *Insect Sci.* 27:133–142.
 - 48 De Luca PA, Bussi ere LF, Souto-Vilaros D, Goulson D, Mason AC, Vallejo-Marín M. 2013. Variability in bumblebee pollination buzzes affects the quantity of pollen released from flowers. *Oecologia.* 172:805–816.
 - 49 Ara Begum H, Idrees A, Afzal A, Iqbal J, Qadir ZA, Shahzad MF, et al. 2023. Impact of different pollen protein diets on the physiology of *Apis mellifera* L. (Hymenoptera: Apidae) workers from essential plant sources. *J King Saud Univ Sci.* 35:102511.
 - 50 Vaudo AD, Tooker JF, Patch HM, Biddinger DJ, Coccia M, Crone MK, et al. 2020. Pollen protein: lipid macronutrient ratios may guide broad patterns of bee species floral preferences. *Insects.* 11:132.
 - 51 Russo L, Vaudo AD, Fisher CJ, Grozinger CM, Shea K. 2019. Bee community preference for an invasive thistle associated with higher pollen protein content. *Oecologia.* 190:901–912.
 - 52 Altieri M, Nicholls C, Montalba R. 2017. Technological approaches to sustainable agriculture at a crossroads: an agroecological perspective. *Sustainability.* 9:349.
 - 53 Altieri MA. 2009. The ecological impacts of large-scale agrofuel monoculture production systems in the Americas. *Bull Sci Technol Soc.* 29:236–244.
 - 54 Horrigan L, Lawrence RS, Walker P. 2002. How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environ Health Perspect.* 110: 445–456.
 - 55 Tosi S, Burgio G, Nieh JC. 2017. A common neonicotinoid pesticide, thiamethoxam, impairs honey bee flight ability. *Sci Rep.* 7: 1201.
 - 56 Tosi S, Nieh JC. 2017. A common neonicotinoid pesticide, thiamethoxam, alters honey bee activity, motor functions, and movement to light. *Sci Rep.* 7:15132.
 - 57 Gauthier M, Aras P, Paquin J, Bioly M. 2018. Chronic exposure to imidacloprid or thiamethoxam neonicotinoid causes oxidative damages and alters carotenoid-retinoid levels in caged honey bees (*Apis mellifera*). *Sci Rep.* 8:16274.
 - 58 Tsvetkov N, Zayed A. 2021. Searching beyond the streetlight: neonicotinoid exposure alters the neurogenomic state of worker honey bees. *Ecol Evol.* 11(24):18733–18742.
 - 59 Brandt A, Gorenflo A, Siede R, Meixner M, B uchler R. 2016. The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (*Apis mellifera* L.). *J Insect Physiol.* 86:40–47.
 - 60 Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, Nazzi F, Gargiulo G, Pennacchio F. 2013. Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. *Proc Natl Acad Sci U S A.* 110: 18466–18471.
 - 61 Tsvetkov N, Bahia S, Calla B, Berenbaum MR, Zayed A. 2023. Genetics of tolerance in honeybees to the neonicotinoid clothianidin. *Iscience.* 26:106084.
 - 62 Richardson RT, Eaton TD, Lin C-H, Cherry G, Johnson RM, Sponsler DB. 2021. Application of plant metabarcoding to identify diverse honeybee pollen forage along an urban–agricultural gradient. *Mol Ecol.* 30:310–323.
 - 63 Charters FJ, Cochrane TA, O’Sullivan AD. 2021. The influence of urban surface type and characteristics on runoff water quality. *Sci Total Environ.* 755:142470.
 - 64 Md Meftaul I, Venkateswarlu K, Dharmarajan R, Annamalai P, Megharaj M. 2020. Pesticides in the urban environment: a potential threat that knocks at the door. *Sci Total Environ.* 711:134612.
 - 65 Mullaney J, Lucke T, Trueman SJ. 2015. A review of benefits and challenges in growing street trees in paved urban environments. *Landsc Urban Plan.* 134:157–166.
 - 66 Koch H, Brown MJ, Stevenson PC. 2017. The role of disease in bee foraging ecology. *Curr Opin Insect Sci.* 21:60–67.
 - 67 Dalmon A, Di evart V, Thomasson M, Fouque R, Vaissiere BE, Guilbaud L, et al. 2021. Possible spillover of pathogens between bee communities foraging on the same floral resource. *Insects.* 12:122.
 - 68 Proesmans W, Albrecht M, Gajda A, Neumann P, Paxton RJ, Pioz M, et al. 2021. Pathways for novel epidemiology: plant–pollinator–pathogen networks and global change. *Trends Ecol Evol.* 36: 623–636.
 - 69 Frago E, Dicke M, Godfray HCJ. 2012. Insect symbionts as hidden players in insect–plant interactions. *Trends Ecol Evol.* 27:705–711.
 - 70 Singh R, Levitt AL, Rajotte EG, Holmes EC, Ostiguy N, Vanengelsdorp D, et al. 2010. RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-*Apis* hymenopteran species. *PLoS One.* 5: e14357.
 - 71 Nanetti A, Bortolotti L, Cilia G. 2021. Pathogens spillover from honey bees to other arthropods. *Pathogens.* 10:1044.
 - 72 Purkiss T, Lach L. 2019. Pathogen spillover from *Apis mellifera* to a stingless bee. *Proc Biol Sci.* 286:20191071.
 - 73 Zayed A, Tsvetkov N. 2018. The poisoned oasis: neonicotinoid spillover harms bees near corn. *theschr.* 04:1–2.
 - 74 De Jong D, De Jong PH, Goncalves LS. 1982. Weight loss and other damage to developing worker honeybees from infestation with *Varroa Jacobsoni*. *J Apic Res.* 21:165–167.
 - 75 Richardson RT, Conflitti IM, Labuschagne RS, Hoover SE, Currie RW, Giovenazzo P, et al. 2023. Land use changes associated with declining honey bee health across temperate North America. *Environ. Res. Lett.* 18:064042.
 - 76 Borba RS, Hoover SE, Currie RW, Giovenazzo P, Guarna MM, Foster LJ, et al. 2022. Phenomic analysis of the honey bee

- pathogen-web and its dynamics on colony productivity, health and social immunity behaviors. *PLoS One*. 17:e0263273.
- 77 Wang J, Leung D. 2009. Determination of 142 pesticides in fruit-and vegetable-based infant foods by liquid chromatography/electrospray ionization-tandem mass spectrometry and estimation of measurement uncertainty. *J AOAC Int*. 92:279–301.
- 78 Schenck FJ, Hobbs JE. 2004. Evaluation of the quick, easy, cheap, effective, rugged, and safe (QuEChERS) approach to pesticide residue analysis. *Bull Environ Contam Toxicol*. 73:24–30.
- 79 Thompson HM. 2021. The use of the Hazard Quotient approach to assess the potential risk to honeybees (*Apis mellifera*) posed by pesticide residues detected in bee-relevant matrices is not appropriate. *Pest Manag Sci*. 77:3934–3941.
- 80 BeeREX. 2015. Version 1.0. United States Environmental Protection Agency. <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#beerex>
- 81 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods*. 13:581–583.
- 82 Henry L, Wickham H. 2020. *purrr: functional programming tools*. RPackage Version 0.3.4. <https://purrr.tidyverse.org/>
- 83 Richardson RT, Sponsler DB, McMinn-Sauder H, Johnson RM. 2020. MetaCurator: a hidden Markov model-based toolkit for extracting and curating sequences from taxonomically-informative genetic markers. *Methods Ecol Evol*. 11:181–186.
- 84 Richardson RT. 2022. Controlling critical mistag-associated false discoveries in metagenetic data. *Methods Ecol Evol*. 13:938–944.
- 85 Oksanen J, et al. 2007. The vegan package. *Community Ecology Package*. 10:631–637.
- 86 Dinno A, Dinno MA. 2017. Package “dunn.test”. CRAN Repository. 10:1–7.
- 87 Bates D, et al. 2015. Package ‘lme4’. *Convergence*. 12(1):2.
- 88 Ripley B, et al. 2013. Package ‘mass’. *Cran r*. 538:113–120.
- 89 Wickham H. 2006. An introduction to ggplot: an implementation of the grammar of graphics in R. *Statistics (Ber)*. 1:1–8.