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RESEARCH ARTICLE

Historic DNA uncovers genetic effects of climate change and landscape alteration in two wild bee species

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Abstract

Historic and contemporary data can shed light on a species' conservation status and work together to address two main goals in conservation biology: (1) identifying species under extinction risk and (2) the forces shaping this process. Museomics is the study of historical DNA acquired from museum specimens that allows researchers to answer myriad questions across many taxa. Museomics is an effective way to understand how populations have been affected by human and climate factors from a historic perspective. Here, our goal is to investigate changes in wild populations of two small carpenter bee species (*Ceratina calcarata* and *C. dupla*) across a 50-year time span. We sampled museum specimens and recent collections to determine their genetic diversity, population structure, effective population size, signatures of selection, and local adaptation. Both species displayed reduced genetic diversity and effective population size through time. We identified signatures of adaptation in both species across human-altered land use and climate change scenarios. We found signatures of selection in genes related to biochemical defense, insecticide, and thermal tolerance, which are consistent with the observed increase in agricultural land use development and rising temperatures over the past 50 years. Our findings suggest that these species are facing population inbreeding, possibly attributable to human land-use change and agrochemicals in their environment. Overall, this study highlights the use of museomics to understand species declines, threats to populations, and targets for remediation.

Keywords Museomics · Adaptation · Population inbreeding · Conservation · Apidae · Pollinators

Introduction

Climate change and human-altered habitats are currently major threats to biodiversity worldwide (Thomas et al. 2004; Parmesan 2006; Bellard et al. 2012; Outhwaite et al. 2022). Changes in land cover and climatic conditions can affect organisms' permanence by modifying their habitat, acting as potential drivers of species population decline and extinction (Thomas et al. 2004). Global warming and altered land cover limits physiological tolerance, decrease or shift species' ranges, and decreases population sizes (Thomas et al. 2004; Franks and Hoffmann 2012; Cahill et al. 2013). In fact, it is estimated that more than 10% of the world's genetic diversity has been lost due to decreasing habitat (Exposito-Alonso et al. 2022). On the other hand, the combined effect of these factors can act as a selective force inducing evolutionary responses, where species would display signatures of adaptation to local climatic and landscape-related conditions that can be identified by genome-wide scans (Franks and Hoffmann 2012). The impact of habitat and climate change may vary according to each species' potential to overcome such changes, including their genetic diversity and potential to adapt to environmental stress. Considering this, monitoring species' genetic responses to climate and habitat change and their potential adaptation becomes a valuable resource for their conservation (Hoffmann et al. 2015; Holmes et al. 2016).

Evolutionary responses to habitat change take time to detect, making it extremely difficult to be directly estimated using only contemporary data (Mikheyev et al. 2015). Typically, studies aiming to identify these changes through time involve compiling records over several decades, which is highly unfeasible due to a dearth of long-term collection efforts (Wandeler et al. 2007; Burrell et al. 2015; Holmes et al. 2016). Museum collections store an extensive and

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invaluable variety of biological specimens dating back many decades, including rare and extinct species (Wandeler et al. 2007; Haile et al. 2009; Burrell et al. 2015). With the recent expansions of molecular technologies, historic samples are emerging as an essential source of DNA for a multitude of studies, including the detection of past pathogens, ancestral population structuring, and species responses to global change (Pääbo et al. 2004; Wandeler et al. 2007; Lozier and Cameron 2009; Vaudo et al. 2018). Historic samples are useful to study the natural history and population health of bees in comparison to present populations that are currently experiencing serious population declines (Grixti et al. 2009; Cameron et al. 2011; Vaudo et al. 2018; Mathiasson and Rehan 2019).

There is a critical worldwide decline in pollinators due to land-use change, climate change, increasing use of agrochemicals, and pathogen infections (Balkenhol et al. 2015; Giannini et al. 2012; Kelemen and Rehan 2021; Parmesan 2006; Potts et al. 2010). Bees are the world's most important pollinators, with over 20,000 species across the globe (Michener 2007). They are extremely dependent on floral resources and as central place foragers, they have specific sites for nesting which can make them sensitive to changes in their environment (Michener 2007; Potts et al. 2010; Goulson et al. 2015). Human land-use changes bee population dynamics by reducing natural habitat, which translates into a reduction of available food, nesting resources, and the spatial isolation of populations (Steffan-Dewenter 2002). During habitat loss, barriers may arise limiting dispersal and colonization, clustering individuals into fragments of suitable habitat, and reducing the foragers' capacity for tracking diverse floral resources (Steffan-Dewenter 2002; vanden Broeck et al. 2017; Pope and Jha 2018). This is especially true for small bees, which usually have a limited foraging distance from their nest (Greenleaf et al. 2007). Long-term genetic effects include inbreeding, caused by a drop in effective population size, resulting in reduced genetic diversity (Frankham et al. 2010). Genetic diversity is critical for evolution and the ability to adapt to environmental changes through selection for different alleles present in populations, where a higher diversity of alleles makes it more likely that some of these will be more suitable under a sudden change in environmental conditions (Reed and Frankham 2003; Hoffmann and Willi 2008; Segelbacher et al. 2010). This is particularly important for bee conservation as changes to their environment are a growing threat and chances of survival could depend directly on their genetic diversity and potential resilience to future changes (Kelemen and Rehan 2021).

Climate change effects on bees are mainly driven by plant–pollinator temporal mismatch, where there is a desynchronization of bees and plants' emergence driven by precipitation or temperature cues (Danforth 1999; Burkle et al. 2013). This can be a major determinant of population decline and species extinction (Cahill et al. 2013). Increasing temperature can harm insects by exceeding their thermal tolerance, leading to excessive water loss and phenological constraints (Garrad et al. 2016). On the other hand, some bees can display signatures of local adaptation to temperature and precipitation if they can tolerate changes in their local climate (Jackson et al. 2020). Genes related to climatic adaptation in insects are mostly associated with heat stress, maintenance of activity during flight, or desiccation resistance at extreme temperatures (Overgaard and MacMillan 2017). An example is the calcium-activated channel gene (*slo*) which has been associated with flight muscle and neurons activity and responses to environmental stimuli, including extreme temperature in *Bombus* (Atkinson et al. 1991; Keyser 2005; Jackson et al. 2020). Insects can also cope with extreme temperatures and pathogen infections by displaying selection for genes involved in the immune response, which is often observed through selection on cytochrome P450s, important detoxication enzymes in insects and protein-coupled receptors, known for their role in insect physiology and insecticide and pathogen resistance (Thorat and Nath 2018; Li et al. 2020; Tsvetkov et al. 2021). Also, former studies have reported signatures of selection associated with urban environments and detected positive selection for genes regulating heat stress, metabolism, and oxidative stress (Theodorou et al. 2018).

In addition to climate change, urbanization and agricultural expansion also contribute to pollinator decline by permanently changing suitable habitats, increasing urban warming (urban heat island), and the presence of non-native plant species (Ayers and Rehan 2021). Land cover across North America has changed radically over the past century (Latifovic et al. 2004). In eastern North American and southern Ontario, colonization by European settlers in the eighteenth and nineteenth centuries has had a dramatic effect (Butt et al. 2005). After European settlement, natural landscapes have converted to agricultural lands and more recently replaced with ever-expanding urbanization (Butt et al. 2005; Puric-Mladenovic et al. 2016). Several studies in temperate bee systems have broadly suggested that agricultural intensification has detrimental impacts on pollinator communities when compared to natural habitats (Kremen et al. 2002). Alongside land-use change, increasing mean temperatures, including extreme heat for this region, are consistent with climate change over the past decades (Fausto et al. 2015).

Small carpenter bees (*Ceratina*) are globally distributed pollinators of over 350 species with important roles in rural and urban habitats (Michener 2007; Kennedy et al. 2013; Dew et al. 2016). *Ceratina calcarata* and *C. dupla* (Hymenoptera: Apidae) are generalist pollinators native to eastern North America and responsible for a wide range of crop and native plant pollination with appreciable ecological

and economical importance (Kennedy et al. 2013; Shell and Rehan 2015; Kleijn et al. 2015; McFrederick and Rehan 2016). Currently, both species are under a secure (G5) global conservation status (NatureServe 2022). Responses of *Ceratina* to both climate and habitat change have been investigated in multiple studies. For example, physiological investigations of Fijian small carpenter bees revealed resistance to thermal and desiccation stress (da Silva et al. 2021). Studies associated intensified land use with a decrease in fitness and the presence of pathogens, as well as a reduced body size related to warming temperatures across North American and Australian small carpenter bee species (McFrederick and Rehan 2019; Nooten and Rehan 2019; Kelemen and Rehan 2021). This group has been also studied in the context of global warming and human land-use change predicting range expansion under future warming temperatures and increasing urbanization (Dew et al. 2019). Considering the myriad responses to environmental change this group displays, examining these two closely related and recently diverged bees (Shell and Rehan 2015) increases the potential to understand the genetic consequences of climate and land-use change, and conserved responses across the genus.

Here we investigate historic and contemporary populations of two *Ceratina* species over a 50-year period to unmask possible effects of land use and climatic changes on genetic diversity, population structure, effective population size, and signatures of selection. The objective of this study is to determine whether changes in natural habitat and climate have genetically affected wild bee populations. We aim to (1) determine changes in population genetic diversity and gene flow from historic to contemporary populations, and (2) examine signatures of selection in both species to identify evidence for local adaptation.

Methods

Sampling

Female *Ceratina calcarata* and *C. dupla* were sampled from two different time points. Historical samples were obtained from The Royal Ontario Museum—Toronto, ON, where 15 females were selected from each species, dating from 1968 with field location recorded at latitude 43° 49′ N and longitude 79° 58′ W, located just southwest of Highway 10 on Forks of the Credit Road, Peel County (MacKay and Knerer 1979). For contemporary samples, 15 adult females from each *Ceratina* species were collected at Forks of the Credit Provincial Park at 43° 49′ N and 80° 0′ W in 2018 (Rubens 2019). All samples were collected using sweep nets, pinned, and dried prior to this study.

Mapping, landscape analysis, and climate data

To investigate how land use changed across time around sampled points, we collected satellite image data from USGS Earth Explorer from 1972 to 2018 (https://earthexplorer.usgs.gov). We used Landsat satellite 1–5 Multispectral Scanner MSS images from 1972 with a 60 m resolution as a proxy for our samples collected in 1968. We used satellite images from 1972 because no image from the sample sites before this date had adequate resolution. For 2018, we downloaded Landsat 8 images with 30 m resolution. To avoid bias due to different resolutions on maps, we changed the 2018 raster images to 60 m using the function *r.resample* from GRASS GIS on QGIS v.3.22.1. We classified both raster images using the dzetsaka classification plugin (Karasiak 2016) in QGIS. First, we identified each Region of Interest (ROI) from the raster image and created a shapefile with all classes to finally create a classified image based on a K-Nearest Neighbours algorithm. We set six land use classes: bare soil, forest, agriculture and pasture, shrub land, urban, and water, within a buffer boundary of 5 km from the sampling points (Fig. 1). We then used the *r.report* function from GRASS GIS to collect percentages of land use for each class.

Historical and contemporary climatic data from the sample site was obtained from the National Climate Archives available at https://climate.weather.gc.ca/. Our climate data set included maximum, minimum, and mean temperature, and total precipitation throughout two 30-year time periods. Historical climate data ranged from 1958 to 1988 and contemporary climate data from 1989 to 2019. A 30-year timeframe was used to ensure the changes were climate-related and not year-by-year fluctuation. We collected the data from three different stations (Orangeville, Orangeville Moe, and Glen Haffy Mono Hill) in order to gather all data needed when the nearest available station missed some of these data. We averaged temperature and precipitation by year onto a compiled dataset for each temporal bin and performed a paired t-test in R to test for significant differences between the historical and contemporary data.

Sequencing, RADseq processing, and mapping

We extracted DNA from pinned specimens with non-destructive whole-body techniques using Quick-DNA Miniprep Plus extraction kits (Zymo Research) and following the protocol of Freitas et al. (2021). With this approach, the whole specimen is incubated rather than grinded for extraction. DNA quality was assessed using agarose gel electrophoresis and a spectrophotometer (Thermo Fisher Scientific). For each bee, DNA concentration was normalized to 20 ng/µL. RAD-seq library preparation was carried out by Floragenex, Inc (Eugene, Oregon, USA), according

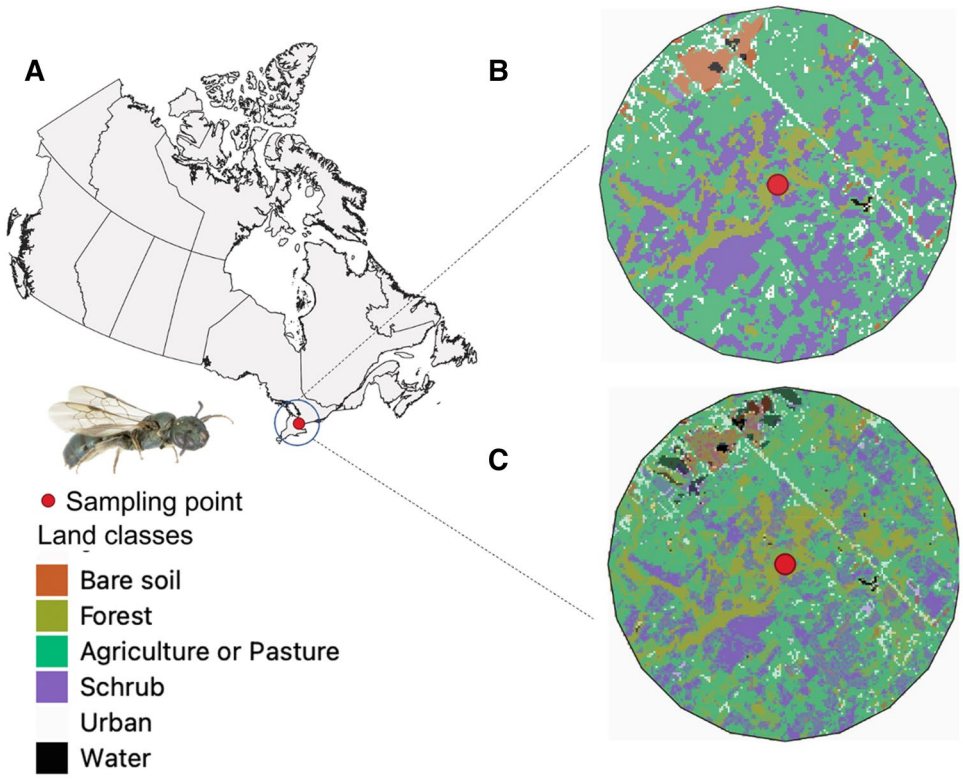

Fig. 1 Maps of *Ceratina* sampling location and land use classes for our study. **A** Map of Canada showing the sampling point location (red dots) in Caledon, Ontario. Land use classification from **B** historic (1972) and **C** contemporary (2018) time points. The bee pictured is a *C. calcarata* female. Photo credit: Sandra Rehan; Map of Canada taken from https://open.canada.ca

to the original RAD protocol described by Baird et al. (2008). DNA from each bee was digested with the restriction enzyme PstI and sequenced on an Illumina NovaSeq 6000. Specimen information and quality of sequencing read for each sample are provided in Table S1.

We demultiplexed FASTQ files and removed multiplexed identifying barcode sequences using process_radtags in Stacks v. 2.3 (Rochette et al. 2019). We excluded reads if they contained an ambiguous PstI cut site, incorrect barcode, or average Phred quality score < 10 within a sliding window (default). All reads were submitted to NCBI's website as BioProject PRJNA728354. RADseq single-end reads were mapped against the reference genome of *Ceratina calcarata* (NCBI Bioproject PRJNA791561) using the bwa *aln* and *samse* functions of the Burrows–Wheeler alignment tool with default parameters (BWA 0.7.10; Li and Durbin 2009). During alignment, we removed the first four bases (-B 4) to prevent any base sequence bias from the adapter PstI. Next, we used SAMtools v.1.12 (Li et al. 2009) to convert mapped reads from SAM to BAM files and to sort BAM files. We also used SAMtools to assess mapping depth and coverage for each sample (Table S1) and removed two contemporary samples from *C. calcarata* due to high missing values. DNA retrieved from museum samples is usually highly degraded due to endogenous nuclease and post-mortem damages caused by a high frequency of cytosine/thymine and adenine/guanine substitutions (Sawyer et al. 2012; Bi et al. 2013; McGaughran 2020). To assess possible damage patterns such as deamination and depurination in our historic DNA data, we used MapDamage 2.0 (Jónsson et al. 2013) with default settings. We removed all SNP positions with a C-to-T and/or G-to-A transformation from our original dataset to avoid biases in further analysis. Analysis of DNA fragmentation and post-mortem deamination of C-to-T and G-to-A are presented in Fig. S1. After MapDamage removal, SNPs were called from the sorted BAM files using the functions *mpileup* and *call* from SAMtools/bcfttools and converted to --vcf format. This resulted in $13{,}589_{(\text{contemporary})}$ and $1319_{(\text{historic})}$ SNPs for *C. calcarata*, and $13{,}413_{(\text{contemporary})}$ and $1793_{(\text{historic})}$ SNPs for *C. dupla*.

Population genetic diversity and structure

To generate genome-wide population statistics, we built catalog loci combining historic and contemporary populations from each species using GSTACKS in Stacks v2.60 (Rochette et al. 2019). Then, we used the POPULATIONS tool in Stacks to generate a dataset containing only shared SNPs in at least 50% of individuals with a minimum allele frequency (--min-maf) of 0.05 and restricted the analysis to only the first SNP per each locus (--write-single-snp). We then quality-trimmed these datasets on VCFTools for genotype filtering (--minDP 2 and minGQ 20), for sites out of Hardy-Weinberg Equilibrium (--hwe 0.001) and to include only biallelic sites (--max-alleles 2). We used the output files to estimate the heterozygosity within a population (H_s) using

Genodive v3.06 (Meirmans 2020) and the Fixation index (F_{is}) using Stacks. We also verified heterozygosity within a population using a stricter dataset (shared SNPs only present in 60%, 70%, and 80% of individuals, Table S2). We also used VCFTools to calculate individual relatedness within a population (--relatedness) to ensure the population's genetic results were not biased by highly related individuals. No related individuals were detected or removed from this dataset.

We estimated the effective population size for *C. calcarata* and *C. dupla* for historic and contemporary populations using a Linkage Disequilibrium and two temporal methods as carried out in NeEstimator v2.1 (Do et al. 2014). We first generated a single-sample estimation using Linkage Disequilibrium method with default parameters. Although historical samples frequently have missing data that could bias the estimation of N_e, NeEstimator v2.1 accounts for this bias by correcting the presence of missing data using a weighted harmonic mean (Do et al. 2014). For the temporal method, we used a two-sample temporal estimation of N_e variance over generations, including two formulations of variance in allele frequency: *F*k (Pollak 1983) and *F*s (Jorde and Ryman 2007). *Ceratina* species might present overlapping generations, but usually express one generation per year (Rehan and Richards 2010). Here we considered *C. calcarata* and *C. dupla* to be univoltine, therefore we estimated sampling across fifty generations for temporal method over the 50-year time span in which we collected historical and contemporary populations. The temporal method generates a single estimate of N_e between samples (Do et al. 2014). Our results were generated based on a jackknifed 95% confidence interval (CI).

For genetic structure analysis, we used two main approaches using the same filtered dataset as described above. First, we generated ped and bed files in PLINK v.2.0 (Chang et al. 2015) for each population to be analyzed on the Bayesian model-based clustering program ADMIXTURE. We evaluated prior clusters from K = 1 to K = 4 to map genetic structure and common kinship. The optimal value of K is designated according to a cross-validation (CV) procedure, where the best value of K will show the lowest CV error (Alexander et al. 2009). Second, we used PLINK v.2.0 (Purcell et al. 2007) to perform a Principal Component Analysis (PCA) by extracting PC coordinates for each individual and visualized the results using the R packages ggplot2 v.3.3.2 and tidyverse v.1.3.0 (Wickham 2016; Wickham et al. 2019). We also estimated the amount of population divergence using the fixation index on Stacks (mean F_{st}).

Signatures of selection and gene ontology

To assess whether our data show candidate genes under selection, we used the dataset containing only SNPs shared between historic and contemporary populations for *C. calcarata* and *C. dupla* aiming to identify selection maintained across generations. First, we created a .bam file for each population, then we used these files to generate a synchronized input containing the allele frequency with a minimum quality of 20 using SAMtools—mpileup flag and popoolation2 Kofler et al. 2011). We then extracted the allele frequency differences of only biallelic sites with a minor allele count of 6 and a coverage ranging from 50 to 200. F_{ST} estimates were calculated for each SNP as a pairwise population comparison using fst-sliding.pl in popoolation2. Based on the pairwise F_{ST}, a locus was considered outlier when falling above the 95th percentile of data distribution ($F_{ST} = 0.234$ for *C. calcarata*, and $F_{ST} = 0.263$ for *C. dupla*). We also used the same approach to investigate candidate genes present only in contemporary populations for both species. This is an important step to understand how temporal changes in habitats have genetically affected recent population for these two species.

To further investigate if our data identified any candidate genes associated with specific biological processes, we performed gene ontology (GO) enrichment analyses on each species SNPs dataset. First, we created a .bed file with only outlier SNPs on bedtools v2.30.0 (Quinlan and Hall 2010). Then, we used the function *intersection* on bedtools to extract gene annotations using the *Ceratina calcarata* genome annotation in gff format. We used the topGO script in R (Alexa and Rahnenführer 2020) to evaluate the overrepresentation of GO terms in individual candidate SNP according to their associated probabilities under Fisher's exact test ($p < 0.05$) using the *C. calcarata* genome annotation. TopGO assigns the weighted gene ontology to a specific gene list by considering parent–child relationships, which suggests an improvement in discovery sensitivity and specificity (Alexa and Rahnenführer 2020).

Results

Land use and climate change

We mapped land use data on QGIS using Landsat images from historic and contemporary sampling dates. We classified both images according to six land-use classes (Fig. 1). For both time periods, agriculture and pasture were the most abundant land-use class, followed by scrubland. Historically the least abundant land cover was water, whereas contemporary land use is least represented by bare soil. Between time points, there was a significant increase in forest and urban

Table 1 Percentage of landscape features for 1972 and 2018 and its variation between time points

Landscape feature	Percentage (%)			Two sample z-test
	1972	2018	Δ	Z-score (p-value)
Bare soil	2.29	4.55	+1.59	−1.53 (0.13)
Forest	9.77	14.14	+4.37	**−3.27 (0.001)**
Agriculture & pasture	54.97	49.49	−5.48	**3.87 (0.001)**
Scrubland	27.16	21.04	−6.12	**4.32 (< 0.001)**
Urban	4.79	7.66	+2.87	**−2.03 (0.04)**
Water	0.35	3.13	+2.78	−1.97 (0.06)

Significant differences in land use cover are shown in bold font

land cover, while agriculture and scrubland significantly decreased (Table 1). We analyzed four climatic variables to quantify possible differences between the two time-periods. For both periods, we analyzed average annual precipitation, and maximum, mean, and minimum temperature (Fig. 2). All variables increased through time, with only mean temperature not showing a significant difference across time periods (Table 2).

Sequence depth and coverage

We generated a high-quality RADseq dataset for two species of *Ceratina* small carpenter bees. The mean coverage for historic samples was 4.13× (2.8–5.8×), whereas contemporary

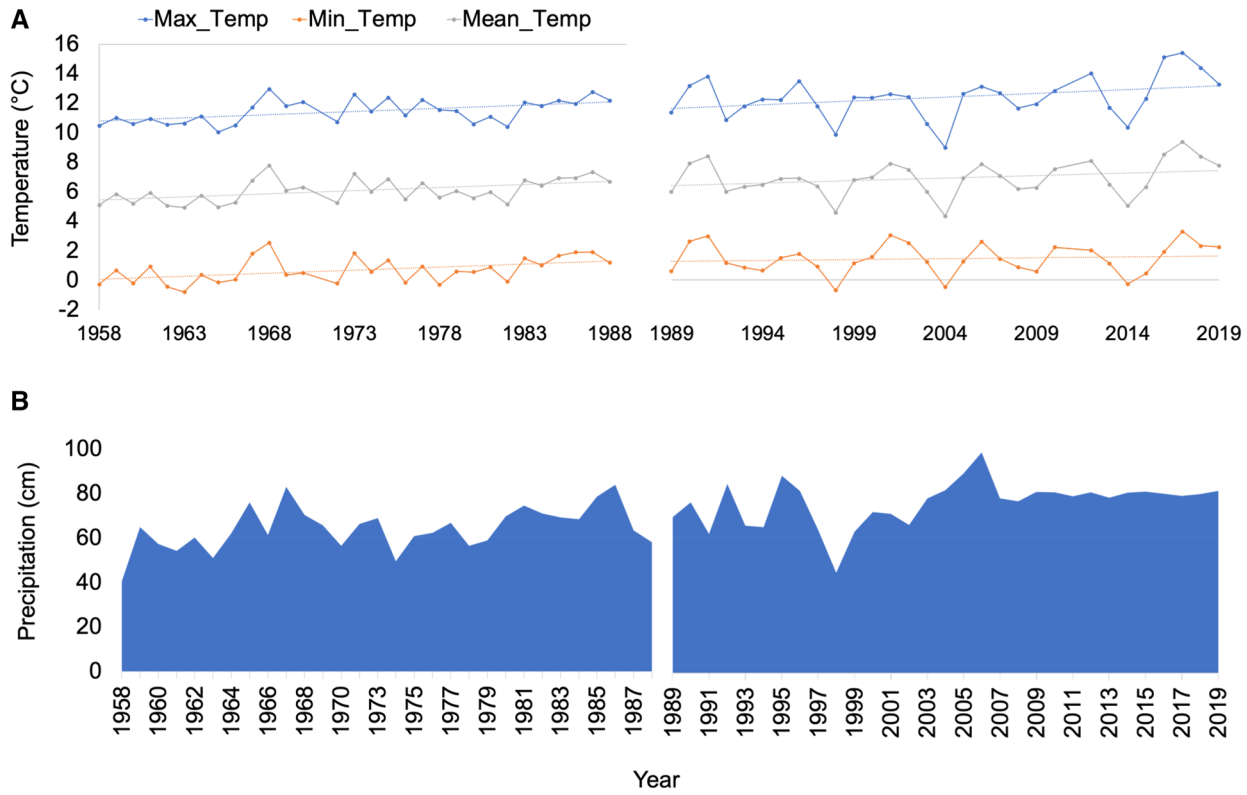

Fig. 2 Climatic variables collected from *Ceratina* sample points. **A** Maximum, mean, and minimum temperatures expressed in Celsius degrees for the historic and contemporary time-bins. On the left is a historic bin ranging from 1958 to 1988. On the right is a contemporary bin ranging from 1989 to 2019. **B** Total annual precipitation expressed in cm for the same two historical and contemporary time bins

Table 2 Paired t-test results for all climatic variables

Variable	Historic $\bar{x}$	Contemporary $\bar{x}$	df	t-value	p
Total precipitation	64.404	72.249	59.870	−3.27	**0.002**
Maximum temperature	11.451	11.990	46.277	−1.85	0.071
Mean temperature	6.078	6.682	52.086	−2.41	**0.020**
Minimum temperature	0.694	1.386	56.675	−2.88	**0.006**

Significant increases in bold font

Table 3 Genetic diversity data from shared SNPs of historic and contemporary samples for *C. calcarata* and *C. dupla*

Species	Population	H_s	F_{is}	N_e LD (95% CI)	N_e temporal F_k (95% CI)	N_e temporal F_s (95% CI)
C. calcarata	Historic	0.157	0.066	Infinite	394.9 (286.8–566.0)	76.7 (59.0–109.4)
	Contemporary	0.101	0.113	771.1 (493.7–1748.2)		
C. dupla	Historic	0.179	0.042	Infinite	99.8 (55.4–175.5)	93.6 (61.9–193.4)
	Contemporary	0.116	0.126	2717.7 (2083.6–3904.5)		

Heterozygosity within populations (H_s), fixation index (F_{is}), and effective population size (N_e) according to single-sample linkage disequilibrium (LD), and two-sample temporal methods (Pollak F_k and Jorde/Ryman F_s)

CI confidence interval based on the jackknife-across samples

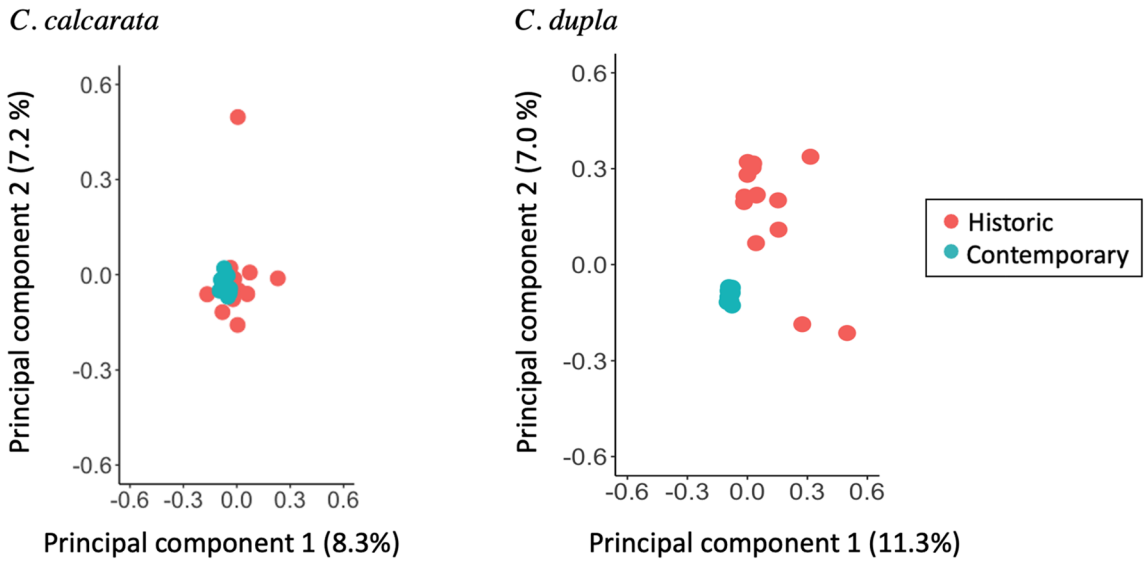

Fig. 3 Principal component analysis (PCA) results from *C. calcarata* and *C. dupla* samples. Individuals from museums are colored as red dots and individuals recently collected are colored as blue dots. PLINK v.2.0 was used to generate PCA and ggplot2 and tidyverse packages were used to create these plots

samples averaged 17.2× (4.4–50.8×; Table S1). The mapping rate according to bcftools across historical samples was 46.85% for *C. calcarata* and 11.17% for *C. dupla*. Comparatively, 89.26% of contemporary samples mapped against the *C. calcarata* reference genome, while *C. dupla* mapped 88.60%. We found 800 shared SNPs between historic and contemporary populations for *C. calcarata* and 1413 SNPs for *C. dupla*. Based on this combined data, the mean effective per-sample coverage was 8.3× (2.9–37.4×) for *C. calcarata*, and 11.8× (3.2–50.1×) for *C. dupla.*

Population diversity and structure

For both species, the heterozygosity was consistently lower across time, exhibiting lower values in contemporary samples (Table 3). This pattern was also followed by the stricter shared SNPs data analysis, where contemporary populations for both species decreased through time (Table S2). F_{IS} had similar values between species, where historical samples exhibit values close to zero and contemporary samples showed higher values (Table 3). Estimates of effective population size (N_e) based on linkage disequilibrium were in the range of 771–2717 for contemporary populations, whereas both historic populations were estimated to be infinitely large (Table 3). Based on the variance of population allele frequency over time, the N_e estimation for *C. calcarata* was 394.9 and 99.8, and for *C. dupla* was 76.7 and 93.6 (F_k and F_s respectively, Table 3).

Population structure data as inferred by PCA revealed contemporary populations with lower divergence when compared to historic populations for both species (Fig. 3). Similarly, genetic differentiation between population pairs (historical × contemporary) was high ($F_{ST} = 0.19$ for *C. calcarata* and $F_{ST} = 0.08$ for *C. dupla*). Our ADMIXTURE analysis revealed the optimal number of clusters as $K = 1$ for both species and time points, according to the lowest cross-validation error (Figs. 4, S2).

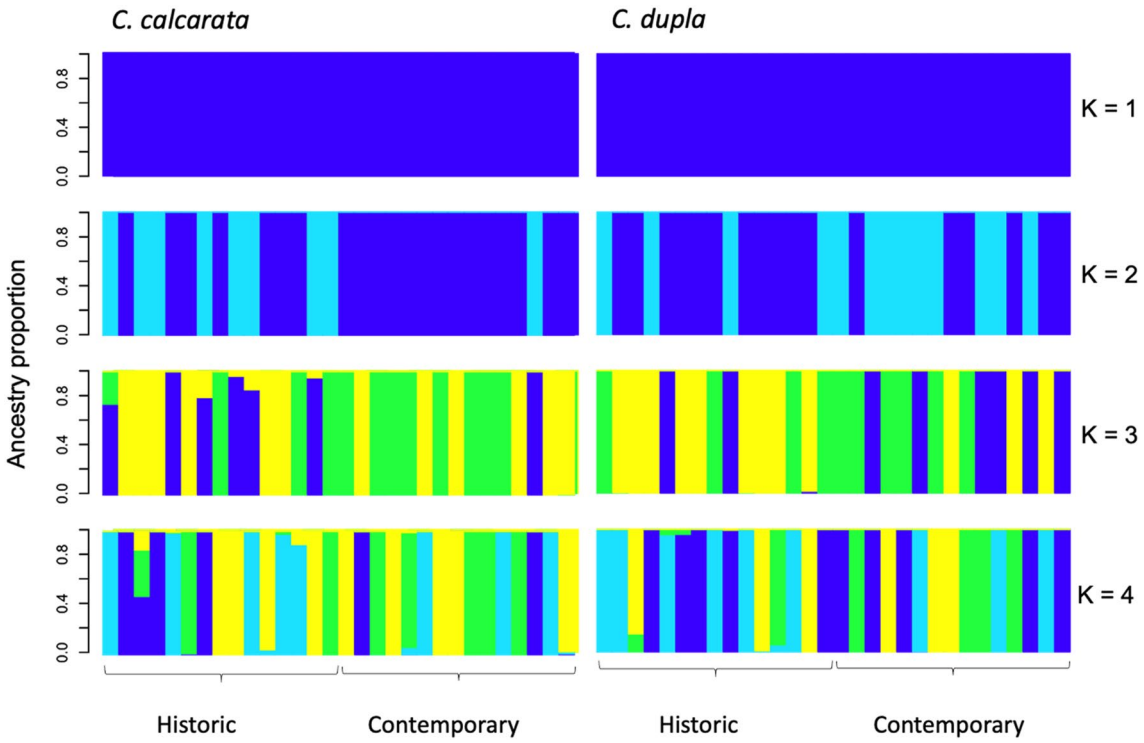

Fig. 4 ADMIXTURE results from *C. calcarata* and *C. dupla* samples show the genetic ancestry proportions for each individual from K = 1 to K = 4. Both are best supported as one breeding population (K = 1; Fig. S2)

Signatures of selection and gene ontology

According to the F_{st} measurements to identify candidate adaptive loci shared between historic and contemporary populations, 23 outlier loci were found for *C. calcarata* and 35 for *C. dupla*. Outliers corresponded to 65 genes for *C. calcarata* with 38 of unknown function, and 55 genes for *C. dupla* with 35 of unknown function. For contemporary populations, we identified 719 shared outlier loci that yielded 158 shared genes under positive selection (excluding genes of unknown function). For *C. calcarata*, we identified genes associated with general transcription factor II-I (*GTF2IRD2*, Table S3), retrovirus-related Pol polyprotein (*pol*), transposon Ty3-G Gag-Pol polyprotein (*TY3B*-I and *TY3B-G*) and putative 115 kDa protein (*R1A1/ORF2*). For *C. dupla*, we also found many genes related to retrovirus-related polyprotein (*pol*) and transposon-derived protein (*TY3B-I* and *TY3B-G*).

For shared genes between species, we found members of the Cytochrome P450 family (*Cyp49a1* and *CYP4C1*, Table S4) which are related to immune response and insecticide tolerance in Hymenoptera (Xing et al. 2021), and the immunity-related genes Cadherin-89 C (*Cad89D*) and Tyrosine kinase receptors (*hop* and *Cad96Ca*). Also, we identified the myosin light chain kinase gene (*MYLK*, Table S4) and sodium-dependent transporters (*CG32669*), with a prominent role in muscle and neural activity. We also found G protein-coupled related receptors (*mth*, *mth2* and *Adgrb3*, Table S3), which is involved in insect physiology and toxicological responses, desiccation stress, and diapause triggering, and more specifically in insecticide resistance (Homma et al. 2006; Terhzaz et al. 2015; Liu et al. 2021), and positively selected genes associated with motor function such as gamma-aminobutyric acid receptor (*GABRA6*, Table S3).

Enrichment analysis for *C. calcarata* revealed a set of 63 GO terms significantly overrepresented associated with the set of 65 candidate genes ($p < 0.05$, Table S5). For *C. dupla*, we identified 81 GO terms associated with 55 candidate genes (Table S5). Enriched GO terms shared between species include binding-related terms such as microtubule binding (GO:0008017) and immune-related terms such as response to antibiotics (GO:0046677) and tyrosine/serine/threonine phosphatase activity (GO:0008138). GO terms uniquely enriched in *C. calcarata* included calcium transport related as calcium chanel activity (GO:0060314), calcium channel complex (GO:0034704) and cuticle related terms such as structural constituent of cuticle (GO:0042302). For *C. dupla*, we detected several GO terms related to viral responses (GO:0039526, GO:0019064 and GO:0019031, Tables 4, S5) and to muscle morphogenesis and development (GO:0060415 and GO:0048743).

Table 4 List of important GO terms ID and annotation under p-value < 0.05 for *C. calcarata and C. dupla* individually and shared between species (*C. calcarata/C.dupla*)

Parental terms	GO ID	GO term	Significant	p value
Ceratina calcarata				
Channel activity	GO:0034704	Calcium channel complex	2	0.002
Binding	GO:0051287	NAD binding	5	0.041
Structural molecule activity	GO:0042302	Structural constituent of cuticle	2	0.096
Ceratina dupla				
Developmental process	GO:0060415	Muscle tissue morphogenesis	2	0.024
Biological regulation	GO:0039526	Modulation by virus of host	2	0.034
Binding	GO:0005525	GTP binding	8	0.015
Shared between species (*C. calcarata//C. dupla*)				
Binding	GO:0008017	Microtubule binding	3//3	0.027//0.025
Response to stimulus	GO:0046677	Response to antibiotics	3//3	0.035//0.012
Catalytic activity	GO:0008138	Tyrosine/serine/threonine phosphatase activity	3//3	0.012//0.011

A full list of GO terms by species is listed in Table S5

Discussion

Here we present a genome-wide comparison of historical and contemporary data to assess how 50 years of changing landscape and climate have affected the population status of two wild bee species in North America. We also present evidence of local adaptation as revealed by the signatures of selection found for *Ceratina calcarata* and *C. dupla* in historic and contemporary populations. Our study is the first to use historical data to detect both signatures of selection and acquaint population status of *Ceratina* species providing a novel understanding of the adaptive evolution over reliable historical data of these bees. Here we also generated data to compare these species and timeframes, although historic samples showed a lower mapping rate, especially *C. dupla*. Low mapping is potentially a consequence of the highly degraded DNA found in historic samples, and similar values have been previously found in studies using insect museomics (Gauthier et al. 2020). This study provides a unique opportunity to directly estimate changes in wild bee populations across time, reinforcing the value of museum specimens for ecological conservation.

Although historical samples had a lower success rate and quality comparing to contemporary data, the approach still produced enough SNP data to allow analysis of population genomics and structure of these two *Ceratina* species. Our data suggests a heterozygosity deficiency from historic to contemporary populations over the past 50 years. It is important to ponder that our results of LD-N_e estimations comparing historical and contemporary populations must be interpreted carefully as our historical samples yielded an infinite population size, which can be an artifact of sampling error or a lack of evidence for variation in the genetic characteristic caused by a finite number of parents (Waples and Do 2010; Do et al. 2014). Other studies have found similar results when comparing historical and contemporary samples for different taxa (e.g. Crates et al. 2019; Lonsinger et al. 2018). According to our data of N_e variation across time, we found that for both species the estimates were relatively small, yielding an N_e of 76–394 individuals. Non-social insects are expected to have a large effective population size. Thus, we believe that the number of effective mating individuals could have been affected in these bees across this period. The problem of populations with dropping N_e is that they tend to become genetically eroded across generations due to a load of rare (generally recessive) deleterious alleles that would be easily spread through a population by an excess of relatives mating (Frankham et al. 2010). Also, populations may lose adaptive alleles through genetic drift decreasing their adaptive potential (Hoffmann and Willi 2008; Segelbacher et al. 2010; Husemann et al. 2016), which is a massive concern for wild bees, reliant on random mating and therefore to such allele exchange to avoid species decline. The low effective population size and high inbreeding have been associated with wild bee declines, including the threatened yellow-banded bumblebee *Bombus terricola* in Canada (Kent et al. 2018) and in the stingless bee *Tetragonisca angustula*, with an N_e yielding only 43–72 individuals, highly associated to changes in amount of forest cover (Barbosa et al. 2022). Additionally, our estimations of low N_e based on allele frequency between generations were similar to those found in the widespread eusocial wasp *Vespula maculifrons* (Dyson et al. 2021) and in the endangered Miami blue butterfly *Cyclargus thomasi bethunebakeri* (Saarinen et al. 2010). A recent report on estimated reduced genetic diversity in many taxa has associated this with reducing habitat and anthropogenic activity worldwide (Exposito-Alonso et al. 2022). Reduced genetic diversity and evidence of high inbreeding have also been found in the Australian small carpenter bee *Ceratina australiensis* (Harpur and Rehan 2021) showing

that *Ceratina* species and all wild bees need careful attention regarding their genetic status. Therefore, although is unclear if the effective population size of these species is decreasing over time, our data suggest a relatively low N_e that can be of a concern to their biological status.

We also detected genetic structure across our contemporary and historic population. A population tends to be structured when there is limited migration and gene flow between subpopulations or when they are entirely isolated (Slatkin 1987). Based on our PCA results, our data shows individuals narrowly clustered together in contemporary populations, suggesting that these individuals are more related and that some resistance to gene flow could have appeared in this sampling population across the last 50 years. Our land-use data revealed an increase in forest and urban land-use types across time and decreased agricultural and pasture areas and scrubland. From a land-use perspective, some features may hinder allele exchange, and ultimately be responsible for these population changes. Although bees have a wide variety of responses to urban landscapes, varying from receiving benefits or been harmed according to their dietary breadth, nesting strategy, body size, and behavior (Ballare and Jha 2020; Ayers and Rehan 2021), several studies have demonstrated that human-altered land, especially impervious cover associated with urbanization, negatively affects small bees (Jha and Kremen 2013; Forester et al. 2018; Birdshire et al. 2020; Conflitti et al. 2022). For example, wild bee communities can show reduced species richness across urban habitats compared to natural environments (Matteson et al. 2008). Additionally, signals of genetic drift, bottleneck, and inbreeding have been found from museum specimens of insects responding to anthropogenic changes to habitat, as is the case of the rare species Adonis blue butterfly (*Polyommatus bellargus*) and other Palearctic butterflies (Gauthier et al. 2020; Harper et al. 2006).

Human-altered land use has increased in our sampled site as shown by our land-cover data, likely affecting the availability of floral and nesting resources for *Ceratina* species. Our data revealed a reduction in scrubland which impacts *Ceratina calcarata* that preferably nest and provision pollen in small trees and shrubs such as *Rhamnus, Rhus*, and *Rubus* genera (Vickruck and Richards 2012; McFrederick and Rehan 2016). Furthermore, a decrease in scrubland may also affect migration and allele exchange, and therefore, may be driving the decrease in genetic diversity observed in both *Ceratina* species. Regarding flight range and mobility, small bees have limited dispersal range due to their size and are thought to be particularly sensitive to land-use change (reviewed in Ayers and Rehan 2021). Both of our focal species are small bees with a narrow foraging range (Greenleaf et al. 2007) that could be affected by detained access to diverse floral resources in an increasingly altered landscape.

We detected signatures of selection in both *C. calcarata* and *C. dupla* populations. From the shared species' loci investigation, we detected positive selection on Cytochrome P450-related genes (*CYP4C1* and *Cyp49a1*), which is closely related to insecticide tolerance in Hymenoptera (Manjon et al. 2018; Xing et al. 2021). Exposure to pesticides has been reported as a crucial issue regarding bees' health and population decline during the past decades (Cameron et al. 2011). For example, when P450s are knocked out the mortality of adult wasps was higher when exposed to chemical insecticides (Xing et al. 2021). Pesticide responses related to cytochrome P450 genes were also recorded in declining wild bumble bee, *Bombus terricola*, emphasizing the link between pesticides and wild bee declines. *C. calcarata* and *C. dupla* are known for their valuable role in pollinating both wild plants and crops and are among the top 20 most economically important bee species by virtue of their pollination services to commercial crops (Kennedy et al. 2013; Kleijn et al. 2015). This relationship might lead to higher exposure to a variety of insecticides. Canada has recently limited the use of neonicotinoids in crops that bees find attractive and prohibited spraying in some crops such as berries and fruiting vegetables (Bhuller et al. 2021). But as these insecticides have been extensively used in agriculture in recent decades, this could be acting as a selective pressure for bees over the last 50 years. Pesticides and pathogens have been broadly linked to bumble bee declines in North America (Grixti et al. 2009; Kent et al. 2018; Tsvetkov et al. 2021). Specifically for investigations on loci maintained across time, we found positive selection for many virus-related genes, which could be indicative of an immune response to increasing viral loads across time. Viral infections are known to cause serious damage to bees at different developmental stages and are an increasing concern for managed and wild bee health (Martin and Brettell 2019; Nanetti et al. 2021; Paxton et al. 2022; Ray et al. 2020; Tehel et al. 2016). Taken together, our results suggest signals of molecular adaptation include responses to immune defense consistent with various studies reporting that wild bees are threatened by lingering pesticide use and increasing viral pathogens.

Our study identified enrichment for calcium activity and transportation, highly associated with muscular and neural function in insects, and is known for its function in flight muscle contraction (Gordon and Dickinson 2006; Overgaard and MacMillan 2017; Keyser 2005). Adaptation related to maintenance of flight activity for bees is essential in a climate change scenario, especially considering that we detected a significant increase in mean and minimum temperature through our 50-year climate data. For small bees such as *C. calcarata* and *C. dupla*, sustaining flight activity over high temperatures can be even more challenging. Interestingly, we also found selection on genes associated

with motor function such as gamma-aminobutyric acid receptor, which could play important roles in foraging efficiency within a landscape with ever-increasing percentages of impervious surface and to climate change. Climate adaptation was also supported by evidence for positive selection of G protein-coupled receptors activity, which are also highly related to the prevention of desiccation in many insect species. For bees, adaptive selection for desiccation tolerance means a remarkable ability to prevent water loss and sustain cellular integrity in a warming scenario (Thorat and Nath 2018). Also, for *C. calcarata*, we found GO enrichment for cuticle structuring. The cuticle that covers the insect's integument helps prevent excess water evaporation and can work as a shield for pathogen invasion (Reynolds and Samuels 1996), and its thickness is also highly related to resistance to some types of insecticides (Wood et al. 2010). Selection for genes related to cuticle composition may help bees cope with pathogen threats, water retention, and insecticide susceptibility (Reynolds and Samuels 1996; Wood et al. 2010; Jackson et al. 2020).

In conclusion, we successfully produced genome-wide data from historic museum samples for two wild bee species that allow for the analysis of population genomics and structure across a 50-year timespan. We detected signatures of selection on genes suggesting local adaptation to a warming climate and urbanized landscape, consistent with the observed increase in temperature, precipitation, and urban land use over time. Alternatively, we found a decrease in scrubland; a potentially vital land type to maintain these populations that live in scrub and dead stems. From a conservation standpoint, it is important to maintain shrub and scrub vegetation throughout landscapes for these and other stem-nesting bees that are highly reliant on such natural vegetation for nesting and foraging. Using an integrative museomics approach incorporating historic DNA and contemporary data, we determined the population genomic status of two important North American pollinators and detected species-level responses to land use and climate change. Our results offer insights into the selective stressors and abiotic pressures underlying these populations, which can improve restoration and conservation planning moving forward. These methods are broadly applicable to a wide range of wildlife species, including the vast diversity of wild bees, and call for similar studies across additional taxa.

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Author contributions SMR conceived and funded the study. EPK obtained specimens, conducted DNA extractions and sent material for sequencing. SNRB analyzed the data and prepared the figures. SNRB and SMR wrote the main manuscript text. All authors reviewed the manuscript.

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Declarations

Competing interests The authors declare no competing interests.

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