

ORIGINAL ARTICLE

Conservation genomics reveals pesticide and pathogen exposure in the declining bumble bee *Bombus terricola*

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Abstract

In recent years, many pollinators have experienced large population declines, which threaten food security and the stability of natural ecosystems. Bumble bees are particularly important because their ability to “buzz” pollinate and tolerate cooler conditions make them critical pollinators for certain plants and regions. Here, we apply a conservation genomics approach to study the vulnerable *Bombus terricola*. We sequenced RNA from 30 worker abdomens, 18 of which were collected from agricultural sites and 12 of which were collected from nonagricultural sites. We found transcriptional signatures associated with exposure to insecticides, with gene expression patterns suggesting that bumble bees were exposed to neonicotinoids and/or fipronil—two compounds known to negatively impact bees. We also found transcriptional signatures associated with pathogen infections. In addition to the transcriptomic analysis, we carried out a metatranscriptomic analysis and detected five pathogens in the abdomens of workers, three of which are common in managed honey bee and bumble bee colonies. Our conservation genomics study provides functional support for the role of pesticides and pathogen spillover in the decline of *B. terricola*. We demonstrate that conservation genomics is an invaluable tool which allows researchers to quantify the effects of multiple stressors that impact pollinator populations in the wild.

KEYWORDS

insecticides, pollinators, transcriptional signs, transcriptomics

1 | INTRODUCTION

Bees play a significant role in the pollination of agricultural crops (Klein et al., 2007; Rucker et al., 2012) and nonagricultural plants (Ollerton et al. 2011; Potts et al., 2016). Bumble bees are particularly important due to their ability to “buzz” pollinate and their ability to fly in low temperatures (Plowright & Lavery, 1984), which makes them better pollinators than honey bees for certain plants (Banda & Paxton, 1990). However, many bumble bee species are experiencing

steep population declines (Cameron et al., 2011; Colla et al., 2012; Colla & Packer, 2008; Williams & Osborne, 2009), threatening both food security and critical ecosystem services (Klein et al., 2007; Steffan-Dewenter et al., 2005). Determining the causes of pollinator decline is crucial for the conservation of bees in general, and bumble bees in particular (Potts et al., 2016). Conservation genomics—the use of next generation sequencing to study the genomes, metagenomes and transcriptomes of species targeted for conservation—is an emerging field that promises to revolutionize conservation

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biology (Grozinger & Zayed, 2020; Lozier & Zayed, 2017; Trapp et al., 2017). This approach is especially useful for the conservation of native bee species, as it allows us to see “the unseen” stressors that impact bee populations (Grozinger & Zayed, 2020).

Bombus terricola, Kirby 1837 is native to North America (Williams et al., 2014) and has experienced major declines in its relative abundance in the last two decades (Cameron et al., 2011; Colla & Packer, 2008). It is now extirpated in Illinois (Grixti et al., 2009) and has experienced significant range declines (Jacobson et al., 2018; Richardson et al., 2019), especially in its southern range (Bartomeus et al., 2013). Currently, it is classified as “vulnerable” on the IUCN Red List (Hatfield et al., 2015). As with other bumble bees, several hypotheses for *B. terricola*'s decline have been proposed. These include habitat loss, climate change, pesticide use and pathogen spillover—the introduction of novel pathogens from commercial honey bee and bumble bee colonies to native species (Goulson et al., 2015; Grixti et al., 2009; Kent et al., 2018; Kerr et al., 2015; Szabo et al., 2012). Kent et al., (2018) recently sequenced the genome of *B. terricola* in an effort to understand the factors underlying its decline. This first population genomic analysis revealed high inbreeding and a low effective population size for *B. terricola*. Moreover, several genes related to immunity exhibited evidence of recent positive selection (Kent et al., 2018), perhaps reflecting an adaptive response to ameliorate stress from pathogens.

Here, we apply a transcriptomic approach to investigate transcriptional signs of stress in *B. terricola*. RNA sequencing can detect changes in global gene expression associated with a wide variety of stressors, including pathogens, pesticide exposure and nutritional stress (Grozinger & Zayed, 2020). We set up a comparison of *B. terricola* workers collected near agricultural crops and away from agriculture. Agriculture exposes bees to multiple stressors including habitat degradation, pesticides and pathogens (Colla et al., 2006; Otterstatter & Thomson, 2008; Sachman-Ruiz et al., 2015; Tsvetkov et al., 2017). We used the transcriptomic data set to test the following hypotheses. (a) If the decline of *B. terricola* is driven by exposure to pathogens, then we would expect to see patterns of differential expression for genes related to immunity. (b) If the decline is related to pesticide exposure, then we would expect to see changes in the expression of genes involved in detoxification. Finally, (c) if the decline is related to nutritional stress, then we would expect metabolism-related genes to show evidence of differential expression. Of course, if bees are experiencing multiple stressors simultaneously, we would expect to see a combination of these signatures. For these hypotheses, we expected stronger signatures of stress in the agricultural sites. Agricultural landscapes probably reduce nutritional resources, increase pesticide exposure, and probably allow for greater spillover of pathogens from managed bees used for pollination (Colla et al., 2006; Otterstatter & Thomson, 2008; Sachman-Ruiz et al., 2015; Tsvetkov et al., 2017). In addition to this transcriptomic analysis, we also examined the unaligned sequencing reads to directly search for specific pathogens that may have infected the sampled *B. terricola* workers using a database of common

bumble bee pathogens (Alger et al., 2019; Hernández-Jarguín et al., 2018; Ngor et al., 2020; Parmentier et al., 2016). Our study sheds light on the current stressors impacting declining *B. terricola* populations, in addition to demonstrating the utility of genomics for wildlife conservation.

2 | METHODS

2.1 | Bee collections

Bombus workers were collected by net between July 19 and August 22, 2016 in southern Ontario, Canada. Those that were identified as *Bombus terricola* in the field were immediately frozen in liquid nitrogen and then transported to York University where they were stored at -80°C . We obtained permission from landowners to sample bumble bees on their property. While *B. terricola*'s conservation status is listed as “vulnerable,” this status does not require researchers to obtain special collecting permits. Additionally, research on insects does not require bio-ethics approval in Canada. Nevertheless, we purposefully chose to sample a relatively small number of nonreproductive workers per site to reduce our study's impact on the population dynamics of this species.

We aimed to sample sites that were far enough apart, relative to typical bumble bee foraging distances, that workers from one site were highly unlikely to originate from the same colony as workers sampled from other sites. While there are no published studies on the foraging range of *B. terricola*, bumble bee foraging distance is related to body size (Greenleaf et al., 2007), and we used data on the similarly sized *Bombus terrestris* to estimate the foraging distance for *B. terricola* (Williams et al., 2014). Foraging distances of *B. terrestris* range from 96 to 800 m away from their colony (Knight et al., 2005; Osborne et al., 1999, 2008; Walther-Hellwig, 2000; and Wolf & Moritz, 2008). Our two closest collection sites are 6.65 km apart. We treated each collection site as independent in our analysis; similarities in gene expression profiles thereby reflect independent changes in gene expression by workers from different colonies in response to similar stressors acting in different sites. We further computed Moran's I (Gittleman & Kot, 1990; Moran, 1950) to test for spatial autocorrelation in our normalized gene counts in the differentially expressed genes based on the longitudinal and latitudinal coordinates. We used the package “ape” (Paradis & Schliep, 2019) in R version 3.2.2 (R Core Team, 2005) to perform the analysis. We found no spatial autocorrelation in the normalized gene counts within the agricultural and nonagricultural sites for all differentially expressed genes reported herein (Moran's I, $p > .1$).

We classified each sampling site as agricultural or nonagricultural (Figure 1) based on land use patterns within a radius of 500–1000 m from the point of collection using GlobCover 2009 (Bontemps et al., 2011). Locations that had no agricultural land use within 500 m and <10% agricultural land use within 1000 m were designated nonagricultural. While our sample size is small, as is the nature of working

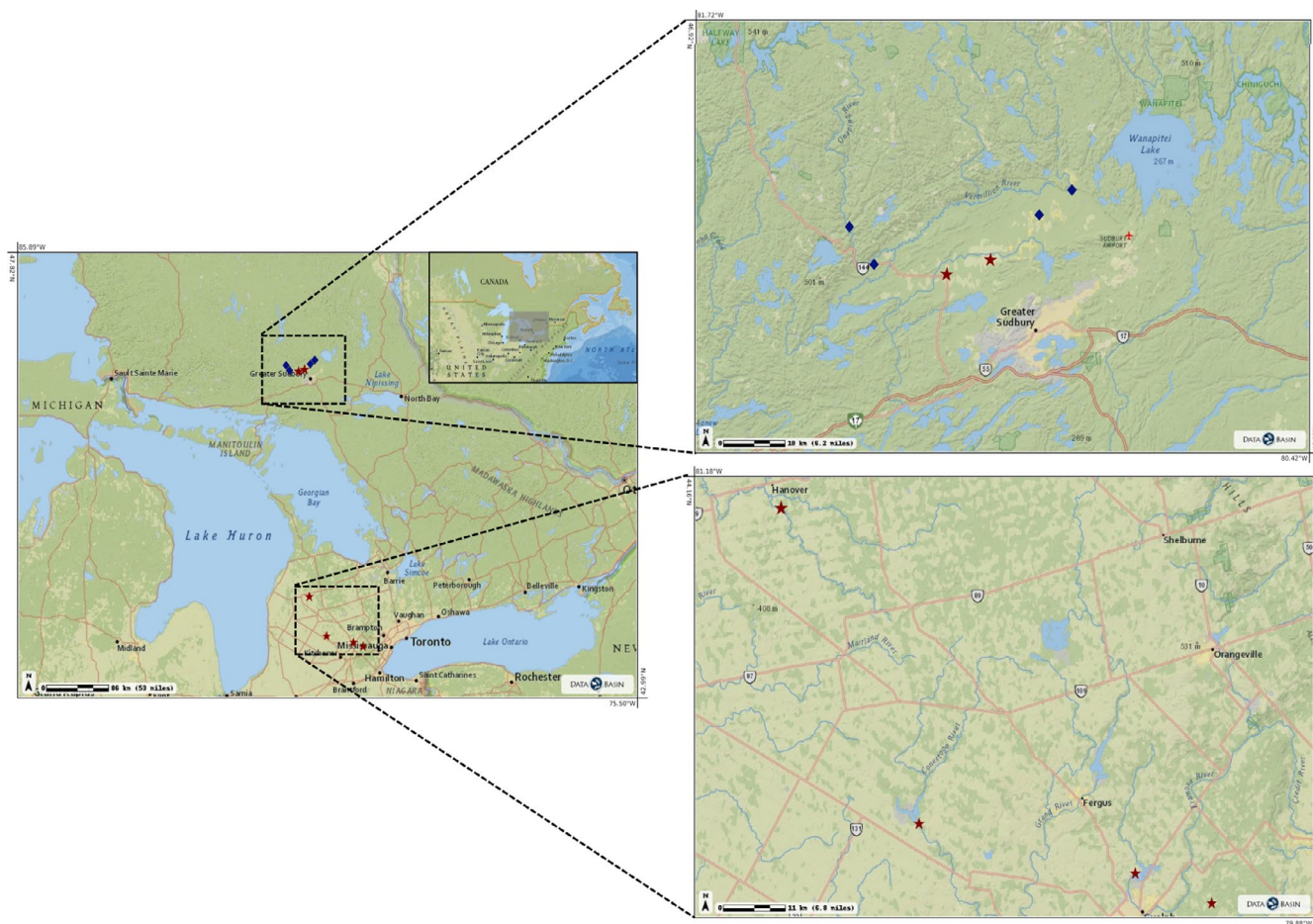


FIGURE 1 *Bombus terricola* workers were collected from agricultural (star) and nonagricultural (diamond) sites in Ontario, Canada

with declining and at-risk species, we note that we are still able to meet minimum sample size requirements for RNA sequencing analyses (Conesa et al., 2016).

2.2 | RNA extraction and analysis

RNA was extracted from the abdomens of three worker bees from each of the 10 sites ($N = 30$) using the Qiagen RNease Mini kit. We used abdomens as it is the tissue most likely to express genes involved in detoxification (Mao et al., 2013), nutrition (Alaux et al., 2011) and immunity (Aufauvre et al., 2014), as well as other stressors that impact hormone levels and ovary activation (Wang et al., 2012). The samples were sequenced at G  nome Qu  bec's Innovation Center using a HiSeq4000 (PE 100 bp; Illumina).

We used TRIMMOMATIC (Bolger et al., 2014) to remove adapters, low-quality bases and low-quality reads. An average of 23,263,068 reads per sample survived the filtering. Quality check was performed using FASTQC (Bioinformatics, 2011). The data successfully passed FASTQC quality checks for all relevant parameters. We then aligned the RNA sequences to the *B. terricola* genome (Kent et al.,

2018) using the Spliced Transcripts Alignment to a Reference (STAR) software (Dobin et al., 2013) to generated gene expression counts. The gene expression counts were then processed using EDGER (McCarthy et al., 2012; Robinson et al., 2010) in R version 3.2.2 (R Core Team, 2005). Any genes that were only expressed in one sample were filtered out, and then the remaining counts were normalized. Differentially excessed genes (DEGs) were determined based on an Exact Test using a Benjamini and Hochberg (1995) p adjustment to account for multiple testing.

Reads that were not mapped onto the *B. terrecola* genome were used to investigate the presence of RNA viruses and other pathogens (Batty et al., 2013; Hernández-Jarguín et al., 2018; Razzauti et al., 2015). We aligned and counted the unmapped reads using STAR (Dobin et al., 2013) using the genomes of common bumble bee pathogens (Table S1; Alger et al., 2019; Parmentier et al., 2016). To ensure specificity, we aligned the unmapped reads using multiple genomes simultaneously, which ensures that ambiguous or multi-mapped reads are not counted. The gene counts were processed using EDGER (McCarthy et al., 2012; Robinson et al., 2010) in R version 3.2.2 (R Core Team, 2005). Any genes that were only expressed in one sample were filtered out. We used a generalized linear model

(GLM; Nelder & Wedderburn, 1972), with site as a nested parameter, with a binomial family structure to analyse the prevalence data.

2.3 | RT-qPCR

To validate pathogens detected by our metatranscriptomic analysis, we diluted the previously extracted RNA to a concentration of 0.7 µg/20 µl. We used the iScript cDNA Synthesis Kit (Bio-Rad) using random primers following the manufacturer's recommended method. A single sample was excluded due to not having sufficient RNA. cDNA was stored at -20°C. All samples were run in triplicate with a negative control for each pathogen/gene. Each replicate contained 1 µl of diluted cDNA, 5 µl of SsoAdvanced SYBR Green Supermix (Bio-Rad), 3 µl of DEPC H₂O, 0.5 µl Forward primer and 0.5 µl Reverse primer of the corresponding pathogen/gene (Table S2). We carried out RT-qPCRs (real-time quantitative polymerase chain reactions) using a Bio-Rad Chromo4 with the following cycle conditions: (a) 30 s at 95°C, (b) 40 cycles of 5 s at 95°C and 30 s at 56°C, and (c) a melt curve analysis starting at 65°C for 5 s repeated for 60 cycles with an increase of 0.5°C each cycle.

We chose to amplify three pathogens: sacbrood virus (SBV), black queen cell virus (BQCV) and *Lotmaria passim*, since they showed different prevalence rates in the metatranscriptomic analysis (see below). We used actin as a reference gene (Alger et al., 2019; McMahon et al., 2015) (Table S2), which was amplified at the same time as the target genes. The actin primer was designed using PRIMER3 web version 4.1.0 (<https://primer3.ut.ee/>), and then we used BLASTN with the blastn-short option (Camacho et al., 2009) to search the primer sequences against the *B. terrecola* genome to ensure that the primer bound to a unique section of the actin gene in *B. terrecola*. Relative quantification (RQ) was obtained using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001; Pfaffl, 2001). We used a sample from an agricultural site, in which all of the pathogens were detected, as the comparator for all other samples (i.e., expression is measured relative to this one sample). Efficiencies for each primer were calculated by diluting a sample known to contain all of the pathogens five times by a factor of 10 and performing qPCR in triplicate as described above (Table S2).

We used a two-step process to analyse the pathogen data. We first tested whether prevalence was different between the agricultural and nonagricultural sites. We used GLM, with site as a nested parameter (Nelder & Wedderburn, 1972) with a binomial family structure to analyse the prevalence data for BQCV, SBV and *L. passim*. Pathogens that show a statistical difference in prevalence (BQCV and SBV, see below) were not analysed for abundance because such comparisons are often not meaningful. For example, samples where a virus was not detected would need to be imputed (typically as 1 + maximum number of PCR cycles) before analysis, but this would lead to a left-skewed distribution. For *L. passim*, since no statistical difference was found for prevalence (see below), we analysed expression levels and imputed Ct values for samples with no visible fluorescence after 40 cycles as 41. We log₂-transformed

the RQ value and performed the nested GLM analysis using r version 3.2.2 (R Core Team, 2005).

2.4 | Gene ontology analysis

Using a best-match BLASTX (Boratyn et al., 2012; Camacho et al., 2009) we mapped all of the *B. terrecola* genes onto the *Drosophila melanogaster* (fruit fly) genome version 6.16 (Adams et al., 2000; Hoskins et al., 2015; Myers et al., 2000) and *Apis mellifera* (honey bee) genome version 4.5 (Consortium, 2006; Elsik et al., 2014). We found 7,845 *D. melanogaster* homologues, of which 54 were DEGs, and 8,495 *A. mellifera* homologues, of which 54 were DEGs. Gene ontology (GO) analysis was performed using DAVID 6.8 (Huang, Sherman, & Lempicki, 2008a, 2008b) using the *D. melanogaster* homologues. We selected the following annotation databases for the analysis: "GO Biological Process All," "GO Molecular Function All," "GO Cellular Component All," as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway and Keywords. This analysis was corrected for multiple testing (Benjamini & Hochberg, 1995).

To gain further insight into the biological relevance of DEGs in *B. terrecola*, we compared our gene lists to a large set of gene expression studies carried out on the honey bee, *A. mellifera*. We chose *A. mellifera* studies that used whole bees or abdomens for their analyses (Alaux et al., 2011; Aufauvre et al., 2014; Badaoui et al., 2017; Brutscher et al., 2017; Corby-Harris et al., 2014; Doublet et al., 2017; Liu et al., 2020; Rutter et al., 2019; Ryabov et al., 2016; Shi et al., 2017; Wang et al., 2012; Wu et al., 2017). Where required, the gene names were converted into the current iteration of the honey bee genome using HYMENOPTERAMINE (Elsik et al., 2016). We used a hypergeometric test (Johnson et al., 2005) to determine if the overlap between published gene lists and our gene list was statistically different from chance after correcting for multiple testing using the Holm-Bonferroni method (Holm, 1979). These tests were performed in r version 3.6.3 (R Core Team, 2005).

3 | RESULTS

We were able to quantify the expression of 9455 genes in the abdomens of *Bombus terrecola* workers. When contrasting gene expression in bees from agricultural vs. nonagricultural sites, we found 61 DEGs, 36 of which were upregulated in bees collected from agricultural areas (Table S3). Our list of DEGs includes homologues of cytochrome P450 4C1 (LOC413833), cytochrome P450 303a1 (LOC727290) and UDP-glucuronosyltransferase 2B18 (LOC411021), all involved in detoxification in insects (Kanehisa & Goto, 2000). Additionally, we found a homologue of nicastrin (LOC552178), which is part of the Notch signalling pathway (Kanehisa & Goto, 2000) and has been implicated in neurodegenerative disorders in vertebrates (Urban, 2016). Our DEGs also included toll-like receptor 4 (LOC724187), which plays a role in immunity (Pålsson-McDermott & O'Neill, 2004).

A GO analysis revealed a statistically significant enrichment of genes involved in myofibril and muscle cell development ($p < .05$, Table S4), muscle protein ($p < .001$, Table S4) and alternative splicing ($p = .035$, Table S4). Genes unregulated in bees collected from agricultural areas were also enriched within the KEGG pathway for biosynthesis of antibiotics ($p = .006$, Table S4). Genes that were up-regulated in nonagricultural areas were not enriched for any annotation terms ($p > .1$, Table S5).

In order to compare our study to previously published research, we converted our *B. terreicola* genes to *Apis mellifera* homologues. We focused on studies that exposed honey bees to various stressors and assayed gene expression in either abdomens or whole bees. We found a statistically significant overlap between the DEGs in *B. terreicola* and those found in a study of common immune responses in *A. mellifera* (Table 1, hypergeometric test, $p < .001$; Doublet et al., 2017). Furthermore, DEGs in *B. terreicola* significantly overlapped with: (i) DEGs of honey bees exposed to *Lotmaria passim* (hypergeometric test, $p = .003$; Liu et al., 2020); (ii) DEGs of honey bees exposed to both SBV and deformed wing virus (DWV; hypergeometric test, $p < .001$) but not DWV alone (hypergeometric test, $p = 1$; Ryabov et al., 2016); (iii) DEGs of honey bees exposed to neonicotinoid insecticides (Shi et al., 2017; hypergeometric test, $p = .029$; Wu et al., 2017; hypergeometric test, $p = .003$) and (iv) DEGs of honey bees exposed to the insecticide fipronil (Aufauvre et al., 2014; hypergeometric test, $p = .023$). We found no statistically significant overlap between *B. terreicola* DEGs and the DEGs of honey bees exposed to *Nosema ceranae*, several viruses (other than SBV) or poor diet (Table 1).

In addition to our transcriptomic analysis, we crossed referenced unaligned transcriptomic reads to a database of 16 bumble bee

pathogens, and discovered five matches (Figure 2). We found that bumble bees from agricultural areas had a marginally higher prevalence of SBV (analysis of deviance type II, $LR \chi^2 = 3.265$, $df = 1$, $p = .071$; Agr: 0.667, NonAgr: 0.333), while bumble bees from non-agricultural areas had a higher prevalence of *Lotmaria passim* (analysis of deviance type II, $LR \chi^2 = 5.999$, $df = 1$, $p = .014$; Agr: 0.0556, NonAgr: 0.417). The other detected pathogens did not have a statistically significant difference in their prevalence rate when comparing agricultural from nonagricultural sites (*Nosema ceranae*: analysis of deviance type II, $LR \chi^2 = 0.456$, $df = 1$, $p = .499$; Agr: 0.833, NonAgr: 0.917; *Crithidia bombi*: analysis of deviance type II, $LR \chi^2 = 0.374$, $df = 1$, $p = .541$; Agr: 0.556, NonAgr: 0.667; BQCV: analysis of deviance type II, $LR \chi^2 = 2.486$, $df = 1$, $p = .115$; Agr: 0.778, NonAgr: 0.500).

In order to confirm our metatranscriptomic analysis, we performed RT-qPCR on previously extracted samples using primers for three of the pathogens: BQCV, SBV and *L. passim* (Figure 3). We found that BQCV and SBV prevalence were statistically higher in the bees collected from agricultural areas (BQCV: analysis of deviance type II, $LR \chi^2 = 8.758$, $df = 1$, $p = .003$; SBV: analysis of deviance type II, $LR \chi^2 = 7.308$, $df = 1$, $p = .007$). *L. passim* prevalence did not differ between bees collected in agricultural and nonagricultural areas (analysis of deviance type II, $LR \chi^2 = 0.832$, $df = 1$, $p = .362$), but bees collected in nonagricultural areas did have higher expression levels of *L. passim* (analysis of deviance type II, $LR \chi^2 = 6.399$, $df = 1$, $p = .011$). There was a strong and significant correlation between prevalence data estimated from metatranscriptomics and RT-qPCR analyses (Figure S1; Pearson's correlation, BQCV: $t = 5.106$, $df = 27$, $p < .0001$, $r = .701$; SBV: $t = 3.840$, $df = 27$, $p < .001$, $r = .594$; *L. passim*: $t = 2.073$, $df = 27$, $p = .048$, $r = .371$).

TABLE 1 Number of differentially expressed genes (DEGs) in *Bombus terreicola* overlapping with previously published transcriptomic studies exploring various stressors in honey bees

Stressor type	Stressor	DEG pverlap	p
Pesticide (Shi et al., 2017)	Thiamethoxam	8	.032*
Pesticide (Wu et al., 2017)	Imidacloprid	7	.003*
Pesticide (Aufauvre et al., 2014)	Fipronil	2	.025*
Pathogen (Doublet et al., 2017)	Immune challenge	10	<.001*
Pathogen (Liu et al., 2020)	<i>Lotmaria passim</i>	10	.003*
Pathogen (Ryabov et al., 2016)	Sacbrood virus + deformed wing virus	24	<.001*
Pathogen (Ryabov et al., 2016)	Deformed wing virus	0	1
Pathogen (Aufauvre et al., 2014)	<i>Nosema ceranae</i>	0	1
Pathogen (Badaoui et al., 2017)	<i>Nosema ceranae</i>	2	.294
Pathogen (Brutscher et al., 2017)	Sindbis virus	13	.104
Pathogen (Brutscher et al., 2017)	Double stranded RNA	3	1
Pathogen (Rutter et al., 2019)	Israeli acute paralysis virus	0	1
Nutrition (Rutter et al., 2019)	Chestnut vs. rockrose (less nutritious) pollen	17	.186
Nutrition (Corby-Harris et al., 2014)	No pollen diet	1	.188
Nutrition (Alaux et al., 2011)	No pollen diet	12	1
Nutrition (Wang et al., 2012)	High and low pollen-hoarding	13	.553

*Statistically significant overlaps ($p < .05$).

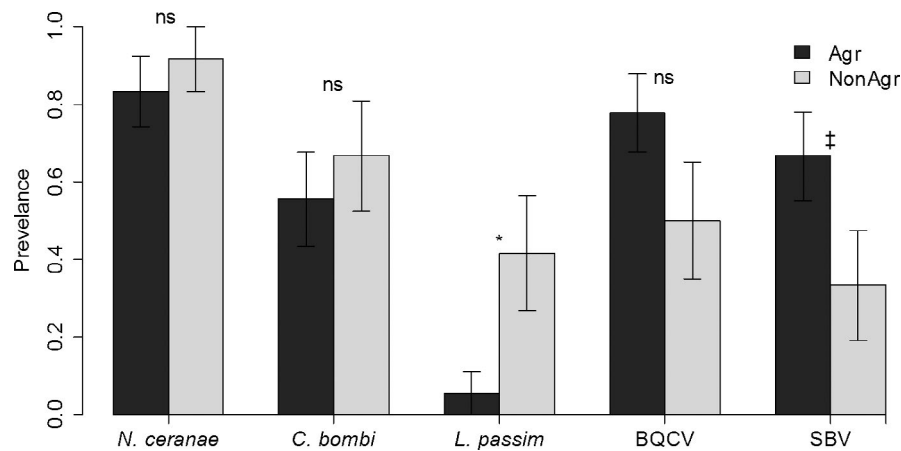


FIGURE 2 Pathogens detected through metatranscriptomics in *Bombus terricola* workers. We found an increased prevalence of *Lotmaria passim* in bees collected from nonagricultural areas (analysis of deviance type II, $LR \chi^2 = 5.999$, $df = 1$, $p = .014$) and a marginally increased sacbrood virus (SBV) prevalence in bees collected from agricultural areas (analysis of deviance type II, $LR \chi^2 = 3.265$, $df = 1$, $p = .071$), but no difference in the prevalence of *Nosema ceranae* (analysis of deviance type II, $LR \chi^2 = 0.456$, $df = 1$, $p = .499$), *Crithidia bombi* (analysis of deviance type II, $LR \chi^2 = 0.374$, $df = 1$, $p = .541$), or black queen cell virus (BQCV; analysis of deviance type II, $LR \chi^2 = 2.486$, $df = 1$, $p = .115$) between agricultural and nonagricultural areas. Mean \pm SE. ns = not significant, $\dagger p < .1$, $*p < .05$

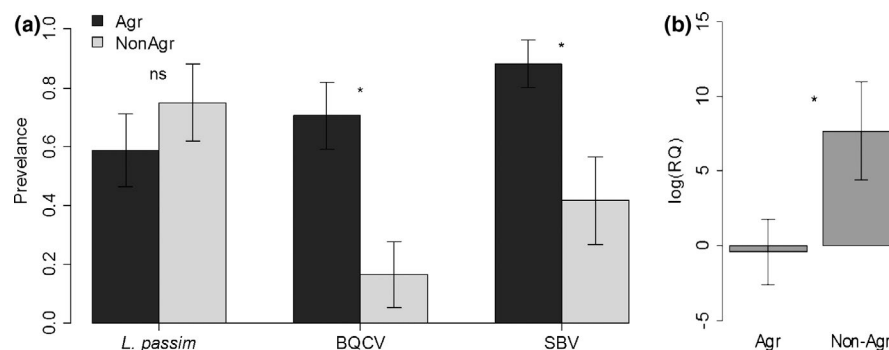


FIGURE 3 Validation of metatranscriptomic analysis of bumble bee pathogens via quantitative PCR. (a) *Bombus terricola* workers collected near agricultural areas had higher prevalence of black queen cell virus (BQCV; analysis of deviance type II, $LR \chi^2 = 8.758$, $df = 1$, $p = .003$) and sacbrood virus (SBV; analysis of deviance type II, $LR \chi^2 = 7.308$, $df = 1$, $p = .007$). *Lotmaria passim* prevalence was not statistically different from bees collected from agricultural and nonagricultural areas (analysis of deviance type II, $LR \chi^2 = 0.832$, $df = 1$, $p = .362$). (b) *L. passim* expression levels were higher in bees collected from nonagricultural areas (analysis of deviance type II, $LR \chi^2 = 6.399$, $df = 1$, $p = .011$). Mean \pm SE. ns = not significant, $*p < .05$

4 | DISCUSSION

We used conservation genomics to gain a better understanding of the stressors experienced by *Bombus terricola* in the field. We found 61 DEGs in abdomens of workers collected in agricultural vs. nonagricultural areas. The genes that were upregulated in the bees collected in agricultural areas were related to muscle function and development, as well as biosynthesis of antibiotics. We then compared our DEGs to previously published studies on transcriptomic responses of honey bees to various stressors. We found statistically significant overlaps with studies that exposed bees to pesticides and certain pathogens. These results point to pesticides as an important stressor affecting bumble bees foraging in agricultural landscapes.

Pesticides have been previously implicated in bumble bee declines (Gill et al., 2012; Whitehorn et al., 2012). Neonicotinoids

negatively impact colony growth, larval development and queen production in bumble bees, but research on the effects of fipronil on bumble bees is limited (Pisa et al., 2015). Overall, whether a link is present between pesticide use and bee decline in the field tends to depend on the bee species and the geographical location (Rundlöf et al., 2015; Szabo et al., 2012; Woodcock et al., 2017). Fipronil exposure depends heavily on environmental conditions and, under certain conditions, can persist for months to years, especially in agricultural landscapes (Bonmatin et al., 2015). Tsvetkov et al., (2017) showed that pollen collected by honey bees from May to August near agricultural areas in Ontario contains neonicotinoid pesticides. It is therefore probable that *B. terricola* workers are also exposed to neonicotinoids during these months in Ontario. Our gene expression data support this. First, we have a statistically significant overlap in DEGs with three separate studies that exposed honey bees to neonicotinoids and fipronil. Second, two of those overlapping genes

are cytochrome P450 genes (LOC551223, LOC413833), which are detoxification genes in honey bees and bumble bees (Berenbaum & Johnson, 2015; Manjon et al., 2018). Finally, the enrichment of muscle development GO terms could be related to neonicotinoid exposure, as these pesticides cause hyperactivity (Boily et al., 2013; Suchail et al., 2001), cause locomotor deficits (Charreton et al., 2015) and impact foraging ability (Henry et al., 2012; Yang et al., 2008).

We also detected a transcriptional signal of pathogenic exposure affecting *B. terricola* workers in agricultural areas. This is supported by the enrichment of genes responsible for the biosynthesis of antibiotics and by the overlap with previous studies that exposed honey bee workers to various immune challenges. In addition, we detected five pathogens in *B. terricola*: two trypanosomatids parasites, *Crithidia bombi* and *Lotmaria passim* (Lipa & Triggiani, 1988; Schwarz et al. 2015); a microsporidian parasite, *Nosema ceranae* (Higes et al., 2006); and two RNA viruses, BQCV and SBV (Chen & Siede, 2007). We confirmed our analysis using qPCR for three of the pathogens: BQCV, SBV and *L. passim*. We found that BQCV and SBV prevalence was higher in workers collected from agricultural areas. This further supports the idea that *B. terricola* workers experience pathogen exposure near agricultural areas.

While there is evidence that some pathogens are more prevalent near agriculture, *L. passim*'s prevalence was actually higher in bees from nonagricultural areas, while *N. ceranae* and *C. bombi* prevalence was the same in both groups of bees. SBV, BQCV and *N. ceranae* are common pathogens in managed honey bee and bumble bee colonies (Bushmann et al. 2012; Chen et al., 2008; Chen & Siede, 2007; Graystock et al., 2014). These findings lend further support to the pathogen spillover hypothesis as a driver of *B. terricola*'s decline (Colla et al., 2006; Kent et al., 2018; Szabo et al., 2012).

We compared our bumble bee DEGs with DEGs that were expressed in honey bees challenged with different stressors. We did this because the availability of literature on honey bees is much greater than that on bumble bees (Trapp et al., 2017). However, we think these contrasts between *Bombus* and *Apis* are justified because many of the stress response pathways, such as detoxification and immunity, are strikingly similar between bumble bees and honey bees (Barribeau et al., 2015; Sadd et al., 2015). Additionally, honey bees and bumble bees are often exposed to the same stressors in the field (Rundlöf et al., 2015; Woodcock et al., 2017), including bumble bees being exposed to honey bee pathogens (Furst et al., 2014; McMahon et al., 2015).

While our work highlights pesticides and pathogens as important stressors acting on current *B. terricola* populations, our study does have some limitations. We were only able to test for a small subset of stressors in a small portion of the species' entire range; expanding the scope of conservation genomic studies will be helpful to fully understand how multiple stressors influence the health of other *B. terricola* populations. Moreover, we can only detect "signatures" of stressors that were explored in previously published research. We look forward to more studies that experientially expose bumble bees to various stressors followed by expression profiling to generate stressor-specific biomarkers (Grozinger & Zayed, 2020).

Our current design also prevents us from detecting stressors that would affect bumble bees in the same manner in both agricultural and nonagricultural sites, such as climate change (Kerr et al., 2015); these would not lead to differentially expressed genes in our analysis. Finally, we cannot detect stressors that exert their effects on queens, males or during larval development (McFrederick & LeBuhn, 2006). However, despite these limitations, we believe that the transcriptomic approach we used here does provide valuable insights into the probable stressors acting on declining *B. terricola* populations, and can be used to inform conservation management of the species. Moreover, the diagnostic power of conservation genomics will only improve for wildlife species as more transcriptomic literature becomes available.

Like several other bumble bee species, *B. terricola* is declining rapidly in North America (Cameron et al., 2011; Colla & Packer, 2008). Using a transcriptomics approach, we found that *B. terricola* workers in agricultural areas exhibit transcriptional signatures of exposure to pesticides and pathogens. Pathogens have been implicated in *B. terricola* previously (Kent et al., 2018; Szabo et al., 2012), but, here, we were able to detect several specific pathogens that may be contributing to *B. terricola*'s decline. We also present the first evidence that *B. terricola* workers are experiencing xenobiotic stressors in the field. This is significant, because pesticides are known to impact colony development and function (Rundlöf et al., 2015; Whitehorn et al., 2012), and impact the individual immune response of workers (O'Neal et al., 2018). We think our study clearly demonstrates the value of genomics in conservation, by allowing researchers to answer previously untraceable questions about the multiple stressors influencing wildlife populations in various habitats.

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AUTHOR CONTRIBUTIONS

N.T., V.J.M., S.R.C. and A.Z. designed the study, N.T. carried out the molecular work, data analysis, and wrote the manuscript. V.J.M. carried out the field sampling. V.J.M., S.R.C. and A.Z. revised the manuscript. S.R.C. and A.Z. provided funding.

DATA AVAILABILITY STATEMENT

The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession no. GSE174536 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE174536>).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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