Whole genome analyses reveal weak signatures of population structure and environmentally associated local adaptation in an important North American pollinator, the bumble bee *Bombus vosnesenskii*.

Running title: WGS analysis of *Bombus vosnesenskii*

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Abstract

Studies of species that experience environmental heterogeneity across their distributions have become an important tool for understanding mechanisms of adaptation and predicting responses to climate change. We examine population structure, demographic history, and environmentally associated genomic variation in *Bombus vosnesenskii*, a common bumble bee in the western U.S.A., using whole genome resequencing of populations distributed across a broad range of latitudes and elevations. We find that *B. vosnesenskii* exhibits minimal population structure and weak isolation by distance, confirming results from previous studies using other molecular marker types. Similarly, demographic analyses with Sequentially Markovian Coalescent (SMC) models suggest that minimal population structure may have persisted since the last interglacial period, with genomes from different parts of the species range showing similar historical effective population size (N_e) trajectories and relatively small fluctuations through time. Redundancy analysis revealed a small amount of genomic variation explained by bioclimatic variables, and environmental association analysis with latent factor mixed modeling (LFMM2) identified few outlier loci that were sparsely distributed throughout the genome. Some outlier loci were in genes with known regulatory relationships, suggesting the possibility of weak selection, although compared to other species examined with similar approaches, evidence for extensive local adaptation signatures in the genome was relatively weak. Overall, results indicate *Bombus vosnesenskii* is an example of a generalist with a high degree of flexibility in its environmental requirements that may ultimately benefit the species under periods of climate change.

Key words: *Bombus*, biogeography, environmental adaptation

Introduction

Studying species that experience environmental heterogeneity is a key component in understanding phenomena like local adaptation and predicting species responses to climate change (Hoffmann, Weeks, & Sgro, 2021; Savolainen, Lascoux, & Merilä, 2013; Sears, Riddell, Rusch, & Angilletta, 2019). The degree of spatial and environmental heterogeneity across a species range can have important effects on gene flow and population structure (Y. Li et al., 2017; Manel, Schwartz, Luikart, & Taberlet, 2003). Spatially complex landscapes have features that might constrain gene flow, increasing population genetic structure of inhabitants compared to more homogeneous landscapes (Rahbek et al., 2019; Wang & Singh, 2019) and also harbor substantial abiotic variation that could drive local adaptation in populations from dissimilar environments (Andrews et al., 2022; Antoniou et al., 2021; Capblancq, Fitzpatrick, Bay, Exposito-Alonso, & Keller, 2020; Heraghty, Rahman, Jackson, & Lozier, 2022). Genomic data can be used to understand current and historical population structure as well as genome-environment associations that might signal local adaptation and can thus provide multiple types of information about evolutionary responses to complex landscapes in widespread species.

Although natural selection is expected to shape genetic variation in species distributed over large biogeographic gradients, not all species experience and respond to spatial or environmental heterogeneity in the same way (Gallegos, Hodgins, & Monro, 2022; Hartke et al., 2020; J. M. Jackson et al., 2020). For instance, species capable of long-distance dispersal or characterized by generalist individual phenotypes across a range of environmental conditions might show weak signals of local adaptation (Lenormand, 2002; Räsänen & Hendry, 2008). This phenomenon has been observed in a variety of taxa including marine invertebrates (Lal, Southgate, Jerry, Bosserelle, & Zenger, 2016), butterflies (Melero et al., 2022), and trees (De la

70 Torre, Wilhite, Neale, & Slotte, 2019). Landscape genomics provides a lens for assessing the
71 challenges of a heterogenous environment by revealing specific environmental features that drive
72 population structure (Capblancq & Forester, 2021), and has also become a widely used tool for
73 detecting signals of environmental adaptation (Capblancq, Morin, Gueguen, Renaud, & Bazin,
74 2020; De la Torre et al., 2019; Heraghty et al., 2022; J. M. Jackson et al., 2020), especially as
75 whole genome data becomes more easily obtained.

76 Demographic responses to past climatic fluctuations can provide another important
77 dimension for understanding environmentally associated genomic variation. In widespread
78 species, for example, distinct demographic histories (e.g. bottlenecks) across a distribution can
79 influence on how populations adapt to their contemporary environment, and testing for parallel
80 or dissimilar effective population size (N_e) trajectories through time can provide context for
81 inferences of contemporary population structure or local adaptation (Ahrens et al., 2018). A
82 common tool for inferring N_e is a class of methods known as Sequential Markovian Coalescence
83 (SMC) models (Lozier, Strange, & Heraghty, 2023; Mather, Traves, & Ho, 2020; Nadachowska-
84 Brzyska, Burri, Smeds, & Ellegren, 2016), which can provide estimates of temporal variation in
85 N_e using genomes from single individuals or populations. SMC approaches can reveal how
86 populations have changed in size over tens of thousands of years, and thus reveal whether past
87 climate conditions may have affected genetic variation in similar or distinct ways across a
88 species range (Lozier et al., 2023; Taylor et al., 2021). Such information may also be helpful in
89 interpreting contemporary patterns of environmental association since complex histories could
90 constrain genetic diversity which ultimately may limit the ability of organisms to adapt to their
91 environment (Reed & Frankham, 2003).

92 In this study, we employ a whole genome resequencing (WGR) approach to evaluate
93 population structure, demographic history, and potential targets of adaptation in a common
94 bumble bee, *Bombus vosnesenskii* Radowski. *Bombus vosnesenskii* is one of the most common
95 bumble bee species in the westernmost parts of North America, with a range extending from
96 southern California, USA through British Columbia, Canada (Cameron et al., 2011; Fraser,
97 Copley, Elle, & Cannings, 2012; J. Koch, Strange, & Williams, 2012; Stephen, 1957). The
98 latitudinal range of *B. vosnesenskii* indicates that the species is capable of inhabiting a broad
99 environmental niche, as well as occurring at a range of elevations from sea level to 2,400 m in
100 elevation (Stephen, 1957). Like many bumble bees, *B. vosnesenskii* provides important
101 pollination services (Greenleaf & Kremen, 2006; Strange, 2015; Velthuis & Doorn, 2006) and is
102 the only native western US bumble bee commercially available for pollination. Thus, in addition
103 to better revealing patterns of evolutionary adaptation to heterogeneous environments in
104 widespread species, improved understanding of regional genomic variation in *B. vosnesenskii*
105 could be of value for breeding or assessing risks associated with bees used in commercial
106 contexts (Lozier & Zayed, 2016), and for understanding potential risks to pollination from range
107 shifts under climate change (H. M. Jackson et al., 2022; Kerr et al., 2012).
108 *Bombus vosnesenskii* is among the most well-studied bumble bees in the United States in
109 terms of its geographic range, habitat use, physiology, and ecology (Heinrich & Kammer, 1973;
110 J. Koch et al., 2012; Mola, Miller, O'Rourke, & Williams, 2020a; Pimsler et al., 2020; Stephen,
111 1957), although no study to date has investigated the species using range-wide whole genome
112 resequencing. Prior information leads to several hypotheses relating to the effects of landscape
113 heterogeneity on genomic variation in *B. vosnesenskii*. Microsatellite and reduced representation
114 (RADseq) single nucleotide polymorphism (SNP) datasets have largely found evidence of

115 minimal population structure and overall genetic homogeneity at the continental scale (J. M.
116 Jackson et al., 2018; S. Jha, 2015; Lozier, Strange, Stewart, & Cameron, 2011). RADseq also
117 revealed relatively few outlier loci associated with bioclimatic or elevational variables compared
118 to a related narrower-niche montane species, *B. vancouverensis* (J. M. Jackson et al., 2020).
119 Foraging in *B. vosnesenskii* appears uninhibited by certain complex landscapes like forests (Mola
120 et al., 2020a) and can benefit from pulses in floral availability associated with wildfire
121 disturbance (Mola, Miller, O'Rourke, & Williams, 2020b), potentially indicating that movement
122 of reproductive castes, and thus gene flow, could also be unimpeded or facilitated by landscape
123 heterogeneity. Consistent with the hypothesis of *B. vosnesenskii* genetic homogeneity, range-
124 wide morphological analyses have also found little evidence of variation in functional traits like
125 body size or wing loading across latitude or altitude compared to *B. vancouverensis* (Lozier et
126 al., 2021). There is evidence, however, that some spatial or environmental landscape features can
127 impact *B. vosnesenskii* demography by influencing nesting density (Shalene Jha & Kremen,
128 2013a) and introducing local or regional barriers to gene flow (J. M. Jackson et al., 2018; S. Jha,
129 2015; Shalene Jha & Kremen, 2013b). Physiological assays have also detected variation in
130 critical thermal minima (CT_{min}) across latitudes and elevation that could indicate adaptive
131 variation associated with local cold temperatures (Pimsler et al., 2020). Overall, *B. vosnesenskii*
132 appears well-suited to occupying heterogenous landscapes, but with some potential for
133 environmental features to shape aspects of the species' evolution that may be clarified using the
134 large genetic marker sets from whole genomes.

135 Here, we generate WGR data from *B. vosnesenskii* workers sampled from diverse
136 environments across California and Oregon to test several hypotheses relating to population
137 structure and local adaptation. WGR data is a powerful tool for detecting adaptation and can

138 overcome potential shortcomings of previously used approaches such as reduced representation
139 sequencing (e.g., RADseq) (Jackson et al. 2020), which may not detect all possible targets of
140 selection when linkage blocks are small (Fuentes-Pardo & Ruzzante, 2017), as in bumble bees
141 (Stolle et al., 2011). Whole genome data enables analyses that infer species' demographic
142 histories which can complement contemporary landscape genomics (Beichman, Huerta-Sanchez,
143 & Lohmueller, 2018; de Greef et al., 2022; Iannucci et al., 2021). First, based on prior results of
144 near-panmixia from other genetic markers, we examine population structure and test for any
145 influence of spatial-environmental variation that may not have been apparent in earlier studies.
146 We also use SMC methods to test whether patterns of genetic structure or homogeneity detected
147 in current populations is likewise reflected by divergent or parallel patterns of historical effective
148 population size trajectories across the *B. vosnesenskii* range. Last, we expand upon previous
149 RADseq data by performing genome scans at the fine resolution afforded by WGR data to
150 identify potential signals of environmental adaptation across the *B. vosnesenskii* range.
151

152 **Materials and Methods**

153 **Sample Collection, DNA extraction and Sequencing**

154 *Bombus vosnesenskii* workers (diploid females) were selected for whole genome
155 resequencing from previously collected samples (J. M. Jackson et al., 2018, 2020) that are
156 representative of elevational extremes sampled across a range of latitudes in California and
157 Oregon (36.5°N – 45.3°N latitude and 49m – 2797m above sea level)(Fig 1, Table 1). Our
158 sampling represents a series of six relatively low and high elevation site-pairs nested across a
159 range of latitudes, which should cover the breadth of environmental conditions experienced by *B.*
160 *vosnesenkii,* such as mean annual temperature from 3°C- 17°C and annual precipitation from 369

161 mm – 2,177 mm (from WorldClim v2 (Fick & Hijmans, 2017). Briefly, samples were collected
162 at each site via sweep netting, placed on ice for identification (being especially careful to exclude
163 the phenotypically similar *B. caliginosus*), and then placed in 100% ethanol on dry ice, before
164 final storage in ethanol at -80°C. Based on prior estimation of relatedness with RADseq data,
165 selected workers should represent independent colonies. See Jackson et al. 2018 for a more
166 complete description of sampling and the study region.

167 DNA was extracted using the Qiagen DNeasy Blood and Tissue kit (Hilden, N.R.W.,
168 Germany). Some samples were sequenced using whole genome shotgun libraries prepared via
169 the NEBnext Ultra II FS DNA kit (Ipswich, MA, USA) with subsequent 150 bp paired-end
170 sequencing using Illumina NovaSeq 6000 technology (Psomagen, Rockville MD). The
171 remaining sample libraries were prepared by the Genomics and Cell Characterization Core
172 Facility at the University of Oregon and sequenced across three separate lanes of an Illumina
173 Hiseq 4000 instrument.

174 **Read mapping, filtering, and variant calling**

175 Sequencing reads were processed with bbduk v37.32 (Bushnell 2020) to remove
176 adaptors, trim low quality bases, and remove short reads (ktrim=r k=23 mink=11 hdist=1 tpe tbo
177 ftm=5 qtrim=rl trimq=10 minlen=25). Read quality was evaluated using FastQC v0.11.5
178 (Andrews 2010) before being mapped to the *B. vosnesenskii* reference genome (NCBI RefSeq
179 ID: GCF_011952255.1) (Heraghty et al., 2020) using BWA mem v0.7.15-r1140 (H. Li &
180 Durbin, 2009). Samtools v1.10 (H. Li et al., 2009) was used to convert the SAM files to BAM
181 files and Picard tools v2.20.4 (Broad Institute 2019) was used to sort, mark duplicates, and index
182 the BAM files. Single nucleotide polymorphisms (SNPs) were called using freebayes v1.3.2
183 (Garrison & Marth, 2012) and filtered following Heraghty et al. (2022). An initial round of

184 filtering was performed to remove low-quality variants using vcftools v0.1.13 (Danecek et al.,
185 2011) with the following flags: --remove-indels --min-alleles 2 --max-alleles 2 --minQ 20 --
186 minDP 4 --max-missing 0.75. Subsequent filtering was designed to remove SNPs with unusually
187 high coverage (> 2x average coverage), excess heterozygosity (--hardy flag in vcftools), located
188 on small scaffolds (< 100 kb in size), and with a minor allele frequency (MAF) ≤ 0.05 to filter
189 SNPs that might have arouse from repeat regions or sequencing artefacts and limit the effects of
190 low frequency variants. A final round of filtering was done to remove differences that might
191 arise from using data generated on different sequencing platforms, as recommended by (De-
192 kayne et al., 2020), using a higher SNP quality filter (min Q of 30 and min GQ of 20).

193 **Environmental Variable Selection and Population Structure**

194 Environmental variables (19 Bioclim variables) at a 0.5 arcminute resolution were
195 obtained from WorldClim v2 (Fick & Hijmans, 2017). To reduce correlation between variables
196 we used an item clustering analysis (*iclust* function with default settings) from the *psych* v.2.1.9
197 package (Revelle 2020) in R v 4.2.0 (R Core Team 2022) and retained a single variable per
198 correlated variable cluster. Retained variables were: BIO1 – annual mean temperature, BIO3 –
199 isothermality, and BIO12 – annual precipitation. Elevation was also included as a variable of
200 interest despite being partly correlated with other environmental variables as it may capture
201 unique environmental information that could be relevant for bumble bees such as air density and
202 oxygen availability (Cheviron & Brumfield, 2012; Dillon, 2006; Heraghty et al., 2022).

203 To assess population structure, the fully filtered vcf file was converted into a genlight
204 object via the *vcfR2genlight* function in the *vcfR* v1.12.0 package in R (Knaus & Grünwald,
205 2017). Global F_{ST} (Weir & Cockerham 1984 method (Weir & Cockerham, 1984)) was calculated
206 using *SNPRelate* 1.30.1 (Zheng et al., 2012) in R. Pairwise F_{ST} was calculated between all

207 sampling localities using the stamppFst function from the *dartR* v1.9.9.1 package in R (Gruber,
208 Unmack, Berry, & Georges, 2018). A geographic distance matrix for all sampling points was
209 created using the *distm* function from the *geosphere* v1.5-14 package in R using coordinates of
210 each site. A Mantel test (statistical testing using 1000 permutations) was performed on pairwise
211 F_{ST} and geographic distance matrices to test for signatures of isolation by distance (Slatkin,
212 1993) using the mantel function from the *vegan* v2.5-7 package in R (Oksanen et al 2020). To
213 visualize population structure, the *gl.pcoa* function from the *dartR* v1.9.9.1 package in R (Gruber
214 et al., 2018) was used to conduct a Pearson PCA. Nucleotide diversity was calculated for each
215 population using the – site-pi flag in vcftools v0.1.13 (Danecek et al., 2011).
216 We also evaluated the relative contribution of spatial and environmental factors on the
217 partitioning of genetic variation in *B. vosnesenskii* using partial redundancy analysis (pRDA)
218 (Capblancq & Forester, 2021). A full RDA model was created with *rda* function in the *vegan*
219 v2.5-7 package in R using the selected environmental variables BIO1, BIO3, BIO12, elevation,
220 and using latitude as a proxy for geographic effects. The pRDA model accounting for
221 environmental effects (hereafter referred to as "Environmental pRDA") used the following
222 model (BIO1 + BIO3 + BIO12 + elevation | latitude) and the pRDA model accounting for
223 geographic distance effects (hereafter referred to as "Geography pRDA") used the following
224 model (latitude | BIO1 + BIO3 + BIO12 + elevation). We also examined models with just
225 environmental variables or latitude separately (i.e. without the partial effects), which we refer to
226 as the "Environmental RDA" and "Geography RDA", respectively. All models were assessed for
227 significance using the *anova* function in R following the protocol from (Capblancq & Forester,
228 2021).
229 **Demographic Inference**

To test whether populations from different parts of the *B. vosnesenskii* range exhibit parallel or distinct recent evolutionary histories, we inferred historical N_e trends using SMC analyses of whole genomes (H. Li & Durbin, 2011; Mather et al., 2020). SMC methods use the distribution of heterozygous and homozygous sites within loci to calculate coalescence rates that provide insight into past demographic events (Beichman et al., 2018; H. Li & Durbin, 2011). This approach is a common tool used when WGR data are available, even for small numbers of individuals, as methods can make use of even single diploid genomes (Mather et al., 2020; Nadachowska-Brzyska et al., 2016). We used PSMC (H. Li & Durbin, 2011) for demographic analyses, which is the original but still widely employed (Lozier et al., 2023; Morin et al., 2021; Nadachowska-Brzyska et al., 2016; Patil et al., 2021; Skovrind et al., 2021) SMC method, and generally followed methods in Lozier et al. (2023). PSMC uses whole genome data from single individual diploid samples to estimate coalescence rates to track N_e history. Because SMC methods generally perform best with high coverage and long scaffolds (Mather et al., 2020), we only ran PSMC for *B. vosnesenskii* workers with ≥18x mean coverage (Nadachowska-Brzyska et al., 2016) and for reference genome scaffolds ≥ 500kb in length (Gower et al., 2018). Following methods described in the PSMC manual, the PSMC input files were constructed from the filtered BAM files using Samtools v1.10 mpileup with base and mapping quality set to 30. The consensus sequence was generated using bcftools call, converted to fastq format with Samtools vcfutils.pl vcf2fq, and converted to the psmcfa format for demographic inference using the psmc program. The default p parameter describing temporal intervals of “4+25*2+4+6" was used, which is typically useful for a range of taxa (Patil & Vijay, 2021). Results were plotted using the plot_psmc.py script provided with PSMC. To convert the results into real time, we used the

252 direct mutation rate estimate of 3.6×10^{-9} per site per year from the bumble bee *Bombus*
253 *terrestris* (Liu et al., 2017) and a generation time of 1 year.

254 **Identifying environmentally associated genomic loci**

255 Environmental Association Analysis (EAA) is often used to detect genetic variants with
256 unusual allele frequency distributions that suggest a possible role in local adaption (e.g., "outlier"
257 loci)(Ahrens et al., 2018). Here we use a common EAA method, latent factor mixed modeling
258 with *LFMM2* from the *LEA* v3.0.0. R package (Gain & François, 2021), which uses a least-
259 squares approach to identify SNPs with a significant association with a given variable while
260 controlling for population structure. The likely number of population clusters (k) used for
261 background population structure control was determined with the *sMNF* function from the *LEA*
262 v3.0.0. R package (Gain & François, 2021), with k representing the value that had the smallest
263 cross-entropy selected from a range of $k = 1 – 10$. To correct for multiple testing with the large
264 number of genetic markers, raw p-values from LFMM2 were transformed into q-values using the
265 *q-value* v2.20.0 R package (Storey et al. 2022). SNPs were considered significantly associated
266 with a given variable at a threshold of $q \leq 0.05$.

267 **Results**

268 **Data summary**

269 A total of 111 individuals were sequenced to an average of 21,053,016 reads per
270 individual and variant calling yielded an initial dataset of 22,105,684 SNPs. The final dataset
271 consisted of 1,091,021 SNPs at a 5% minor allele frequency with an average coverage of 13.39x
272 SNP^{-1} $individual^{-1}$ and < 25% missing data SNP^{-1} (98.02% complete data across all samples).

273 **Population structure and demographic history**

Consistent with prior results in *B. vosnesenskii*, there was minimal population structure, with global F_{ST} = 0.001. The PCA also revealed little population structure and weak spatial clustering, but with a trend towards individual loadings related to the latitude at which the samples were collected (Fig 2a). This trend likely reflects the weak but significant isolation by distance at a range-wide scale (Mantel *r*: 0.374, *P* = 0.006; Fig 2b). The RDA models also supported minimal population structure, with all models explaining little variation, with the full model explaining only ~5% of the variation (Table 2). Neither of the partial RDA models were significant, but all three RDA models without partial effects (Full model, Environment RDA, and Geography RDA) were significant. The Geography RDA indicates that most variation is likely attributable to weak isolation by distance as suggested by above analyses, with samples clustering together by approximate sampling latitude along the x-axis (Fig. 2), but with a significant amount of unexplained variation (captured by the y-axis).

Consistent with the minimal overall population structure in the species, PSMC indicated similar N_e trajectories across samples that indicate a shared demographic history (Fig 3). Inferred population sizes were fairly stable over time, being smallest at the oldest time scales (before the last interglacial period), changing little from the Last Interglacial period to the Last Glacial Maxima aside from a modest increase just before the LGM and decline during or immediately following the LGM (Fig 3). All populations showed some degree of recent increases in N_e, although the variation among genomes in the most recent time interval is likely in part driven by challenges to modeling the most recent time segments with SMC methods (Beichman et al., 2018).

Environmentally associated outlier SNPs

296 Only a single population cluster was identified as optimal with sMNF based on the lowest
297 cross entropy, so *k*=1 was used for LFMM2. A total of 81 loci in 72 genes were found to be
298 significantly associated with at least one environmental variable. Significant SNPs were sparsely
299 spread across the genome with few genes having multiple outlier SNPs and no clear peaks of
300 association (Table 2, Fig 4). Most outlier SNPs fell within genes (60 of 81) and were principally
301 associated with annual precipitation (n = 66, BIO12), with smaller numbers of SNPs associated
302 with annual mean temperature (n = 4, BIO1) and isothermality (n = 16, BIO3). No outliers were
303 associated with elevation. BIO3 and BIO12 shared 9 outlier SNPs, but BIO1 outliers were all
304 unique.

305 Several notable genes from the LFMM2 results included LOC117231927
306 (*calcium/calmodulin-dependent protein kinase type II alpha chain*, homologous to *CaMKII* in *D.*
307 *melanogaster*) which had the most outlier SNPs (n=3; two with BIO3 and one with BIO12). This
308 gene has roles in neuronal growth, calcium signaling, as well as learning associated with appetite
309 (Gillespie & Hodge, 2013). There were also several other genes with functions relating to
310 neuronal/neuromuscular function such as LOC117231202 (*hemicentin-1*, homologous to *nrm* in
311 *D. melanogaster*) (Kania, Han, Kim, & Bellen, 1993) and LOC117241676 (*bone morphogenetic*
312 *protein 1-like*, homologous to *tok* in *D. melanogaster*) (Serpe, Ralston, Blair, & O'Connor,
313 2005). Interestingly, there were also several outliers in gene pairs with some form of known
314 interaction; LOC117231348 (*potassium voltage-gated channel protein eag*, homologous to *eag*
315 in *D. melanogaster*) is regulated by LOC117231927 (*CaMKII* in *D. melanogaster*) (Bronk et al.,
316 2018) and LOC117233891 (*dorsal-ventral patterning protein sog*, homologous to *sog* in *D.*
317 *melanogaster*) has its product cleaved by LOC117241676 (*tok* in *D. melanogaster*) (Serpe et al.,
318 2005).

319

320 **Discussion**

321 Whole-genome resequencing of *B. vosnesenskii* across large latitude and altitude
322 gradients in the western USA confirmed the presence of weak overall population structure and
323 patterns of environmentally associated genomic variation that match prior results with other
324 genetic markers (J. M. Jackson et al., 2018, 2020; Lozier et al., 2011). However, WGR data did
325 provide some new insights, indicating that populations appear to have shared fairly concordant
326 demographic histories (reflected by N_e) over time, suggesting that the minimal population
327 structure apparent today has persisted for thousands of years. Gene flow appears extensive across
328 the study area and may be a likely explanation for the relatively small amount of genomic
329 variation associated with the environmental variables and homogenous evolutionary histories
330 reflected in genomes from different regions. Although there were relatively few and sparsely
331 distributed environmentally associated outlier SNPs, there were some notable patterns
332 highlighting the potential importance of ion homeostasis and neuromuscular function that is
333 consistent with other bumble bee studies (Heraghty et al., 2022; Huml et al., 2023; Sun et al.,
334 2020). Overall, the results suggest that *B. vosnesenskii* may be an example of a species with
335 minimal population structure impeding adaptation or with weak selection over gene networks
336 that will require much more extensive sampling to resolve.

337 Analysis of population structure suggests *Bombus vosnesenskii* is nearly but not
338 completely panmictic, with weak isolation by distance and much of the explainable genetic
339 variation associated with latitudinal separation, which is consistent with previous studies
340 (Cameron et al., 2011; J. M. Jackson et al., 2018; Lozier et al., 2011). We were interested in
341 determining whether the greater resolution afforded by WGR data would provide additional

342 evidence for gene flow barriers across the sampled regions, but no major differences emerged
343 compared to prior studies. Low genetic differentiation over large spatial scales, is often observed
344 in bumble bee species in the absence of obvious physical dispersal barriers (Christmas et al.,
345 2022; Heraghty et al., 2022; J. B. Koch, Looney, Sheppard, & Strange, 2017; Lozier et al.,
346 2011). Although long distance dispersal of individual reproductives (gynes or drones) may be
347 rare (Williams et al. 2022), stepping stone dispersal through suitable habitat is likely in bumble
348 bees (Williams et al., 2022; Williams, Lobo, & Meseguer, 2018) and would contribute to weak
349 genetic structure when species inhabit continuous geographic ranges. The isolation by distance
350 observed here is consistent with such a model of stepping-stone population structure. *Bombus*
351 *vosnesenskii* is abundant and can be found at low and high elevations throughout California and
352 Oregon, and thus its range in these regions is composed almost entirely of contiguous suitable
353 habitat. Not all bumble bees show this pattern, however, and data from other species in the
354 region have higher levels of population structure. For example, *B. vancouverensis,* a species in
355 the same subgenus (Williams, Cameron, Hines, Cederberg, & Rasmont, 2008) with a similar
356 latitudinal range, but more narrow higher elevation niche at any given latitude (J. Koch et al.,
357 2012), has much greater range-wide F_{ST} based on whole genome and RADseq data (Heraghty et
358 al., 2022; J. M. Jackson et al., 2018). Importantly, however, our sampling did not include
359 strongly isolated populations, such as those on islands, or populations from many coastal regions,
360 which may harbor unique genetic variation (S. Jha, 2015). Incorporating whole genome data
361 from such populations may provide evidence of the potential for greater population structure in
362 *B. vosnesenskii*.
363 PSMC historical demographic analyses were also consistent with contemporary patterns
364 of population structure. All individuals showed highly similar historical N_e trajectories, likely

365 indicating a relatively high degree of homogeneous population structure through much of the
366 recent past. Again, results in *B. vosnesenskii* can be compared to *B. vancouverensis*, where SMC
367 analyses inferred markedly different bottleneck and expansion magnitudes across the species
368 range that were linked to major climate fluctuations and consequent changes in suitable habitat
369 areas over time that may have promoted genetic divergence (Lozier et al., 2023). For *B.*
370 *vosnesenskii*, there were relatively small signatures of genomic bottlenecks or other major N_e
371 fluctuation associated with glacial-interglacial periods, possibly suggesting that flexibility in
372 environmental requirements may facilitate population stability and near-panmixia over time.
373 This would also be consistent with the minimal environmentally associated genomic variation
374 observed here and in earlier RADseq results (Jackson et al. 2020).

375 **Environmentally associated outliers**

376 The lack of major population structure and parallel N_e histories across the *B. vosnesenskii*
377 range is mirrored by a lack of clear influence of environmental variables on overall population
378 structure or on allele frequencies at individual loci that might be produced by local adaptation.
379 Interestingly, most of the detected outliers were associated with precipitation, with smaller
380 numbers of loci associated with other variables, and none with elevation. Although, the outlier
381 loci were sparsely spread throughout the genome (Table 2, Fig 4), there were several interesting
382 cases where outlier-containing genes interacted, possibly suggesting the potential for weak
383 selection on members of gene networks. This is supported by several instances where outlier
384 genes have known regulatory interactions (e.g., LOC117231927 and LOC117231348 or
385 LOC117233891 and LOC117241676). The overall effect of selection across networks can be
386 evaluated by looking at the roles of the individual genes with detected outliers, such as the
387 number of outlier-containing genes related to neuromuscular function.

388 Given the broad latitudinal and elevational range sampled for this study, the scarcity of
389 outlier SNPs associated with thermal variables (BIO1 and BIO3) in these whole genomes was
390 somewhat unexpected but does match the less dense RADseq SNP set results from Jackson et al.
391 (2020) that included even more individuals and populations. One possible explanation is that *B.*
392 *vosnesenskii* is a noted elevation generalist (J. Koch et al., 2012; Lozier et al., 2021; Thorp,
393 Horning Jr., & Dunning, 1983), which, combined with high gene flow, may not produce strong
394 or consistent signatures adaptations for elevation or temperature stressors like isothermality
395 across the species range. The scarcity of outliers tied to elevation or temperature is similar to the
396 absence of correlations between relevant functional morphological traits (e.g., body size and
397 wing loading) with temperature or elevation observed in this species (Lozier et al., 2021).
398 However, a lack of genome regions that show clear associations with temperature is still
399 surprising given recent work on thermal tolerance physiology in *B. vosnesenskii* (Pimsler et al
400 2020) that found populations from cold, high elevation habitats had significantly lower critical
401 thermal minima (CT_{min}) compared to populations from warmer localities (Pimsler et al., 2020). It
402 is possible that selection is weak on individual genes associated with a complex trait like thermal
403 tolerance (Yang, Crossley, Schrader, & Dubovskiy, 2022), that different genes may be targeted
404 by selection in different regions (Yeaman, 2022), or that another non sequence-based mechanism
405 (e.g., epigenetic) could facilitate variation in cold tolerance among populations (Mccaw,
406 Stevenson, & Lancaster, 2020). Detecting selection under the high levels of gene flow that seems
407 prevalent in *B. vosnesenskii* will also be challenging, and more independent studies, additional
408 sampling, and experimentation will be needed to fully understand any mechanisms of genetic
409 adaptation in this species.

410 In addition to the general challenges of sample size when selection is weak on individual
411 genes, there are several other important considerations when evaluating adaptive potential of
412 individual outliers. First, environmental variation is spatially correlated, so models need to
413 consider species demography (Hoban et al., 2016). Here, population structure is weak and the
414 statistical method employed (LFMM2) accounts for population structure, which may limit major
415 effects here, but at the same time LFMM2 is a relatively conservative method (Luo, Tang,
416 Schoville, & Zhu, 2021), which might contribute to reduced power to detect outliers with weak
417 environmental associations with our modest sample size (Ahrens et al., 2018). Second,
418 correlation between environmental variables can complicate linking environmentally associated
419 loci to driving pressures. For instance, annual precipitation, which had the most outlier loci,
420 could be associated with a number of different potential pressures such as desiccation tolerance,
421 changes in biotic interactions (e.g., flowering phenology), or a number of other factors (see
422 (Chown, Sørensen, & Terblanche, 2011) for a more thorough discussion). The presence of
423 outliers that are common to multiple environmental variables (e.g., BIO12 and BIO3) highlights
424 this challenge, and indicates that even a strong signal of possible selection could be associated
425 with multiple (even unsampled) variables.
426 The outlier signatures in *B. vosnesesnskii* can be summarized by once again comparing to
427 *B. vancouverensis* from similar geographic regions (Heraghty et al., 2022; J. M. Jackson et al.,
428 2020). From both whole genome resequencing and RADseq SNPs, *B. vancouverensis* has much
429 stronger signals of environmental association across its genome, with several large peaks of
430 genomic divergence in genes with putatively relevant functions (Heraghty et al., 2022). Thus, in
431 comparison to *B. vancouverensis*, *B. vosnesenskii* has less substantial population structure, a lack
432 of population-specific demographic histories from SMC analyses, and much sparser evidence for

local adaptation. That said, our results here do reveal a few parallels between these species with respect to the functions of genes with identified outlier SNPs. For instance, the gene LOC117231202 (*hemicentin-1*) was identified as containing outlier SNPs in both species. This gene reflects the shared trend towards genes involved in neuromuscular function (Kania et al., 1993) in outlier sets for both species, which has also been observed in outlier analysis of other bumble bees (Huml et al., 2023). Although elevation and thermal variables were not as associated with outliers in *B. vosnesenskii*, given correlations among environmental variables discussed above, such processes could nonetheless reflect shared selection pressures on traits such as thermal adaptation and flight. Bumble bee flight is crucial for foraging, dispersal, and overall colony fitness (Mola et al., 2020a), while the capacity for thermogenesis by shivering their flight muscles is well-recognized in *Bombus* (Heinrich 1975). These overlaps may suggest there may be some common tools important for bumble bees that occupy spatial-environmental heterogeneity across their ranges, even if such species exhibit different intensities in evidence for selection, which would be well worth additional research.

Broader Implications

As one of the most common bees in the western U.S.A., *B. vosnesenskii* is an important pollinator in natural ecosystems as well as agricultural systems in California and Oregon (Fisher, Watrous, Williams, Richardson, & Woodard, 2022; Greenleaf & Kremen, 2006). Thus, ensuring the health of *B. vosnesenskii* populations in a changing environment is of great interest. Our results contribute to the current understanding of how *B. vosnesenskii* may fare under climate change. Contemporary analysis of *B. vosnesenskii* suggests that this species has remained stable over recent decades (Cameron et al., 2011). The historical demographic inferences from SMC modeling suggest that populations sizes across our study region have likewise been minimally

affected by the change in environmental conditions from the LIG to the LGM (Fig 3). During the LIG, conditions were warmer than present by ~1.2 °C relative to the 1998-2016 average (Lüning & Vahrenholt, 2017) whereas LGM conditions were colder by ~6 °C (Von Deimling, Ganopolski, Held, & Rahmstorf, 2006). The species' success under warmer conditions is particularly encouraging since it may indicate stability in future climates (IPCC). Recent work suggests that higher temperatures may even be beneficial to *B. vosnesenskii* and a possible factor in range expansions into British Columbia (Fraser et al., 2012; H. M. Jackson et al., 2022), with *B. vosnesenskii* representing a possible "climate winner", at least to date (Jackson et al. 2022). In addition to temperature, precipitation patterns will also shift in future climate change (IPCC). Our results suggest that annual precipitation is associated with most genomic selection signatures, and prior results found precipitation to be a key predictor of *B. vosnesenskii* range limits in species distribution models (Jackson et al. 2018). Although recent work has indicated that changes in precipitation are less likely to have an impact on future distributions of North American bumble bees, there are some exceptions (H. M. Jackson et al., 2022), and our genomic data indicate that water limitation may represent an important consideration under future climates that may warrant further study.

In summary, we have confirmed previous findings indicating there is relatively little population structure in *B. vosnesenskii* (J. M. Jackson et al., 2018) and identified relatively few environmental associated outliers which could underlie adaptation in this species. The lack of outliers could be associated with a gene swamping effect resulting from the high observed gene flow (Lenormand, 2002), although contradicts data that suggests biogeographic variation in key thermal performance metrics (Pimsler et al. 2021). For the environmentally associated outliers that were identified, future work should be conducted to evaluate their potential role in local

adaptation. Additionally, future work should be aimed at teasing apart the environmental variation that is captured by precipitation (BIO12) and how that might impact adaptation and future species distributions, given changes in precipitation patterns forecasted under climate change. Finally, more work is needed to examine other avenues for adaptation in this species, such as epigenetic modifications which may explain observed differences in cold tolerance across the range.

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Data Accessibility

Raw sequencing data is available on NCBI SRA (Bioproject PRJNA909611; accession numbers SAMN32298940- SAMN32298956, SAMN32230877 - SAMN32230970 SAMN32298940). Variant data for SNPs as well as scripts used in data filtering and analysis are available on FigShare.

Author Contributions

S.D.H. processes samples, performed statistical analysis, wrote the manuscript, and edited the manuscript. J.M.J. collected samples and helped to process samples. J.D.L. designed the study, obtained funding, conducted field work, helped with statistical analysis, and edited manuscript.

825 **Tables**

Site	Number of samples	Latitude	Longitude	Elevation (m)	π
CA01.2015	8	36.458	-118.879	313	0.2745
CA04.2012	11	41.356	-122.208	2261	0.2743
CA06.2015	11	37.012	-119.732	131	0.27478
CA11.2013	12	39.316	-120.326	2164	0.2745
CA11.2015	7	39.237	-121.451	49	0.2747
CA12.2015	10	40.508	-122.322	138	0.2746
CA13.2015	8	36.619	-118.811	2268	0.2751
CA29.2015	9	37.291	-119.102	2796	0.2746
OR02.2015	5	45.238	-123.063	53	0.2741
OR05.2016	10	45.333	-121.670	1698	0.2743
OR08.2012	2	42.076	-122.717	2055	0.2747*
OR09.2012	8	42.073	-122.754	2134	0.2747*
OR11.2012	10	42.118	-123.085	518	0.2746

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827 Table 1: Summary of sampling sites and average nucleotide diversity (π) per population. * OR08.2012 and OR09.2012 were pooled

828 together as a single population when calculating nucleotide diversity.

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A)

Model	Inertia	R^2	*p*	Proportion of explainable variance	Proportion of total variance
Full (Env + Lat)	13985	0.037	0.001	1	0.0486
Environment pRDA	11140	0.009	0.09	0.7966	0.0387
Latitude pRDA	2781		0.503	0.1989	0.0097
Confounded	64			0.0046	0.0002
Unexplained	273535				0.9514
Total	287520				1

B)

Model	Inertia	R^2	*p*	Variance Explained	Total Variance
Full (Env + Lat)	13985	0.037	0.001	0.0486	287520
Environment RDA	11205	0.009	0.001	0.0386	290301
Geography RDA	2845		0.001	0.0095	298660

Table 2: A) Results of the pRDA analysis reporting the results of the full RDA model (BIO1 + BIO3 + BIO12 + Elevation + Latitude), the results of the Environment pRDA (BIO1 + BIO3 + BIO12 + Elevation | Latitude) and the results of the Latitude pRDA (Latitude | BIO1 + BIO3 + BIO12 + Elevation). It also shows that amount of confounded variation (variation that could be associated with either pRDA model), and the amount of unexplained variation. B) Results of different the full RDA model (BIO1 + BIO3 + BIO12 + Elevation + Latitude) (note this is the same as in table 1A), the results of the Environment RDA (BIO1 + BIO3 + BIO12 + Elevation) and the result of the Latitude RDA (Latitude).

SCF	POS	LOC	Name	Fly homolog	Environmental variable
NW_022882922.1	3208914	LOC117230477	fat-like cadhherin-related tumor suppressor homolog	kug	BIO3,BIO12
NW_022882923.1	104464	LOC117232989-LOC117234423	uncharacterized - uncharacterized	#N/A - #N/A	BIO3
NW_022882923.1	713637	LOC117232142	uncharacterized	#N/A	BIO12
NW_022882923.1	4077534	LOC117234457	tropomodulin	tmod	BIOI3
NW_022882923.1	7217833	LOC117232441	protein spliit ends-like	sba	BIO12
NW_022882925.1	6861603	LOC117242995-LOC117243288	trehalase - uncharacterized	Treh - #N/A	BIO12
NW_022882926.1	2932739	LOC117243561	lysine-specific histone demethylase 1A	Su(var)3-3	BIO3,BIO12
NW_022882927.1	1136770	LOC117230390	collagen alpha chain CG42342	CG42342	BIO12
NW_022882927.1	5589210	LOC117230819	conserved oligomeric Golgi complex subunit 1	Cog1	BIO1
NW_022882927.1	7339228	LOC117230712	max dimerization protein 1-like	#N/A	BIO3,BIO12
NW_022882928.1	1315015	LOC117230941-LOC117230955	uncharacterized - protein adenylyltransferase Fic	Sox14 - Fic	BIO12
NW_022882928.1	1771666	LOC117231000	nephrin-like	#N/A	BIO12
NW_022882928.1	2109466	LOC117231079	nephrin-like	#N/A	BIO12
NW_022882930.1	642634	LOC117231202	hemicentin-1	nrm	BIO12
NW_022882930.1	1400678	LOC117231243	collageen alphda-1 (XVIII) chain	Mp	BIO12
NW_022882930.1	1701967	LOC117231281	tRNA dimethylallytransferase	CG31381	BIO12
NW_022882930.1	4543233	LOC117231348	potassium voltage-gated channel protein eag	eag	BIO3,BIO12
NW_022882930.1	5271102	LOC117231280-LOC117231225	fringe glycosyltransferase - uncharacterized	fng - CG32432	BIO12
NW_022882932.1	911460	LOC117231809	uncharacterized	#N/A	BIO12
NW_022882933.1	211778	LOC117231908-LOC117231891	innexin inx2-like - innexin inx7-like	Inx2 - Inx7	BIO12
NW_022882934.1	701863	LOC117231927	calcium/calmoodulin-dependent ptoetin kinase type II alpha chain	CaMKII	BIO3
NW_022882934.1	701882	LOC117231927	calcium/calmoodulin-dependent ptoetin kinase type II alpha chain	CaMKII	BIO3

SCF	POS	LOC	Name	Fly homolog	Environmental variable
NW_022882934.1	738415	LOC117231927	calcium/calmoodulin-dependent ptoetin kinase type II alpha chain	CaMKII	BIO12
NW_022882938.1	2188879	LOC117232181	uncharacterized	#N/A	BIO12
NW_022882938.1	2192345	LOC117232181	uncharacterized	#N/A	BIO12
NW_022882938.1	3280509	LOC117232145-LOC117232141	pre-mRNA 3'-end-processing factor FIP1 - pumilio homolog 2	#N/A - pum	BIO12
NW_022882939.1	813717	LOC117232387-LOC117232337	zinc finger MIZ domain-containing protein 1 - uncharacterized	tna - #N/A	BIO12
NW_022882942.1	154820	LOC117232587	uncharacterized	#N/A	BIO1
NW_022882945.1	670729	LOC117233040	small conductance calcium-activated potassium channel protein	SK	BIO12
NW_022882945.1	1365986	LOC117233045	class E basic helix-loop-helix proteiini 23	Oli	BIO12
NW_022882946.1	5568048	LOC117233408	lysophoospholipid acyltransferase 1	oys	BIO12
NW_022882946.1	6135136	LOC117233164	serine/threoonine-proteiin phosphatase 4 regulatory subunit 1-like	#N/A	BIO12
NW_022882947.1	1392144	LOC117233588	integrin alpha-PS2	if	BIO3
NW_022882947.1	1928801	LOC117233584	putatiive polypeptide N-acetylgalactosaimnyltransferase 9	Pgant9	BIO12
NW_022882947.1	2031308	LOC117233584	putatiive polypeptide N-acetylgalactosaimnyltransferase 9	Pgant9	BIO12
NW_022882947.1	2169738	LOC117233552-LOC117233534	multiple coagulation factor deficiency protein 2 homolog - homeobox protein prophet of Pit-1	#N/A - CG32532	BIO12
NW_022882949.1	1126918	LOC117233891	dorsal-ventral patterning protein Sog	sog	BIO12
NW_022882951.1	567652	LOC117234079-LOC117234069	uncharacterized - uncharacterized	#N/A - CG5756	BIO3,BIO12
NW_022882951.1	1054364	LOC117234181	uncharacterized	#N/A	BIO12
NW_022882953.1	2335188	LOC117234317	uncharacterized	#N/A	BIO3,BIO12
NW_022882953.1	3616066	LOC117234263	potassium voltage-gated channel subfamily KQT member 1	KCNQ	BIO1
NW_022882957.1	72400	LOC117234678-LOC117234677	BET1 homolog - KN motif and ankyrin repeat domain-containing protein 2	Bet1 - Kank	BIO12
NW_022882957.1	515680	LOC117234759-	protein lethal(2)essential for life-like - high affinity cAMP-	l(2)efl -	BIO3

SCF	POS	LOC	Name	Fly homolog	Environmental variable
		LOC117234755	specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8-like	Pde8	
NW_022882958.1	204189	LOC117234852	uncharacterized	#N/A	BIO12
NW_022882958.1	1005699	LOC117234833-LOC117234847	SAGA-associated factor 29 - uncharacterized	Sgf29 - #N/A	BIO12
NW_022882959.1	1208400	LOC117234939	microtubule-assoociated protein futsch-like	futsch	BIO12
NW_022882960.1	3778493	LOC117235117	protein cortex-like	cort	BIO12
NW_022882961.1	265994	LOC117235314-LOC117235313	uncharacterized - alpha-glucosidase-like	#N/A - #N/A	BIO12
NW_022882962.1	236847	LOC117235573	long-chaiin fatty acid transport protein 4	Fatp2	BIO12
NW_022882962.1	236867	LOC117235573	long-chaiin fatty acid transport protein 4	Fatp2	BIO12
NW_022882962.1	4277581	LOC117235535-LOC117235540	receptor-type guanylate cyclase Gyc76C-like - somatostatin receptor type 2-like	CG42637 - Astc-R2	BIO12
NW_022882964.1	1850900	LOC117235795	uncharacterized	#N/A	BIO3
NW_022882964.1	1850938	LOC117235795	uncharacterized	#N/A	BIO12
NW_022882972.1	399855	LOC117235909	ras-responsive element-biinding protein 1	peb	BIO12
NW_022882977.1	723073	LOC117236241	band 3 anion transport protein	Ae2	BIO3,BIO12
NW_022882985.1	1602088	LOC117236599	midasin	CG13185	BIO3
NW_022882988.1	1948816	LOC117236799	transient receptor potential-gamma protein	Trpgamma	BIO12
NW_022882989.1	355203	LOC117237000-LOC117237016	E3 ubiquitin-protein ligase RBBP6-like - uncharacterized	#N/A - #N/A	BIO12
NW_022882990.1	345704	LOC117237067	uncharacterized	#N/A	BIO12
NW_022882990.1	345853	LOC117237067	uncharacterized	#N/A	BIO12
NW_022882990.1	952989	LOC117237065	histamine H2 receptor-like	#N/A	BIO12
NW_022882990.1	992602	LOC117237065	histamine H2 receptor-like	#N/A	BIO12
NW_022883009.1	908150	LOC117237571	sperm flagellar protein 2-likee	#N/A	BIO12
NW_022883019.1	226656	LOC117237628-LOC117237625	uncharacterized - protein slit	#N/A - sli	BIO12
NW_022883022.1	213524	LOC117237685-	LHFPL tetraspan subfamily member 6 protein-like -	#N/A -	BIO12

SCF	POS	LOC	Name	Fly homolog	Environmental variable
		LOC117237701	uncharacterized	#N/A	
NW_022883036.1	516302	LOC117237948-LOC117237935	serine/threonine-protein kinase 17B-like - probable serine/threonine-protein kinase MARK-A	Drak - #N/A	BIO12
NW_022883058.1	145023	LOC117238319	homeotic protein spalt-major-like	salm	BIO3,BIO12
NW_022883144.1	503689	LOC117238692-LOC117238739	vesicular glutamate transporter 1 - uncharacterized	Vglut - #N/A	BIO3,BIO12
NW_022883264.1	1402456	LOC117239068-LOC117239065	uncharacterized - uncharacterized	#N/A - #N/A	BIO12
NW_022883289.1	248365	LOC117239335	uncharacterized	#N/A	BIO12
NW_022883289.1	248458	LOC117239335	uncharacterized	#N/A	BIO12
NW_022883317.1	14976	LOC117239422	uncharacterized	#N/A	BIO12
NW_022883371.1	3253233	LOC117239509	phosphatase and actin regulator 4B	CG32264	BIO12
NW_022883452.1	410515	LOC117239853	protein madd-4-like	nolo	BIO12
NW_022883531.1	1137597	LOC117240238	odorant receptor 13a-like	#N/A	BIO12
NW_022884330.1	20490	LOC117241676	bone morphogenetiic protein 1-like	tok	BIO1
NW_022884342.1	679964	LOC117242267	homeotic protein antennapedia-like	Antp	BIO12
NW_022884342.1	1078031	LOC117242285	uncharacterized	#N/A	BIO3
NW_022884343.1	3681294	LOC117242443	cAMP-specific 3',5'-cyclic phosphodiesterase-like	dnc	BIO3
NW_022884344.1	1195029	LOC117242689-LOC117242710	uncharacterized - uncharacterized	#N/A - #N/A	BIO12
NW_022884348.1	2233766	LOC117242787	uncharacterized	#N/A	BIO12

Table 3: SNPs that were identified as environmentally associated outliers noting the scaffold the SNP is located on (SCF), the position of the of the SNP (POS), the NCBI identifier (LOC), the NCBI gene name (Name), the homologous gene in *D. melanogaster* if available (Fly homolog), and the environmental variable(s) associated with the SNP.

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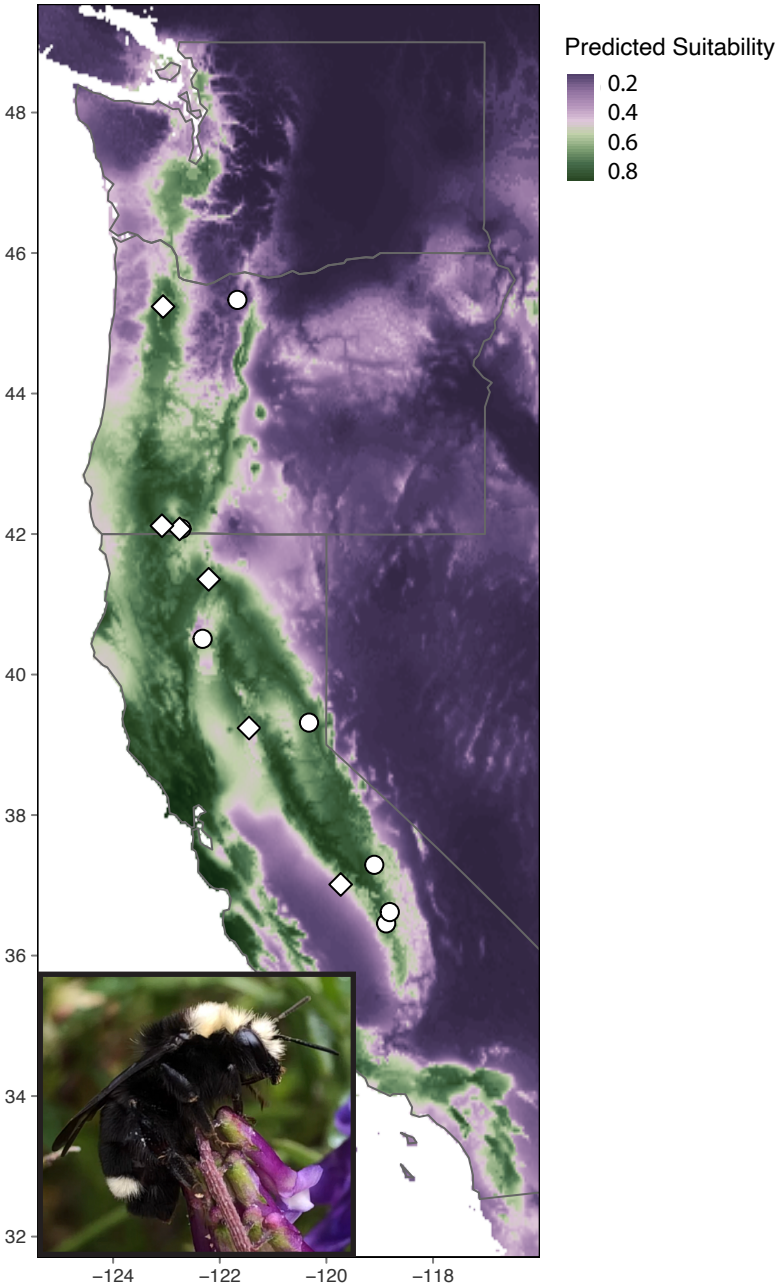
Figures

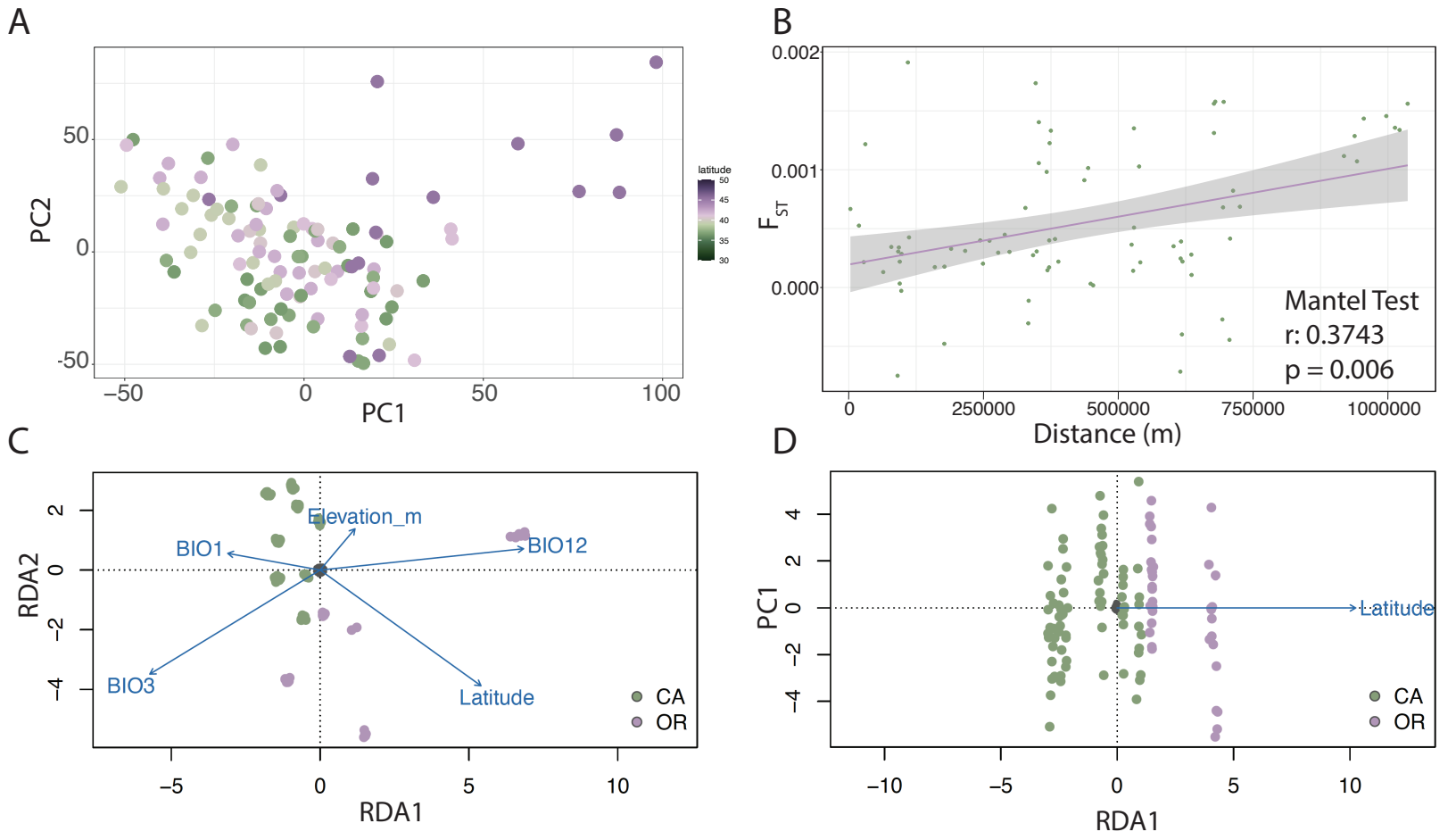
Figure 1: Map showing Maxent (Phillips, Anderson, Dudík, Schapire, & Blair, 2017) range of the *B vosnesenskii* using select environmental variables (BIO1,BIO3, and BIO12) with presence absence data from (Cameron et al., 2011). White diamonds indicate sites that were used for PSMC analysis.

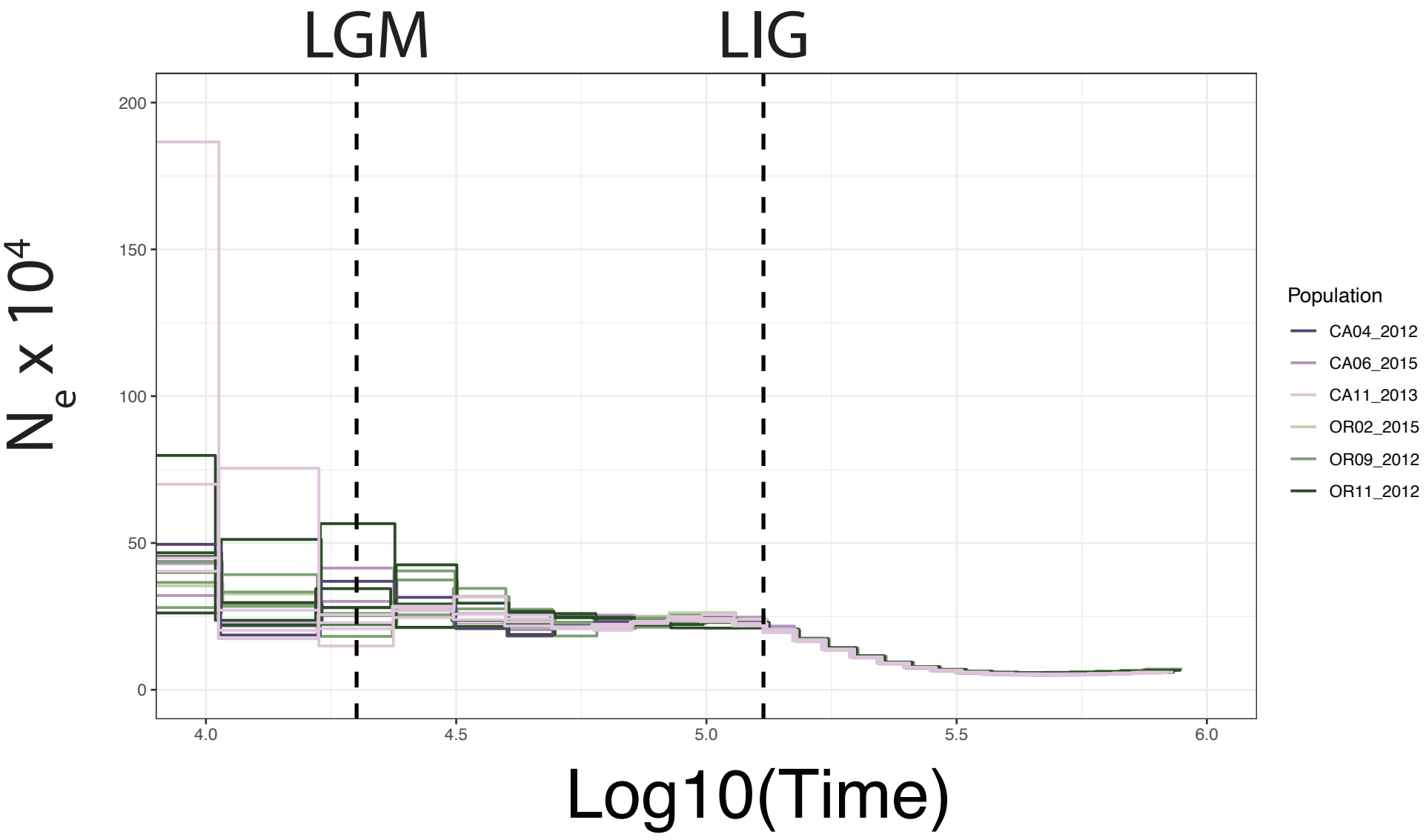
Figure 2: A) Pearson Principal Component analysis with samples colored by latitude of origin. B) Results of the isolation by distance analysis. C) The full RDA model containing all selected environmental variables as well as latitude (a proxy of distance) with samples colored by state of origin. Note this model is essentially identical to the RDA with only environmental variables (see supplemental Fig 1). D) The geography RDA model with samples colored by state of origin.

Figure 3: Demographic inference from PSMC models, marked with the approximate dates of the last glacial maximum (LGM - 20,000 years ago) and last interglacial period (LIG – 130,000 years ago)

Figure 4: Manhattan plot of LFMM2 *q*-values for each of the four selected environmental variables (BIO1, BIO3, BIO12, and elevation). The black line represents a *q*-value threshold of 0.05, which is the threshold for significance.


Predicted Suitability
0.2
0.4
0.6
0.8
48
46
44
42
40
38
36
34
32
−124
−122
−120
−118

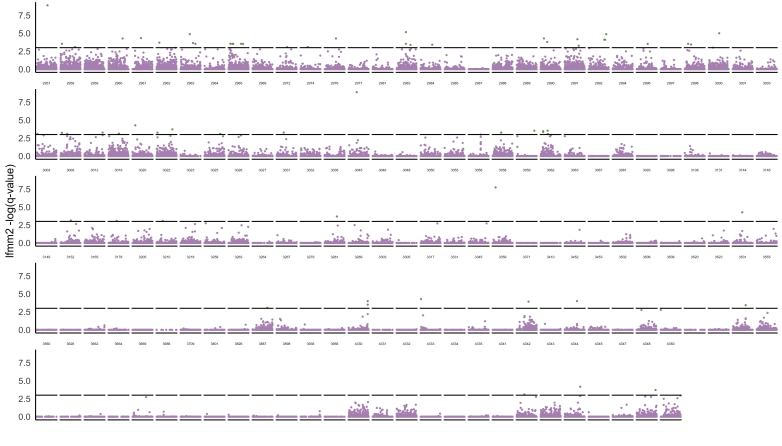
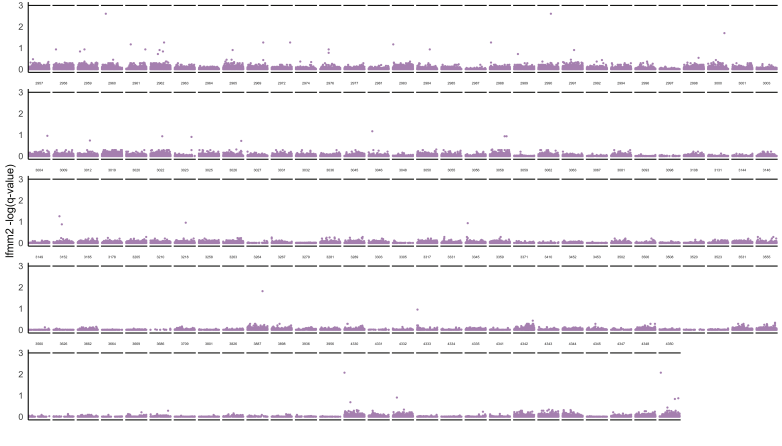

A
PC2
PC1
latitude
50
45
40
35
30
B
F_{ST}
Distance (m)
Mantel Test
r: 0.3743
p = 0.006
C
RDA2
RDA1
Elevation_m
BIO1
BIO12
BIO3
Latitude
CA
OR
D
PC1
RDA1
Latitude
CA
OR


LGM
LIG
200
150
100
50
0
4.0
4.5
5.0
5.5
6.0
$N_e \times 10^4$
Log10(Time)
Population
CA04_2012
CA06_2015
CA11_2013
OR02_2015
OR09_2012
OR11_2012

BIO1: Mean Annual Temperature - 4

BIO3: Isothermality - 16

BIO12: Annual Precipitation - 66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