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Contemporary human-altered landscapes and oceanic barriers reduce bumble bee gene flow

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Abstract

Much of the world's terrestrial landscapes are being altered by humans in the form of agriculture, urbanization and pastoral systems, with major implications for biodiversity. Bumble bees are one of the most effective pollinators in both natural and cultivated landscapes, but are often the first to be extirpated in human-altered habitats. Yet, little is known about the role of natural and human-altered habitats in promoting or limiting bumble bee gene flow. In this study, I closely examine the genetic structure of the yellow-faced bumble bee, *Bombus vosnesenskii*, across the southwestern US coast and find strong evidence that natural oceanic barriers, as well as contemporary human-altered habitats, limit bee gene flow. Heterozygosity and allelic richness were lower in island populations, while private allelic richness was higher in island populations compared to mainland populations. Genetic differentiation, measured for three indices across the 1000 km study region, was significantly greater than the null expectation ($F_{ST} = 0.041$, $F'_{ST} = 0.044$ and $D_{est} = 0.155$) and correlated with geographic distance. Furthermore, genetic differentiation patterns were most strongly correlated with contemporary (2011) not past (2006, 2001) resistance maps calibrated for high dispersal limitation over oceans, impervious habitat and croplands. Despite the incorporation of dramatic elevation gradients, the analyses reveal that oceans and contemporary human land use, not mountains, are the primary dispersal barriers for *B. vosnesenskii* gene flow. These findings reinforce the importance of maintaining corridors of suitable habitat across the distribution range of native pollinators to promote their persistence and safeguard their ability to provide essential pollination services.

Keywords: *Bombus*, dispersal, landscape genetics, microsatellites, pollinator

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Introduction

More than 38% of earth's ice-free terrestrial surface is managed by humans as agriculture or pasture (FAO 2011), and urban and suburban areas continue to grow rapidly (Grimm *et al.* 2008), with an estimated 60% of the global population (4.9 billion) expected to inhabit urban areas by 2030 (UN 2005). In the United States alone, land-use patterns have changed dramatically over the last century (Sisk 2008), with sharp increases in agricultural land development until the 1950s, followed by subsequent increases in urban, suburban and exurban growth, especially in coastal areas (Brown *et al.* 2005). These increases in human-dominated land cover largely occur at the expense of natural land cover and result in declines in biodiversity levels (Loreau 2002; Brown *et al.* 2005; McKinney 2006) that ultimately lead to declines in ecosystem service and function (Hooper *et al.* 2005; Wardle *et al.* 2011). While there are clear impacts of human land use on species richness, relatively less is known about the effects of land use on the population genetics of native species, although interest and research on the topic has grown rapidly in the past decade (reviewed in Manel *et al.* 2003; Keyghobadi 2007; Aguilar *et al.* 2008). Understanding the impacts of land use on populations is particularly critical for the conservation of species that provide valuable ecosystem services, such as pollination.

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Pollination is an essential ecosystem service that contributes to the reproduction of 87% of all flowering plant species (Ollerton *et al.* 2011). Pollination is also responsible for the increased quantity, quality and stability of over 60% of world crops (Klein *et al.* 2007), worth an estimated $300 billion annually (Gallai *et al.* 2009). Although pollinators are recognized as ecosystem service providers in many natural and agricultural landscapes, a number of native pollinator taxa are exhibiting declines (Kearns *et al.* 1998; Goulson *et al.* 2008a; Bartomeus *et al.* 2013), often associated with natural habitat destruction and human development (e.g. Biesmeijer *et al.* 2006; reviewed in Winfree *et al.* 2011). One potential mechanism for pollinator decline in human-altered landscapes is a loss in critical nesting and foraging habitat (Williams *et al.* 2010). Most bees nest in soil, woody stems and cavities, and all species depend on floral resources to feed themselves and their offspring (Michener 2000). Agriculture and other types of human development often result in tilling and paving of natural lands, which makes habitat less available for bee nesting and often precludes the growth of flowering plants. This loss of nesting and food resources may be partially responsible, along with other factors, for the recent global decline in bumble bees (Williams 1982; Goulson *et al.* 2008a; Cameron *et al.* 2011).

Bumble bees are one of the most effective native pollinators on a per visit basis (e.g. Stubbs & Drummond 2001; Kremen *et al.* 2004) and are often considered 'keystone' species within plant–pollinator communities because of their tendency to pollinate both rare and abundant plant species (reviewed in Goulson *et al.* 2008b). Given this generalist behaviour, plant–pollinator network studies have suggested that a loss of bumble bees in the community could potentially lead to immediate declines in native plant reproduction and long-term losses in plant diversity (Memmott *et al.* 2004; Cusser & Goodell 2013). Unfortunately, bumble bee declines have been documented across a wide range of geographic regions (e.g. Williams 1982; Kosior *et al.* 2007; Cameron *et al.* 2011), with at least four species in North America exhibiting range contractions and population declines over the past 20–30 years (Cameron *et al.* 2011). Interestingly, these four declining species (*B. occidentalis*, *B. pensylvanicus*, *B. affinis* and *B. terricola*) and four co-occuring nondeclining species (*B. bifarius*, *B. vosnesenskii*, *B. impatiens* and *B. bimaculatus*) also exhibit considerable gene flow across their species range (Cameron *et al.* 2011). Despite high levels of gene flow, a number of species, including *B. bifarius,* appear to exhibit dispersal limitation across natural barriers such as elevation gradients and water bodies (Lozier *et al.* 2011, 2013). Recent studies have also indicated that human land use, such agricultural and urbanized land, can limit bumble bee gene flow at small spatial scales (Jha & Kremen 2013b), but it remains unclear whether human land-use barriers prevent gene flow at larger spatial scales. Given increasing global urbanization and agricultural expansion patterns, and high levels of human dependence on pollinators for food security, it is essential to advance understanding about how human-induced land-use changes impact critical native pollinators.

While previous studies have documented some negative impacts of human-altered landscapes on gene flow and nest densities, these studies have been conducted at relatively small spatial scales (200–600 km) and have examined land use across only single time periods (Davis *et al.* 2010; Goulson *et al.* 2010; Jha & Kremen 2013a). Examining population declines related to any single time period can be challenging, given that biodiversity declines post-land alteration may exhibit time lags (Krauss *et al.* 2010). Such delayed extinction events, or so-called 'extinction debts' (Tilman *et al.* 1994), are important to consider for pollinators (Bommarco *et al.* 2014) and may be especially critical to consider for bumble bees given that bumble bee queens potentially exhibit high levels of natal nest site fidelity (Jha & Kremen 2013b), typically establish a single nest site per generation and exhibit longer generation times (usually one per year, Thorp *et al.* 1983) compared to many other bees, and thus, they may take longer to respond to land-use change. One examination of bumble bee population genetic response to land use in northern California found that contemporary land use (2006) was more predictive of genetic structure than past (1987) and historic (1900) land-use patterns (Jha & Kremen 2013b). However, this study was conducted across a small region (~ 200 km) located in the northern Central Valley and did not encompass the larger and more landscape-heterogeneous coastal, island and mountain areas.

In this study, I examine three critical components of gene flow for the yellow-faced bumble bee, *Bombus vosnesenskii*, across major natural and man-made barriers and multiple time points in the southwestern USA. First, I examine the existence of distinct genetic groups and differences in allelic richness, inbreeding and heterozygosity levels across three major biogeographic areas, the Channel Islands, the Coastal Mountains and the Sierra Nevada Mountains (Fig. 1A). Second, I examine the impact of geographic distance, elevation (mountains) and land use (oceans, urban areas and agricultural areas) on gene flow. Third, given evidence that contemporary land use impacts bumble bee gene flow (Jha & Kremen 2013b), I investigate the relative influence of land use at three different contemporary timescales (0 years, 5 years and 10 years prior to

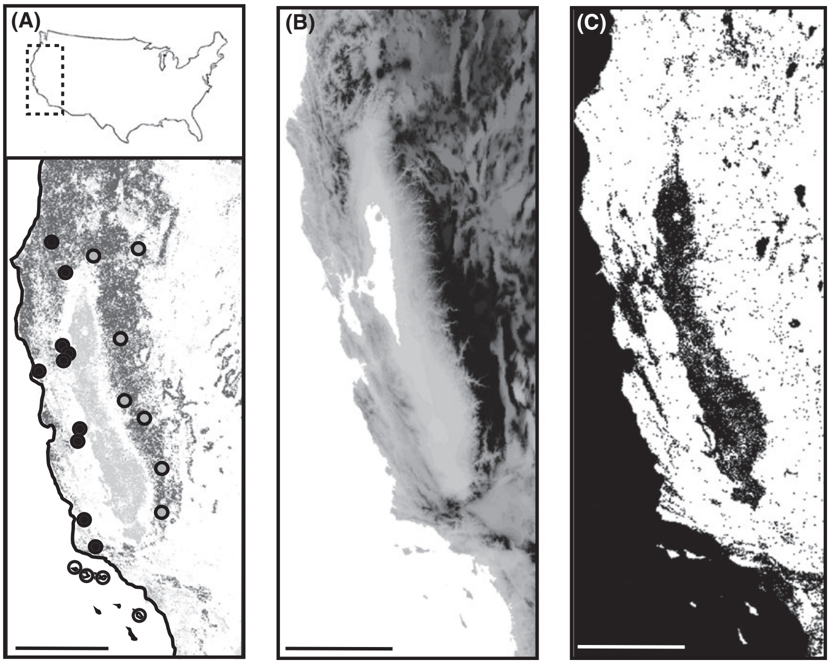

Figure 1 (A) Study area in the USA (dashed rectangle in top panel) and detailed map for 2011 (bottom panel) where increasing darkness represents increasing density of woody vegetation, with Coastal populations (black circles), Sierra populations (grey circles) and Island populations (open circles) indicated. (B) Elevation resistance map where increasing darkness represents increasing elevation (higher resistance) and (C) Land Use resistance map where white represents low resistance (0.1), designated for Grassland and Forest habitat types, and black represents high resistance (0.9), designated for Open water, Croplands and Impervious cover (Table 1). Horizontal scale bar at the bottom of each map represents 200 km.

sampling). Specifically, I hypothesize that (i) island populations of *B. vosnesenskii* are genetically isolated from mainland populations, (a) experience greater historic population bottlenecks and greater ongoing inbreeding and (b) exhibit a lower influx of alleles over time, than mainland populations; (ii) across the entire study region, *B. vosnesenskii* populations engage in decreasing gene flow with increasing geographic distance and experience lower levels of gene flow across oceans, urbanized areas and agricultural landscapes; and (iii) *B. vosnesenskii* gene flow responds quickly to land-use change and is characterized by short time lags.

Methods

Study species and region

This study focused on the yellow-faced bumble bee, *Bombus vosnesenskii*, one of the most effective native pollinators for crops on the US west coast (Kremen *et al.* 2002). Although *B. vosnesenskii* is not currently exhibiting declines in relative abundance (Cameron *et al.* 2011), it is often the first bee species to be extirpated with increasing human land-use intensity within agricultural areas (Larsen *et al.* 2005). Moreover, *B. vosnesenskii* exhibits many of the life history traits shared by other *Bombus* species, including univoltine reproductive cycles, high foraging demands and a primarily subterranean nesting strategy (Thorp *et al.* 1983), making it a model species for the study.

This study was conducted across the southwest coast of the United States, from 2011 to 2012 (Fig. 1A). Bees were sampled across 21 populations separated by 7.15 km–963.24 km (mean 376.4 km ± 218.47), which constitutes more than half of the species range (Thorp *et al.* 1983) and covers an area with a −85 m to 4330 m elevation gradient. The sampling for this study encompasses three main biogeographic ranges of the state of California: the Sierra Nevada foothills and mountain range (hereafter Sierra populations), the Coastal and Transverse foothills and mountain range (hereafter Coastal populations), and the Channel Islands (hereafter Island populations) (Fig 1A). Population sampling areas were selected based on the presence of past records of *B. vosnesenskii* in the study region (sensu Thorp *et al.* 1983; R. Thorp, Personal communication) and an effort to separate sampling sites beyond the most conservative bumble bee queen dispersal distances, estimated to span approximately 9 km (Lepais *et al.* 2010; Jha & Kremen 2013b); however, it should be noted that *B. vosnesenskii* is distributed over a wide geographic area (Thorp *et al.* 1983) with unsampled populations in existence. Given these circumstances, it is theoretically never possible to have a complete understanding of the geographical pattern of genetic variation (Beerli 2004), and thus, our sampling represents an estimate of this genetic variation.

To examine recent land-use change in the study region, I obtained land-cover data from the National Land Cover Database (NLCD, http://www.mrlc.gov/) for 2011, 2006 and 2001. For this study, I classified the NLCD data into five main bumble bee habitat types at 1-km resolution using the majority Resampling function in ARCGIS 9.2 (ESRI 2006) (with NLCD classification terms in parentheses): Open water (open water and perennial ice, the latter of which comprised <0.05% of the pixels), Impervious cover (high- and moderate-intensity commercial/industrial/transportation land with >20% impervious cover), Cropland (crops and wetlands, the latter of which comprised <15% of pixels), Grassland and pasture (developed space with <20% impervious cover, bare ground, grassland and pasture), and Forest and shrubland (all forests and shrub/scrub). In 2001, the study region was comprised of 23.05% Open water, 2.00% Impervious cover, 5.20% Cropland, 14.09% Grassland and pasture, and 55.68% Forest and shrubland. Examining human-induced land-cover change from 2001 to 2006, and 2006 to 2011 revealed that Impervious cover increased by 4.04% and 4.85%, Cropland increased by 0.01% and 1.77%, Grassland and pasture increased by 0.31% and 0.58%, and Forest and shrubland decreased by 0.32% and 0.20% in the study region.

Sampling and genotyping

Within each of the 21 populations, an average of 41.14 (±2.36 SE) worker bees were collected. DNA was extracted from the tarsal segment of each bee sample using the HotShot protocol (Truett *et al.* 2000) and was screened at 12 microsatellite loci, B96, B100 and B119 (Estoup *et al.* 1995), and BT33, BT43, BT65, BT124, BT125, BT128, BT131, BT132 and BT136 (Stolle *et al.* 2009), which are located on 10 different chromosomes, based on the *B. terrestris* genome v1.1 (Stolle *et al.* 2011). Multiplex polymerase chain reactions (PCRs) were performed in a final volume of 20 μL, containing approximately 2 ng of DNA, 2 μL of 10× PCR buffer, 1.5 mM $MgCl_2$, 300 μM of each dNTP, 1U of Taq Polymerase and 0.25 μM of each primer. The thermal cycle began with a 5-min denaturation step at 95 °C and was followed by 37 cycles: 30 s at 94 °C, 60 s at the locus-specific annealing temperature and 30 s at 72 °C, followed by a final extension at 72 °C for 20 min. One primer from each pair was labelled with 6-FAM, NED, VIC or PET, and genotyped on an ABI 3730 Sequencer. Alleles were scored manually using GENEMARKER® (Softgenetics), and only samples with ≥8 loci scored per individual were included in the population genetic analyses.

Colony identity, Hardy–Weinberg equilibrium (HWE), allelic richness and STRUCTURE analyses

Full-siblings collected from each study region were first assigned to colonies using COLONY 2.0 (Wang 2004). In this assignment, the genotyping error rate was set to 0.001, based on error rates documented in previous studies (Knight *et al.* 2005; Jha & Kremen 2013b). Because bumble bee species are largely assumed to be monandrous (Estoup *et al.* 1995) and because we are interested in genetic structure unbiased by sibship (repeated surveying of a single colony), I randomly removed colony mates (or full-siblings), leaving just one representative per colony in the data set. This removal of all colony mates and individuals with <8 scored genotypes resulted in an average population sample size of 35.90 (±2.19 SE) worker bees (Table S1, Supporting information).

The probability of null alleles was calculated using the software MICRO-CHECKER (van Oosterhout *et al.* 2006), and deviations from HWE and linkage disequilibrium (LD) were tested in GENEPOP v 4.0.10 (Raymond & Rousset 1995) with 1000 dememorizations, 100 batches and 1000 iterations per batch using the Markov chain approximation for the exact tests and likelihood ratio tests, respectively. Allelic richness (AR) and private allelic richness (PAR) per population were estimated using rarefaction, standardized to 10 gene copies per population (as per Lozier *et al.* 2011; Jha & Kremen 2013b), in HP-RARE (Kalinowski 2005). I estimated heterozygosity using Nei's gene diversity, H_E (Nei & Kumar 2000) (Table S1, Supporting information) and compared AR, PAR, H_E and F_{IS} for the three main biogeographic regions using a Kruskal–Wallis rank sum test followed by a post hoc Wilcoxon rank sum tests within the R platform (R Development Core Team 2005). I also examined the relationship between AR, PAR, H_E and F_{IS} and Latitude and Elevation using Spearman rank correlation in R. Populations were also examined for evidence of population bottlenecks using the program BOTTLENECK (Piry *et al.* 1999) with 1000 replications under the assumption of the stepwise mutation model and the two-phase mutation model, instead of the infinite alleles model, which is often less conservative in estimates of heterozygote excess (Luikart & Cornuet 1998), testing significance using a Wilcoxon test. Finally, I examined population structure using two methods, the clustering method in STRUCTURE 2.3.3 (Falush *et al.* 2003) and in TESS 2.3 (Durand *et al.* 2009) the latter of which differs from STRUCTURE in that it uses the geographic coordinates of the samples as prior information (Francois & Durand 2010). For both methods, I utilized the admixture model, which assumes that individuals comprise K unknown populations (K ranged from 1 to 10) to which fractional or individual genotypes can be assigned. Both models were run with 20,000 burn-in steps and 100 000 samples, with 10 iterations for each K.

Patterns of genetic differentiation

I calculated pairwise and overall genetic differentiation using the classic F_{ST} index modified for multi-allelic markers (Weir & Cockerham 1984), another index generalized for multiple alleles, F'_{ST}, (also known as G_{ST}) (Nei 1973), and an index especially appropriate for multi-allelic markers when heterozygosity levels are high, Jost's D_{est} (Jost 2008). F_{ST} and F_{IS} were calculated in the software GENEPOP (Rousset 2008), and the remaining indices were calculated with the software DEMETICS (Gerlach *et al.* 2010) within the R platform. For all estimates, I calculated the 95% confidence intervals using 10 000 bootstrap repetitions.

In addition to examining the role of geographic distance on genetic differentiation (IBD), I am also interested in examining the effects of elevation, given that it has been suggested to correlate with bumble bee genetic differentiation in some studies (Lozier *et al.* 2011; *but see* Jha & Kremen 2013b). This is because higher elevations and the associated reduced oxygen and air density make insect flight more difficult (Dillon *et al.* 2006), and thus, *Bombus* populations at higher elevations may be less well-connected than those at lower elevations (Lozier *et al.* 2011). Therefore, I created an elevation resistance map to test the hypothesis that higher elevations are more limiting for bumble bee gene flow. In landscape genetics, resistance surfaces can be created in a variety of ways (Spear *et al.* 2010); one fundamental approach is to develop resistance maps that test hypotheses regarding landscape composition and gene flow (Storfer *et al.* 2007; Holderegger & Wagner 2008). However, analyses can be particularly sensitive to the relative cost assigned to each surface, with greater deviations from null hypotheses documented when the relative differences in assigned resistance are greater (reviewed in Rayfield *et al.* 2010). Thus, I use a relatively narrow scale of resistance cost, from 0–1 for all surface types investigated, where 0 represents no resistance and 1 represents the highest resistance. To create the elevation resistance map, I used a digital elevation map of the study region, available through the National Elevation Dataset (NED, http://ned.usgs.gov/). I divided all elevation values by the maximum elevation of 4330 m to create a raster ranging from <0.01 to 1 in resistance value.

Finally, I created a resistance surface to test for the effects of land use, calibrated based on existing literature and past genetic studies of bumble bees. Previous studies have shown that bumble bee nesting densities are lower in some human-altered landscapes (Svensson *et al.* 2000; Goulson *et al.* 2010; Jha & Kremen 2013a) and gene flow may be limited by open water, impervious land and crops (Jha & Kremen 2013b). Because the resistance maps for Open water, Impervious land and Cropland are derived from the same land-use raster, they are inherently correlated and thus are investigated as a single high resistance surface in this study. Furthermore, because spatial genetic structure appears to be influenced more by contemporary (2000s) than historic (1900s) land-use patterns (Jha & Kremen 2013b), I examined the influence of land use across three recent time periods: the year of sampling (2011), 5 years prior to sampling (2006) and 10 years prior to sampling (2001). Therefore, I utilized the 2011, 2006 and 2001 NLCD land-use maps, classified into the five main bumble bee habitat types. I assigned Open water, Impervious land and Cropland types a resistance of 0.9 and Grassland and Forest types a resistance of 0.1.

Using both the elevation and land-use resistance maps, I calculated pairwise Elevation and Land Use Resistance Distances (RD, McRae 2006). Specifically, the RD was calculated utilizing the software CIRCUITSCAPE v 3 (McRae 2006) using 1-km resolution Elevation and Land Use resistance maps (Fig. 1B–C). The 1-km scale is appropriate for the resistance maps in this study, given the large size of the study region and the long-distance dispersal ability of queen *B. vosnesenskii* (~ 9 km minimum, Jha & Kremen 2013b; Lepais *et al.* 2010; ~ 200 km maximum, Schmid-Hempel *et al.* 2013). Geographic distances between each pair of populations were calculated using Euclidean (straight line) distance.

While there is little consensus on the best statistical approach to examine relationships between genetic distance and environmental variables, some methods are more prone to inflation of Type I error than others (e.g. Mantel tests, Balkenhol *et al.* 2009; Guillot & Rousset 2013), and multiple statistical approaches can provide a more robust set of results (Balkenhol *et al.* 2009). Therefore, I used a combination of two approaches including multiple regression on distance matrices (MRDM, Legendre & Legendre 1998) and a maximum-likelihood population-effects model (MLPE, Clarke *et al.* 2002), the latter of which accounts for multiple population membership. In both approaches, I examined the role of the three explanatory variables: (i) Geographic Distance, (ii) Elevation RD and (iii) Land Use RD (2001, 2006 and 2011), on the dependent variable, Genetic Distance (F_{ST}, F'_{ST}, D_{est}). Due to collinearity between the Land Use RD for the three focal study years, the different Land Use RD were investigated in three separate models for 2001, 2006 and 2011. Furthermore, I examined all possible combinations of the three independent variables (Geographic Distance, Elevation RD and Land Use RD). None of the matrices within a model were collinear (Pearson's correlation coefficient <0.50).

The MRDM models were implemented within the R package ECODIST (Goslee & Urban 2007). The MLPE models were implemented using linear mixed effects models, where the random effect term is used to account for the dependency between pairwise observations in a distance matrix (Yang 2004). Specifically, the random effect term includes a covariate structure where a proportion, ρ_τ, of the total variance, σ^2, is the result of the correlation between two pairwise distances involving a common population (Clarke *et al.* 2002). The intercept, slopes, ρτ and σ2 of this MLPE model are estimated with REML, which is desirable for unbiased estimates of the variance components of mixed models (Clarke *et al.* 2002; Gurka 2006). For both the MRDM and MLPE models, I centred all explanatory variables around their mean. Thus, for the MLPE models, the REML estimates of the intercept were the same as estimates obtained from simple linear regression (Clarke *et al.* 2002). I fitted MLPE models with REML estimation using the 'gls' function in the package NLME (Pinheiro *et al.* 2014) utilizing the corMLPE R package (https://github.com/nspope/corMLPE, Supporting Information) to account for multiple memberships (where each pairwise distance was associated with two populations). For the MLPE approach, I conducted model selection via BIC and model averaging using the R package MuMIn (Barton 2013), including all models within ΔBIC < 3. For all results, ± values represent standard error.

Results

Colony identity, HWE, allelic richness and population structure across biogeographic regions

Micro-Checker results indicated that one locus (BT136) exhibited substantial evidence of null alleles (>60% of populations with evidence of null alleles); therefore, I chose to exclude BT136 from the analysis. The remaining loci exhibited either low or no signs of null alleles (<35% of populations). Populations exhibited deviations from HWE at 1–5 loci (mean = 38.0% loci, SD = 16.2%), likely due to small sample sizes. Based on the Kruskal–Wallis rank sum test, biogeographic areas did not differ significantly in deviance from HWE ($P = 0.532$). Significant LD was detected for multiple loci, but within <10% of the populations and not consistent for any loci pair; therefore, I elected to retain all markers, except for BT136 (excluded for null alleles), for the analyses.

Average allelic richness across populations was 4.660 (±0.305), average private allelic richness across populations was 0.041 (±0.027), average heterozygosity across populations was 0.720 (±0.053), and average inbreeding (F_{IS}) was 0.133 (±0.174) (Table S1, Supporting information). Based on the Kruskal–Wallis rank sum test, the three biogeographic areas exhibited significantly different H_E ($P < 0.015$) and AR ($P < 0.016$), but no significant differences in PAR ($P = 0.111$) or F_{IS} ($P = 0.756$). Post hoc Wilcoxon rank sum tests showed that H_E in Island populations was marginally significantly lower than in Sierra populations ($P = 0.088$) and significantly lower than Coastal populations ($P = 0.008$), while AR in Island populations was significantly lower than both Sierra populations ($P = 0.042$) and Coastal populations ($P = 0.008$) (Fig. 2). I found no relationship between AR, H_E and F_{IS} for either Elevation or Latitude, but a significant relationship between PAR and Latitude ($P = 0.023$). Less than 25% of all populations showed evidence of a bottleneck (excess heterozygosity, Wilcoxon test $P < 0.05$) for the SMM model (20% of Coastal populations, 42% of Sierra populations and 0% of Island populations). No populations showed evidence of a bottleneck for the TPM model. For the STRUCTURE analysis, the Evanno method (Evanno *et al.* 2005) suggests $K = 5$ clusters, while for the TESS analysis, the DIC criterion (Spiegelhalter *et al.* 2002) suggests $K = 4$ clusters

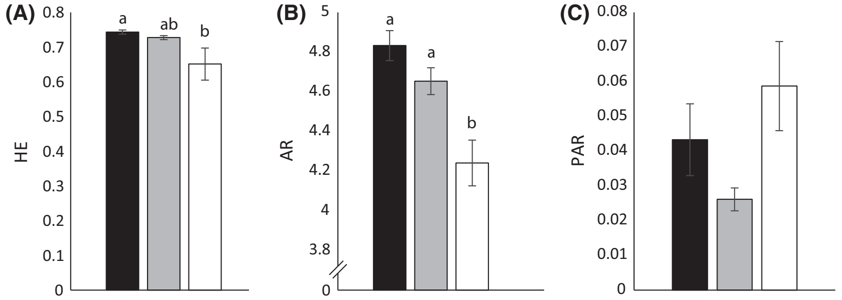

Figure 2 (A) Gene diversity (H_E), (B) allelic richness (AR) and (C) private allelic richness (PAR) across the three major biogeographic regions: the Coastal populations (black), the Sierra populations (grey) and the Island populations (white). For indices with overall significant differences, lowercase letters represent significant difference between groups using a Wilcoxon rank sum test ($P < 0.05$). Error bars represent standard error.

for the sampled study region. However, I document very high levels of coancestry within each population, with populations including individuals from a mean of 2.62 (±0.24) and 2.33 (±0.16) different clusters, for $K = 5$ and $K = 4$ clusters, respectively (individual cluster identity based on >50% assignment to a cluster) (Fig. S1, Supporting information).

Patterns of genetic differentiation

Overall, the populations were significantly differentiated from one another using all three indices, F_{ST} ($F_{ST} = 0.041$, 95% CI = 0.021–0.056), F'_{ST} ($F'_{ST} = 0.044$, 95% CI = 0.042–0.046) and D_{est} ($D_{est} = 0.155$, 95% CI = 0.147–0.162).

Pairwise population differentiation and relationship to geographic distance, elevation and land use were similar across all three differentiation indices; therefore, I only discuss results for F_{ST} (results for F'_{ST} and D_{est} reported in Supplementary Information). MRDM models with single variables (Geographic distance, Elevation RD and Land Use RD) demonstrated marginal support for isolation by Geographic distance ($P = 0.100$) and significant support for isolation by Land Use RD ($P = 0.005$–0.026, for the three Land Use periods) (Table 1). For both MRDM and MLPE, examining models including all three variables or a combination of the variables revealed that the most predictive explanatory factor was Land Use RD (Tables 1 and 2). Furthermore, for both MRDM and MLPE, the best model included only 2011 Land Use RD (MRDM model, $P = 0.005$, Table 1; MLPE model, lowest BIC, Table 2, Fig. 3).

Discussion

In this study, I closely examine the genetic structure of the yellow-faced bumble bee, *B. vosnesenskii*, across the western US coast and find strong evidence that human land use limits bee dispersal. Although I found weak evidence of distinct genetic groups in the study range, many measures of genetic diversity were lower in island populations compared to mainland populations. Across the study region, genetic differentiation was significantly predicted by oceans and two human-altered land-use types: impervious cover and cropland. Despite the incorporation of dramatic elevation gradients, the analyses reveal that oceans and current human land use, not elevation, are the primary dispersal barriers for *B. vosnesenskii* gene flow.

Biogeographic regions

Differentiation patterns were significantly greater than zero across indices, but low; likewise, there was evidence of distinct genetic groups for *B. vosnesenskii,* but these groups exhibited high levels of coancestry, likely due to frequent long-distance dispersal events. Patterns of little to no genetic structuring have been noted for the species at smaller scales (Jha & Kremen 2013b) as well as larger scales (Cameron *et al.* 2011; Lozier *et al.* 2011), like those examined in this study. This lack of genetic structuring has also been documented for most North American bumble bee species (Cameron *et al.* 2011; Lozier *et al.* 2011) with the exception of *B. bifarius,* which is comprised of multiple subspecies with distinct colour forms

Table 1 (A) MRDM results investigating Geographic Distance (Geo), Elevation Resistance Distance (Elev) and Land Use Resistance Distance (Land Use) for 2001, 2006 and 2011 and their relation to differentiation, F_{ST} ($N = 210$ pairs). (B) Coefficients for the full 2011 model and (C) the model with the highest *F*-test value. The *F*-test value (*F*-test), coefficient of determination (R^2), *P*-value (*P*) and Coefficient (Coef) are listed

(A)	No Land Use RD			2001 Land Use RD			2006 Land Use RD			2011 Land Use RD		
Model	*F*-test	R^2	*P*	*F*-test	R^2	*P*	*F*-test	R^2	*P*	*F*-test	R^2	*P*
Elev + Geo	10.340	0.091	0.143									
Elev	12.365	0.056	0.227									
Geo	6.007	0.028	0.100									
Elev + Geo + Land Use				10.912	0.137	0.164	11.033	0.138	0.156	20.251	0.228	0.036
Elev + Land Use				16.419	0.137	0.095	16.605	0.138	0.098	29.578	0.222	0.022
Geo + Land Use				16.025	0.134	0.053	16.212	0.135	0.052	30.523	0.228	0.010
Land Use				32.169	0.134	0.026	32.541	0.135	0.027	59.174	0.221	0.005

(B)	Coef	*P*	(C)	Coef	*P*
Elev	0.001	0.984	—		
Geo	<0.001	0.517	—		
2011 Land Use	0.123	0.034	2011 Land Use	0.123	0.005

Table 2 MLPE results investigating Geographic Distance (Geo), Elevation Resistance Distance (Elev) and Land Use Resistance Distances (Land Use) for 2001, 2006 and 2011 and their relation to differentiation, F_{ST} ($N = 210$ pairs). The Bayesian information criterion (BIC), change in BIC (ΔBIC) and Bayesian weight (w_i) are listed for each model. Indices are calculated independently for models with different Land Use years (2001, 2006 and 2011) and indices reported for models without Land Use (first 3 rows) are compared with the best (2011) Land Use model. (B) Coefficients for the full 2011 model, (C) the model with the lowest BICc and (D) all models with ΔBIC < 3 averaged

(A)	No Land Use RD			2001 Land Use RD			2006 Land Use RD			2011 Land Use RD		
Model	BICc	ΔBIC	w_i	BICc	ΔBIC	w_i	BICc	ΔBIC	w_i	BICc	ΔBIC	w_i
Elev + Geo	−1110.9	10.08	0.003									
Elev	−1114.9	6.16	0.020									
Geo	−1114.9	6.16	0.020									
Elev + Geo + Land Use				−1110.8	8.12	0.006	−1111.3	7.91	0.007	−1114.8	6.20	0.019
Elev + Land Use				−1114.4	4.55	0.036	−1114.6	4.54	0.038	−1116.5	4.47	0.046
Geo + Land Use				−1116.2	2.80	0.086	−1116.6	2.59	0.101	−1120.2	0.86	0.279
Land Use				−1119.0	0.00	0.350	−1119.2	0.00	0.367	−1121.0	0.00	0.428

(B)	Coef	SE	(C)	Coef	SE	(D)	Coef	SE
Elev	<0.001	0.055	—		—			
Geo	<0.001	<0.001	—		—	Geo	<0.001	<0.001
2011 Land Use	0.124	0.040	2011 Land Use	0.028	0.012	2011 Land Use	0.045	0.025

(Lozier *et al.* 2013). Overall, the weak level of genetic structuring for most North American bumble bees suggests that many species, including *B. vosnesenskii,* are actively engaging in gene flow across massive continental scales or are engaging in 'stepping-stone' gene flow, crossing mountains and large water bodies. The mechanism for this extensive dispersal is not known. While past studies have measured queen bumble bees dispersing approximately 9 km to find nest sites in their native range (Lepais *et al.* 2010; Jha & Kremen 2013b), estimates of dispersal across invasion frontiers suggest movement up to 200 km per generation (Schmid-Hempel *et al.* 2013), suggesting the potential for frequent long-distance dispersal events. Further, because male bumble bees exhibit longer foraging flight distances than female workers (Kraus *et al.* 2009), it is possible that males may be especially capable of long-distance dispersal, although few studies have attempted to quantify male bumble bee dispersal (*but see* Wolf *et al.* 2012).

Despite the lack of distinct genetic groups, the three focal biogeographic areas in this study show significant differences in *B. vosnesenskii* genic and allelic richness. Specifically, the Channel Island populations exhibit lower genic diversity (H_E) and allelic richness (AR) and higher private allelic richness (PAR) than the mainland populations, located in the Coastal and Sierra Nevada mountains and foothills. Past studies have documented high F_{ST} values for a *B. vosnesenskii* population on San Juan Island relative to the mainland and similar findings of distinctly greater F_{ST} values have been recorded for other *Bombus* species of nearby islands in the United States (Lozier *et al.* 2011). However, while I documented lower genetic diversity and allelic richness in the island populations, I did not find that islands made up distinct genetic groups, nor did they show evidence of bottlenecks, revealing that bumble bees can at least occasionally cross the 30–45 km oceanic barrier between the islands and the mainland, either passively, via strong wind currents, or actively by foraging in high-wind environments, which bumble bees are capable of navigating (Riley *et al.* 1999). While the population sizes investigated in this study were small (~ 40 individuals), making inbreeding and bottlenecks harder to detect, the lack of significant inbreeding and bottleneck signal across the island populations suggests that bumble bee flight and survival may be higher than expected when crossing large expanses of open water and high-wind conditions.

Patterns of genetic differentiation

Across three different indices ($F_{ST} = 0.041$, $F'_{ST} = 0.044$ and $D_{est} = 0.155$), the results demonstrate that genetic differentiation for *B. vosnesenskii* is significantly greater than the null expectation across the study system, an area that comprises more than half of the species range. Estimates from the current study are similar to those of smaller scale (200 km) studies of *B. vosnesenskii* in the USA ($F_{ST} = 0.019$, Jha & Kremen 2013b) and other *Bombus* species in the UK ($F_{ST} = 0.034$–0.16, Darvill *et al.*

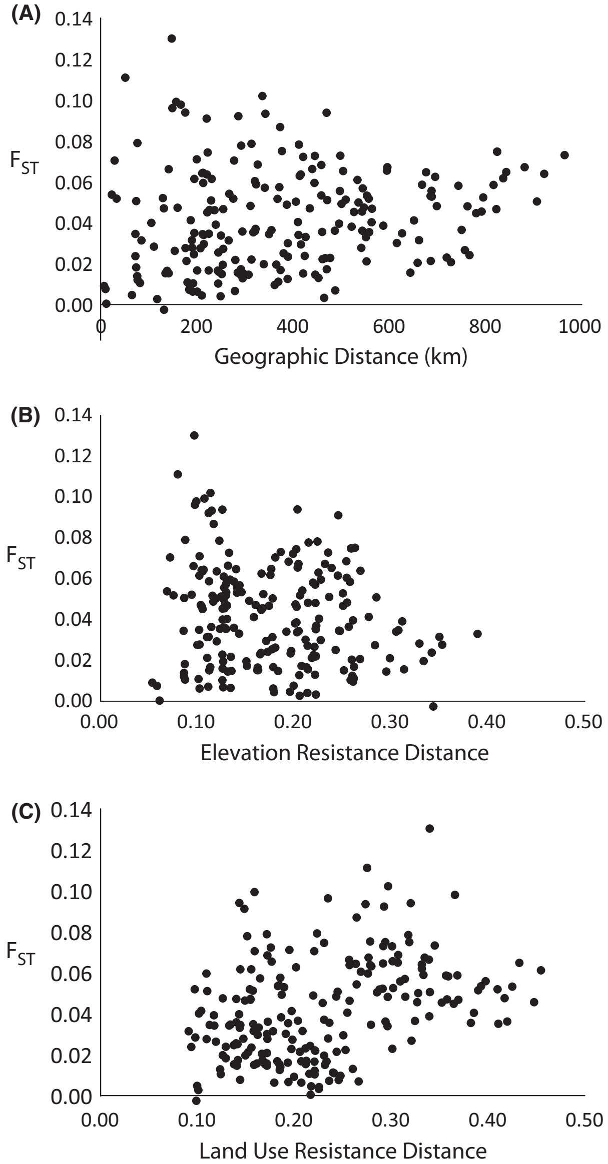

Figure 3 Isolation by Geographic Distance and Land Use Resistance Distance. Pairwise comparisons of genetic differentiation (F_{ST}) as a function of (A) Geographic Distance, (B) Elevation Resistance Distance and (C) Land Use Resistance Distance for 2011 (Table 1).

2010; Goulson *et al.* 2011), but higher than those measured for the same species in the central and northern portion of the species range (F_{ST} = 0.005, Cameron *et al.* 2011; e.g. D_{est} = 0.018, Lozier *et al.* 2011). Given the similar spatial scale for these past studies (Cameron *et al.* 2011; Lozier *et al.* 2011) and current study (1000 km), one potential reason for differences in estimated genetic differentiation includes a greater focus on the central and southern portion of the species range in the current study, which includes greater areas of human-developed land than the northwestern USA (Brown *et al.* 2005; Sleeter *et al.* 2013). Additionally, the current study utilizes a more intensive sampling of individuals per populations (~ 40 vs. ~ 20 individuals), a greater number of populations and a greater number and different selection of microsatellite markers, all of which could result in higher levels of estimated genetic differentiation for the species.

Furthermore, by investigating genetic differentiation patterns in response to geographic and resistance distances, this study shows that oceans and human-altered land use are the strongest barriers for *B. vosnesenskii* gene flow. Despite evidence from past studies that elevation may correlate with genetic differentiation for *B. bifarius* and may potentially limit gene flow (Lozier *et al.* 2011), I found no support for elevation as a predictor of differentiation patterns. The current study provides marginal evidence of isolation by distance (IBD), but reveals that the strongest correlations exist when incorporating oceans and human land-use types into the genetic differentiation models. These results are similar to the findings of Lozier *et al.* (2013) who use environmental niche modelling to reveal high gene flow levels for *B. bifarius* across forested mountain ranges across the western USA.

Finally, in this study, I show that resistance maps based on 2011 land use are better at predicting genetic differentiation patterns than maps based on 2006 and 2001 land use. Closer examination of the maps reveals that the low-resistance land-use types, Forest and Grassland, did not change substantially in the study region from 2001 to 2006, and 2006 to 2011 (the former decreasing by 0.20% and 0.32%, the latter increasing by 0.31% and 0.58%), while the high-resistance land-use types, Cropland and Impervious land, increased proportionally in cover. Specifically, Cropland increased by 0.01% and 1.77% and Impervious cover increased by 4.04% and 4.85%. Given the stronger model fit for 2011 land use, the results suggest that recent increases in the amount of urban and crop cover (between 2006 and 2011) played an important role in limiting bumble bee gene flow. While past studies have suggested that land-use maps created 5 years prior to sampling adequately predict bumble bee gene flow (Jha & Kremen 2013b), this study reveals that bumble bee genetic differentiation patterns are responding to land use at even shorter timescales than previously believed, in as little as months or single years since the time of land-use change.

Overall, this study provides strong support for the theory that urbanization, agricultural intensification and open water limit bumble bee gene flow, likely due to (i) the largely ground-nesting strategy of bumble bees, (ii) the lower level of floral resources in these landscapes and (iii) bumble bee aversion to dispersal across impervious surfaces. There are a number of past studies that support these three mechanisms of land-use-mediated bumble bee decline. First, since the 1950s, the extent of suburban and exurban development has increased rapidly in North America, especially in the study region (Brown *et al*. 2005). These landscapes often have large amounts of impervious cover, which limit bumble bee nesting densities (e.g. Jha & Kremen 2013a), probably due to reductions in the availability of optimal nest sites, such as tussock grasses and abandoned rodent nests (e.g. Svensson, Lagerlof & Svensson 2000). Second, empirical studies have also shown that bumble bee colony growth is directly impacted by floral resource availability (Williams *et al.* 2012), which is often lower in human-altered habitat, such as intensively farmed land, potentially leading to reduced survival in human-altered habitat. Finally, it is also likely that impervious cover limits bumble bee foraging effort, given that bumble bee worker foraging activity is markedly hindered by the presence of roads (Bhattacharya *et al*. 2003).

In addition to bumble bees, various taxa have been reported to exhibit limited gene flow in the presence of urban and agricultural areas. Urban and impervious areas appear to act as dispersal barriers for solitary bees (e.g. Davis *et al.* 2010), nonpollinating insects (e.g. Watts *et al.* 2004) and larger organisms such as small mammals (e.g. Munshi-South 2012); likewise, predominantly agricultural lands have been documented to reduce gene flow in beetles (e.g. Marchi *et al.* 2013), amphibians (e.g. Zellmer & Knowles 2009) and large mammals (e.g. Kelly *et al.* 2014). Correlations between genetic differentiation and human-altered land use can be the result of a variety of forces, such as population bottlenecks, or changes in inbreeding and/or demographic processes, such as recurrent extinction or colonization in the altered habitat (e.g. Zellmer & Knowles 2009). In this study, the analyses reveal low levels of inbreeding and limited evidence of bottlenecks for *B. vosnesenskii* across the western US coast; therefore, I suggest that increased genetic differentiation across oceans, impervious landscapes and agriculturally modified landscapes is the result of reduced nest site availability in combination with dispersal limitation and natal site fidelity.

Conclusions

Recent declines in bumble bee abundance have been well-documented in human-altered habitats, including cities and agricultural areas (reviewed in Goulson *et al.* 2008a). In support of these abundance declines, the analyses from this study reveal that bumble bee gene flow is limited by two human-altered land-use types: impervious cover and croplands. Given the coarse scale of this analyses (1-km cells), it is possible that small patches of natural habitat within cities and agricultural areas are providing bee habitat despite classification of these cells as 'highly developed' or 'intensely farmed' land. Regardless, measures can be taken to reduce intensification and encourage the additional incorporation of small green spaces in both of these land types. Recent studies have documented relatively high densities of bumble bees (McFrederick & LeBuhn 2006) and bumble bee nests (Goulson *et al.* 2011) in urbanizing areas, if green space and semi-natural woodland are available, respectively. Past research on pollinator community conservation has likewise supported the notion of improving the agricultural or urban 'matrix' by incorporating natural elements that provide resources or facilitate movement (e.g. Ricketts 2001; Perfecto & Vandermeer 2010). While putting a complete stop to the tilling and paving of agricultural or urbanizing landscapes is unlikely, actions that improve matrix quality or create spatially linked refugia within urban and agricultural areas can yield two major potential benefits: improved pollinator dispersal and survivorship, and increased spatial extent of crop pollination services (Lonsdorf *et al.* 2009; Ricketts & Lonsdorf 2013), for which bumble bees are key providers.

Finally, the results from this study clearly demonstrate that bumble bee genetic differentiation patterns respond to land use at very short timescales, approximately months or single years since the land-use change. While these results indicate that current human land-use practices (such as paving and tilling) are having immediate negative effects on bumble bee gene flow, possibly due to the annual generation time of the species, it is also possible that conservation efforts may have positive impacts within short timescales. Bumble bee abundance has been documented to increase substantially within just a few years of wild flower restoration plantings (Carvell *et al.* 2006; Pywell *et al.* 2006; Heard *et al.* 2007). While little is known about the ability of bumble bee populations to recolonize disturbed landscapes following a restoration, if increased bee abundances post-restoration are truly representative of local increases in nest density, then bumble bee gene flow and dispersal may also rebound rapidly post-restoration. Overall, this study suggests that the enhancement of urban and agricultural areas is a critical component of future bumble bee conservation aimed at securing gene flow and long-term population persistence.

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S.J. designed the research, performed the research, analysed the data and wrote the manuscript.

Data accessibility

Microsatellite and geographic collection data: Dryad digital repository entry: doi:10.5061/dryad.hr4 g0.

corMLPE script: available at Github: https://github.com/nspope/corMLPE.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Estimated population structure of *B. vosnesenskii* using STRUCTURE (Pritchard & Wen 2000) and TESS (Durand *et al.* 2009) (top and bottom panels, respectively) for (A) $K = 4$ and (B) $K = 5$ groups, with biogeographic region of sampling (Island, Coastal, and Sierra) and population IDs labeled (see Table S1, Supporting information).

Table S1 Genetic diversity within populations.

Table S2 (A) MRDM results investigating Geographic Distance (Geo), Elevation Resistance Distance (Elev), and Land Use Resistance Distance (Land) for 2001, 2006, and 2011 and their relation to differentiation, F'_{ST} (N=210 pairs).

Table S3 (A) MRDM results investigating Geographic Distance (Geo), Elevation Resistance Distance (Elev), and Land Use Resistance Distance (Land) for 2001, 2006, and 2011 and their relation to differentiation, D_{est} (N=210 pairs).

Table S4 MLPE results investigating Geographic Distance (Geo), Elevation Resistance Distance (Elev), and Land Use Resistance Distances (Land Use) for 2001, 2006, and 2011 and their relation to differentiation, F'_{ST} (N=210 pairs).

Table S5 MLPE results investigating Geographic Distance (Geo), Elevation Resistance Distance (Elev), and Land Use Resistance Distances (Land Use) for 2001, 2006, and 2011 and their relation to differentiation, D_{es}t (N=210 pairs).