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Biogeography, climate, and land use create a mosaic of parasite risk in native bumble bees

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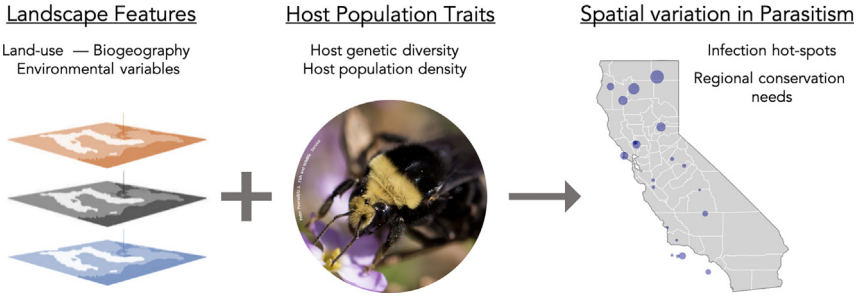
HIGHLIGHTS

- Bumble bees show high prevalence of three gut parasites along Western U.S. coast.
- Northern latitudes have higher prevalence of two closely related parasites.
- Environmental variation and host population structure drive parasitism patterns.
- Land use dually shapes host population genetic structure and parasitism risk.

GRAPHICAL ABSTRACT



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ABSTRACT

Host-parasite interactions are crucial to the regulation of host population growth, as they often impact both long-term population stability and ecological functioning. Animal hosts navigate a number of environmental conditions, including local climate, anthropogenic land use, and varying degrees of spatial isolation, all of which can mediate parasitism exposure. Despite this, we know little about the potential for these environmental conditions to impact pathogen prevalence at biogeographic scales, especially for key ecosystem service-providing animals. Bees are essential pollination providers that may be particularly sensitive to biogeography, climate, and land-use as these factors are known to limit bee dispersal and contribute to underlying population genetic variation, which may also impact host-parasite interactions. Importantly, many native bumble bee species have recently shown geographic range contractions, reduced genetic diversity, and increased parasitism rates, highlighting the potential importance of interacting and synergistic stressors. In this study, we incorporate spatially explicit environmental, biogeographic, and land-use data in combination with genetically derived host population data to conduct a large-scale epidemiological assessment of the drivers of pathogen prevalence across >1000 km for a keystone western US pollinator, the bumble bee *Bombus vosnesenskii*. We found high rates of infection from *Crithidia bombi* and *C. expoekii*, which show strong spatial autocorrelation and which were more prevalent in northern latitudes. We also show that land use barriers best explained differences in parasite prevalence and parasite community composition, while precipitation, elevation, and *B. vosnesenskii* nesting density were important drivers of parasite prevalence. Overall, our results demonstrate that human land use can impact critical host-parasite interactions for native bees at massive spatial scales. Further, our work indicates that disease-related survey and conservation measures should take into account the independent and interacting influences of climate, biogeography, land use, and local population dynamics.

1. Introduction

Host-parasite interactions can play a key role in ecology by altering host population growth (Anderson and May, 1986), health (Rutrecht and Brown, 2009), behavior (Hutchings et al., 2003), interactions with other

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species (Altizer et al., 2013), and subsequent ecological function (Hatcher et al., 2006). For example, infected animals may forage for longer durations (Wolf et al., 2014) and visit resources outside of their typical preferences (Richardson et al., 2016), thus impacting the availability of those resources in the local environment (Hatcher et al., 2006). Given their host mobility, animal parasites may be particularly responsive to environmental context across organizational and spatial scales, leading to variation in prevalence across host populations. Indeed, past work has shown that across large spatial scales, animal hosts may be differentially susceptible to pathogens due to underlying population genetic differences (Parsche and Lattorff, 2018; Huth-Schwarz et al., 2012), differences in host density and contact among susceptible host species (Graystock et al., 2020), and differences in underlying abiotic conditions, including biogeography (Escobar and Morand, 2021), land-use (Gottdenker et al., 2014), and climate (Altizer et al., 2013). Despite the likely role of abiotic environmental conditions and host population characteristics in mediating parasite transmission, few studies have simultaneously characterized both sets of forces and their potential impact on parasite prevalence at biogeographic scales.

Recent work on pathogens of widely distributed species suggest that biogeography, elevation, climate, and land use may be more important drivers of spatial variation in parasitism than previously believed (Stephens et al., 2016). Historically, studies have often hypothesized that parasite diversity is highest near the equator due to higher host diversity per unit of area (*e.g.*, Guernier et al., 2004). However, more recent studies have suggested that this relationship is more host-dependent, where the intensity of parasitic interactions may actually increase at higher latitudes likely due to differences in host susceptibility and immune priming in more diverse parasite communities at low latitudes (Zvereva and Kozlov, 2021). Biogeographic, elevational, and climatic gradients can also create variation in habitat suitability, which could influence interactions between hosts and parasites. For example, precipitation has a fundamental impact on plant biomass and plant-based resource availability, and this can affect an animal's interaction with plant-based resources (Lawson and Rands, 2019). Climate can also directly impact animal communities by shifting the geographic range of hosts and pathogens, leading to novel species interactions (Altizer et al., 2013), or by altering the susceptibility of hosts, which may occur more in stressful environments with limited resources (Gottdenker et al., 2014). Anthropogenic land-use change often creates conditions of stress that may impact wildlife disease (Łoś et al., 2020; Guo et al., 2019; Gottdenker et al., 2014; Goulson et al., 2012; but see Ceballos et al., 2006), with documented increases in infectious disease prevalence within wildlife and higher risk of zoonotic outbreaks within humans following land conversion (Jones et al., 2013). Land use change can also modify ecological niches and can ultimately alter the spatial dynamics of disease incidence through changes to host movement and the composition of hosts and pathogens (Gottdenker et al., 2014).

Interestingly, these biogeographic, elevation, climate, and land use forces also impact host genetic composition, which can underlie a hosts' ability to respond to stressors (Manlik et al., 2022). For example, elevation and land use can create resistance to dispersal for many host organisms (Jha, 2015), leading to population genetic isolation, as organisms are either unable to encounter the resources they need, or their movements are diverted to avoid stressful conditions. From a population genetics perspective, isolated host populations are more likely to experience inbreeding depression and a decrease in local genetic diversity (Hedrick and Kalinowski, 2000). Genetic diversity is closely associated with host immune function (reviewed in King and Lively, 2012), and may also predict prevalence, where populations with higher genetic diversity often experience lower parasite prevalence (Lively et al., 1990; Shykoff and Schmid-Hempel, 1991; Whitehorn et al., 2011). Landscape-level genetic structure (differentiation) also captures gene flow resulting from population connectivity, which may correlate with the long-term host population stability and its impact on infectious disease status (Kozakiewicz et al., 2018). A large-scale study comparing disease incidence and landscape genetics of several North American bee species showed that declining host species and those undergoing range contractions experienced higher incidences of parasitism compared to stable co-occurring species (Cameron et al., 2011). Further, a positive feedback loop may emerge between population decline, genetic inbreeding, and increased parasitism (McCallum, 2012). Thus, it is essential to quantify both the population-level genetic diversity and genetic differentiation between host populations in order to best characterize landscape-level parasite incidence.

Finally, these host-parasite interactions and their ecological consequences are especially important for mobile organisms that forage across space to provide key ecosystem functions (Kremen et al., 2007). Pollinators provide great value to terrestrial ecosystems through the transport of pollen necessary for the reproduction of more than 85 % of all plant species (Ollerton et al., 2011). Over 80 % of crop species are also dependent on pollinators, with bees providing the majority of pollination services (Bartomeus et al., 2014). Bumble bees are some of the most ecologically and economically important pollinators, and as central place foragers, they are tied to the local landscape where they have constructed a nest. Thus, they are often limited by suitable habitat (Koh et al., 2016) and may be particularly sensitive to the synergistic stressors of land use change, climate change, and pathogen pressure (Goulson et al., 2015). Host-parasite interactions are also fascinating for bees given that many bee parasites are transmitted through shared use of floral resources (Graystock et al., 2015), as well as *via* intra-colonial transmission through contact with nest-mates (Otterstatter and Thomson, 2007; Pinilla-Gallego et al., 2020) and brood (Folly et al., 2017). Both of these forces could work to amplify parasite abundance, which in turn can influence pollinator foraging with negative consequences for the reproductive success of both plants and pollinators (*i.e.*, Gillespie and Adler, 2013).

In this study, we investigate the key drivers of spatial variation in parasite prevalence, focusing on one of the most important native pollinators in the Western US (Kremen et al., 2002), the yellow-faced bumble bee, *Bombus vosnesenskii*. We quantify pathogen prevalence across nearly 1000 kms, allowing us to evaluate the impact of natural environmental variation and population level differences across roughly half of the species' range. We hypothesize that spatial variation in parasitism is related to both landscape context (land-use and biogeography), and the habitat preferences and dispersal patterns of the host. Additionally, given the small but significant degree of population genetic structure in our samples (Jha, 2015), we hypothesize that populations with high genetic diversity would have low rates of parasitism and that genetic diversity would impact the overall distribution of parasitism. We implement a large-scale epidemiological and spatially-explicit modeling approach to understand variation in parasitism, identify potential hotspots, and describe the relationship between environmental conditions, host dynamics, and resulting host-parasite interactions.

2. Methods

2.1. Study region and sampling

We sampled *Bombus vosnesenskii* from 21 sites (Fig. 1), separated by a mean of 298.9 km (7.15–963.24 km), throughout the state of California along the southwestern coast of the US, encompassing large gradients in precipitation, elevation, and land use (as described in Jha, 2015). The sampling scheme was established to maximize detection of potential patterns of genetic differentiation and study sites were selected to represent the three main biogeographic regions of California including the Sierra Nevada foothills and mountain range, the Coastal and Transverse foothills and mountain range, and the Channel Islands. An average of 41.14 (SE = 2.36) bees were collected by aerial netting within 25 m of the centroid of each site, and were immediately submerged in 95 % ethanol within individual glass vials (as described in Jha, 2015).

2.1.1. GIS

Regional land use was characterized using the National Land Cover Database (NLCD) (Homer et al., 2015) and measured at a 1-km resolution using the majority resampling function in ArcGIS v.9.2 (ESRI, 2006).

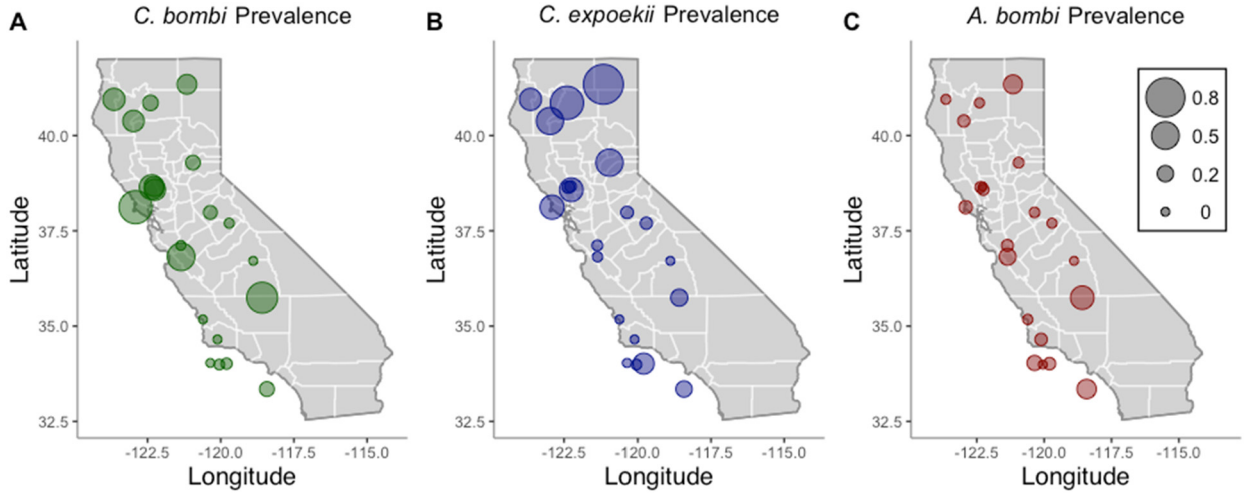

Fig. 1. Maps of the study regions showing the location and parasite prevalence at each of the 21 collection sites. The relative size of circles at each site indicates the prevalence of *Crithidia bombi* (A), *Crithidia expoekii* (B), and *Apicystis bombi* (C),

Land use was classified into 5 main categories of bumble bee habitat based on nest and food availability (as in Jha, 2015): Open water (open water and surface ice) (0–26.1 % cover), Forests and scrubland (all categories of forest and shrub/scrub) (0–96.3 % cover), Impervious land (developed surfaces including commercial and industrial land with >20 % impervious cover) (0–17.7 % cover), Grassland and pasture (including developed land with <20 % impervious cover, grassland, barren land, and pasture) (3.5 %–80 %), and Cropland (crops and wetlands) (0–14.2 % cover). Land use was calculated from the 2011 NLCD land use surface which was concurrent to the first year of bee sampling.

Mean precipitation was extracted from the publicly available PRISM dataset (Daly et al., 2007; as in McNeil et al., 2020). As per past studies examining climate impacts on bee pathogen dynamics (McNeil et al., 2020), we used a long term mean precipitation score, in this case the 30-year Normal mean annual precipitation (800 m resolution) and averaged all point values within a 2-km radius of the site. The elevation of each collection site was calculated from the National Elevation Dataset (NED, httl://ned.usgs.gov/). The absolute latitude was taken from the geographic coordinates for the center-point of each collection site.

2.2. Molecular analyses

2.2.1. Genotyping and nest assignment

Tarsal snips were taken from collected bees, and DNA was extracted from each individual for genotyping (see Jha, 2015). DNA was extracted using the HotShot protocol (Truett et al., 2000) and 12 microsatellite loci were screened, including B96, B100, B119 (Estoup et al., 1995), and BT33, BT43, BT65, BT124, BT125, BT128, BT131, BT132, and BT136 (Stolle et al., 2009). Multiplex polymerase chain reactions (PCRs) contained 2 mg of DNA, 2uL of 10× PCR buffer, 1.5 mM $MgCl_2$, 300 uM of each dNTP, 1 U of Taq polymerase, and 0.25uM of each primer with a final reaction volume of 20uL. The reaction began with a 5-min denaturation step at 95 °C, followed by 37 cycles of: 30 s at 72 °C, 60 s at the primer specific annealing temperature, and 30 s at 72 °C, ending with a final 20-min extension period at 72C (Jha, 2015). One primer in each pair was also labelled with a dye (6-FAM, NED, VIC or PET) to aid in genotyping with an ABI3730 Sequencer (Jha, 2015). Alleles were scored using GENEMARKER ® (Softgenics). In order to optimize the accuracy of patterns of population genetic structure (Landguth et al., 2012) and genetic differentiation (ie. Arthofer et al., 2018), only specimens with at least 8 viable markers were included in the study. To prevent pseudo-replication at the colony level, we assigned individuals to colonies using the program COLONY 2.0 (Wang, 2004) with the error rate set to 0.001 (as in Jha and Kremen, 2013b). We then randomly excluded individuals until only one representative of each colony remained, leaving a total of 762 bees with a mean of 35.81 bees per site (± 2.15 SE) for the pathogen screen.

2.2.2. Site-level nesting density and genetic variation

Next, the density of colonies nesting at each site was estimated based on the distribution of recapture events for each colony at a site using the software CAPWIRE (Miller et al., 2005). We used the Two Innate Rate Method (TIRM) which has been found to best model the expected truncated Poisson distribution of nest densities (as in Goulson et al., 2010). Previous analysis of this dataset revealed small but significant genetic differentiation using several measures (Jha, 2015). Given our emphasis on site-level host-pathogen landscape dynamics, we assessed site-level nesting density and site-level genetic variation (H_E; Nei and Kumar, 2000).

2.3. Pathogen screen

We briefly removed the specimens from ethanol storage and dissected the gut tissue by making a lateral incision using iris scissors and forceps. We removed all gut tissue and dried it at room temperature for 15 min to remove ethanol that may interfere with subsequent molecular reactions. We used a motorized pestle to grind the tissue, extracted DNA through isopropyl precipitation, and finally resuspended the DNA in 50uL TE buffer.

We used a multiplex PCR approach to simultaneously detect three parasite taxa, *Crithidia*, *Vairimorpha*, and *Apicystis* (Mullins et al., 2020; Tripodi et al., 2018). The PCR reaction mix consisted of 0.8 μM of each parasite detection primer, 0.4 μM of a bee positive control primer set, 1.3× buffer, 2.0 mM MgCl2, 0.8 μM total dNTPs, 1 unit Taq (Genesee Scientific, Apex polymerase), 1.2 μL DNA sample, with DEPC treated water to adjust the volume to a total of 25 μL (Mullins et al., 2020). The PCR reaction followed an initial denaturation step of 94 °C for 2 min, followed by 10 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 45 s then 30 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 45 s and finally an extension at 72 °C for 5 min (Mullins et al., 2020). Samples were run in batches, and included one positive control for each of the parasite taxa, as well as a negative control with water substituting DNA. PCR products were visualized with gel electrophoresis, where each primer set amplified a unique DNA fragment length (*Crithidia* = 584 bp, *Apicystis* = 357 bp, *Vairimorpha* = 270–316 bp;; Mullins et al., 2020). We had difficulty reliably amplifying our *Vairimorpha* positive controls, and ultimately excluded this taxon from the analysis. Samples which contained *Crithidia* DNA were then identified based on fragment length using a secondary multiplex PCR to distinguish between *Crithidia bombi* (279 bp), *Crithidia expoekii* (163 bp), and a sequence conserved across *Crithidia* (584 bp) to account for non-target parasites (Mullins et al., 2020). Samples which contained

Apicystis DNA were secondarily sequenced using a primer set to distinguish between *Apicystis bombi* and the recently described *Apicystis cryptica* (Schoonvaere et al., 2020).

2.4. Statistical analyses

2.4.1. Spatial autocorrelation

We mapped and analyzed spatial variation in parasitism (Fig. 1) using Moran's I to test for spatial autocorrelation. We conducted all statistical analyses using the R statistical computing software. Specifically, we calculated and compared geographic distances and dissimilarity in the rate of parasitism for each parasite species at a given site and calculated Moran's I using the *ape* package and the Moran.I function (Paradis et al., 2004).

2.4.2. Generalized linear mixed effect models

To analyze linear relationships between local and landscape attributes and site-level parasitism, we ran generalized linear mixed effect models (GLMM) (*lme4* package; Bates, 2010) for prevalence of each of the three parasites. Specifically, predictor variables included site-level nesting density, genetic variation (H_E), elevation, mean precipitation, latitude, and nesting suitability (combined Forest and Grassland cover, as in Jha and Kremen, 2013a) within 2 km. Response variables included prevalence (presence or absence) of a given parasite species as well as the overall 'Health Status' of individual bees (parasitized or not parasitized) (as in McNeil et al., 2020). For all GLMMs, we used a binomial distribution and included site as a random effect given repeated sampling (Zuur et al., 2009). To test for collinearity between response variables, the *car* package (Fox et al., 2013) was used to ensure Variance Inflation Factor (VIF) scores for all predictor variables were below a conservative cutoff of 2.5 for all models. Models underwent a strict model selection protocol using the *MuMIN* package in R (Barton, 2020) which generates models with all combinations of predictor variables and ranks each model using Akaike Information Criteria (AIC). The 'top model' was selected as the model with the lowest AIC score. In cases where multiple models fell within 2 AIC, these were incorporated into a weighted 'averaged best model' (Table 2).

2.4.3. Multiple regression on distance matrices

To analyze pairwise differences in parasitism between all pairs of study sites, we utilized multiple regression on distance matrices (MRDM), a common approach in landscape ecology (Cusser et al., 2018) which is increasingly used to understand pathogen dynamics across large spatial scales (Poulin et al., 2011). MRDMs allow for multiple environmental matrices to be evaluated in the same model, rather than collapsing variables into a single environmental dissimilarity score (Lichstein, 2007). MRDMs use non-parametric regression methods where significance of each distance matrix is tested using permutation (Lichstein, 2007). Therefore, specific hypotheses for environmental metrics, geographic metrics, and land use resistance metrics may be tested in the same model (Cusser et al., 2018).

The response variables used in this analysis were parasitism distance matrices, where each response variable was calculated as site-by-site pairwise dissimilarity in parasite prevalence, where sites with more similar parasite prevalence have lower distances and sites with less similar rates of parasitism have greater distances. Distance matrices were calculated for the site-level prevalence of *C. bombi*, *C. expoekii*, and *A. bombi* using the Bray-Curtis dissimilarity metric. We then calculated a Bray-Curtis dissimilarity matrix for overall parasite pressure by including all three parasite taxa in a single matrix (as in Poulin et al., 2011).

The predictor variables used in this analysis were the geographic distance between sites, resistance distance between sites, elevation dissimilarity (as in Jha, 2015), host genetic distance (F_{ST}), host nesting density dissimilarity, and mean precipitation dissimilarity. Geographic distance was calculated as the Euclidian distance between the center-point of each site. Resistance distance was calculated using resistance surfaces generated in CIRCUITSCAPE v3 based on land-use classifications for nesting suitability (*sensu* Jha, 2015) where high resistance land-use represents unsuitable habitat for host nesting and low resistance land-use represents suitable habitat for nesting. Specifically, using the 2011 NLCD land use classifications, we created a single resistance raster with a resistance score of 0.9 applied to water cover, cropland cover, and impervious cover, and a resistance score of 0.1 applied to grassland and forest cover, and then calculated a pair-wise Euclidian distance between each site (as in Jha, 2015). Elevation dissimilarity matrices were calculated from the National Elevation Dataset (NED, httl://ned.usgs.gov/), by taking the elevation difference between sites and centering them between 0 and 1 by dividing all values by the maximum elevation of 4330 m (as in Jha, 2015). To create host genetic distance matrices based on pairwise genetic differentiation between sites, we calculated F_{ST} modified for multi-allelic markers (Weir and Cockerham, 1984) using weighted analysis of variance (weighted for sample size) in the software FSTAT (Goudet, 1995) as in Jha and Kremen (2013b). Dissimilarity matrices for host nesting density and mean precipitation were calculated as the Euclidean distance between site level values (as in Šurinová et al., 2019). Because geographic distance and resistance distances were highly correlated (Pearson's $R = 0.942223$; $P = 2.2e\text{-}16$), they were explored in independent models alongside the remaining matrices.

The MRDMs were conducted using the *ecodist* package in R (Goslee and Urban, 2007) using a backwards selection procedure (Numerical Ecology 3rd ed., 2012), where the initial model includes the greatest number of variables and subsequent models exclude the least predictive variable (highest *P*-value) at each step (as in Cusser et al., 2018). Model selection stopped when all variables in the model had a significant effect, or when the model retained only a single predictor variable after excluding all other variables in previous steps. We ran 10,000 permutations in all models, and compared models based on the goodness of fit measured as R^2 values, given that other metrics such as AIC, AICc, and BIC are limited in their ability to compare MRDMs with different numbers of variables (Franckowiak et al., 2017). Because the resistance distance models consistently had greater predictive power than the geographic distance models (Supplemental Table 2), we chose to focus on the resistance distance models in remaining sections.

3. Results

3.1. Parasitism rates

The most common parasites in the study were *Crithidia expoekii* ($n = 177$; 23.53 %) and *Crithidia bombi* ($n = 173$; 23.0 %), followed by *Apicystis bombi* ($n = 83$; 11.03 %). Site-level parasite prevalence was highly variable (Fig. 1), with *C. expoekii* ranging from 0 to 82.2 % (mean site level prevalence = 8.43 %, SD = 9.57), *C. bombi* ranging from 0 to 64.2 % (mean site level prevalence = 8.24 %, SD = 8.66), and *A. bombi* ranging from 0 to 39.0 % (mean site level prevalence = 3.95 %, SD = 4.33) (Fig. 1).

3.2. Spatial autocorrelation

There was strong evidence of spatial autocorrelation for both *C. expoekii* and *C. bombi*, but not for *A. bombi*, which was much less prevalent in this study. Moran's I test for spatial autocorrelation showed significant spatial autocorrelation for *C. expoekii* ($P = 0.0047$), and *C. bombi* ($P = 0.048$) but was non-significant ($P = 0.926$) for *A. bombi.*

3.3. Generalized linear mixed effect models

After model selection, the top model for *C. bombi* (Table 2) included a significant positive relationship with latitude ($P = 0.00239$; Fig. 2A). After model selection, the top model for *C. expoekii* (Table 2) included a significant positive relationship with latitude ($P \leq 2e\text{-}16$; Fig. 2B), and a significant negative correlation with host genetic diversity ($P = 0.0199$). After model selection, the top model for *A. bombi* prevalence (Table 2) included a significant positive relationship with estimated nesting density ($P = 0.01947$; Fig. 4A), and a significant negative relationship with precipitation ($P = 0.00503$; Fig. 4B). The top Health Status model, where individual bees

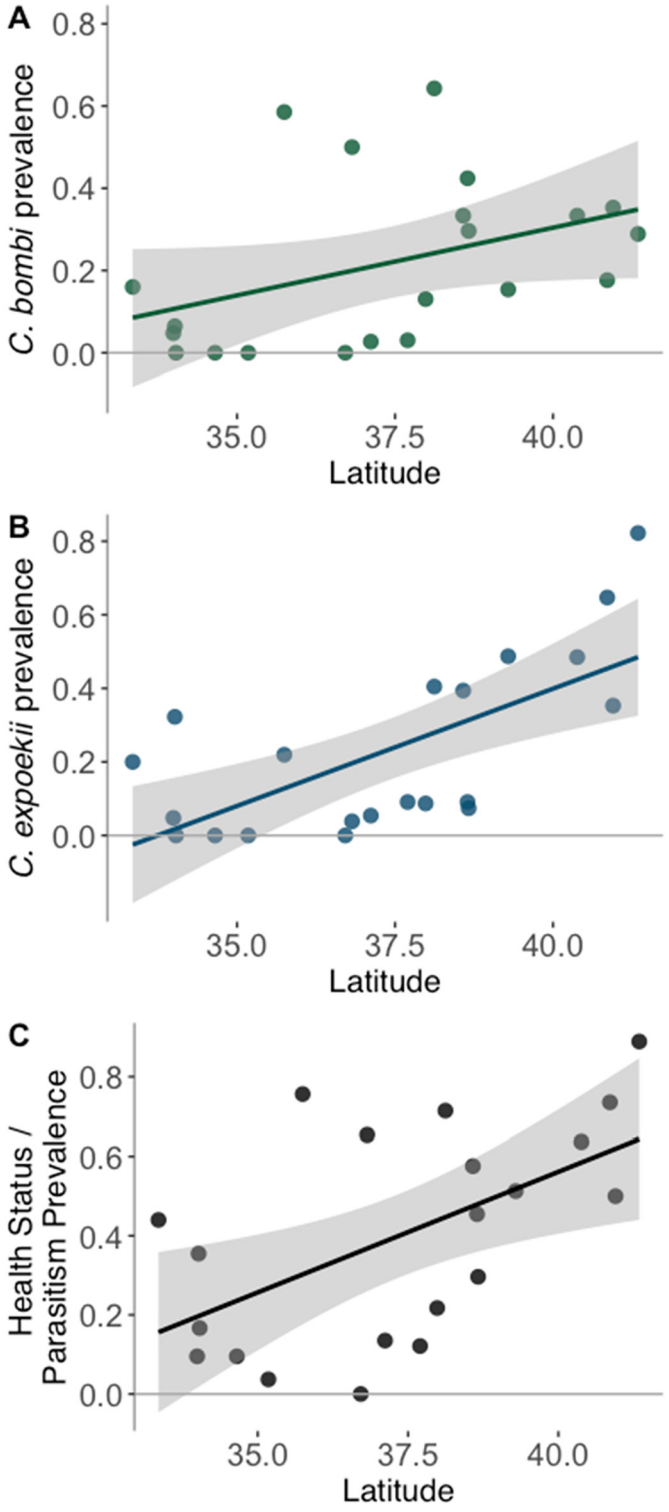

Fig. 2. Regression plots from GLM models, showing significant correlations between latitude and *C. bombi* prevalence (A), *C. expoekii* prevalence (B), and the Health Status (parasitized or not parasitized) metric (C). All three show significantly higher prevalence in Northern latitudes.

were either 'parasitized' or 'not-parasitized', included latitude as the single significant predictor variable ($P = 0.014$; Fig. 2C). The full models and averaged top models (within delta AIC 2) for all three pathogens were very similar to their respective top models, including all of the same significant variables (Table 1).

Table 1
Summary of GLM results in the full model average after model selection, including all models within delta 2 AIC. Response variables are the presence or absence of *C. bombi*, *C. expoekii*, or *A. bombi* in individual bees. The 'Health Status' response variable indicates the presence or absence of any parasite in an individual bee (ie. parasitized or not parasitized individuals). Significant terms in the full model are indicated in bold.

Response	Predictor	Estimate	Standard Error	*Z*-value	P-value
Crithidia bombi	Nest Density	0.01234	0.12582	0.098	0.922
	Mean Heterozygosity	0.02351	0.14108	0.166	0.867
	Elevation	−0.07724	0.21711	3.55E-01	0.722
	Precipitation	−0.05702	0.21372	0.267	0.789
	Latitude	**0.89275**	**0.36934**	**2.413**	**0.015**
	Nest Site Suitability	0.00639	0.11627	0.055	0.956
Crithidia expoekii	Nest Density	0.01709	0.12574	0.136	0.892
	Mean Heterozygosity	**−0.66595**	**0.28566**	**2.328**	**0.019**
	Elevation	−0.00353	0.10369	0.034	0.972
	Precipitation	−0.01341	0.13679	0.098	0.922
	Latitude	**1.44804**	**0.32987**	**4.383**	**1.17E-05**
	Nest Site Suitability	0.04847	0.16422	0.295	0.768
	Nest Density	**0.43652**	**0.19023**	**2.291**	**0.021**
	Mean Heterozygosity	0.01223	0.08076	0.151	0.879
Apicystis bombi	Elevation	0.05821	0.14316	0.406	0.684
	Precipitation	**−0.61641**	**0.20411**	**3.015**	**0.002**
	Latitude	−0.00247	0.00664	0.373	0.709
	Nest Site Suitability	0.02878	0.11235	0.256	0.798
	Nest Density	0.22297	0.31307	0.313	0.476
	Mean Heterozygosity	−0.00661	0.09099	0.091	0.942
Health Status	Elevation	−0.06601	0.18143	0.181	0.716
	Precipitation	−0.10312	0.25033	0.251	0.681
	Latitude	**0.79455**	**0.32305**	**0.323**	**0.014**
	Nest Site Suitability	0.08219	0.21639	0.216	0.704

3.4. Multiple regression on distance

The most important predictor variable explaining pair-wise differences in parasite prevalence across our sites was resistance distance (Fig. 3). After backward model selection (Supplemental Table 2), the top MRDM model for *Crithidia bombi* distance (Table 3) included both a significant positive effect of resistance distance ($P = 0.0002$; Fig. 3A) and a significant negative effect of precipitation ($P = 0.0472$). The top MRDM model for *C. expoekii* distance (Table 3) included significant positive effects from resistance distance ($P = 0.0076$; Fig. 3B) and elevation ($P = 0.0499$). The top MRDM model for *Apicystis bombi* distance (Table 3) did not include any significant predictors; model selection removed all possible variables, and stopped at Step 5 ($R^2 = 0.0301$) with a non-significant effect from nesting density ($P = 0.0616$). The top MRDM model for overall parasite community dissimilarity (Step 5; $R^2 = 0.0816$) included a significant positive effect of resistance distance ($P = 0.0014$; Fig. 3C).

4. Discussion

In this large-scale epidemiological study of a keystone bumble bee species along the Western US coast, we document substantial spatial variation in parasitism across the region, with strong spatial autocorrelation for both *Crithidia* species, which were found at significantly higher prevalence in northern latitudes. Identifying this hot-spot for native bee parasitism demonstrates the value of a landscape-scale approach to disease ecology, especially for key ecosystem-service providing organisms. The spatial patterns seen in both *Crithidia* species were not found in *A. bombi*, which was more influenced by local conditions, including precipitation and host population density, likely due to differences in transmission mechanism between the parasite taxa. We also show that biogeography and landscape composition, in particular the availability of nesting resources, impacts

Table 2
Results from the GLM models found with the lowest AIC scores during MuMIN model selection. Significant terms in each model are included in bold.

Response	Predictor	Estimate	Standard Error	Z-value	P-value	MarginalR^2	AIC
Crithidia bombi	**Latitude**	0.8583	0.333	2.576	0.0099	0.1242	677.6
Crithidia expoekii	**Latitude**	1.4512	0.312	4.643	3.43E-06	0.2952	625.2
	Mean Heterozygosity	−0.6724	0.282	−2.383	0.0172		
Apicystis bombi	**Nest Density**	0.4463	0.191	2.336	0.0194	0.1202	491.2586
	Precipitation	−0.5968	0.212	−2.805	0.005		
Health Status	**Latitude**	0.7739	0.261	2.962	0.003	0.1154	839.6

Table 3
Result of MRDM model selection showing the best model for each parasite and hypothesis. The backwards model selection for *A. bombi* the models for 'Isolation by Distance' and 'Isolation by Resistance' hypotheses each removed geographic and resistance distances, respectively, where both models indicate estimated nesting density is the top predictor. Significant terms in each model are included in bold.

Response	Predictor	Estimate	Predictor P-value	Model F-test	Model P-value	R^2
Crithidia bombi	**Precipitation**	−0.0017	0.0472	11.655	0.016	0.1012
	Resistance Distance	2.4178	0.0018			
Crithidia expoekii	**Elevation**	0.0885	0.0499	21.166	0.008	0.1697
	Resistance Distance	2.1986	0.0076			
Apicystis bombi	**Estimated Nesting Density**	0.00005	0.0605	6.472	0.061	0.0301
Bray-Curtis Dissimiliarty	**Resistance Distance**	2.9155	0.0014	16.711	0.001	0.0816

spatial variation in parasitism. Finally, we found that populations with lower genetic diversity had higher prevalence of *C. expoekii,* indicating that genetic diversity may reduce infection risk in local populations. All considered, we show that both abiotic environmental factors and host population characteristics impact spatial variation in parasitism across the landscape.

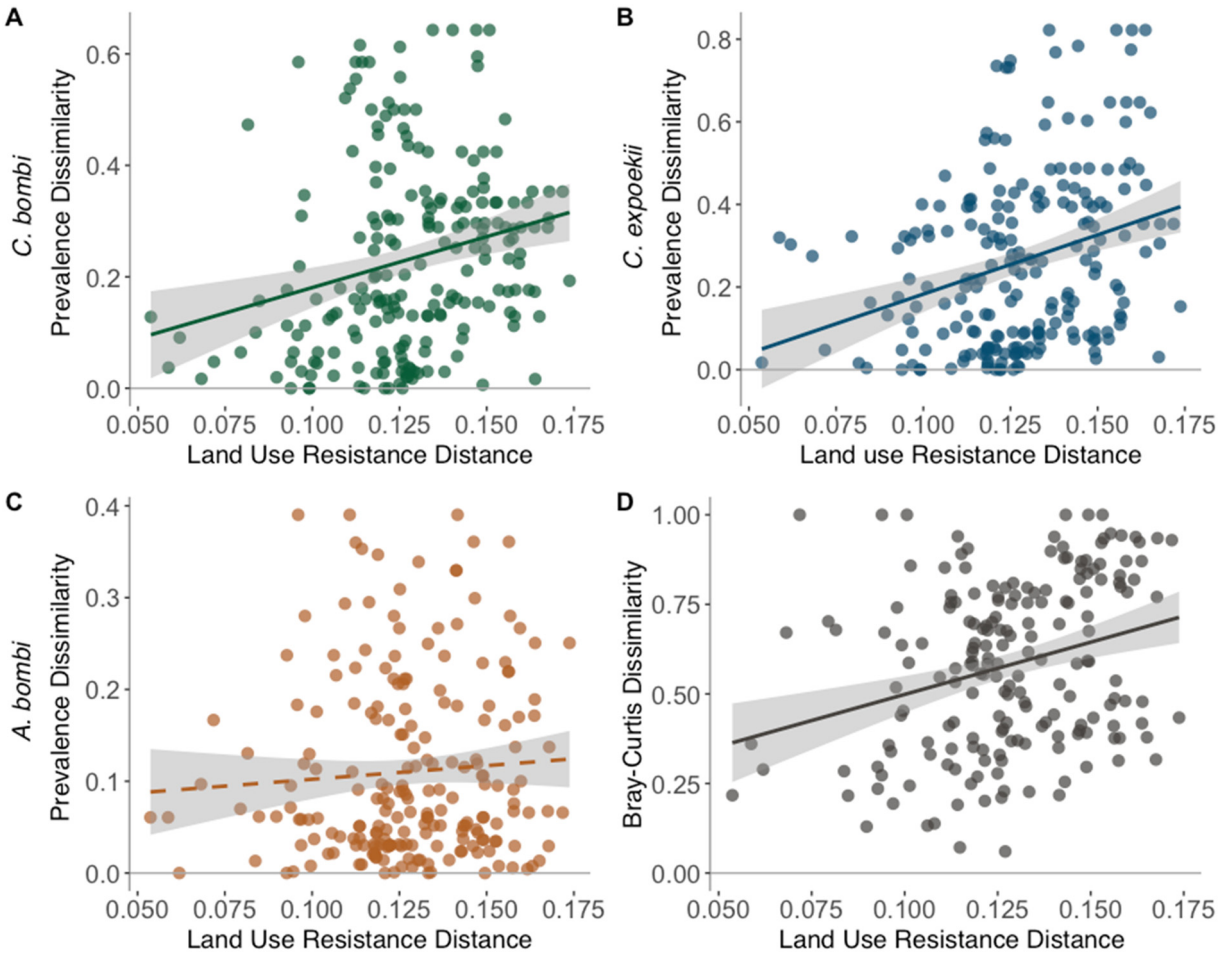

Fig. 3. Regression plots the resistance distance surface and dissimilarity in parasite abundance for *C. bombi* (A), *C. expoekii* (B), and *A. bombi* (C), and Bray-Curtis parasite community dissimilarity (D). Solid lines indicate significant trends, and dashed lines are non-significant.

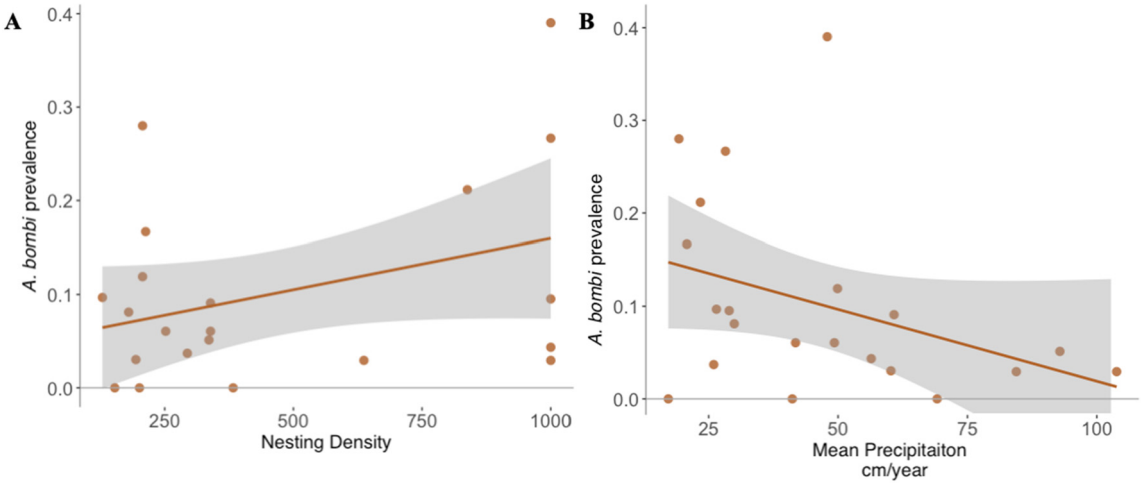

Fig. 4. Regression plots for the top GLM model for *A. bombi* prevalence. *A bombi* prevalence is positively correlated with nesting density (A), here represented as site level nesting density estimated through genetic recapture. *A. bombi* also shows a negative correlation with local mean precipitation (B).

4.1. Pathogens exhibit spatial autocorrelation and greater prevalence at higher latitudes

The parasites in our study exhibited substantial spatial variation in prevalence. We found strong evidence for spatial autocorrelation in both *C. bombi* and *C. expoekii*. Both of these *Crithidia* species were significantly more prevalent in Northern latitudes, and our 'health status' response variable, which accounts for prevalence across all parasites, showed the same patterns of spatial autocorrelation. We found the highest documented prevalence of *C. expoekii* at our highest latitude site, and all sites above 40° latitude had >50 % prevalence. Similarly, most of the sites with the highest *C. bombi* prevalence were at the highest latitude. Overall, this provides some of the first compelling evidence that bumble bees at higher latitudes experience significantly higher *Crithidia* infection rates. It has been suggested that social bees may experience higher parasite prevalence at higher latitude due to extremes in temperature and precipitation that reduce the number of active foraging days and increase within-colony contact rates (Retschnig et al., 2017; Lawson and Rands, 2019). Further, the duration and timing of flowering periods differs greatly across the latitudinal gradient with later snowmelt causing truncated flowering seasons at higher latitudes (Wielgolaski and Inouye, 2013). Because the quantity and quality of foraging resources impacts both plant-pollinator networks and contact among bees and their parasites (Koch et al., 2017), it is possible that both within-colony and external foraging patterns driven by latitude may be responsible for greater parasite prevalence at higher latitudes.

4.2. Geographic and resistance distance explain prevalence differences across taxa

We found evidence that both geographic distance and anthropogenic land use were mediating differences in parasite prevalence and community composition. Specifically, we found that land-use resistance distance models had greater predictive power than geographic distance models for *C. bombi, C. expoekii,* and for overall parasite composition. Few studies have included resistance distances in models of spatial variation in parasitism, but this approach often improves predictive power by incorporating biologically meaningful dispersal limitation (Nobert et al., 2016). For example, Chronic Wasting Disease risk in white-tailed deer has been effectively modeled using resistance distance, and also showed that incorporating land use connectivity more effectively explained the spatial distribution of infected deer (Nobert et al., 2016).

In our study, resistance distance captures limitations to bumble bee nesting due to unsuitable land cover, and was previously shown to be a predictor of host population genetic structure, likely due to dispersal limitation across unsuitable nesting habitat (Jha, 2015). Impervious cover can limit nest establishment by ground nesting bees such as *B. vosnesenskii* (Jha and Kremen, 2013a) and greater impervious cover in the local landscape is a known driver of higher bumble bee parasite prevalence in community gardens along the central coast of California (Ivers et al., 2022). Land cover which limits bumble bee dispersal may also negatively impact bumble bee health forcing bumble bees to forage further in order to access nesting and foraging resources (Ivers et al., 2022; McNeil et al., 2020). Land-cover types can be predictive of bee health across landscape scales (Clermont et al., 2015), where habitats with abundant floral resources and nesting habitat support bumble bees with greater health and lower levels parasitism (McNeil et al., 2020).

We acknowledge that land use resistance is tightly correlated with geographic distance in our study, which has been studied to a greater extent in the past. For example, the relationship between Euclidean distance and parasite community similarity has previously been explored within freshwater (Poulin and Morand, 1999) and marine fish (ie. Poulin et al., 2011), mites (Vinarski et al., 2007), fleas (van der Mescht et al., 2018), and helminth parasites of bats (Krasnov et al., 2010), with mixed relationships between community similarity and Euclidean distance. Parasite communities typically become isolated due to the dispersal movements of their hosts (reviewed in Poulin et al., 2011), especially for non-motile and directly transmitted parasites (Vinarski et al., 2007). In other words, distance is a key factor when parasites are heavily dependent on the host for dispersal (Poulin et al., 2011); in contrast, when dispersal can happen indirectly, such as wind dispersal or phoresy, distance is less important (Vinarski et al., 2007). For example, mite and flea assemblages are macroscopic, motile, and capable of phoresy, which may explain why community similarity remains high even across large distances (Vinarski et al., 2007; van der Mescht et al., 2018). *C. bombi* and *C. expoekii* are non-motile outside of the host and have a limited window for viable indirect transmission at flowers (Figueroa et al., 2019). Therefore, dispersal is highly dependent on bee host movement, which we know can be responsive to the biogeographic and land-use barriers included in our resistance surfaces.

4.3. Elevation and precipitation also explain prevalence dissimilarity

Beyond resistance distance, we found that local environmental conditions, specifically elevation and precipitation, also explained spatial variation in *C. bombi* and *C. expoekii* prevalence. Interestingly, sites with the greatest difference in mean yearly precipitation experienced the most similar rates of parasitism. Our sites varied in precipitation by >85 cm/year, with sites ranging from 17.2 to 103.82 cm annual rainfall; we hypothesize that both high rainfall and low rainfall sites exhibit high variability in foraging opportunities, concentrating foraging effort both spatially and

temporally. Regions with high precipitation may experience limited foraging days due to high precipitation frequency, whereas low precipitation regions may be characterized by intermittent rainfall shaping more ephemeral flowering phenologies and resulting bee foraging (eg. Minckley et al., 2013). Looking forward, it is 'virtually certain' that drought in the region will increase, and it is also possible that the disparity in precipitation between dry and wet regions will increase with climate change over time (Collins et al., 2013). Based on these predictions, we can expect to see greater dissimilarity in *C. bombi* prevalence as the spatial variation in precipitation becomes more extreme.

Past work also shows that sites with similar elevations have similar plant assemblages, bee foraging behaviors, and subsequently transmission dynamics (Inouye, 2008). Montane plant and pollinator assemblages are known to be experiencing rapid climate-driven changes (Inouye, 2008) which may impact contact rates among bee individuals in the local population. These unique foraging conditions inherently alter pathogen transmission dynamics and likely contribute to the unique parasite prevalence seen at a given altitude (Hickling et al., 2006; Morgan et al., 2004). Elevation is especially likely to impact patterns of infection when parasites are physiologically limited in their altitudinal range, and while *Crithidia* was found at all elevations, there is experimental evidence that *Crithidia* infection levels are significantly different at distinct temperature ranges (Palmer-Young et al., 2019), as would be seen along elevation gradients. For example, *Batrachochitridium dendrobatidis* (*Bd*), a fungal pathogen of frogs, is found at greater prevalence at higher elevation due in part to their thermal tolerance levels (Cohen et al., 2019). Similarly, elevation also drives patterns of smut disease in flowering plants (Abbate and Antonovics, 2014). Given that dissimilarity in parasite prevalence was associated with elevation in our study, it is possible that elevation impacts contact rate or physiological conditions in the host or parasite.

4.4. Response to precipitation is parasite specific

We found that *A. bombi* prevalence declined with increasing precipitation. Few studies have evaluated precipitation as a driver of parasite prevalence in wild bee populations, and the results to date have included positive (ie. McNeil et al., 2020), negative (ie. Rowland et al., 2021), and neutral trends depending on the pathogen. Early evidence documenting prevalence and intensity of *Vairimorpha* (formerly *Nosema*) infection in managed honey bees in Turkey indicated higher prevalence in regions with greater precipitation (Aydin et al., 2005), presumably due to higher intracolonial transmission. McNeil et al found a positive relationship with spring rainfall and *Vairimorpha* infection in bumble bees (2021), indicating this pattern continues in other hosts. Rainfall is thought to restrict bee foraging activity (Lawson and Rands, 2019), keeping colony mates in close contact within the nest and amplifying intracolonial disease transmission. In contrast, Rowland and colleagues evaluated climatic drivers of several honey bee diseases at landscape scales, and precipitation was negatively correlated with Varroosis (Rowland et al., 2021). *Varroa* mites depend on honey bees to infect new colonies, and if flight is restricted by rainfall, intercolonial transmission also declines. Parasites which depend on intracolonial transmission may benefit from rainfall mediated contact rates, whereas parasites which depend on intercolonial transmission are likely limited by rainfall (Rowland et al., 2021).

A. bombi may be dependent on intercolonial transmission due to its' unique biology and potential to be transmitted across the community through plant-pollinator-parasite networks (Figueroa et al., 2020). *A. bombi* is known to infect a large number of solitary nesting bee species (Figueroa et al., 2020) which do not have nest mates, indicating that community wide transmission events outside of the nest are also important to this parasite. Rainfall may also reduce transmission potential outside of the nest by washing flowers and physically removing spores from flowers. Rainfall reduces nectar sugar concentrations in flowers (ie. Tadey and Aizen, 2001), and may similarly reduce the concentration of infective spores in nectar and limit successful transmission and establishment in new hosts. Additional research into precipitation-mediated changes in viability and transmission should be explored on a parasite-specific basis, as the morphology and physiology of each parasite species is unique and likely relevant to our understanding of landscape-level disease dynamics.

4.5. Pathogen prevalence increases with nesting density

We found that *A. bombi* was significantly more abundant at sites with higher *B. vosnesenskii* nesting density. This is likely due to the fact that higher nesting density leads to higher contact rate among colonies, and by extension parasite transmission (Durrer and Schmid-Hempel, 1994). Studies that have investigated the influence of nesting density on bumble bee parasitism in natural populations have consistently found higher parasite prevalence in populations with higher nesting density (Parsche and Lattorff, 2018; Huth-Schwarz et al., 2012). Huth-Schwarz and colleagues found that *Vairimorpha* (*Nosema) bombi* prevalence increased with local nesting density of the host *Bombus terrestris* (2012). Parsche and Lattorf show a similar pattern for *C. bombi* prevalence, with higher prevalence in sites that support higher nesting densities (2018). We note that host nesting density serves as a proxy for other key risk factors for multi-host parasite establishment including host density, host contact rate, and the density of potential transmission sites (ie. colonies and flowers; Graystock et al., 2020). Our results resonate with past work and provide novel evidence that host density positively impacts the prevalence of the less well-understood parasite *A. bombi.*

4.6. Host genetic diversity may reduce parasitism

One of our principal hypotheses was that more genetically diverse populations would experience lower parasite pressure, as has been seen in many other taxonomic groups (reviewed in King and Lively, 2012) including mammals (O'Brien et al., 1985; Thorne and Williams, 1988), fish (Lively et al., 1990), crustaceans (Altermatt and Ebert, 2008), and insects including Drosophila (Spielman et al., 2004), and many social insects including bumble bees (Baer and Schmid-Hempel; Shykoff and Schmid-Hempel, 1991; Parsche and Lattorff, 2018; Whitehorn et al., 2011). In this study, we also found a negative relationship between *C. bombi* presence and genetic diversity. Past studies on bumble bees have also found that populations with higher genetic variation experience lower rates of *C. bombi* infection (Shykoff and Schmid-Hempel, 1991; Parsche and Lattorff, 2018; Whitehorn et al., 2011). For example, Whitehorn et al. (2011) found a strong negative correlation between *Bombus muscuorum* heterozygosity and *C. bombi* prevalence in wild caught bees. Interestingly, while Parsche and Lattorff (2018) found that *Bombus terrestris* exhibited a significant negative correlation between heterozygosity and *C. bombi* prevalence, the co-occurring *Bombus lapidarius* exhibited no relationship between genetic diversity and prevalence, likely due to differences in contact driven by host abundance (Parsche and Lattorff, 2018) or differences in host competence (Stewart Merrill and Johnson, 2020). Taken together, past work and our results indicate that host genetic variation plays an important role in mediating parasitism in natural populations, but additional biotic and abiotic factors are also critical in rapidly changing landscapes. Host populations become isolated when the surrounding landscape prevents dispersal (Jha, 2015), leading to lower genetic variation in local bumble bee populations, and creating hot-spots with higher parasite prevalence.

5. Conclusion

By examining pathogen prevalence at biogeographic scales, our study reveals that parasitism rates in wild bumble bees are a function of multiple abiotic environmental traits and host traits, including human land use, precipitation, elevation, host nesting density, and host genetic diversity. Further, we show that different parasite species exhibit unique responses to abiotic environmental and host conditions. Overall, our results indicate that large-scale disease dynamics are heavily impacted by human landscape modification, biogeography, climate context, and host population demographic history. We also highlight the potential role of shifting

environmental conditions for host-parasite interactions, where climate-driven changes in precipitation and pollinator elevational distributions may play a key role in mediating future landscape-level disease dynamics.

Lastly, when planning for future conservation challenges, we must bear in mind that factors in many environmental spheres influence key ecosystem service providing organisms, including parasites which simultaneously pose conservation challenges to their hosts while also regulating population growth and maintaining long-term population stability. Future work which incorporates projections about future climate and land-use scenarios as well as host population responses to parasites and other health risks will be especially valuable in balancing conservation priorities.

CRediT authorship contribution statement

Nicholas A. Ivers: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Shalene Jha:** Conceptualization, Methodology, Funding acquisition, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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