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Distance, elevation and environment as drivers of diversity and divergence in bumble bees across latitude and altitude

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Abstract

Identifying drivers of dispersal limitation and genetic differentiation is a key goal in biogeography. We examine patterns of population connectivity and genetic diversity using restriction site-associated DNA sequencing (RADseq) in two bumble bee species, *Bombus vosnesenskii* and *Bombus bifarius*, across latitude and altitude in mountain ranges from California, Oregon and Washington, U.S.A. *Bombus vosnesenskii*, which occurs across a broader elevational range at most latitudes, exhibits little population structure while *B. bifarius*, which occupies a relatively narrow higher elevation niche across most latitudes, exhibits much stronger population differentiation, although gene flow in both species is best explained by isolation with environmental niche resistance. A relationship between elevational habitat breadth and genetic diversity is also apparent, with *B. vosnesenskii* exhibiting relatively consistent levels of genetic diversity across its range, while *B. bifarius* has reduced genetic diversity at low latitudes, where it is restricted to high-elevation habitat. The results of this study highlight the importance of the intersect between elevational range and habitat suitability in influencing population connectivity and suggest that future climate warming will have a fragmenting effect even on populations that are presently well connected, as they track their thermal niches upward in montane systems.

KEYWORDS

Bombus, gene flow, population genomics, RADseq, resistance modelling, SNPs

1 | INTRODUCTION

Heterogeneous landscapes can impede dispersal and isolate individuals in ways that reduce genetic connectivity and diversity and lead to reproductive isolation (Kokko, 2006; Lowe & McPeek, 2014). Identifying the drivers of dispersal limitation and genetic differentiation is a major goal in biogeography because of their conservation, ecological and evolutionary implications (Manel & Holderegger, 2013; Manel, Schwartz, Luikart, & Taberlet, 2003; Moritz, 2002; Storfer et al., 2007). Landscape genetics seeks to understand gene flow across heterogeneous landscapes by identifying how spatial genetic discontinuities relate to characteristics such as topographic barriers or environmental clines (Manel et al., 2003). Under a simple isolation-by-distance (IBD) model (Slatkin, 1993; Wright, 1943), spatially structured populations separated by increasing geographic distances are also increasingly genetically divergent. More recently, statistical approaches such as least-cost path (Arnaud, 2003; Cushman, McKelvey, Hayden, & Schwartz, 2006) and isolation-by-resistance (IBR) (McRae, 2006) analyses have been developed to provide a more functional view of connectivity and identify features that shape gene flow in realistic landscapes. Such advances allow researchers to test the effects of factors such as environment, topography and range dimensions on population divergence to determine their ecological and evolutionary significance (Manel & Holderegger, 2013; McRae & Beier, 2007).

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Determining how heterogeneous landscapes affect population connectivity is vital for understanding the biogeographic factors that generate biodiversity and its conservation, as major contributors to population declines like climate change or habitat loss isolate populations by altering the spatial arrangement of suitable habitat or environmental conditions (Butchart et al., 2010; Wilson et al., 2015). Under climate change, one way in which organisms are expected to compensate is through thermal niche tracking (DeChaine & Martin, 2004; Graham et al., 1996; Parmesan, 2006; Schoville, Roderick, & Kavanaugh, 2012; Tingley, Monahan, Beissinger, & Moritz, 2009), which might be accomplished through poleward migration or upslope migration for species in mountainous landscapes. Montane species may face specific challenges if populations become isolated in high-elevation "sky islands," unable to disperse through unsuitable intervening habitat and impeding poleward niche tracking (Brown, 1971; Lomolino, Brown, & Davis, 1989; Lozier, Strange, & Koch, 2013; Shafer, Cullingham, Côté, & Coltman, 2010). Thus, studies investigating effects from latitude, elevation and related environmental variation on genetic structure in widespread species will be critical for understanding impacts of global change on species that occur in montane habitats.

Bumble bees (Hymenoptera: Apidae: *Bombus*) are ecologically and commercially important pollinators (Garibaldi et al., 2013; Kremen, Williams, & Thorp, 2002) and have increasingly become model species for studies in ecology, biogeography and evolution (reviewed in Woodard et al., 2015). As a genus, the ~250 *Bombus* species are distributed across temperate, arctic, alpine and tropical zones (Cameron, Hines, & Williams, 2007; Williams, 1998; Woodard et al., 2015), and individual species commonly have large geographic ranges that encompass diverse environmental conditions. The social life cycle of bumble bees requires exposure of different life stages to environmental conditions from early spring through autumn that may make them particularly susceptible to the spatial–environmental heterogeneity that can influence population structure. The sequencing of two bumble bee genomes (Sadd et al., 2015) has opened the door to powerful landscape genetics studies to investigate the influence of spatial and environmental influences on genetic structure and diversity at a high genomic and spatial sampling resolution (Lozier & Zayed, 2016).

In this study, we perform a comparative population genomic analysis of two bumble bees: *Bombus vosnesenskii* Radowski and *Bombus bifarius* Cresson (Figure 1). The species belong to the subgenus *Pyrobombus* Dalla Torre (Williams, Cameron, Hines, Cederberg, & Rasmont, 2008) and are separated by about 4 million years (Cameron et al., 2007; Hines, 2008). Both species have broad altitudinal and latitudinal ranges in western North America (Koch, Strange, & Williams, 2012; Stephen, 1957; Thorp, Horning, & Dunning, 1983). We focus on mountain regions of California (CA), Oregon (OR) and Washington (WA), U.S.A., where both species are common (Cameron et al., 2011; Stephen, 1957; Thorp et al., 1983). This region is of particular utility because the north–south mountain orientation presents environmental gradients with both latitude and elevation and provides an ideal system for investigating how spatial and environmental variations interact to influence genetic structure and diversity (Halbritter, Alexander, Edwards, & Billeter, 2013; Keller, Alexander, Holderegger, & Edwards, 2013).

Studies using microsatellites have shown that *Bombus* genetic diversity is likely shaped by spatial and environmental factors in a species-specific manner across this region (Koch, Looney, Sheppard, & Strange, 2017; Lozier, Strange, Stewart, & Cameron, 2011). To investigate factors underlying such differences, we take advantage of slight differences in the abiotic and elevational niche of these broadly overlapping species. Both *B. bifarius* and *B. vosnesenskii* latitudinal ranges encompass the study area, and both species have similar altitudinal ranges overall and are often sympatric. However, *B. bifarius* is generally found at a somewhat higher and narrower band of elevations at any particular latitude in our study region. As a result, they experience a somewhat narrower and less variable bioclimatic niche, especially for temperature (Figure 1), which we hypothesize may lead to more restricted gene flow (Lozier et al., 2013). Gene flow within *B. vosnesenskii* is also sensitive to land use and cover characteristics (Jha, 2015; Jha & Kremen, 2013), despite high range-wide population connectivity (Lozier et al., 2011).

Previous studies have illuminated the role of abiotic factors on genetic diversity of bumble bees in the western United States (Jha, 2015; Koch et al., 2017; Lozier et al., 2013), however, have several limitations. For example, Lozier et al. (2011, 2013) found that genetic connectivity and diversity in *B. bifarius* were shaped by elevation and environment across western North America, but inferences are confounded by unexpectedly large phylogeographic divergence (Lozier, Jackson, Dillon, & Strange, 2016). Other studies (e.g., Jha, 2015; Koch et al., 2017) have revealed differences in genetic connectivity that correlate with elevational range, niche specificity, oceanic barriers or anthropogenic landscape modifications, but have focused on relatively small geographic scales, single species or relatively coarse sampling across latitudinal or altitude gradients. Finally, nearly all studies have relied on microsatellite or mitochondrial markers, which may have limited power to resolve subtle landscape genetic patterns (Lozier, 2014). Given the likely importance of high-elevation habitats under climate change for a wide diversity of organisms, we aim to build upon this previous work to elucidate how topographic and environmental heterogeneity within a montane region influences genetic diversity and gene flow on a large geographic scale.

We present RADseq data (Baird et al., 2008) from fine-scale sampling of latitude and elevation transects designed to distinguish between the effects of space, elevation and environmental niche dimensions. Our major hypothesis is that lower-elevation populations will be more well connected and have higher genetic diversity than high-elevation populations that might be isolated spatially or environmentally. We expect that *B. vosnesenskii* will exhibit weaker structure than *B. bifarius* given the former's generally lower and broader altitudinal range, and that the distribution of suitable habitat will be more important driver of population differentiation than distance alone, especially in *B. bifarius*. Finally, we expect that differences in niche preference and sensitivity to elevation may produce

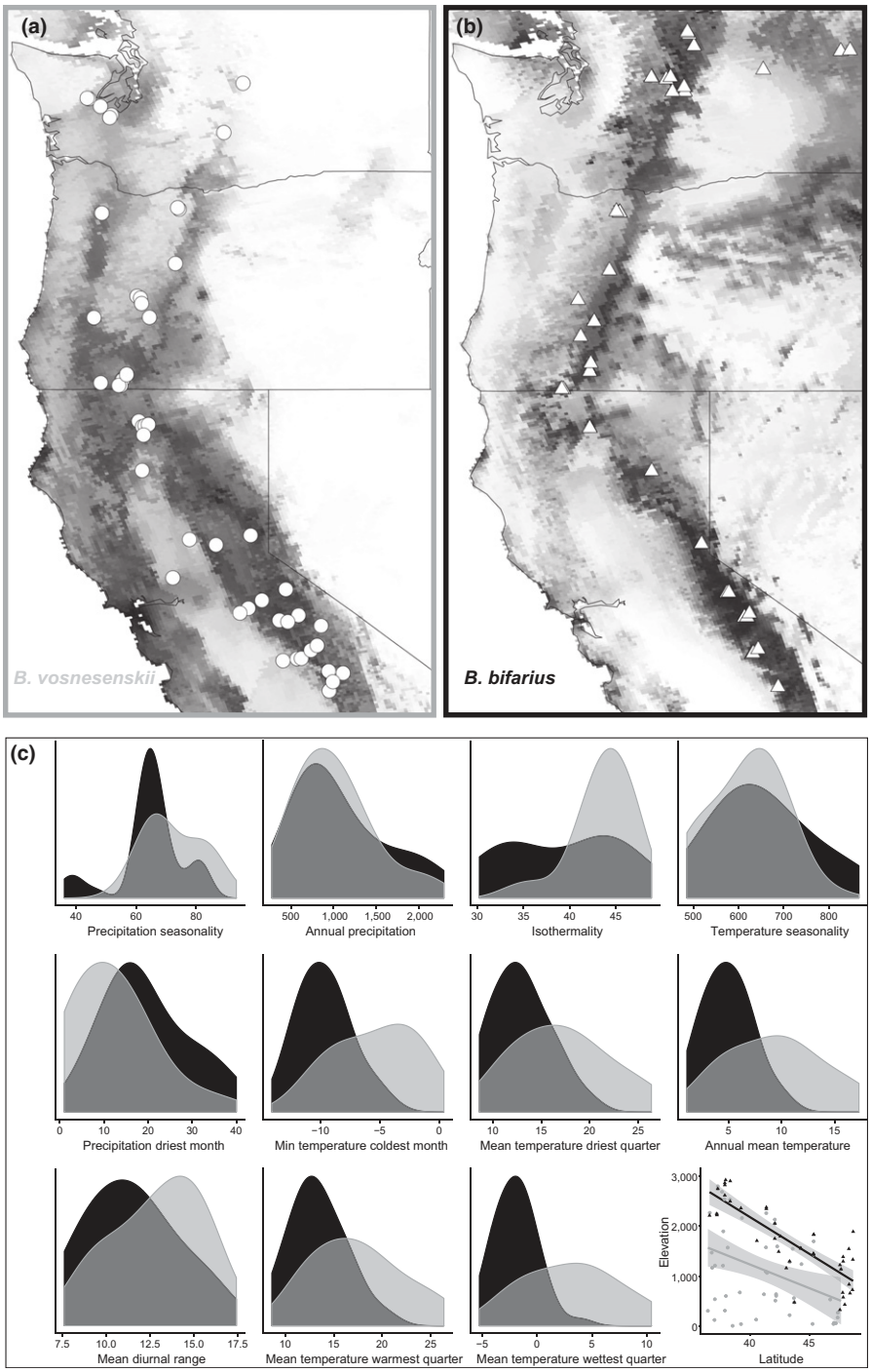

FIGURE 1 Collection sites represented in the final data set for (a) *Bombus vosnesenskii* and (b) *Bombus bifarius* displayed on the Maxent model for the species (darker shades indicate more suitable predicted habitat), with (c) density histograms of each environmental variable included in the final niche model for each species and the latitudinal by elevational range of collection sites with 99% CIs (data for plots from sites sampled in a and b)

contrasting population genomic signatures across the north–south distribution of these species.

2 | METHODS

2.1 | Study species and sampling

Bombus vosnesenskii is one of the most common bumble bee species in the Pacific coast states of the United States that comprise its range (Thorp et al., 1983). With its broad latitudinal and altitudinal distribution, from southern CA to northern WA and from the coast eastward into the Sierra-Cascades up to ~3,000 m in elevation, *B. vosnesenskii* encounters multiple environmental clines and complex topographical regimes over a large geographical area. *Bombus bifarius*, a closely related species, broadly overlaps with *B. vosnesenskii* but inhabits a larger range including much of the western United States and Canada from the Pacific coast to the Rocky Mountains and north into Alaska. In much of its range, especially in our study area, *B. bifarius* largely occupies higher elevations, although in parts of its range, outside of our focal region can be found at low

elevations. *Bombus bifarius* comprises two subspecies, *B. bifarius nearcticus* in the western portion of the range and *B. bifarius bifarius* in the east, distinguishable most clearly by abdominal coloration (Stephen, 1957). Our previous work suggests that these lineages are largely independent and may be separate species, with no obvious gene flow from *B. b. bifarius* into *B. b. nearcticus* (Lozier et al., 2016). Only *B. b. nearcticus* is found in our study area, and all samples used here belong to the "black-banded" lineage (Lozier et al., 2016; Pimsler, Jackson, & Lozier, 2017), which should limit the influences of phylogeographic structure, but for simplicity, we refer to our focal populations as *B. bifarius*, the currently accepted nomenclature.

We collected worker females from CA, OR and WA across latitude and elevation transects (Figure 1; sampling site data are provided in Supporting Information Table S1, including coordinates, sample sizes and other summary statistics). Sampling was conducted using a hierarchical approach such that each latitudinal region was sampled at both relatively low and high elevations for each species wherever possible to facilitate separation of spatial and environmental effects associated with distance, latitude and elevation. Specimens were collected via sweep-netting, placed in pure ethanol on dry ice and ultimately stored at −80°C. In total 1,124 samples ($N_{bifarius}$ = 435, $N_{vosnesenskii}$ = 689) from 87 sites ($N_{bifarius}$ = 42, $N_{vosnesenskii}$ = 50) were analysed.

2.2 | DNA extraction and RADtag sequencing

DNA extractions from thoracic muscle (Lozier, 2014) were sent to Floragenex (Portland, OR, U.S.A.) for RADseq library preparation and sequencing. Single-end libraries (size-selected to 200–400 bp, or 300–500 bp with adapters) were prepared as in Baird et al. (2008) following digestion with PstI. Roughly, half the samples were sequenced on Illumina HiSeq 2000 and half on HiSeq 4000 instruments (single-end 100-bp reads, multiplex panels of 95 uniquely indexed bees on both platforms). Replicated samples run on both platforms detected no quality issues apart from differences in sequencing coverage (approximately 40% more reads on the HiSeq 4000) and differences in base quality scores at 5′ or 3′ ends of reads, both of which were standardized during sample processing (see Section 2.3). When multiple plates were processed together, samples from each species were organized diagnostically and samples from populations and species were distributed across sequencing runs. The last sequencing plate contained four replicated individuals from each previous multiplex batch for genotyping accuracy and sample processing and sequencing quality control. We directly compared the genotypes between replicated libraries to obtain percentage similarity as an accurate assessment. Unfortunately, single-end RADseq does not permit detection of PCR duplication, which will potentially inflate mean sequencing coverage and genotype accuracy per site per sample (Andrews et al., 2014; Tin, Rheindt, Cros, & Mikheyev, 2015). However, our replicate sample library controls provide a measure of the good reliability of genotyping in this study even without paired-end sequencing (see below).

2.3 | RADtag sequence processing

FASTQ files were demultiplexed with *process_radtags* in STACKS v1.42 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). Reads were excluded if they contained an ambiguous PstI cut site, incorrect barcode or average Phred quality score <10 within a sliding window (default). Raw 100-bp reads produced for all libraries were trimmed to 79 bp using *process_radtags* and cutadapt 1.13 (Martin, 2011) by removing 16 bp at the beginning (10 bp barcode, 5 bp PstI cut site and one additional base that commonly had reduced quality scores) and 5 bp at the end of reads due to deteriorating quality in the last few bases in some HiSeq 2000 samples. Prior to mapping, we normalized library size for samples sequenced on the Illumina HiSeq 4000, which had ~40% more reads than HiSeq 2000 libraries. We used the R 3.3.3 (R Core Team 2017) package SEQINR (Charif & Lobry, 2007) to standardize high-coverage samples by randomly sampling reads without replacement such that the final number fell within one standard deviation of the mean library size for the remaining samples (Supporting Information Table S2).

2.4 | Read mapping

Libraries were aligned to the *Bombus impatiens* 2.0 genome (Sadd et al., 2015) using bwa-mem (Li, 2013). *Bombus impatiens* is a close relative of the target species (all *Pyrobombus*), and bumble bee genomes have high synteny (Sadd et al., 2015), so cross-species mapping performs well (Harpur et al., 2017; Lozier & Zayed, 2016). SAM outputs were converted to BAM and sorted with SAMTOOLS 1.2 (Li et al., 2009). We identified indels and performed local realignment (requiring ≥ 3 reads per locus) using the Genome Analysis Toolkit (GATK) (McKenna et al., 2010). Samples were assigned read groups based on sample number, locality and sequencing batch.

2.5 | SNP genotyping and analysis

Variants were called using two approaches: SAMtools *mpileup* and GATK. For GATK, we followed recommended best practices using individual and joint genotyping. Individual.g.vcf files were generated using *HaplotypeCaller* with minimum map quality of 30 and minimum confidence threshold of 10 to emit and call variant sites, and then joint-genotyped using *GenotypeGVCFs* for each species. SNP calling with SAMtools *mpileup* and BCFtools followed Lozier et al. (2016) using a map quality minimum of 30 as above. Ultimately, *mpileup* produced data sets with greater consistency between replicates, and thus, we primarily present *mpileup* results, although results were highly similar for both SNP sets (see Section 3).

We used VCFTOOLS (0.1.15; Danecek et al., 2011) for filtering of variants (Supporting Information Table S3) to ensure that RADtag data sets contained clean, high quality, single-copy loci. Sites with an individual sequencing depth with <8 in an individual were marked as missing data, as were genotypes with genotype quality scores <20. Sites with >20% missing data were removed. Second, average sequencing depth per site was ~21 in both species for both SNP

calling methods; however, a small fraction of SNPs had much higher coverage; as a step to remove paralogous regions with unusually high coverage, we removed SNPs with mean sequencing depth >42 (twice the average coverage). As a second step to eliminate paralogous loci, we removed a small number of sites with greater than 60% observed heterozygosity (e.g., Lozier et al., 2016 and Taylor, Curry, White, Ferretti, & Lovette, 2014) as calculated with the VCFtools *hwe* function; all SNPs above this threshold had highly significant heterozygosity excess, and this level of observed heterozygosity represented an empirical inflection point above which Hardy-Weinberg *p*-values decreased dramatically (Hardy-Weinberg statistics for the final data set are available on DRYAD). Of the remaining sites, those with minor allele frequency (MAF) <1% across all individuals were removed. SNPs were annotated to the *Bombus impatiens* 2.0 genome (Sadd et al., 2015) using SNPEff (Cingolani et al., 2012). Using these annotations, we then manually assigned SNPs to broader categories (synonymous, nonsynonymous, intron and intergenic) to examine results across different SNP classes within the genome.

2.5.1 | Identifying colony-mate siblings and analysing replicate samples

Bumble bees are social insects, and sampling multiple workers in close proximity can produce multiple full-siblings from the same colony. We identified probable sibling groups using pairwise relatedness from *relatedness2* (Manichaikul et al., 2010) in VCFtools. Pairs with relatedness >0.20 (output values can range from 0 to 0.5) were considered as probable sibs; all such pairs occurred within sites, and no between-site pairs approach this threshold, which was found to be suitable based on analysis of SNP data from siblings in a related study of laboratory colonies (personal observation; also see Lozier et al., 2016). In all sib-pairs, the bee with lower mean sequencing depth was removed from the final data set. Likewise, for replicated samples, the individual replicate with the lowest mean sequencing depth was removed. After removal of siblings and replicates, the data sets were refiltered using the above criteria to remove artefacts to produce final data sets with unique, nonsibling individuals (383 bees from 42 sites for *B. bifarius* and 587 bees from 50 sites for *B. vosnesenskii*).

2.5.2 | Genetic diversity

To test how genetic diversity varies spatially across the study region, we calculated mean nucleotide diversity (π) at each SNP. For this analysis, we created a "thinned data set" including populations with ≥5 samples and only one SNP per 1,000 bp. To test the relationships between π and spatial variables for each population, we used linear mixed-effect models (LMM) to account for among nucleotide specific variation by specifying genomic position as a random effect, for example, Koch et al. (2017). We constructed the models with the *lmer* function in the LME4 v4 1.1-14 (Bates, Mächler, Bolker, & Walker, 2015) package for R, specifying nucleotide position as the random effect and comparing models with latitude, elevation, latitude and elevation; and latitude, elevation and their interaction as fixed effects, with LMERTEST 2.0-33 (Kuznetsova, Brockhoff, & Christensen, 2017) used to determine significance and MUMIN 1.40.0 (Bartoń, 2018) to calculate R^2.

2.5.3 | Genetic differentiation

Individual-based population structure was determined with the Bayesian population assignment algorithm sNMF (Frichot, Mathieu, Trouillon, Bouchard, & François, 2014) distributed with the R package LEA 2.0 (Frichot & François, 2015). The number of putative clusters (*K*) was allowed to range from 1 to 10, with two repetitions of each for a maximum of 200 iterations. The minimum cross-entropy was used to decide the most suitable value of *K*. We also performed principle components analysis in ADEGENET 2.1.0 (Jombart, 2008; Jombart & Ahmed, 2011) to visualize genomic similarity among individuals. Input files were prepared for R using VCFR (Knaus & Grünwald, 2017).

To explore spatial effects on population differentiation, we ran GESTE 2.0 (Foll & Gaggiotti, 2006) on the thinned SNP sets (for computational efficiency). GESTE estimates a population-specific F_{ST} for each population using the *F*-model (Gaggiotti & Foll, 2010) and tests for effects of variables on population structure using generalized linear models. We elected to include only elevation and latitude as variables for this analysis, as they are the principle spatial dimensions in which our samples vary, encompass a large amount of environmental variation and also enable specification of an interaction term (GESTE only allows interactions for models with two main factors).

Populations separated by increasing geographic distances are often expected to exhibit increasing genetic differentiation (IBD). However, over the study area, elevation and environmental conditions vary considerably and, in cases, rapidly, which should create a nonuniform spatial projection of the species' environmental niches. Therefore, we hypothesize that elevation and the distribution of suitable habitat (as determined by environmental conditions) would both have an effect on genetic differentiation. To investigate effects of geographic distance, elevation and niche distribution on genomic differentiation, we test for IBD and IBR (McRae, 2006). For each species, we calculated pairwise Weir and Cockerham's (1984) F_{ST} among populations with ≥5 genotyped bees using GENEPOP 4.7.0 (Rousset, 2008).

We tested for the presence of IBD using a Mantel test in R package ecodist 2.0.1. We used circuit theory, implemented in CIRCUITSCAPE 4.0 (McRae, 2006; Shah & McRae, 2008), to generate pairwise resistance distances for testing IBR, which effectively reflects the predicted gene flow potential between the populations. To parameterize Circuitscape for testing the influence of spatial ecological niche dimensions on genetic connectivity, we developed an ecological niche model (ENM) for both species using 11 bioclimatic variables at 30-s resolution from WORLDCLIM 2.0 (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). The variables represent a subset of the available 19 bioclimatic variables selected by excluding one from each pair having a

Pearson's correlation coefficient >0.9, as calculated using SDM TOOLBOX 2.2 (Brown, 2014) in ARCMAP 10.2 (ESRI) (Supporting Information Table S4). We developed the model using MAXENT 3.4.1 (Phillips, Anderson, & Schapire, 2006) with default parameters, with 10 replicate runs and cross-validation used to test the model and jackknife analysis performed to determine variable importance. The area under the receiver operator characteristic curve (AUC) was used to evaluate model results. Unique occurrence data (573 *B. bifarius* and 825 *B. vosnesenskii* records with no more than one per raster grid cell per species) were compiled from collections for this study and extensive digitized natural history records from Cameron et al. (2011). We constructed models using the Alber's equal area North America conic projection to minimize model biases produced by nonrandom background sampling that occurs when map cells are not uniform in size (Elith et al., 2011). The logistic output raster from the ENM was used as a conductance surface for Circuitscape, which should represent the effects of both distance and environmental habitat suitability between each site pair. To test for IBD within the Circuitscape resistance framework, analyses were also performed with a conductance layer where all land cells are given values of 1.0 (uniform conductance). We also ran separate analyses examining the effect of elevation on population connectivity via an elevation resistance map. A digital elevation layer of the study region was obtained from worldclim.org (30-s resolution), and all cell values divided by the maximum cell value to produce a range of 0–1, following Jha (2015).

To compare models for the distance matrices, we used maximum-likelihood population effects (MLPE) (Clarke, Rothery, & Raybould, 2002). MLPE modelling accounts for the nonindependence of pairwise comparisons by allowing population-specific effects with random effect terms for each population (Clarke et al., 2002). MLPE modelling was performed using NLME 3.1-133 (Pinheiro, Bates, DebRoy, & Sarkar, 2018) with CORMLPE (Jaffé et al., 2016; Reid, Mladenoff, & Peery, 2017; available at https://github.com/nspope/corMLPE). Models were run for each independent variable (environmental resistance, flat resistance and elevation resistance) against $F_{ST}/(1 - F_{ST})$. Model selection for the MLPE method was conducted via Bayesian information criterion (BIC).

Finally, we wanted to test the robustness of results to genome location of SNPs, which might influence their neutrality and resulting demographic inference. First, we examined how genetic patterns vary across SNP classes in the genome by calculating expected heterozygosity (H_E), Weir & Cockerham's F_{ST} (average F_{ST}) and their 95% confidence intervals in the R package HIERFSTAT 0.4-22 (Goudet, 2005) with all populations pooled but SNPs divided into synonymous, nonsynonymous, intron and intergenic classes based on SNPeff annotations. We also repeated IBD/IBR analyses with these divisions. Next, we repeated analyses on a "neutral" SNP set by removing putative targets of selection via outlier analysis. The goal is not to perform functional analysis of SNPs under selection (see Section 4), but rather to create a filtered data set with potential targets of selection removed to investigate effects on demographic analyses. This neutral set was constructed by removing potential outlier SNPs detected with latent factor mixed modelling in LFMM (part of LEA package in R) (Frichot, Schoville, Bouchard, & François, 2013). Preliminary analysis suggested LFMM was a highly sensitive outlier detection strategy likely to remove true targets of selection (and inversely retain truly neutral SNPs), although at the risk of removing false positives (see Section 4). For each species, we tested for significant SNP associations with the top five environmental variables (percentage contribution to the model) in the respective ENM (Supporting Information Table S4). To control for population structure, we specified a number of latent factors (K) equal to the number of clusters identified by sNMF plus one ($K = 2$ for *B. vosnesenskii* and $K = 3$ for *B. bifarius*). We ran LFMM for 10,000 iterations with 5,000 iterations as burn-in, adjusted p-values using the genomic inflation factor and consider environmental associations significant at a false discovery rate of 0.05.

3 | RESULTS

3.1 | Data set characteristics

We sequenced 1,124 samples ($N_{bifarius} = 435$, $N_{vosnesenskii} = 689$) from 87 sites ($N_{bifarius} = 42$, $N_{vosnesenskii} = 50$). 93.97% of reads per sample passed quality processing with Stacks and approximately $1.59 \times 10^6 \pm 4.94 \times 10^5$ *SD* reads per sample were passed to bwa-mem after quality control and normalization of HiSeq 4000 runs (Supporting Information Table S2). Initial variants were filtered to produce 16,430 and 18,700 SNPs for *B. vosnesenskii* and 32,073 and 37,474 SNPs for *B. bifarius* from GATK and *mpileup*, respectively (Supporting Information Table S3). Relatedness analysis resulted in removal of 34 and 59 individuals for *B. bifarius* and *B. vosnesenskii* as putative siblings, with 92% (*SD* = 12%) and 90% (*SD* = 16%) of bees at each site from unique colonies for *B. bifarius* and *B. vosnesenskii*, respectively. Final data sets comprised 383 bees from 42 sites for *B. bifarius* (bees per site = 9 ± 4 *SD*) and 587 bees from 50 sites (bees per site = 12 ± 4 *SD*) for *B. vosnesenskii*.

For replicated samples, the original and duplicate had similar genotypes recovered from both SNP calling methods: 99.14% ± 0.2% *SD* and 99.08% ± 0.2% *SD* for *B. bifarius* from *mpileup* and GATK and 98.06% ± 2.5% *SD* and 97.78% ± 2.6% *SD* for *B. vosnesenskii* from *mpileup* and GATK (Supporting Information Table S5), suggesting that library preparation and sequencing were highly repeatable. Because *mpileup* was slightly more consistent than GATK (Supporting Information Table S5), we primarily present these results, although results were highly similar for GATK (Supporting Information Figures S1–S4 and Table S6).

3.2 | Genetic diversity over latitude and elevation

Variation (H_E from Hierfstat) was lower in *B. vosnesenskii* than in *B. bifarius*, with nonoverlapping 95% CIs (Table 1). Average π per SNP per population ranged from 0.105 to 0.116 in *B. vosnesenskii* and from 0.122 to 0.140 in *B. bifarius*. For *B. vosnesenskii*, π declined slightly with increasing latitude, whereas π increased more strongly with latitude in *B. bifarius*. The two species also showed differing patterns with respect to elevation. Average population π decreased

TABLE 1 F_{ST} and H_E estimates with 95% confidence intervals calculated in hierfstat

Species	SNP category	F_{ST}	H_E	N_{SNPs}
B. bifarius	All SNPs	0.0187 (0.0182–0.0193)	0.1430 (0.1416–0.1445)	37,474
	Intergenic	0.0193 (0.0183–0.0202)	0.1429 (0.1407–0.1453)	14,729
	Intron	0.0181 (0.0174–0.0189)	0.1434 (0.1414–0.1454)	19,328
	Nonsynonymous	0.0227 (0.0169–0.0301)	0.1369 (0.1262–0.1485)	598
	Synonymous	0.0194 (0.0174–0.0218)	0.1435 (0.1378–0.1496)	2,117
	Neutral (LFMM outliers removed)	0.0109 (0.0105–0.0112)	0.1061 (0.1049–0.1073)	31,887
B. vosnesenskii	All SNPs	0.0027 (0.0025–0.003)	0.1207 (0.1189–0.1224)	18,700
	Intergenic	0.0029 (0.0025–0.0034)	0.1201 (0.1170–0.1233)	7,099
	Intron	0.0024 (0.002–0.0027)	0.1220 (0.1195–0.1245)	9,513
	Nonsynonymous	0.0029 (0.001–0.0049)	0.1014 (0.0899–0.1143)	356
	Synonymous	0.0035 (0.0025–0.0044)	0.1239 (0.1172–0.1312)	1,312
	Neutral (LFMM outliers removed)	0.0017 (0.0014–0.0020)	0.0948 (0.0933–0.0965)	16,548

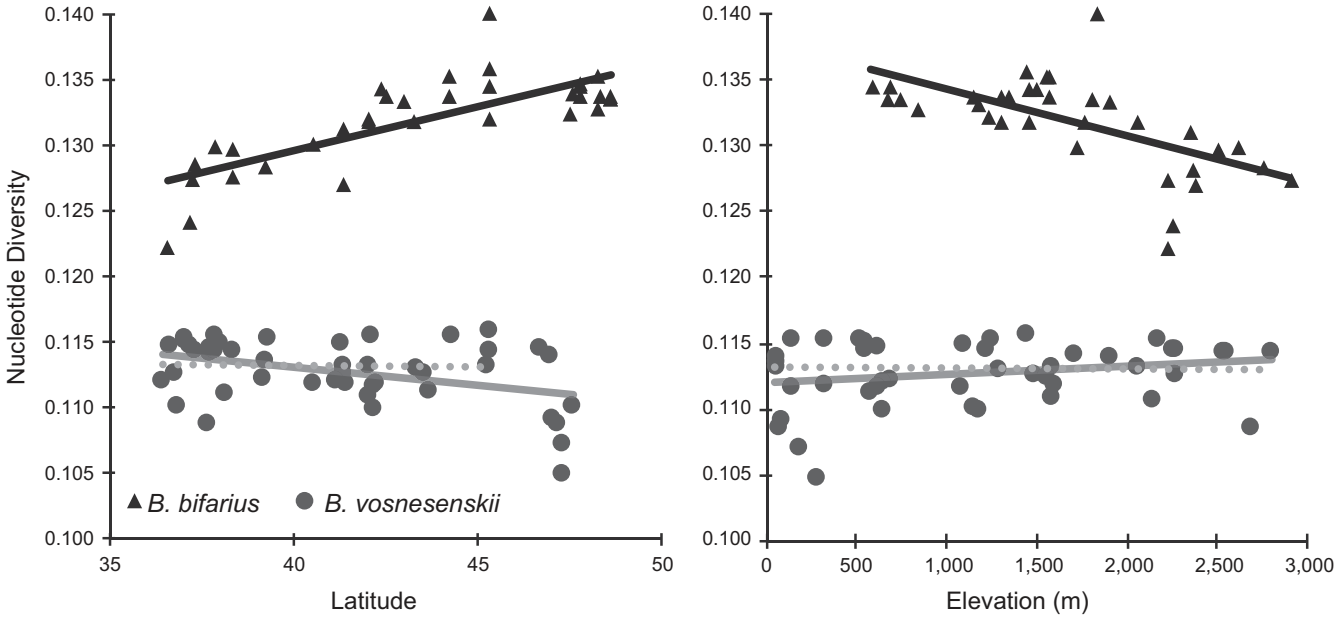

FIGURE 2 Population average nucleotide diversity (π) of thinned data sets (only one SNP per kb) for both species by elevation and latitude. Solid lines are trend lines for each species. The dotted line represents the trend line with WA populations omitted for *Bombus vosnesenskii* and shows that the relationship with spatial variables disappears when omitting these populations (see Table 2)

with elevation in *B. bifarius* but increased slightly with elevation in *B. vosnesenskii* (Figure 2). For both species, LMMs including latitude, elevation and their interaction provided the best explanation for π (Table 2). Unlike *B. bifarius*, where the diversity trend was consistent across the full range of both spatial variables; however, slopes in *B. vosnesenskii* were flatter and diversity declines appear largely restricted to a subset of populations in WA (also see Section 3.3 for a comparable result). A similar pattern was observed in WA populations in a previous microsatellite study, which hypothesized recent nonequlibrium dynamics (e.g., population expansion) in these historically rare northernmost populations (Lozier et al., 2011; Stephen, 1957). We thus repeated analyses without WA sites for *B. vosnesenskii* to avoid potential bias of more general conclusions by a geographically restricted phenomenon. In this reduced data set, the full model is still recovered as best by AIC; however, latitude and elevation effects are not significant, only their interaction, and the models are not as clearly distinguishable (e.g., ΔAIC between the full model and the random effect only model was 98 in the full population set and 8 with WA excluded), suggesting a reduced relationship between nucleotide diversity and latitude or elevation.

3.3 | Population structure

Bombus vosnesenskii exhibited weaker population structure (F_{ST} = 0.003, $p < 0.001$) than *B. bifarius* (F_{ST} = 0.019, $p < 0.001$) (Table 1), with nonoverlapping confidence intervals. For both species,

TABLE 2 LMM values for nucleotide diversity with random effect of SNP position. Models are ordered by Δ AIC within each species

Species		Δ AIC	Parameter estimate	*SE*	*df*	*t*	Pr(>|*t*|)
B. bifarius	Formula: pi ~ elevation scaled × latitude scaled + (1|chrom_pos)						
	(Intercept)	0	1.32×10^{-1}	1.08×10^{-3}	1.60×10^{4}	1.23×10^{2}	$<2.00 \times 10^{-16}$
	Elevation		1.13×10^{-4}	2.38×10^{-4}	5.13×10^{5}	4.73×10^{-1}	6.36×10^{-1}
	Latitude		2.58×10^{-3}	2.44×10^{-4}	5.13×10^{5}	1.06×10^{1}	$<2.00 \times 10^{-16}$
	Elevation × Latitude		1.06×10^{-3}	1.56×10^{-4}	5.13×10^{5}	6.8	1.11×10^{-11}
	Formula: pi ~ latitude scaled + (1|chrom_pos)						
	(Intercept)	44	1.31×10^{-1}	1.07×10^{-3}	1.56×10^{4}	1.23×10^{2}	$<2.00 \times 10^{-16}$
	Latitude		2.74×10^{-3}	1.30×10^{-4}	5.13×10^{5}	2.11×10^{1}	$<2.00 \times 10^{-16}$
	Formula: pi ~ latitude scaled + elevation scaled + (1|chrom_pos)						
	(Intercept)	44	1.31×10^{-1}	1.07×10^{-3}	1.56×10^{4}	1.23×10^{2}	$<2.00 \times 10^{-16}$
	Latitude		2.99×10^{-3}	2.37×10^{-4}	5.13×10^{5}	1.26×10^{1}	$<2.00 \times 10^{-16}$
	Elevation		2.98×10^{-4}	2.37×10^{-4}	5.13×10^{5}	1.26	2.7×10^{-1}
	Formula: pi ~ elevation scaled + (1|chrom_pos)						
	(Intercept)	202	1.31×10^{-1}	1.07×10^{-3}	1.56×10^{4}	1.23×10^{2}	$<2.00 \times 10^{-16}$
	Elevation		-2.10×10^{-3}	1.30×10^{-4}	5.13×10^{5}	-1.70×10^{1}	$<2.00 \times 10^{-16}$
	Formula: pi ~ (1|chrom_pos)						
		489					
B. vosnesenskii	Formula: pi ~ elevation scaled × latitude scaled + (1|chrom_pos)						
	(Intercept)	0	1.13×10^{-1}	1.20×10^{-3}	1.03×10^{4}	9.39×10^{1}	$<2.00 \times 10^{-16}$
	Elevation		4.90×10^{-4}	1.36×10^{-4}	4.51×10^{5}	3.61	3.02×10^{-4}
	Latitude		-5.37×10^{-4}	1.38×10^{-4}	4.51×10^{5}	−3.89	1.04×10^{-4}
	Elevation × Latitude		7.95×10^{-4}	1.30×10^{-4}	4.51×10^{-5}	6.1	1.07×10^{-9}
	Formula: pi ~ latitude scaled + elevation scaled + (1|chrom_pos)						
	(Intercept)	35	1.13×10^{-1}	1.20×10^{-3}	1.02×10^{-4}	9.37×10^{1}	$<2.00 \times 10^{-16}$
	Latitude		-8.54×10^{-4}	1.28×10^{-4}	4.51×10^{5}	−6.67	2.62×10^{-11}
	Elevation		2.19×10^{-4}	1.28×10^{-4}	4.51×10^{5}	1.71	8.75×10^{-2}
	Formula: pi ~ latitude scaled + (1|chrom_pos)						
	(Intercept)	36	1.13×10^{-1}	1.20×10^{-3}	1.03×10^{4}	9.37×10^{1}	$<2.00 \times 10^{-16}$
	Latitude		-9.40×10^{-4}	1.18×10^{-4}	4.51×10^{5}	−7.97	1.55×10^{-15}
	Formula: pi ~ elevation scaled + (1|chrom_pos)						
	(Intercept)	78	1.13×10^{-1}	1.20×10^{-3}	1.03×10^{4}	9.37×10^{1}	$<2.00 \times 10^{-16}$
	Elevation		5.53×10^{-4}	1.17×10^{-4}	4.51×10^{5}	4.69	2.76×10^{-6}
	Formula: pi ~ (1|chrom_pos)						
		98					

(Continues)

latitudinal separation appeared to be the major axis of genetic differentiation. The PCA for *B. vosnesenskii* produced some weak latitudinal separation on PC1, consistent with the low global F_{ST}. The PCA for *B. bifarius* more clearly separated individuals geographically, with clusters corresponding to nearby mountains and structured by latitude along PC1. sNMF indicates a single ancestral cluster for *B. vosnesenskii*, while *B. bifarius* is split into two clusters, with membership again corresponding strongly with latitude (Figure 3). No additional subdivision was apparent with separate analyses of northern and southern clusters (separating individuals based on >50% cluster membership in $K = 2$ analysis) (e.g., Janes et al., 2017).

We used GESTE to test for specific contributions of latitude and elevation on population structure (Figure 4). *F*-model F_{ST}'s were also lower in *B. vosnesenskii* (mean $F_{ST} = 0.005$ with a range of 0.000–0.036 per site) than *B. bifarius* (mean $F_{ST} = 0.018$ with a range of 0.004–0.054 per site). WA sites account for the upper portion of the range for *B. vosnesenskii* (average WA $F_{ST} = 0.023 \pm 0.011$ *SD*), with the values outside of WA being much lower (average CA/OR $F_{ST} = 0.002 \pm 0.002$ *SD*). The most probable model was the latitude-only model, although with weak support (constant + latitude; posterior prob. = 0.56). With WA populations removed as above, the latitude-only model was still the best supported, but with much

TABLE 2 (Continued)

Species		Δ AIC	Parameter estimate	*SE*	*df*	*t*	Pr(>|*t*|)
B. vosnesenskii (no WAsites)	Formula: pi ~ elevation scaled × latitude scaled + (1|chrom_pos)						
	(Intercept)	0	1.20×10^{-1}	9.30×10^{-4}	1.84×10^{4}	1.29×10^{2}	$<2.00 \times 10^{-16}$
	Elevation		-5.39×10^{-5}	9.87×10^{-5}	6.80×10^{5}	-5.46×10^{-1}	5.85
	Latitude		-1.36×10^{-4}	9.30×10^{-5}	6.80×10^{5}	−1.47	1.42×10^{-1}
	Elevation × Latitude		3.03×10^{-4}	1.03×10^{-4}	6.80×10^{5}	2.95	3.14×10^{-3}
	Formula: pi ~ latitude scaled + elevation scaled + (1|chrom_pos)						
	(Intercept)	7	1.20×10^{-1}	9.30E−04	1.84×10^{4}	1.29×10^{2}	$<2.00 \times 10^{-16}$
	Latitude		-1.66×10^{-4}	9.24E−05	6.80×10^{5}	−1.79	7.30×10^{-1}
	Elevation		-1.56×10^{-4}	9.24E−05	6.80×10^{5}	−1.69	9.20×10^{-1}
	Formula: pi ~ latitude scaled + (1|chrom_pos)						
	(Intercept)	8	1.20×10^{-4}	9.30×10^{-4}	1.84×10^{4}	1.29×10^{2}	$<2.00 \times 10^{-16}$
	Latitude		-1.42×10^{-4}	9.14×10^{-5}	6.80×10^{5}	−1.55	1.21×10^{-1}
	Formula: pi ~ elevation scaled + (1|chrom_pos)						
	(Intercept)	8	1.20×10^{-1}	9.30×10^{-4}	1.84×10^{4}	1.29×10^{2}	$<2.00 \times 10^{-16}$
	Elevation		-1.31×10^{-4}	9.14×10^{-5}	6.80×10^{5}	−1.43	1.53×10^{-1}
	Formula: pi ~ (1|chrom_pos)						
		8					

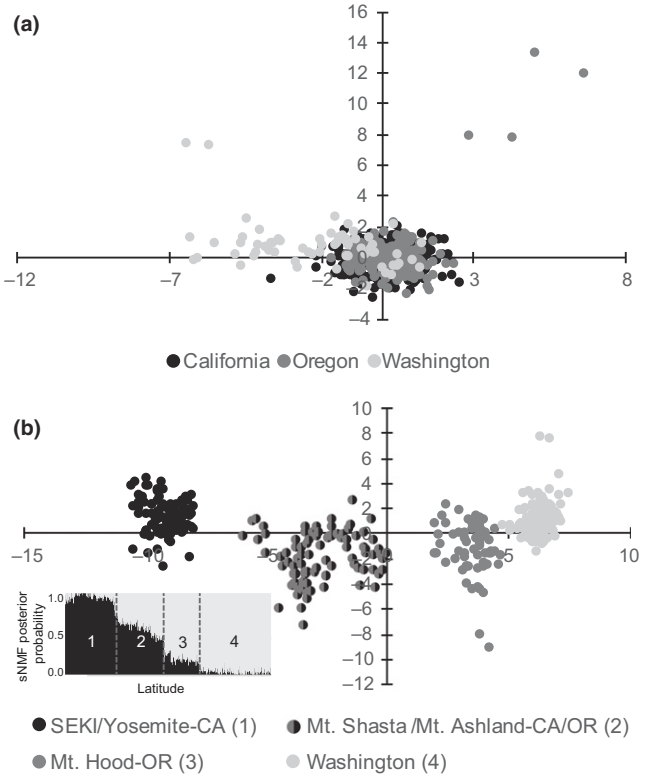

FIGURE 3 (a) PCA for *Bombus vosnesenskii* colour coded by state, showing a slight relationship with latitude but overall a large cloud of points. (b) PCA for *Bombus bifarius* with points coloured based on geographic region corresponding to the state in which they are located according to the colour scheme from (a) and sNMF plot as inset and geographic regions number coded and delimited by vertical dashed lines

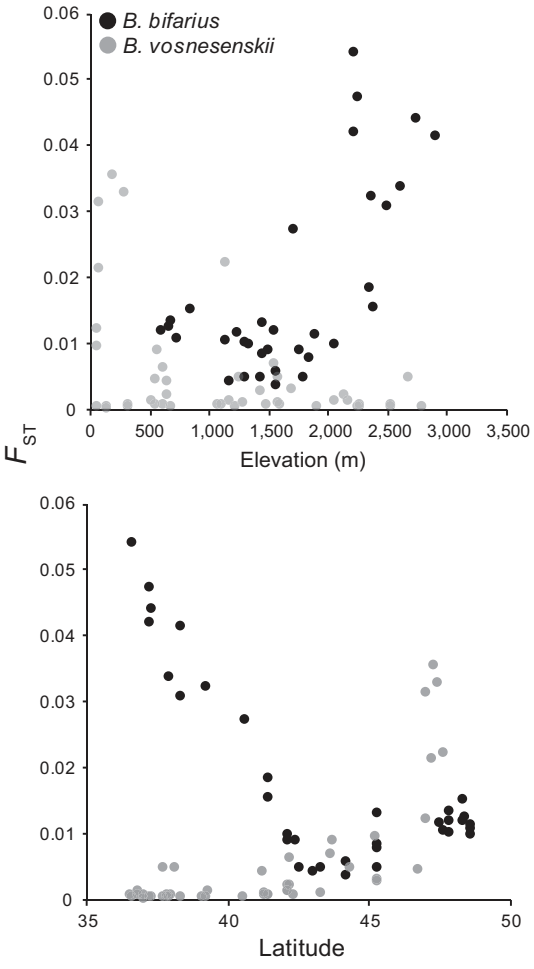

FIGURE 4 *Bombus bifarius* and *Bombus vosnesenskii* GESTE *F*-model F_{ST} by elevation and latitude

TABLE 3 GESTE model probabilities for *B. vosnesenskii*, *B. bifarius* and *B. vosnesenskii* without WA sites

Model	*B. bifarius* probability	*B. vosnesenskii* probability	*B. vosnesenskii* probability without WA
Constant	0.000	0.000	0.004
Constant, latitude	0.000	0.555	0.914
Constant, elevation	0.000	0.000	0.000
Constant, elevation and latitude	0.000	0.038	0.061
Constant, latitude × elevation, elevation and latitude	1.000	0.407	0.022

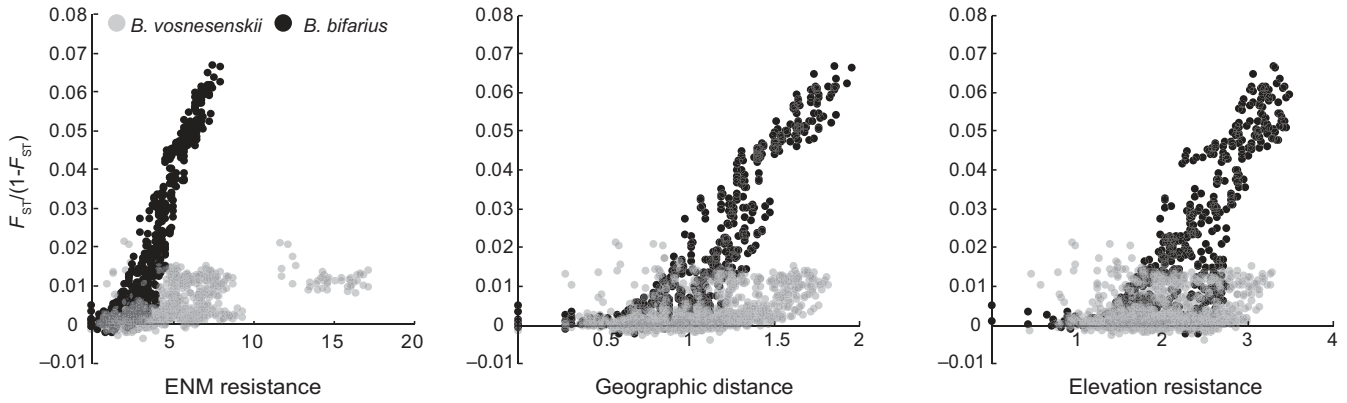

FIGURE 5 Pairwise linear F_{ST} by Circuitscape ENM resistance, "flat" resistance (i.e., geographic distance) and elevation resistance

stronger support (posterior prob. = 0.91). The *F*-model F_{ST} pattern was more complex for *B. bifarius*, varying with both latitude and elevation, especially >2,000 m, and the most highly supported model was the full model (constant + latitude + elevation + latitude × elevation; posterior prob. = 1.0) (Table 3).

Pairwise linearized F_{ST} increased with pairwise geographic distance in both species, indicating a degree of IBD that was clearly stronger in *B. bifarius* (Mantel $r = 0.94$ for *B. bifarius* and 0.40 for *B. vosnesenskii*, both $p < 0.001$). Pairwise F_{ST} also increased with pairwise ENM resistance distance, pairwise elevation resistance and pairwise flat resistance (a proxy for geographic distance in Circuitscape) (Figure 5). MLPE analysis found that ENM resistance models were best supported in both species, suggesting that the configuration of suitable habitat across the species ranges explains the degree of genomewide genetic differentiation better than geographic distance alone (Table 4). *Annual Precipitation* contributed

TABLE 4 MLPE model selection BIC for each resistance model and SNP Class

Species	Category	Δ BIC	ENM	Flat	Elevation
B. bifarius	All SNPs	243	−3,946[a]	−3,703	−3,602
	Intergenic	237	−3,895[a]	−3,658	−3,560
	Nonsynonymous	187	−3,515[a]	−3,328	−3,247
	Intron	245	−3,984[a]	−3739	−3,638
	Synonymous	194	−3,821[a]	−3,627	−3535
	Neutral	210	−4,347[a]	−4,137	−4,058
B. vosnesenskii	All SNPs	52	−10,675[a]	−10,623	−10,617
	Intergenic	32	−10,111[a]	−10,079	−10,076
	Nonsynonymous	18	−7,473[a]	−7,455.0	−7,452
	Intron	32	−10,338[a]	−10,306	−10,301
	Synonymous	7	−8,879[a]	−8,872	−8,869
	Neutral	0.15	−10,596	−10,601	−10,601[a]
B. vosnesenskii no WA	All SNPs	4.00	−8,054[a]	−8,050	−8,048

Note. The Δ BIC value is the difference between the best-supported model, marked with superscript letter a) and the second highest supported model (which was the "flat" landscape model in all SNP categories).

most to the Maxent ENM for *B. bifarius* and also had the highest training gain when used alone and reduced the gain most when not included. The second most informative variable was either *Annual Mean Temperature* or *Minimum Temperature of the Coldest Month*, depending on method of evaluation, followed by *Mean Temperature of the Wettest Quarter* and *Mean Diurnal Range*. *Precipitation Seasonality* had the highest contribution and influences on gain in the *B. vosnesneskii* model, followed by *Annual Precipitation*, *Isothermality* and *Temperature Seasonality*.

3.4 | Intragenomic variation in genetic patterns across SNP classes

Both species showed similar intragenomic patterns. Most SNP classes were similar to the average values, and diversity and population structure results were consistent with the full results presented above, indicating that conclusions are robust with respect to sampling of loci (Table 1). Although there were slight differences, diversity estimates were similar to the full SNP sets for each class, with the exception of nonsynonymous SNPs, which had much greater variance and lower mean diversity. In terms of genetic structure, both species again showed similar intragenomic patterns as the full data set, although for *B. bifarius*, the nonsynonymous SNPs showed slightly elevated mean F_{ST} values. For all SNP class-specific IBD and IBR analyses, the ENM resistance model was still the best-supported model (Table 4), and overall patterns were similar to Figure 5.

After removal of putative outliers with LFMM, the filtered "neutral" SNP set retained 16,548 SNPs for *B. vosnesenskii* and 31,887 SNPs for *B. bifarius*, with 2,116 and 5,587 SNPs identified as outliers, respectively. Data sets were filtered for specific analyses as described above. Major patterns above were retained in these neutral data sets, with minor exceptions. Heterozygosity was lower than other SNP classes (Table 1), but spatial patterns (Supporting Information Figure S5) and model comparisons were similar (Supporting Information Table S7). The main exception was for *B. vosnesenskii* with WA excluded, where the random effect only model is best supported by AIC with no significant latitude or elevation effects (Supporting Information Table S7 and Figure S5). In terms of population structure, F_{ST} was also lower in the neutral SNPs (Table 1); however, linearized F_{ST} still increased with geographic distance in both species (Mantel $r = 0.91$ and 0.31 for *B. bifarius* and *B. vosnesenskii*, both $p < 0.001$). The ENM resistance model is still the best-supported model for *B. bifarius* in the MLPE analyses, but maximum differentiation was reduced, and relationships with spatial and environmental factors were thus shallower (Supporting Information Figure S5). For *B. vosnesenskii*, models were less distinguishable than in the full SNP set (maximum $\Delta BIC = 5$) but the flat resistance and elevation models were slightly better supported than the ENM model for neutral SNPs (Table 4; Supporting Information Figure S5). GESTE recovered highly similar patterns as in the full SNP set, although no model had strong support for *B. vosnesenskii* (Supporting Information Table S8 and Figure S5).

4 | DISCUSSION

Montane habitats can present considerable challenges for their inhabitants. Steep clines in environmental variables can isolate populations in suitable habitat patches surrounded by inhospitable environmental conditions that can reduce population sizes, genetic diversity and gene flow (Brown, 1971; Halbritter, Billeter, Edwards, & Alexander, 2015; Murphy, Dezzani, Pilliod, & Storfer, 2010; Polato et al., 2017). Comparing species that are broadly geographically sympatric but with differences in abiotic niche that produce slight differences in local elevational distributions can be useful for investigating spatial and environmental factors contributing to differentiation and diversity across montane systems (Phillipsen et al., 2015). Using high-resolution genomic data and fine-scale spatial sampling, this study expands our understanding of how natural landscape structure influences population structure in highly mobile species by investigating the interplay of space, elevation and environmental niche on bumble bees of the Sierra-Cascade mountain region in North America. We find that a bumble bee species that exists at low latitudes throughout most of its range is likely to be characterized by high genetic connectivity and little variation in genetic diversity even when populations also occur at high elevations within a region. In contrast, we see that a species will show reduced diversity and gene flow in parts of its range where its environmental niche is shifted upslope and lower-elevation populations are rare. Results have key implications for understanding the effects of habitat availability and arrangement on population connectivity in topographically complex regions and will be of value for understanding potential effects of global change.

Overall, population structure was weak in *B. bifarius* and *B. vosnesenskii* ($F_{ST} < 0.02$ for both species), consistent with most studies in bumble bees (reviewed in Woodard et al., 2015). The major axis of structure from individual (PCA, sNMF) and population (F_{ST})-based analyses in both species exhibited a relationship with latitude, reflecting the effect of distance on gene flow. However, *B. bifarius* was clearly more structured than *B. vosnesenskii*. Both IBD and IBR models show not only that pairwise F_{ST} values have a higher maximum in *B. bifarius* but that they also increase more rapidly with increasing niche resistance and distance, highlighting the increased challenges to population connectivity between elevationally and environmentally restricted sites in our study region. In contrast, *B. vosnesenskii*, which inhabits lower elevations and a broader elevational range than *B. bifarius* across most latitudes, appears capable of relatively free movement across this range, with much weaker structure apparent in both individual and population-based analyses.

It is inherently difficult to untangle the effects of elevation from those of latitude (which should largely be a proxy for distance effects on gene flow) because for many montane species, including *B. bifarius*, in certain regions, populations only occur at relatively high altitudes. Our sampling design, intended to sample as broad a gradient of elevations as possible across latitudes, was able to resolve several patterns. *Bombus bifarius* populations at higher elevations and lower latitudes exhibited relatively greater differentiation, with a

significant interaction term reflecting the overall increase in the species elevational range with decreasing latitude and the resulting isolation of these high-elevation low-latitude sites. Similar patterns were reflected in genetic diversity. Although *B. bifarius* has more diversity than *B. vosnesenskii*, likely because of its much broader global distribution (also see Lozier et al., 2011), *B. bifarius* is clearly more sensitive to effects of spatial factors on this diversity. While *B. vosnesenskii* exhibited little variation in diversity across latitude or altitude, southern high-elevation *B. bifarius* showed lower π and elevated F_{ST}, suggesting that low latitude populations restricted to the highest elevations are smallest and most isolated.

The role of environmental conditions for shaping these population genetic patterns, especially in *B. bifarius*, is apparent from our ENM-based resistance model testing, signifying the importance of habitat availability and arrangement over geographic distance or elevational heterogeneity alone. This does not discount the importance of elevation for structuring genetic variation. Rather, we propose that the ENM resistance model outperforms the elevation model because elevation becomes most important in regions where the environmental niche is shifted upslope and low-elevation sites are inhospitable, and is thus implicitly reflected in the Maxent model. In other words, the spatial distribution of the *B. bifarius* niche is broader in the north, allowing for larger more well-connected populations across elevations, while in lower latitudes for our study area, *B. bifarius* occupies a narrow range of habitat exclusively at high elevation (Figure 1). Such low-permeability barriers are especially apparent in the PCA clustering (Figure 3).

Some general patterns are emerging about the effects of elevation and environmental niche on biogeography of bumble bees in western North America. Previous studies of *B. bifarius* suggested that high-elevation populations exhibit greater isolation (Lozier et al., 2011, 2013); however, these studies included populations from independent phylogeographic lineages that are now known to be distinct (Lozier et al., 2016). Our results suggest that the effects of abiotic niche associated with this species are also observed within a lineage (*B. b. nearcticus*); however, the effect is most important in regions where the species occurs exclusively at high elevations. Koch et al. (2017) likewise found support for the role of elevational niche restriction on population connectivity among four broadly sympatric bumble bee species in the Pacific Northwest, showing that species with a mainly high-elevation niche displayed significant IBD, which was absent for species distributed across a wider range of elevations. This result supports the importance of niche in predicting genetic structure and mirrors our larger-scale patterns. In contrast to *B. bifarius*, previous studies have found that elevation is not an important driver of differentiation within *B. vosnesenskii* (Jha, 2015; Jha & Kremen, 2013), which we replicate here with higher resolution sampling. Upon initial consideration, *B. vosnesenskii* appears to conflict with our general hypothesis that populations at high elevation would be more structured. However, this result fits with the larger overall pattern that elevation per se is not a significant challenge for bumble bees, but rather that the combined effect of environmental niche and elevation can promote habitat islands that influence population structure.

Patterns observed here are not unusual in montane regions, where inhospitable lowlands can produce "sky island" archipelagos that isolate populations of habitat specialists that may be well-connected in other times or places (Brown, 1971; Lomolino et al., 1989; Manthey, Klicka, & Spellman, 2011; Phillipsen et al., 2015; Schoville, Stuckey, & Roderick, 2011). It is interesting that such patterns transfer to strong fliers like bumble bees, for which many species exhibit high gene flow and near-panmixia (Woodard et al., 2015). Ultimately, the same ongoing processes that drive intraspecific variation in montane *Bombus* may be important for speciation in the long term, where high-elevation isolation and range fragmentation during periods of climate change have generated present species diversity and demographic patterns in cold-adapted *Bombus* (Dellicour et al., 2017; Duennes, Lozier, Hines, & Cameron, 2012; Hines, 2008; Williams, Lobo, & Meseguer, 2017). Periods of global cooling can drive high-elevation populations lower to provide dispersal corridors and increased species ranges. Warming temperatures then force populations back to high elevations, isolating high-elevation populations and promoting divergence. Patterns observed in *B. bifarius* thus likely reflect processes that have been important for diversification in bumble bees over longer timescales.

Results also have implications for predicting the ease with which organisms will be able to track spatial niche shifts with changing temperature and precipitation under contemporary global climate change. Under climate change, species can respond in several ways, including adaptation, range shifts to track spatial changes in conditions, or local extinction if adaptation or dispersal is not possible (Moritz & Agudo, 2013; Parmesan, 2006). Diverse taxa are already shifting ranges poleward or upslope, with consequences for genetic diversity, species ranges and community assembly (Parmesan, 2006; Rubidge et al., 2012; Steinbauer et al., 2018; Tingley et al., 2009). Interestingly, bumble bees do not appear to be tracking their thermal niches poleward as global temperatures increase; however, southern populations seem to be undergoing a retreat to higher elevations, especially in North America (Kerr et al., 2015). Although genetic patterns in our study demonstrate that elevation alone is not responsible for reduced genetic diversity and gene flow, it is likely that these mountains will become stronger barriers if species respond to climate change with upslope niche shifts and fail to adapt to local changes in abiotic conditions at low elevations (Moritz & Agudo, 2013). Thus, population structure in species like *B. vosnsensekii*, which are currently well-connected, could begin to resemble the type of isolation observed in southern *B. bifarius* moving forward if they follow the overall trend (Kerr et al., 2015). Populations already restricted to higher elevations, like southern *B. bifarius*, may become even more vulnerable.

The potential for adaptation to climate change, or local adaptation to existing abiotic variation across bumble bee species ranges more generally, requires further study. We show that population genomic patterns reported here are largely consistent across SNP classes within the genome and for putatively "neutral" marker sets,

although the strength of responses varies in ways that may reflect interesting evolutionary dynamics. Consistent with other high-resolution population genomic analyses (e.g., Dutoit, Burri, Nater, Mugal, & Ellegren, 2016), the highest diversity was observed in the intergenic, intron and synonymous sites, with the lowest diversity at nonsynonymous sites. Both species have higher average differentiation, and much greater variance, among nonsynonymous sites than the genome average, although the number of nonsynonymous sites was small. Montane regions present numerous abiotic clines that should promote adaptive divergence (Endler, 1977; Haldane, 1948; Keller et al., 2013; Yi et al., 2010), and thus, patterns at nonsynonymous sites are suggestive. Statistical methods like LFMM can potentially uncover relationships between environmental variables and local adaptation at individual SNPs using genomic data (e.g., Coop, Witonsky, Di Rienzo, & Pritchard, 2010; Frichot & François, 2015); however, detecting selection in this system could prove challenging because of the confounding effects of latitude (Novembre & Di Rienzo, 2009). Indeed, the large number of SNPs detected as outliers by LFMM suggests the potential for many false positives and calls for caution before attempting to assign functional significance to putative targets of selection. Because of this, we limited our objective for outlier detection in this study to evaluating the robustness of results to the inclusion of SNPs under selection, and the large number of LFMM outliers was viewed as a conservative approach from the perspective of retaining a neutral set for demographic inference. The major results relating to differences in diversity and structure between species were retained in the neutral SNP set, suggesting that main conclusions are robust to the effects of selection. However, the reduced fit of the ENM resistance model in *B. vosnesenskii* and the weaker *B. bifarius* differentiation patterns might suggest that some sensitivity to the environmental niche distribution in the full SNP data set could stem from selection acting on a subset of loci. Ultimately, our nested elevational sampling design may prove useful for untangling effects of environment from those of space, and efforts to evaluate optimal approaches for modelling selection and better controlling for population structure in this system are underway. Together with the present results on factors influencing genetic structure and diversity, understanding the response to selection across this region will prove useful in predicting responses to climate change, including the relative importance of niche tracking and adaptation (Bay et al., 2018; Fitzpatrick & Keller, 2015).

We elected to consider environmental niche models as an objective representation of environmental variation across the landscape, rather than individual bioclimatic variables, because bees likely respond to individual variables and their interactions in complex ways that may vary over a species range. However, evaluating Maxent performance can yield some tentative insights into variables that might be important for shaping gene flow in contemporary and future species ranges. For both species, precipitation variables were most important, followed by temperature variables. The use of precipitation as the key driving variable is somewhat surprising, as thermal variables seem to show the greatest differences between the species (e.g., *B. bifarius* is far more restricted for mean, minimum and maximum temperatures; Figure 1). It may be the case that precipitation reflects an indirect effect that is important for bumble bees generally, perhaps via effects on floral resource availability, with temperature variables most relevant only for particular species like those that dominate in high-elevation montane habitats. Bumble bees are unique compared with most insects in their capacity for thermoregulation and maintenance of thoracic temperatures across abiotic conditions (Heinrich, 1979), but it is largely unknown to what degree species vary in thermal sensitivity. Comparative physiology analyses across species will ultimately be required to clarify the roles of temperature and precipitation in determining the limits of different bumble bee species distributions. Differences in the relative importance of precipitation and temperature for species at different elevation zones are known in other groups (e.g., Tingley et al., 2009), and it will be especially important to determine the differential sensitivity to abiotic variables among bumble bee species before we can fully understand the consequences for species ranges and genetic connectivity. In this context, it will also be interesting to expand this study to other systems including mountain ranges with different spatial, geological and environmental characteristics, such as the lower-elevation north–south oriented Appalachians in the eastern United States. Determining how species may respond differently to elevation gradients in more east–west-oriented ranges like those in Europe would also be interesting, as such mountain ranges have had distinct consequences for Pleistocene phylogeography (Hewitt 2000) and may likewise pose different challenges for contemporary populations under climate change.

Finally, there are a few caveats to the present results. First, factors other than bioclimatic variables may influence bumble bees. For example, studies have suggested that such recent land use changes may reduce dispersal and impact nesting in some species (Jha, 2015; Jha & Kremen, 2013). We did not test the effects of land use characteristics such as urbanization, as our sampling was not designed to capture those effects with suitable replication. This could easily be incorporated in the future to examine fine-scale effects of urbanization leveraging the power of population genomic data. Second, population structure patterns are influenced both by demographic dynamics of species in modern landscapes but also by broader phylogeographic processes. Although some *Bombus* species in North America are experiencing range reductions, both *B. vosnesenskii* and *B. bifarius* are stable (Cameron et al., 2011), so our results are not likely influenced by recent declines. However, populations of *B. vosnesenskii* north of the Columbia River in WA were historically rare (Stephen, 1957) but are now fairly common (Koch et al., 2012). Lozier et al. (2011) speculated that nonequilibrium processes might explain unusual patterns in microsatellite diversity in these northern *B. vosnesenskii*. Based on the repeated observation of these unusual signatures, a recent expansion of WA populations seems plausible. Whatever the explanation, it is clear that these populations behave differently than more southern sites and do not likely reflect more general patterns about the interaction of elevation and environmental conditions on population structure. Future work should, however, specifically be designed to evaluate genetic evidence for potential northern range expansions in bumble bees, especially in the context

of the concerning lack of northward range shifts in the genus as a whole from natural history collection record data (Kerr et al., 2015).

5 | CONCLUSIONS

Our results highlight the importance of the amount and arrangement of suitable habitat in determining population connectivity in montane insects. We see that absence of low-elevation populations is more important for increasing population isolation than occupancy of high elevations alone, and that the amount of isolation can vary within the same species if the elevational range, and thus the spatial distribution of a suitable abiotic niche, varies. High-elevation habitat can, therefore, drive neutral differentiation and potentially facilitate local adaptation. These observations provide contemporary support for the role that high-elevation areas have played in shaping *Bombus* biodiversity over long timescales. Our findings also have implications for predicting and managing species loss in the future. Certain parts of species ranges will likely be more vulnerable as populations that must track their niches upslope inhabit reduced altitudinal areas and become isolated in these regions, even if other parts of the species range maintain connectivity.

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DATA ACCESSIBILITY

Raw sequence data as demultiplexed fastq files are available on GENBANK SRA (SRP149031). Various input data files (VCF files, GESTE inputs) and results' summaries (Hardy–Weinberg statistics, pairwise F_{ST}, distances and resistances) are available on DRYAD (https://doi.org/10.5061/dryad.st7gm24).

AUTHOR CONTRIBUTIONS

The study was conceived and the manuscript was written by J.M.J. and J.D.L. Data were generated and most statistical analyses were performed by J.M.J. Many of the bioinformatics analyses were developed and relevant Methods' sections were written by M.L.P. All authors conducted field work and made intellectual contributions to the study design and text.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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