Comparative Phylogeography of the Mexican Rock Fig (*Ficus petiolaris*) and its Fig Wasp Pollinator: Effects of Abiotic Versus Biotic Factors

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Abstract

Numerous studies have tested geographically congruent spatial genetic structures and population units in codistributed species. Yet, few have elucidated the relative importance of biogeographic influences versus ecological interactions in determining the congruence of genetic structure in coevolving species. Here, we present the first study testing for genetic codifferentiation in a widely distributed and highly-coevolved mutualism, in which symbiont gene dispersal is expected to be positively correlated. In the fig, *Ficus petiolaris*,and its host-specific *Pegoscapus* pollinating wasp, we evaluated the extent to which the geographical patterns of differentiation in each species are similar and explained by shared sources of vicariance, codispersal, or species-dependent factors. In both species, the Trans-Mexican Volcanic Belt was a major source of vicariance differentiating southern and northern phylogroups. Within these phylogroups, however, fig and pollinator showed surprisingly different population genetic structure. In *F. petiolaris*, the Gulf of California was a strong phylogeographic break in the northern phylogroup. In contrast, within its northern phylogroup, *Pegoscapus sp.* showed no genetic structure and only weak isolation by distance over a range of 1500 km. In the southern phylogroup, exceptional genetic differentiation was observed between populations separated by as little as 300 km. Despite mutual selective pressure between figs and fig wasps, and the role of fig wasps in fig gene flow, we conclude that range-wide patterns of genetic differentiation are primarily influenced by biological features unique to each species rather than by shared sources of vicariance or correlated gene dispersal.

Keywords: pollination mutualism, coevolution, phylogeography, gene flow, fig and fig wasp

1 Introduction

Species responses to landscape history can be complex, leading to varied phylogeographic patterns within the same biota [Bowen and Avise, 1990; Lamb et al., 1992; Zink, 1996; Carstens et al., 2005; Soltis et al., 2006; Moussalli et al., 2009]. Nonetheless, geographical features of a landscape can generate congruent phylogeographic patterns across taxa by posing common physical barriers to gene flow [Hewitt, 2000; Nason et al., 2002; Pyron and Burbrink, 2010; Garrick et al., 2013]. Similarities and differences in the biology of species, such as dispersal ability, reproductive biology, and niche requirements, can also influence their degree of phylogeographic congruence, as can the intimacy, specificity, and longevity of their ecological interactions [Carstens and Richards, 2007; Moussalli et al., 2009; Smith et al., 2011]. Co-evolutionary patterns are often considered on macro-evolutionary timescales, but they can be affected by ongoing landscape-level abiotic and biotic processes [Ehrlich and Raven, 1964; Thompson, 1994, 1999],including the interplay between geographic variation in reciprocal selection, the demographics of local populations, and the homogenizing effects of gene flow – a predication supported by empirical studies [Thompson and Cunningham, 2002] and theoretical models [Nuismer et al., 1999; Gomulkiewicz et al., 2000; Hochberg et al., 2000; Nuismer et al., 2000; Gomulkiewicz et al., 2003]. Although advances in genomic data acquisition and analytical tools continue to enhance our understanding of genetic structure [Knowles, 2009; Papadopoulou and Knowles, 2016; Satler and Carstens, 2017, 2019], debate remains concerning the relative importance of abiotic [Hewitt, 2000; Nason et al., 2002;

Pyron and Burbrink, 2010; Garrick et al., 2013] versus biotic [Carstens and Richards, 2007; Moussalli et al., 2009; Smith et al., 2011] in the evolution of this structure. Here we address how biogeographical, ecological, and coevolutionary processes influence population genetic structure within - and the spatial congruence of this structure between - symbiont species in an obligate pollination mutualism. Examples of these highly specialized interactions include yuccas and yucca moths, leafflowers and leafflower moths, globeflowers and globeflower flies, and palms and weevils [Cruaud et al., 2012; Hembry and Althoff, 2016; de Medeiros and Farrell, 2020; Pellmyr et al., 2020], as well as figs and fig wasps, which are the focus of this study.

1.1 Co-diversification of Figs and Fig Wasps

The prolonged ($\sim$ 80 Ma) ecological interaction between figs (genus *Ficus*) and fig wasps (family *Agaonidae*) has resulted in deep-time codiversification between these lineages [Janzen, 1979; Ramírez, 1970; Weiblen, 2002; Anstett et al., 1997; Cook and Rasplus, 2003; Rønsted et al., 2005; Machado et al., 2005; Cruaud et al., 2012]. *Ficus* comprises over 800 species employing elaborate floral fragrances to attract female fig wasps, which pollinate fig flowers and oviposit in fig ovules, leading to gall formation by developing larvae [Chen and Song, 2008; Wang et al., 2013]. Consequently, the mutualism has coevolved due to reciprocal selective pressures for reproductive success Janzen [1979]; Weiblen [2002]. Although exceptions are known [Molbo et al., 2003; Herre et al., 2008; Su et al., 2008; Wang et al., 2016; Satler et al., 2019].

Despite a brief adult lifespan (<60h) [Kjellberg et al., 1988; Dunn et al., 2008], pollen-bearing female fig wasps disperse over long distances, using wind currents to reach receptive fig trees frequently located several kilometers away [Ramírez B., 1969; Nason et al., 1998; Harrison and Rasplus, 2006; Ahmed et al., 2009]. While vertebrate frugivores can disperse fig seeds over long distances[Galil and Neeman, 1977; Staddon et al., 2010], aiding the maintenance of extensive species ranges [Hernández-Esquivel et al., 2020], rates of gene flow via pollen migration are generally much higher than via seed migration in flowering plants [Ennos, 1994; Petit et al., 2005], including figs [Yu et al., 2010; Liu et al., 2015]. Recent studies have shown that figs and fig wasps exhibit complex phylogeographical patterns influenced by past climatic and geographical events shared by host and pollinator [Chen et al., 2012; Honorio Coronado et al., 2014; Vieira et al., 2015; Yu and Nason, 2013; Cooper et al., 2020]. Nonetheless, the co-dispersal of fig wasps and fig pollen should promote symmetries in gene flow, leading to the *a priori* expectation that associated fig and wasp species should exhibit spatial congruence in population structure.

1.2 Biogeography of Baja California Peninsula and Western Mexico

The rock strangling fig (*F. petiolaris*) and its species-specific fig-wasp pollinator (*Pegoscapus* sp.) are endemic to the Baja California peninsula (BCP) and the western Mexican Transition Zone (MTZ) [Piedra-Malagón et al., 2011, 2019]. BCP was originally thought to have a dispersal-dominated biotic history [Savage, 1960], but our greater understanding of plate

tectonics has facilitated more complex vicariant hypotheses [Hess, 1962; Murphy, 1983; Grismer, 1994; Graham et al., 2014]. Shared signals of vicariance associated with two ancient transpeninsular seaways and the formation of the Gulf of California [Sedlock, 2003] (Figure 1 Hypotheses A–C) have been detected in the spatial genetic structures of several animal and plant species [Riddle et al., 2000a,b,c; Nason et al., 2002; Hurtado et al., 2004; Ross and Markow, 2006; Pfeiler et al., 2007; Garrick et al., 2009, 2013]. Encompassing much of western mainland Mexico, the MTZ includes two major highland provinces that interrupt the ranges of *F. petiolaris* and its pollinator: the Trans-Mexican Volcanic Belt (TVB) [Ferrari et al., 2012] and the Sierra Madre del Sur [Ferrari et al., 2014] (SMS, Figure 1 hyptheses D and E, respectively). Numerous taxonomic and phylogeographic studies have investigated biogeographical patterns within the TVB and SMS regions, frequently finding similar patterns of dispersal and vicariance across taxa. [Halffter, 1964, 1987; Becerra, 2005; Bryson Jr et al., 2011; Gutiérrez-Ortega et al., 2018; Rocha-Méndez et al., 2019; Anguiano-Constante et al., 2021]. The distributions of *F. petiolaris* and its pollinator traverse several potential sources of vicariance that, with correlated gene flow, could impact the spatial genetic structure of both species.

Here we adopt a multifaceted approach to uncover how abiotic and biotic factors shape the genetic structure of interacting fig and fig wasp species. First, we examine the population genetic structure of each species to determine the extent to which they coincide with biogeographical patterns hypothesized for the BCP and MTZ (Figure 1). Second, we compare the genetic structure of these two co-evolving species to determine the extent to which they are geographically congruent. Finally, we evaluate the relative importance of historical biogeography versus biotic factors (reproductive interactions and co-dispersal) in generating shared phylogeographic patterns between host and pollinator.

2 Materials & Methods

2.1 *Ficus petiolaris*

2.1.1 Host Fig Sampling and Sequencing

Because of the geopolitics of western mainland Mexico, our sampling of *F. petiolaris* within this area was restricted to regions we could access safely. This resulted in sparse sampling so not all our *a priori* vicariance hypotheses could be tested for the host plant. We sampled 247 *F. petiolaris* individuals from 19 sites spanning the range of the species in the BCP and mainland Mexico between 2012-2017 (Figure 1, Table 1). To provide an outgroup for phylogenetic analyses, we also sampled three *F. aurea* individuals from Oaxaca, Mexico. Leaf samples were preserved in silica gel and then stored at -80°C until DNA extraction and library construction at Iowa State University.

To identify single nucleotide polymorphisms (SNP) for population genetic analysis, DNA was extracted from leaf tissue using a cetyltrimethylammonium bromide (CTAB) and chloroform protocol, including polyvinylpyrrolidone and proteinase K to increase DNA quality. DNA was then precipitated in 2-propanol. A modified Peterson et al. [2012] dd-RADseq protocol was used to select double digested DNA fragments cut with the restriction en-

zymes PstI and MspI (dd-RADseq). After ligation of Illumina adapters, polymerase chain reaction (PCR) amplified products were size-selected to 300–800 bp using BluePippin.. After sequencing at the Iowa State University DNA Facility with an Illumina Hiseq 3000 raw reads were processed and *de novo* assembled with Ipyrad v0.9.31 [Eaton and Overcast, 2020] using these parameters: a maximum of five low–quality base calls with the quality score offset of 33, minimum depth for statistical base calling of six, minimum depth for the majority-rule calling of six, and a maximum cluster depth within samples of 10,000. The loci were further filtered to remove those found in less than 50% of the samples, having a maximum of 25% heterozygous sites, five or more SNPs, five or more indels or a maximum of five low quality sites.

2.1.2 Host Fig Population Genetic Structure

A principal component analysis (PCA) was performed to assess the clustering of individual multilocus genotypes of *F. petiolaris* in multivariate space using R v4.0.3 [R Core Team, 2020] and the *dudi.pca* function from ade4 v1.7.19 [Dray and Dufour, 2007; Thioulouse et al., 2018]. For this analysis, we randomly subsampled two individuals per BCP collection site for our input data set due to the greater density of collection sites there compared to other regions. This subsample was used as input for all subsequent *F. petiolaris* analyses, unless stated otherwise. Further, to reduce complexity, we limited the dataset to a single biallelic SNP per locus. Allele frequencies were calculated using the R package adegenet v2.1.3 [Jombart, 2008; Jombart and Ahmed, 2011; R Core Team, 2020] and missing data were imputed by using average allele frequencies within local sample sites.

Next, we used STRUCTURE Pritchard et al. [2000] to identify genetic clusters (K) and to estimate membership coefficients of individual samples within each cluster. We applied an admixture model with $500,000$ iterations, preceded by a $100,000$ iteration burn-in. Analyses covered $K = 1 - 8$ with 15 replicates per K, and the optimal K was determined using the maximum posterior log–likelihood and ΔK via the Evanno method [Evanno et al., 2005] in the pophelper R package v2.3.0 [Francis, 2017]. We iteratively subset the data by the inferred K and re-ran independent STRUCTURE analyses to test potential sub-structuring. Continuing until discrete clusters were no longer evident. To mitigate overestimation of clusters due to isolation by distance (IBD), our final K determination considered all genetic analysis evidence and geographical coherence of clusters. If putative hybrids were detected in the PCA or STRUCTURE analyses, we utilized *snapclust* [Beugin et al., 2018] within the R package adegenet to classify hybrids into either first-generation (F1) or back-cross.

To assess the genetic differentiation between inferred genetic clusters and populations adjacent to hypothesized barriers to gene flow (as indicated in Figure 1), we estimated Wright's *Fst* [Weir and Cockerham, 1984] and 95% confidence limits (999 permutations) using the functions *pairwise.WCfst* and *boot.ppfst* in the R package hierfstat version 0.5.11 [Goudet, 2005]. Additionally, using hierfstat we computed site-specific estimates of F_{IS}, which serve as a measure of local inbreeding among relatives. Based on a mating system analysis of *F. petiolaris* conducted by Gates and Nason [2012], we expected substantial outcrossing across and F_{IS} estimates close to zero.

2.1.3 Host Fig Phylogenetic Reconstruction of Phylogroups

We used a phylogenetic approach to infer the evolutionary relationships among the inferred genetic clusters. SVDquartets [Chifman and Kubatko, 2014], as implemented in PAUP* (version 4.0a163) [Swofford, 2003], was utilized to construct a population tree based on genetic clusters identified the PCA and STRUCTURE. These analysis used the complete SNP dataset as input, exhaustively evaluating quartets. To assess the robustness of our results,we executed 100 bootstrap replicates to attain node support for the inferred trees.

2.1.4 Host Fig Geographical Patterns of Isolation by Distance and Diversity

We conducted a Mantel test (999 iterations) to assess the correlation between genetic and geographic distances to quantify the genetic differentiation resulting from IBD among host populations. Within and between the genetic clusters identified by PCA and STRUCTURE analyses. Population pairwise genetic distances were computed using two metrics: Nei's standard distance [Nei et al., 1983] and Cavalli-Sforza and Edwards chord distance [Cavalli-Sforza and Edwards, 1967], which make different assumptions about the roles of mutation, genetic drift, and population size in population divergence through time. These genetic distances were estimated using genet.dist from hierfstat [Goudet and Jombart, 2020] and great circle geographic distances were determined using rdist.earth the fields R package[Douglas Nychka et al., 2017]. To explore geographical patterns in within-population genetic diversity, we plotted expected heterozygosity (H_e) against latitude for each sample population. H_e ws estimated using the function basicstats in hierfstat.

2.1.5 Host Fig Demographic Model Selection

Using the inferred phylogeographic structure, we tested historical demographic models with PHRAPL [Jackson et al., 2017]. Analyzing competing models of differentiation and gene flow across major phylogeographic breaks. The models tested included isolation-only, isolation with migration, and migration-only. For input, we randomly sub-sampled 400 RAD loci without replacement. Per locus, we calculated maximum likelihood gene trees using RAxML v8.2.11, using the GTR substitution model with gamma and proportion of invariant sites [Stamatakis, 2014]. Upon rooting each gene tree, we removed outgroup taxa and then performed 20 rounds of random subsampling of empirical gene trees with replacement. Subsequently, we conducted simulations of 70,000 gene trees by varying parameter values for divergence time (τ) and migration (m). Akaike weights (wAIC) were used for model comparison and to calculate metrics analogous to model probabilities that go from 0 (low support) to 1 (high support).

We opted for PHRAPL over an allele frequency spectrum (AFS) approach for demographic model selection. Unlike PHRAPL, AFS approaches cannot handle missing data, which is typical of RAD-seq datasets, necessitating further data downsampling. Moreover, a comparison of allele frequency and gene tree-based approaches in model selection accuracy has not yielded conclusive results [Ruffley et al., 2018]. Both approaches incorporate coalescent theory, and model selection accuracy may depend more on the information available in AFS or gene trees.

2.2 The *Pegoscapus* Pollinator

2.2.1 Pollinator Sampling and Sequencing

We collected near-mature (late interphase) and mature *F. petiolaris* syconia (commonly "fruit") from trees across 29 collecting sites between 2012-2019 (Figure 1 and Table 1). The syconia were placed in plastic vials and, pollinators were allowed to emerge before being preserved in 95% ethanol or RNALater. If wasps had not emerged within 24 hours, or a syconium was immature, then in some cases, galled flowers were preserved for later removal of wasp larva or pupae. In total, we genotyped 102 individual *Pegoscapus* sp. wasps, as well as one outgroup pollinator collected from *F. crocata*.

To generate genome-wide sequence data for the wasps, we used targeted enrichment of ultra-conserved elements (UCEs) following the workflow outlined in Faircloth et al. [2012]. A single wasp was selected per syconium to ensure independence among samples, and genomic DNA was extracted with a Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA). Samples were fragmented to an average size range of 450 bp using a Covaris ME220 focused-ultrasonicator (Covaris Inc., Woburn MA) and Illumina libraries were prepared using a KAPA HyperPrep Kit (Roche Sequencing and Life Science). After library construction, samples were grouped into eight sets and hybridized with biotinylated RNA probes to capture targeted loci. For targeting UCE loci, we used the hymenopteran probe set *v2* [Branstetter et al., 2017]. After probe hybridization and library amplification, we confirmed size distributions using a Bioanalyzer and pooled the libraries into equimolar concentrations for sequencing. Sequencing was performed by GeneWiz (South Plainfield, NJ) on two full lanes of an Illumina Hiseq 3000 using 150 base pair paired-end sequencing.

To process raw sequence reads, we used Phyluce *v1.6.7* [Faircloth, 2016] in combination with SAMtools *v1.10.2* [Li et al., 2009; Cock et al., 2015]. Raw sequence reads were cleaned with illumiprocessor [Faircloth, 2013; Bolger et al., 2014] and then assembled into contigs using SPAdes *v3.12.0* [Bankevich et al., 2012]. Contigs were aligned to the hymenopteran probe set to filter out nonspecific sequences. Generated UCE loci were aligned using MAFFT *v7.407* [Katoh and Standley, 2013], edge trimmed with trimAl Capella-Gutierrez et al. [2009], and ambiguously aligned internal sites were removed using Gblocks version 0.91b [Castresana, 2000]. To retain a locus, a minimum of 50% sample coverage was required.

Omitting the outgroup sample, phased alleles were generated for downstream analysis following the pipeline outline by Andermann et al. [2018]. We took our aligned loci, before the use of Gblocks, and used the *phyluce_snp_bwa_mulitple_align* function to map cleaned sequenced reads for each sample to the loci using *BWA-MEM* [Li, 2013] in bwa *v0.7.17* [Li and Durbin, 2010]. *phyluce_snp_phase_uces* was then used to phase the mapped reads, resulting in two alleles per individual per locus [Andermann et al., 2018]. The loci were then realigned and cleaned as previously described, with a minimum requirement of 50% sample coverage.

Additionally, owing to the prevalence of mitochondria in animal tissues, UCE sequence capture probes can recover maternally-inherited mitochondrial DNA sequences. The mitochondrial sequences of the cytochrome oxidase subunit I (COI) were assembled using *Novoplasty* using a reference COI (JN103329) sequenceDierckxsens et al. [2016]; Cruaud et al.

[2012]. The alignment of COI sequences was done using MAFFT.

2.2.2 Pollinator Population Genetic Structure

We used a custom Python script to extract unlinked SNPs from our phased and unphased UCE loci as input data. We primarily used the phased data for our analyses, however phasing was not very effective for every sample. In instances where subsetting the dataset resulted in excessive missing data, we chose to utilize the unphased data. The phased dataset was used for identifying genetic clusters using PCA and STRUCTURE, the latter employing the same approach as for *F. petiolaris* Potential hybrid individuals were classified using *snapclust*, and the results were validated using COI gene trees inferred through RAxML.

In a survey of recently pollinated *F. petiolaris* syconia at various locations in Baja California and Coastal Sonora, we observed an average of 1.48 pollinator foundresses per fruit (based on 245 fruits, unpublished data). Given the frequent occurrence of a single foundresses and their broods in fruits, inbreeding is anticipated to be common and F_{IS} estimates should be substantially greater than zero. It is important to note that F_{IS} could not be estimated for three locations with only one sampled pollinator each (sites 214, 220, 228 in Table 1).

2.2.3 Pollinator Phylogenetic Reconstruction of Phylogroups

We used SVDquartets to infer phylogenetic relationships among all *Pegoscapus* sp. phylogroups. For this analysis, we concatenated all recovered UCE loci into a single data matrix and exhaustively evaluated quartets, with 100 bootstrap replicates used to assess nodal support values.

2.2.4 Pollinator Geographical Patterns of Isolation by Distance and Diversity

As for the host fig, we identified the extent of spatial genetic differentiation due to IBD by performing a Mantel test of the relationship between population pairwise estimates of genetic distance (Nei's and CavalliSforza and Edwards distances) versus geographical distance, both within and between inferred phylogroups. We also plotted population-level H_e against latitude to investigate spatial trends in genetic diversity.

2.2.5 Pollinator Demographic Model Selection

Using the approach previously described, we assessed the fit of demographic models to inferred genetic clusters using PHRAPL. For *Pegoscapus sp.* we used 767 unphased UCE loci containing our outgroup taxon (pollinator of *F. crocata*). After estimating gene trees in RAxML and trimming outgroup taxa from each rooted tree, gene trees were subsampled at random with replacement 15 times to reduced computation load. After simulating 50,000 gene trees under a range of model (τ) and m values, the lnL and AIC of each model were calculated with respect to the empirical gene trees. As with the host fig, wAIC was used to compare models and calculate metrics analogous to model probabilities.

2.3 Congruence of Genetic Structure between Host and Pollinator

We used population graphs to assess the spatial symmetry of genetic structures in *F. petiolaris* and *Pegoscapus* by measuring the congruence of their population graph topologies [Dyer and Nason, 2004; Dyer, 2015]. Population graphs use graph theory to visually represent

population genetic structure, creating a model-free network based on conditional genetic covariance. Populations are depicted as geometric nodes, with their size proportional to the within-population component of genetic variance. Connections between populations are represented by edges, the magnitude of which corresponds to inter-population variance [Dyer, 2015].

To assess topological congruence within the *popgraph* R package [Dyer, 2021], it is required to generate population graphs using sites that are co-sampled in both species. We assumed that nearby Oaxacan sites 209 (*Pegoscapus*) and 210 (*F. petiolaris*) are equivalent for this analysis, as were the sites 214 (*F. petiolaris*) and 222 (*Pegoscapus*). We tested the topological congruence of the population graphs in two ways. First, we asked if nodes that are close in the *F. petiolaris* graph are also close together in the *Pegoscapus* graph by measuring the correlation between the shortest path matrices of the two graphs. For this, we used the *test_congruence* function of the *popgraph* package to calculate the non-parametric correlation of pair-wise path distances through the graph between sites. Second, we used custom code to examine the congruence of connectivity pattern between sites. Using permutation ($n = 10000$), we tested whether the *F. petiolaris* and *Pegoscapus* population graphs share more edges in common than expected by chance. Finally, we overlayed a population graph of *Pegoscapus sp.* onto a raster map of Mexico to determine whether pollinators preferentially travel through lower elevations. To assess this, we used a permutation test with 999 iterations, randomly reassigning connections (edges) between sites (nodes). We computed the mean elevation of edge set configurations by extracting elevation data along edges from our raster map [Dyer et al., 2012]. Statistical significance was evaluated by comparing the observed mean elevation to the distribution of mean elevation values generated through permutation. Under the null hypothesis, this approach assumes no differences in mean elevation across edge set configurations.

3 Results

3.1 *Ficus petiolaris*

3.1.1 Host Fig Sampling and Sequencing

We generated 137,216,883 single–end raw reads. After *de novo* assembly, 25,997 loci were discovered. We filtered out loci found in less than 50% of samples, which removed most of the loci, indicating a large amount of missing data across individuals. To prevent the use of linked SNPs, only one SNP per locus was kept for downstream analyses. We later removed outgroups and individuals with less than 30% of genomic data across loci, which resulted in somem polymorphic loci becoming monomorphic in *F. petiolaris* only. Removing monomorphic loci resulted in a final data set of 1,192 biallelic SNPs across 247 individuals.

3.1.2 Host Fig Population Genetic Structure

The initial two principal components of the PCA demonstrated large eigenvalues, collectively explained 52% of the variation in the data. Based on genetic differences, individual samples formed five distinct and well-separated clusters in multivariate space, which

we interpret as comprising five distinct genetic clusters. PC axes one and two separated Baja California, Coastal Sonora, Inland Sonora + Central Mexico (Jalisco and Sinaloa), and Southern Oaxaca (Figure 2B), while PC one and PC three further separated Inland Sonora and Central Mexico (Figure 2C). The Coastal Sonora samples were intermediately spread between the Baja California versus Inland Sonora and Central Mexico genetic clusters in PCA space, suggesting admixture between well-differentiated Baja and northwestern Mexico populations.

The optimal number of genetic clusters for STRUCTURE analysis was $K = 2$ based on maximum posterior log–likelihood and ΔK (Figure 2A). STRUCTURE supported the differentiation of Baja California from mainland Mexico and identified samples from Coastal Sonora as admixed between these two regions. However, it did not distinguish Inland Sonora or Central Mexico as separate from Oaxaca. We subset samples based on the two inferred STRUCTURE clusters and ran STRUCTURE on each subset. No geographically associated samples were further identified.

Multilocus estimates of F_{st} (Table S1) revealed significant differentiation between all five regional genetic clusters of *F. petiolaris* represented in the PCA (Figure 2B & C). Southern Oaxaca showed high genetic differentiation from all other clusters ($F_{st} = 0.32 - 0.69$). Similarly, Baja California exhibited significant differentiation from the other clusters ($F_{st} = 0.28 - 0.69$). Despite their close geographic proximity, Coastal Sonora was strongly differentiated from both Baja California ($F_{st} = 0.28$) and Inland Sonora ($F_{st} = 0.16$). In contrast, Inland Sonora and Central Mexico showed weaker differentiation from each other, with an F_{st} value of 0.07, despite their larger geographic span.

Given our density of sampling in northwestern Mexico, we were able to test vicariance hypotheses A-C in Figure 1. Genetic differentiation between sites adjacent to the MPS (Ho Figure 1A) and ILP (Ho Figure 1B) seaways was not significant (F_{st} = -0.019 and 0.006, respectively). In contrast, differentiation between the Baja California and Coastal Sonora genetic clusters adjacent to the Gulf of California (Ho Fig.1C) was large and significant ($F_{st} = 0.28$, as noted above). Consistent with expectations based on prior research in Baja California [Gates and Nason, 2012], inbreeding in *F. petiolaris* populations was found to be low. Averaged across sites, F_{IS} was 0.043 (range -0.069 to 0.118) with nine of 19 locations having estimates significantly greater than zero. These results indicate that *F. petiolaris* is highly outcrossing across its range, regardless of the genetic cluster or geographic region.

3.1.3 Host Fig Phylogenetic Reconstruction of Phylogroups

The population tree showed that the genetic clusters of northern and central Mexico Baja California, Coastal Sonora, Inland Sonora, Central Mexico formed a single monophyletic clade closely sister to Southern Oaxaca (Figure 3A). The SVDquartets analysis generally supported the results of the population genetic analyses. The SVDquartets population tree (Figure 3A) placed Southern Oaxaca sister to all other genetic clusters, while Baja California and Coastal Sonora (in north-central Mexico) formed a well-supported clade (BS = 100) sister to a weakly supported Inland Sonora plus Central Mexico clade (BS = 50). However, these findings should be interpreted cautiously as a bifurcating tree is not optimal for handling admixture.

3.1.4 Host Fig Geographical Patterns of Isolation by Distance and Diversity

Sampling of *F. petiolaris* in mainland Mexico was relatively sparse, so we limited testing of IBD to Baja California, spanning *a priori* sources of vicariance (Figure 1A and B). Using all individuals from Baja California, a significant positive ($R = 0.352$, $p = 0.029$) correlation was observed between Nei's genetic distance and geographical distance across the peninsula (Figure 4), with similar results obtained using Cavalli-Sforza and Edwards chord distance (results not shown). Though historical vicariance cannot be ruled out, the continuous IBD indicates no lasting signals of vicariance associated associated with the ancient transpeninsular seaways. Figure 5 shows that both Baja California ($0.03 < H_e < 0.042$) and Southern Oaxaca ($0.045 < H_e < 0.0475$) genetic clusters have relatively low population genetic diversity. These clusters represent the northern and southern limits of our geographic sampling. In contrast, populations in the Coastal Sonora, Inland Sonora, and Central Mexico genetic clusters had substantially higher genetic diversity ($H_e = 0.069 - 0.082$).

3.1.5 Host Fig Demographic Model Selection

We tested 46 demographic models based on the inferred north-central and southern Mexico phylogroups of *F. petiolaris* indicated in the SVDquartets population tree. These models included: 1) migration from the north-central to southern phylogroup, 2) migration from the southern to north-central phylogroup, and 3) a coalescent event between these two phylogroups. The two highest-ranked demographic models had wAIC values greater than 0.39, while the remaining models all had much lower support (Table S3). These two top models both indicate divergence with gene flow between the north-central and southern Mexico phylogroups. More specifically, these models share the same bidirectional gene flow parameters of $m = 4.64$ (in units of $4Nm$) while differing in the timing of the coalescent event, which was deeper in the top-ranked model, $t = 7.691$, than in the second, $t = 0.300$ (in units of $4N$ generations). Surprisingly, the PHRAPL models ranked 3 and 5 have the same migration parameters and very similar coalescent times to models 1 and 2, respectively, yet have substantially lower wAIC values (Table S3).

3.2 The *Pegoscapus* Pollinator

3.2.1 Pollinator Sampling and Sequencing

We generated 390,571,240 raw reads resulting in an average of 5,139,095 reads per individual ($\pm 3,334,310$). After processing and filtering, our unphased data set with outgroup taxa had a pool of 2359 loci with an average of 1940 loci per individual ($\pm$ 392.02). Requiring a minimum of 50% taxon coverage to retain a locus, the final data set consisted of 2053 loci. Loci had an average length of 1135 bp ($\pm$ 519.12), ranging from 262 bp to 4534 bp. For the data set without outgroup taxa, we recovered a pool of 2332 loci with an average of 1952 loci per individual ($\pm$354.62). Requiring a minimum of 50% taxon coverage, the unphased data set consisted of 2057 loci with an average length of 1134 bp ($\pm$522.53), ranging from 302 bp to 4534 bp. The phased data set (50% taxon coverage) consisted of 1886 loci with an average length of 552.19 bp. One individual did not phase well (FW261) and was removed from the final phased data set. After extracting unlinked, biallelic SNPs using a custom script, the

unphased data set had 1921 SNPs, and the phased data set had 1414 SNPs. Additionally, we were able to recover and assemble COI sequences for 82 individuals.

3.2.2 Pollinator Population Genetic Structure

The first two principal components of the PCA accounted for 49.95% and 7.40%, respectively, of the variation in PCA space (Figure 6B). We identified three primary and well-separated genetic clusters: Northern Mexico, Morelos + Northern Oaxaca, and Southern Oaxaca. The Northern Mexico cluster included individuals from Baja California, Sonora, Sinaloa, Zacatecas, and Jalisco. One individual from Jalisco appeared to be a putative hybrid, which was excluded from subsequent PCA. We analyzed a subset of the data to explore genetic sub–clustering within the main inferred clusters. There was no additional sub-clustering revealed for individuals from Northern Mexico through PCA. For southern Mexico, PC axe 1 versus 2 revealed three clusters representing individuals from Morelos, Northern Oaxaca, and Southern Oaxaca (Figure 6D).

STRUCTURE supported a strong division between northern and southern Mexico with an optimal $K = 2$ genetic clusters based on both the maximum posterior log–likelihood and ΔK (Figure 6A). Evidence of admixture between these two clusters was minimal, except for the inferred hybrid individual from Jalisco. As with PCA, additional STRUCTURE runs on these two genetic clusters showed no additional sub-structure in the north, but revealed $K = 3$ clusters for southern Mexico, corresponding to Morelos, Northern Oaxaca, and Southern Oaxaca (Figure 6C).

Both PCA and STRUCTURE identified a pollinator individual (P342) from Jalisco as a putative hybrid. *Snapclust* modeling inferred the putative hybrid to be an F1 hybrid between north-central and southern Mexico genetic clusters The COI gene tree placed the F1 hybrid within the Morelos + Northern Oaxaca clade, indicating maternal transmission from that region and paternal contribution from Jalisco. However, since the maternal ancestor originally dispersed from the south to Jalisco, their descendants had an equal chance of inheriting the maternal haplotype or a local one. Consequently, the geographic origin of the hybrid's haplotype is not highly informative.

To avoid inflating Wright's F_{st} estimates, the pairwise calculations (Table S4) excluded the F1 hybrid from Jalisco. Within the north-central Mexico genetic cluster, genetic differentiation between nested regional populations was generally low, with most pairwise F_{st} estimates <0.07, and only five significantly greater than zero. Low differentiation was observed between regional populations around the Gulf of California ($F_{st} \leq 0.021$). Higher differentiation was found between Zacatecas and Coastal Sinaloa ($F_{st} = 0.134$, sig.), and Inland Sinaloa ($F_{st} = 0.060$, n.s.), as well as between Jalisco and Coastal Sinaloa ($F_{st} = 0.064$, sig.). While southern populations were geographically closer than northern ones, the differentiation among the three inferred genetic clusters exhibited greater variability. For example, F_{st} between Morelos and Northern Oaxaca was 0.057. However, F_{st} increased to 0.577 and 0.558 when comparing Morelos and Northern Oaxaca with Southern Oaxaca, respectively. Differentiation was consistently high between regional populations in the north-central phylogroup versus the southern phylogroups ($F_{st} = 0.715 - 0.809$).

Consistent with expectations based on the observation of multifoundress fruit, deviations

from HWE in *Pegoscapus sp.* populations were found to be substantial. Averaged across the 26 sites with sample sizes greater than one, F_{IS} was 0.393 (data not shown). Further, 20 sites had estimates significantly greater than zero. The remaining six sites had an even larger mean $F_{IS} = 0.512$ but also had very broad confidence limits on their site-specific estimates. These results indicate that the pollinator maintains a highly inbred mating system throughout its range, irrespective of genetic cluster or geographical area.

3.2.3 Pollinator Phylogenetic Reconstruction of Phylogroups

The SVDquartets population tree supported the division between north-central and southern Mexico (BS = 100, Figure 3). Southern Mexico clade had subclades Morelos + Northern Oaxaca (BS = 93) and southern Oaxaca (BS = 100). Southern Oaxaca branch was sister to Morelos + Northern Oaxaca.

3.2.4 Pollinator Geographical Patterns of Isolation by Distance and Diversity

In north-central Mexico, there was weak but significant IBD (R = 0.281, p = 0.035, Figure 7A), even though populations were separated by distances of up to 1500 km. In the GOC region, no significant IBD was found, indicating genetic connectivity over a 1000 km range (R = 0.158, p = 0.124, Figure 7B). In contrast, within southern Mexico, despite the close proximity of populations (<= 400 km), strong and significant IBD was observed (R = 0.847, p = 0.021, Figure 7C) consistent with Morelos, northern and southern Oaxaca comprising three distinct genetic clusters. When comparing between northern and southern Mexico populations, no significant IBD was detected (R = -0.318, p = 0.735, Figure 7D), indicating the TVB acts as a strong barrier to gene flow. An analysis specifically between populations bracketing the TVB (Sites 215 and 221) yield inconclusive results, attributed to a limited sample size. Genetic diversity in the pollinator did not show a clear latitudinal pattern like the host fig with H_e varing widely among populations within and across regions (Figure 5). The analysis excluded the F1 hybrid individual from Jalisco to avoid inflating H_e.

3.2.5 Pollinator Demographic Models Selection

Using PHRAPL, we tested the same 46 demographic models as we did for *F. petiolaris*. We initially ran PHRAPL models on populations immediately north (Jalisco and Zacatecas) and south (Morelos and Northern Oaxaca) of the TVB. Due to limited sample sizes, PHRAPL failed to differentiate between models. Thus, we included all northern and southern populations for the analysis. Additionally, we excluded the F1 hybrid individual from Jalisco to assess whether gene flow played a significant role in our demographic models, independent of any recent migration event's influence. The highest-ranked PHRAPL models ($wAIC = 0.85$, Table S6) included gene flow and a coalescent event. These results indicate divergence with gene flow between the northern and southern Mexico *Pegoscapus sp* phylogroups. The leading model indicated an asymmetrical gene flow pattern, with estimated migration from south to north being approximately twice as much as from north to south (m1_2.1 vs. m1_1.2, Table S6).

3.3 Congruence of Host and Pollinator Population Structure

In the estimated population graph, *F. petiolaris* sites were grouped into four distinct clusters (Fig. 8A). These clusters represent independently evolving units, categorized as follows: (1) Baja California sites, (2) coastal Sonora sites, (3) central Mexico and inland Sonora sites, and (4) Oaxacan sites. The population graph for *Pegoscapus sp.* revealed two primary clusters (Fig. 8 B). The first consisting exclusively of Oaxacan sites and the second cluster encompassing the remaining sites (Baja California, Coastal Sonora, Inland Sonora, and Central Mexico)

Our test for topological congruence revealed a weak and statistically non-significant correlation (R=0.1457, p=0.5515, t=0.6075, df=17, 95% CI of [-0.3303, 0.5627]) between the shortest path matrices of *F. petiolaris* and *Pegoscapus sp.* graphs. The weak "distance congruence" correlation between the two graphs suggests differences in their respective inter-population variance. The test of "structural congruence," which examined connectivity patterns between sites, did show a significance number of shared edge connections between the *F. petiolaris* and *Pegoscapus* population graphs (p=0.0048, rep=10,000, total possible edges = 78, edge correlation = 0.2696). Considering the node and edge count in *F. petiolaris* (11 edges) and *Pegoscapus sp.* (15 edges) population graphs, the five shared edges between these graphs are more than expected by chance. These shared edges (100-104, 158-39, 172-39, 201-95, and 209/210-214/222) show a distribution across geographic locations, with three located in Baja California, one in Coastal Sonora, and one in Oaxaca. All shared edges connected neighboring sites, with the exception of site 39 in Baja California, which is the southernmost BCP site yet shares edges with the two northernmost BCP sites, 172 and 158.

Our last test investigated whether pollinators demonstrate a preference for traveling through lower elevations compared to those encountered through out their species distribution. Our analysis revealed a statistically significant difference between the observed mean elevation and the distribution of mean elevation values generated through permutation ($p-value = 0.037$, $mean_{obs} = 1559.69$ m, $n = 999$). This suggests that the arrangement of edges we observed in the population graph of *Pegoscapus sp.* is associated with lower elevations compared to the complete range of potential edge connections between nodes. Importantly, these results hold even when considering variations in both population geographical distribution and graph structure.

4 Discussion

The interplay of historical gene flow and vicariance events is a key determinant of contemporary patterns of genetic variation, providing insights into evolutionary processes and biogeographical scenarios. Biotic associations, particularly in obligate pollination mutualists, also affect species spatial genetic structure. Here, we explored how historical gene flow, vicariance, and contemporary biogeography patterns influence genetic variation by investigating these dynamics in Western Mexico's complex geography. Despite the strong selective pressure of their mutualistic relationship, species-specific biological traits strongly influence the population genetic structure of figs and fig wasps.

4.1 Genetic Patterns in Baja California Peninsula and Mexican Highlands

The Baja California Peninsula (BCP) and the Mexican Highlands have played pivotal roles in shaping the genetic patterns and distribution of fauna and flora [Riddle et al., 2000c; Mastretta-Yanes et al., 2015]. In the BCP, we focus on three hypothesized sources of vicariance: the mid-peninsular seaway (MVP), the formation of the Isthmus La Paz (ILP), and the Gulf of California (GOC, Fig. 1 A-C) [Upton and Murphy, 1997; Riddle et al., 2000c]. Within the Mexican Highlands, we focused on two prominent features as potential sources of vicariance: the Trans-Mexican volcanic belt (TVB) and the Sierra Madre del Sur (SMS, Fig. 1 E-F).

We identified four distinct genetic clusters in *F. petiolaris*: (1) Baja California, (2) Inland Sonora, (3) Central Mexico, and (4) Southern Oaxaca – with a fifth zone of admixture, Coastal Sonora, located between Baja California and Inland Sonora (Fig. 2B & C). This genetic structure is unlikely to be the result of restricted gene flow due to local inbreeding, as low estimates of F_{IS} (Table S2) suggest highly outcrossing populations. For *Pegoscapus sp.*, we identified four distinct genetic clusters: (1) northern Mexico (Baja California + Sonora + Sinaloa + Zacatecas + Jalisco), (2) Morelos, (3) Northern Oaxaca, and (4) Southern Oaxaca (Fig. 6). There is no evidence of admixture between northern Mexico and the genetic cluster south of the TVB. Despite a large estimate of F_{is} (Table S5), *Pegoscapus* interpopulation genetic connectivity remains high throughout northern and central Mexico (*i.e.*, low F_{st} values; Table S4).

4.2 Lack of Vicariance Within the Baja California Peninsula

Long–term habitat stability influences the spatial distribution of intraspecific genetic diversity [Vasconcellos et al., 2019], leading to expected phylogeographic congruence between taxa. However, recent range expansion can obscure the signatures imposed by long–term habitat stability. The evolutionary history of BCP is often cryptically integrated into widespread species and species-groups genetic structure[Upton and Murphy, 1997; Riddle et al., 2000c]. Not all BCP taxa exhibit such shared vicariance signals, with some species showing limited or no genetic structure [Vázquez-Miranda et al., 2022]. Similarly, our results indicate that MVP and ILP formation has not left a vicariance signal in the genetic structure of *F. petiolaris* and *Pegoscapus* Weak IBD was observed in *F. petiolaris* BCP populations (Figure 4). High genetic connectivity was observed despite an 800 km distance between sites, which was supported by the low genetic differentiation of pollinators between BCP sites (Table S2 and Table S4).

Nason et al. [2002] proposed that frost-sensitive plant systems and their associated insects experienced a contraction of their geographical ranges into southern refugia during the last glacial maximum (LGM), followed by a subsequent northward expansion to their present distributions in the Holocene. The absence of vicariance signals in *F.petiolaris* and *Pegoscapus sp.* could be attributed to the contraction of its range into the southern BCP refugia during the LGM. However, sampling is insufficient to reach a definite conclusion. Although the BCP genetic cluster shows the lowest genetic diversity among the inferred genetic clus-

ters (as shown in Figure 5A and Table S2), it is difficult to determine whether *F.petiolaris* expanded from a central refugee population, as southern Oaxaca also has low genetic diversity. Even if insects possess moderate frost-tolerance, their genetic structure is expected to be affected by the frost-sensitivity of their host. Consequently, glacial-interglacial cycles of the Pleistocene are likely to have impacted the genetic structure of the frost-sensitive desert flora and their associated insects [Nason et al., 2002; Garrick et al., 2009, 2013].

4.3 Divergent Phylogeographic Patterns and Contrasting Life History Strategies

Despite sharing some phylogeographic patterns, there are points of incongruence between *F. petiolaris* and *Pegoscapus sp.* around the BCP. PCA (Fig. 2B & C), along with the population tree from the SVDquartets (Fig. 3 A) highlight the GOC as a factor contributing to the separation between *F. petiolaris* populations in Baja California and the mainland (Fig. 1C). The effectiveness of GOC as a barrier to genetic flow is limited due to detection of gene admixture in coastal Sonora (Fig. 2A). Baja California had the lowest genetic diversity among clusters, whereas coastal Sonora exhibited higher H_e, akin to Inland Sonora and Central Mexico (Figure 5A), suggesting that Inland Sonora and Central Mexico predominantly contribute to the genetic variance in Coastal Sonora.

This signal of vicariance and admixture is absent in *Pegoscapus* (Fig. 6), and genetic differentiation is relatively low in north central Mexico (Table S1). Weak IBD was detected (Fig. 7A & B), but there was no genetic structuring in *Pegoscapus* (Fig. 6A & B). Differences in life-history strategies between *F. petiolaris* and *Pegoscapus sp.* may explain the contrasting vicariance signals. Classical coevolutionary models predict that obligately interacting species adhere to the same adaptive principles and operate within similar time frames [Alvarez et al., 2010]. We propose considering their ecological relationships and idiosyncrasies, including two life-history traits that influence distinct vicariance signals: 1) life-span and 2) gene flow. When a pollinator has a shorter generation time and greater dispersal capabilities than its host, signs of vicariance are expected to diminish more rapidly in the pollinator species than in its host. *Ficus petiolaris* and its pollinator have considerably different generation times (years to decades vs. 44-77 days, respectively) [Piedra-Malagón et al., 2019]. As such, the pollinator *Pegoscapus* possesses traits that promote gene flow among its populations more effectively than its host.

Pollen migration contributes to total gene flow by at least an order of magnitude more than seed migration, a pattern similarly observed in the genus *Ficus* [Petit et al., 2005; Yu et al., 2010; Liu et al., 2015]. Fig wasps can travel long distances exceeding 100 km, aided by prevailing wind currents [McKey, 1989; Ahmed et al., 2009]. Consequently, pollinator dispersal plays a substantial role in influencing the gene flow and genetic structure of host fig populations. This does not ensure a congruent phylogeographic signal between the host and its pollinator. Cross-pollinated *F. petiolaris* seeds often fail to produce viable offspring, while female wasps that access fig syconia tend to have more successful reproduction [Crawley and Ross, 1990; Bronstein and Hossaert-McKey, 1996].

Extreme events, such as droughts, can further increase incongruence in genetic structure

by causing local fig wasp extinction while leaving host-plant populations intact [Harrison, 2000]. High summer temperatures can reduce syconium volume, leading to smaller wasps with fewer resources for long-distance pollination [Krishnan et al., 2014]. These phenomena are likely to become more prevalent due to climate change. Given these factor two scenarios emerge: (1) reduced genetic structure of the pollinator compared to the host due to long-distance pollinator dispersal or (2) pronounced genetic structure in fig wasp populations due to bottlenecks and founder effects from limited colonizers. Our data, along with the results from Liu et al. [2015], support the first scenario, which estimated that pollinators (*W. pumilae*) introduced genetic variability (cpDNA and nuclear DNA) into their populations at a rate of 14:1 compared to genetic variability introduced into the populations of the host plant (*F. pumila*). This discrepancy suggests that generation time, dispersal, and abiotic factors influences play a crucial role in shaping discordant population genetic structure patterns between *F. petiolaris* and *Pegoscapus*.

4.4 Barriers to Gene Flow and Hybridization Dynamics

The genetic landscape of *F. petiolaris* populations across the TVB exhibit strong genetic differentiation. Minimal IBD was observed in BCP *F. petiolaris* samples, so IBD is unlikely to explain the significant genetic differentiation across the TVB. Futhermore, the genetic differentiation between inland Sonora and central Mexican *F. petiolaris* populations remains relatively low, despite a geographic distance of $\sim$ 1000 km. The genetic differentiation observed between north-central and southern Mexico is more likely due to vicariance than IBD.

Our demographic modeling indicated strongly supported isolation with migration, suggesting ongoing gene flow between these regions (see Table S3). This gene flow was quantified at $4Nm = 4.64$, which translates to an $Fst \approx 1/(4Nm + 1) = 0.177$, assuming an infinite island model operating at migration-drift equilibrium. Typically, such *Fst* values (ranging from 0.15 to 0.25) indicate substantial genetic differentiation [Wright, 1949]. The top PHRAPL models shared identical migration parameters, but their coalescent parameter estimation varied. This variability can be attributed to the limited phylogenetic signal obtained from ddRAD gene trees and PHRAPL's inherent bias towards models that emphasize divergence, making distinguishing between isolation-only and isolation with migration models challenging. This bias persists even with an increasing number of loci [Jackson et al., 2017]. Considering all factors, PHRAPL results suggest a short divergence time, with limited gene flow homogenizing the observed differentiation between *F. petiolaris* populations separated by TVB ($F_{st} \geq 0.292$; see Table S1).

The *Pegoscapus* populations exhibit strong genetic differentiation throughout the TVB, characterized by F_{st} values commonly seen between species. The top PHRAPL model supported an isolation-with-migration scenario, which was confirmed by the detection of an F1 hybrid. *snpclust* verified that the F1 hybrid originated from north-central or southern Mexico. Identifying this F1 hybrid within a sample of 102 wasps suggests the prevalence of long-distance dispersal and hybridization among genetic groups. However, detecting migration events does not always equate to effective gene flow between source populations.

Since the hybrid was found in Jalisco, it suggests that a female *Pegoscapus* traveled from

Morelos or northern Oaxaca. Based on field observations, single-foundress broods (65% prevalence) in *F. petiolaris* often produce multiple generations before admixture. When source populations are deeply divergent genetic incompatibilities result in infertile first-generation hybrids [Orr, 1995]. Therefore, the absence of second-generation hybrids in our 102 samples is unsurprising. The divergence between north-central and southern Mexico highlights their restricted effective gene flow. Genetic differentiation is less pronounced north of the TVB, possibly due to the Sierra Madre Occidental orientation along the western coast, which allows for migration corridors aided by seasonal wind patterns [Adams and Comrie, 1997].

Although Morelos and Oaxaca are less than 300 m apart, there is a high level of genetic differentiation between their populations, similar to the pairwise F_{st} values seen in populations separated by the TVB. The variation in genetic connectivity may be due to physiological limitations experienced during traversal of environmental gradients. Heat and humidity constrain fig wasps [Jevanandam et al., 2013; van Kolfschoten et al., 2022; Aung et al., 2022], limiting their navigation abilities. Altitude gradients on Mount Wilhelm in Papua New Guinea have been shown to affect the composition and diversity of wasp communities [SoutoVilarós et al., 2020]. Elevation changes hinder gene flow between *Ficus* and pollinator populations, causing genetic divergence with a 500m elevation shift over 4km. [Segar et al., 2017; Souto-Vilarós et al., 2019]. *F. petiolaris* pollinators in high-altitude environments experience lower humidity levels, which increase fig wasp mortality rates [Dunn et al., 2008; Jevanandam et al., 2013]. Our data shows that pollinators prefer lower elevations (Figure S1) despite the altitude range available within their environment.

4.5 The Spatial Scale of Gene Flow

We found differences in the spatial symmetry of genetic structures between *F. petiolaris* and *Pegoscapus* based on a topological test that identified significant disparities in inter-population variance (Figure 8). Furthermore, a structural test of population connectivity patterns revealed a significant number of shared edge connections between the host and pollinator population graphs. Considering the population graph results along with PCA and STRUCTURE, there is significant population genetic structure differences between *F. petiolaris* and *Pegoscapus*. However, our results also suggest localized genetic connectivity similarities between the host and pollinator. These observed phylogeographic patterns are not unique to our system. The absence of IBD and the lack of spatial genetic structure over extensive geographical ranges are seen in other *Ficus* host-pollinator pairs [Molbo et al., 2004; Zavodna et al., 2005; Yu et al., 2010; Kobmoo et al., 2010; Lin et al., 2008; Yu and Nason, 2013; Tian et al., 2015; Heer et al., 2015; Bain et al., 2016; Honorio Coronado et al., 2019; Wilde et al., 2021].

Various factors contribute to the differences in broad-scale population genetic structure between host and pollinator, These include specific traits and their environment. Life-history traits, physiology, and environment influence fig wasp dispersal, longevity, and community composition. Behavioral factors, such as flight height and emergence times can exert strong influences on the dispersal capabilities of pollinators. Some fig wasp species have adopted a nocturnal flight strategy to avoid high air temperatures during the day, thereby expanding their dispersal range [Warren et al., 2010]. Fig wasps also exhibit behaviors that optimize

resource allocation to enhance their fitness [Greeff and Kjellberg, 2022]. Lack of pollination harms fig wasp larvae since figs entered by pollen-free wasps are more likely to abort [Jousselin et al., 2003; Jansen-González et al., 2012; Borges, 2021]. Pollen collection and deposition have an energy cost, so balancing oviposition with pollination is necessary [Kjellberg et al., 2001; Anstett et al., 1997]. Long-distance dispersal should lead to increased allocation of energy resources towards oviposition instead of pollination. Therefore, pollinators are more likely than their host plant to introduce genes within their own populations. [Liu et al., 2015].

The growth patterns of fig tree species can also contribute to the incongruent host and pollinator genetic structure. [Chen et al., 2011]. Dioecious figs are typically small trees or shrubs that are sparsely distributed across the landscape, yet they frequently form relatively dense local populations [Wang et al., 2009]. Additionally, individual plants of dioecious species tend to bear fruit more frequently compared to their monoecious counterparts [Harrison, 2003]. As a result, dioecious figs pollinators do not need to disperse as far, leading to a strong population structure among dioecious figs [Wang et al., 2009; Chen et al., 2011; Dev et al., 2011]. In contrast, monoecious figs are thinly scattered (<1 individual per hectare) and predominantly depend on pollinators that rely on wind currents to disperse their pollen. Consequently, monoecious figs tend to exhibit limited or no genetic structure across their species distribution [Kobmoo et al., 2010; Bain et al., 2016; Honorio Coronado et al., 2019; Wilde et al., 2021].

At a local level, we observed similar population connectivity patterns between *F.petiolaris* and *Pegoscapus* likely driven by seed dispersal Heer et al. [2015]. In the case of *F. petiolaris*, the dispersion of seeds is facilitated by frugivorous bats, which are commonly recognized as effective long-distance seed dispersers [Piedra-Malagón et al., 2019; Thornton et al., 1996]. It is unlikely that seeds ingested by bats are dispersed over long distances as they typically spend less than 30 minutes in the digestive tracts of bats [Morrison, 1980; Laska, 1990]. Most fig seeds are expected to be dropped close to their maternal fig tree [Heer et al., 2015]. It is possible that some *Pegoscapus* pollinators disperse shorter distances, which could contribute to the similar localized population connectivity between host and pollinator. This signal is likely to be very weak in comparison to the gene flow occurring at broader scales.

4.6 Implications for Host Fig and Pollinator Classification

Initially described as a single species, ongoing taxonomic debates resulted in the recognition of a species complex consisting of four morphologically distinct species, including *F. petiolaris*, *F. jaliscana*, *F palmeri*, and *F. brandegei*. Piedra-Malagón et al. [2011] found only gradual morphological variations among these species, indicating a potential single species. Our results support the classification of *F. petiolaris* subsp. *petiolaris* and *palmeri*, demonstrating significant genetic differentiation between Baja California and mainland Mexico clusters. The classification of subsp. *brandegii* is not supported by our findings. We recognize three distinct allopatric subspecies of *F. petiolaris*: one in Southern Oaxaca, another in BCP, and a third widespread across northern and central mainland Mexico.

There has been no formal taxonomic analysis of its associated *Pegoscapus* pollinator. Similar to the host, genetic differentiation across TVB indicates the presence of distinct pollinator subspecies. Fig populations in Baja California, Inland Sonora, and Central Mexico

comprise two subspecies, while minimal pollinator population genetic differentiation indicates a single subspecies. We recognize three subspecies of the *F. petiolaris* pollinator: one widespread in Baja California and northern+central mainland Mexico, a second in Morelos and northern Oaxaca, and a third southern Oaxaca.

4.7 Conclusion

The phylogeography of *F. petiolaris*, and its pollinator, *Pegoscapus sp.*, demonstrates that biological traits, such as life history strategies, dispersal capabilities, and physiological constraints, are important factors that shape their respective population genetic structure. This study contributes to our understanding of the complex dynamics that shape the genetic variation in obligate mutualistic interactions and highlights the need to consider historical factors along side biological traits to unravel their complex genetic patterns.

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6 Author Contributions

KQ, FP and JDN conceived of the study. KQ, FP and JDN collected the fig and fig wasp samples. KQ, JDS and FP generated and processed the sequence data. KQ and JDN conducted all analyses. KQ, FP, JDS, TAH and JDN wrote the paper, and all authors contributed to revised versions of the manuscript and approved of the final version.

7 Data Availability Statement and Benefit-sharing

Raw sequence data are available from the NCBI Sequence Read Archive (SRA) under BioProject ID: XXX (BioSample accessions: XXX). NCBI BioSample accession numbers for individual wasps, Ipyrad and Phyluce assemblies, along with their related script datasets are available on Dryad (`https://doi.org/10.5061/dryad.fbg79cnwk`). Scripts for figures and data analysis are available in a GitHub repository (`https://github.com/kquinteros/ficus-phylogeography.git`).

8 Benefit-Sharing

Benefits Generated: Benefits from this research accrue from the sharing of our data and results on public databases as described above.

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9 Figures

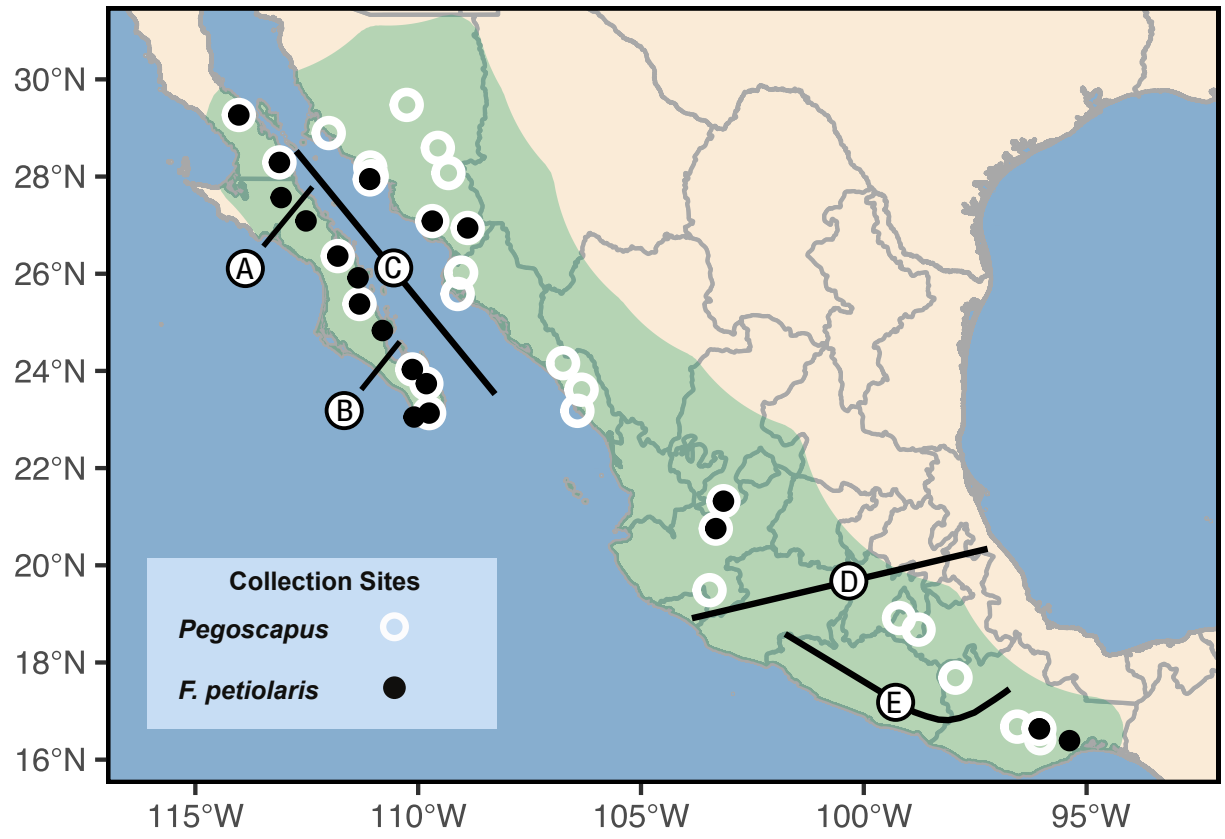

Figure 1: *Ficus petiolaris* and *Pegoscapus sp.* sample sites and known biogeographical barriers in Mexico. The geographical distribution of the fig and its obligately-associated pollinator are indicated by the green shading. *A priori* vicariance hypotheses are based on biographical barriers: (A) ancient mid-peninsular seaway (MPS, ca. 1 Mya), (B) formation of the Isthmus of La Paz (ILP, ca. 3 Mya), (C) the Gulf of California (GOC, ca. 5 Mya), (D) the Trans-Mexican Volcanic Belt (TVB, ca. 11-3 Mya), and (E) the Sierra Madre del Sur (SMS, ca. 48-23 Mya). Ficus petiolaris geographical distribution is based on Global Biodiversity Information Facility (GBIF) occurrence data.

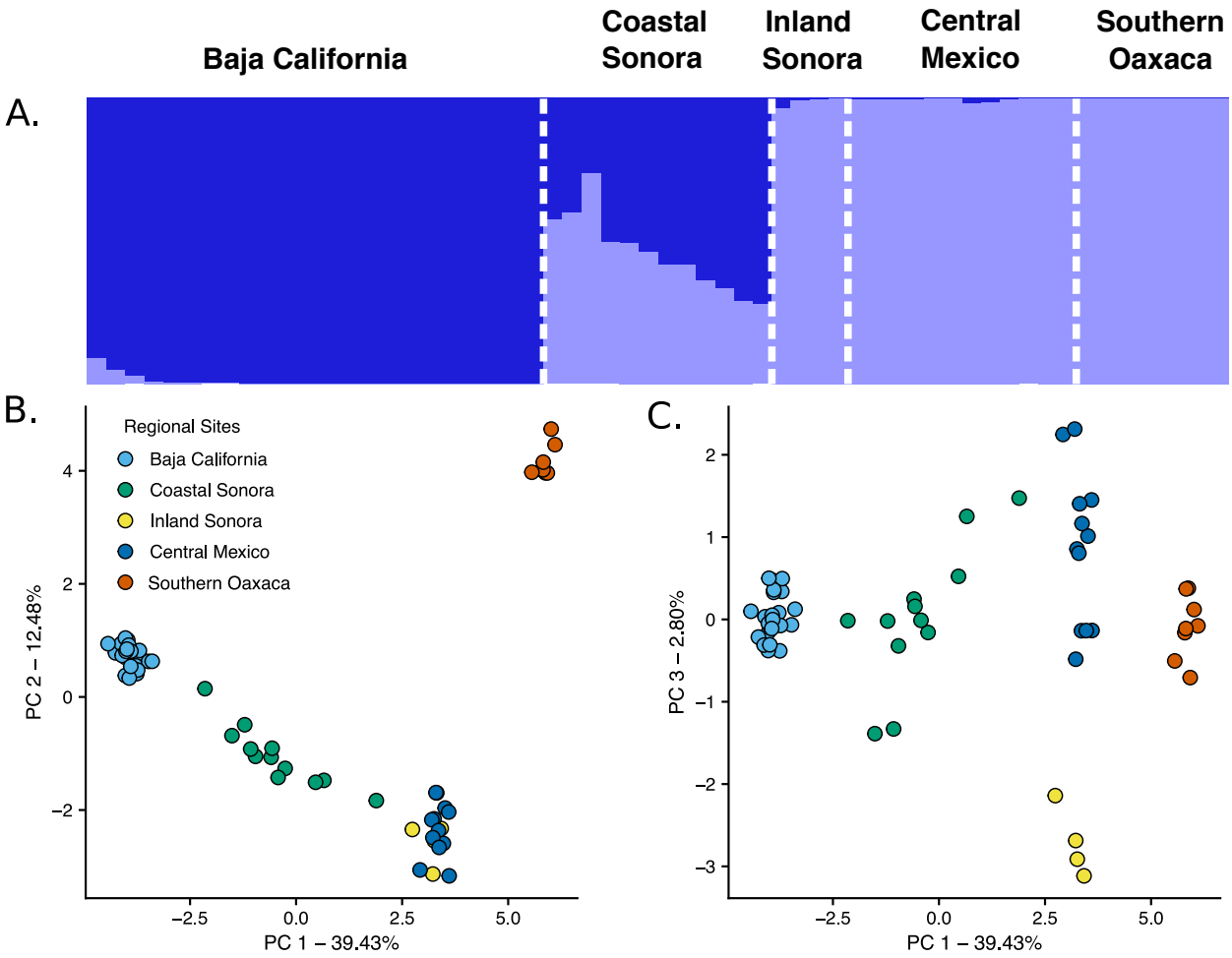

Figure 2: Population genetic structure of *Ficus petiolaris* individuals, colored according to the region in which they were sampled. (A) STRUCTURE analysis revealed $K = 2$ genetic clusters corresponding to Baja California and Inland Sonora + Central Sonora + Oaxaca, with coastal Sonora admixed between them. Subsequent STRUCTURE analyses of each genetic cluster revealed no additional subdivision. Principal component analysis (PCA) revealed five distinct genetic clusters with Baja California, coastal Sonora, inland Sonora + central Mexico, and Oaxaca separated by PC 1 versus PC 2, which together explained more than 50% of the variation among samples (B), and inland Sonora and central Mexico further differentiated by PC 1 versus PC 3 (C). As in the STRUCTURE analysis, coastal Sonora is intermediate between Baja California and mainland clusters, specifically inland Sonora and Central Mexico. Both STRUCTURE and the PCA support the hypothesis that the Gulf of California is a significant source of vicariance in this species (Figure 1C). The PCA further identifies the Trans-Mexican Volcanic Belt (Figure 1D) as a significant source of vicariance. Pairwise analyses using Wright's F_{st} subsequently showed the five clusters identified by the PCA to be significantly genetically differentiated from each other (Table S1).

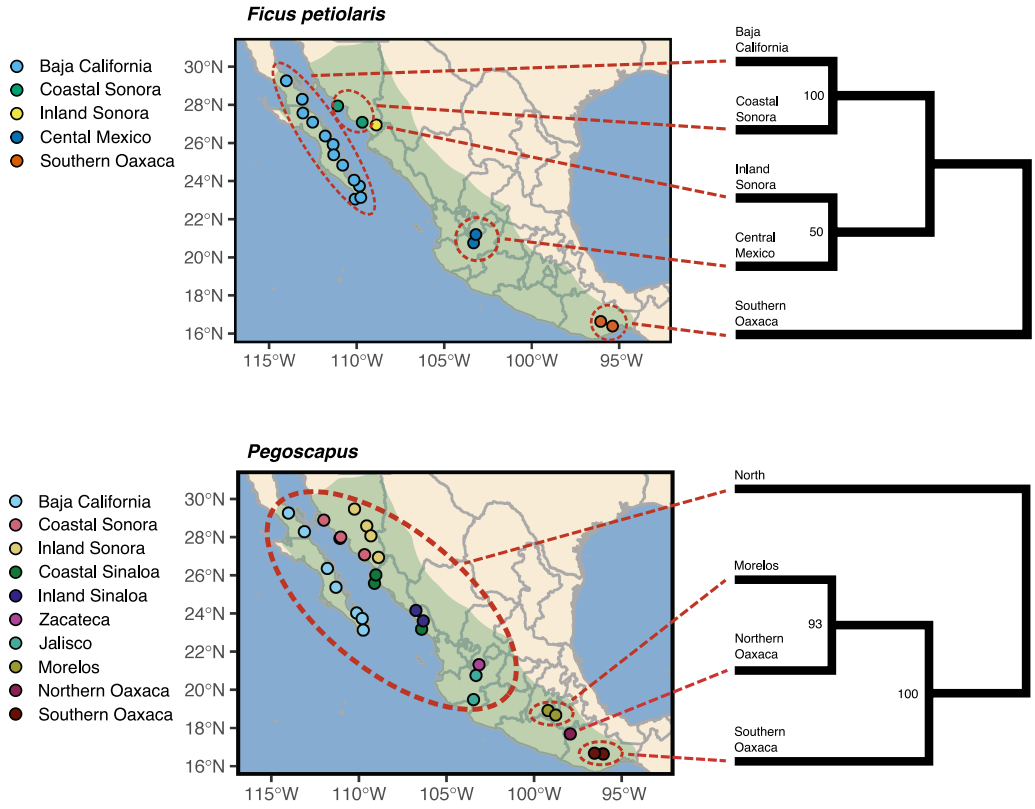

Figure 3: SVDquartets population trees for *Ficus petiolaris* and its *Pegoscapus sp.* pollinator, in which individuals are grouped into populations corresponding to genetic clusters inferred from PCA and STRUCTURE analyses (Figures 2 and 3, respectively). Bootstrap values are shown at the nodes. In the host fig tree (top), consistent with SNAPP results (Figure 4), southern and north-central Mexico form monophyletic clades and, within the latter, coastal Sonora is sister to Baja California while inland Sonora is sister to central Mexico. In the pollinator (bottom), all northern and central Mexico clades were grouped into a single North (Mexico) population based on our PCA and STRUCTURE results The North Mexico phylogroup was sister to all southern Mexico phylogroups. Among the southern Mexico phylogroups, Oaxaca was sister to Northern Oaxaca and Morelos. SVDquartets also provide high support for Northern Oaxaca being sister to Morelos.

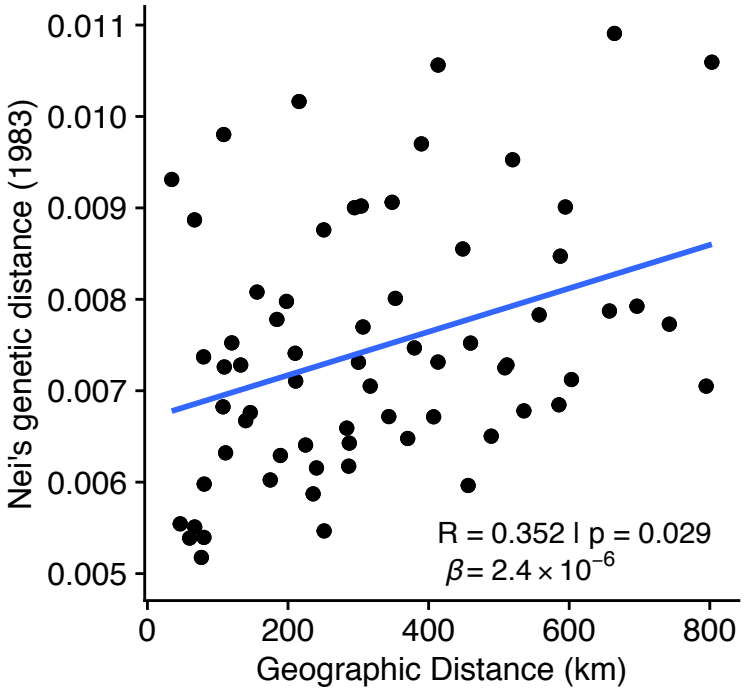

Figure 4: In *F. petiolaris*, weak but significant isolation by distance (IBD) was observed across Baja California. This result does not support hypotheses A and B in Figure 1, that two ancient trans-peninsular seaways have been lasting sources of vicariance in this region.

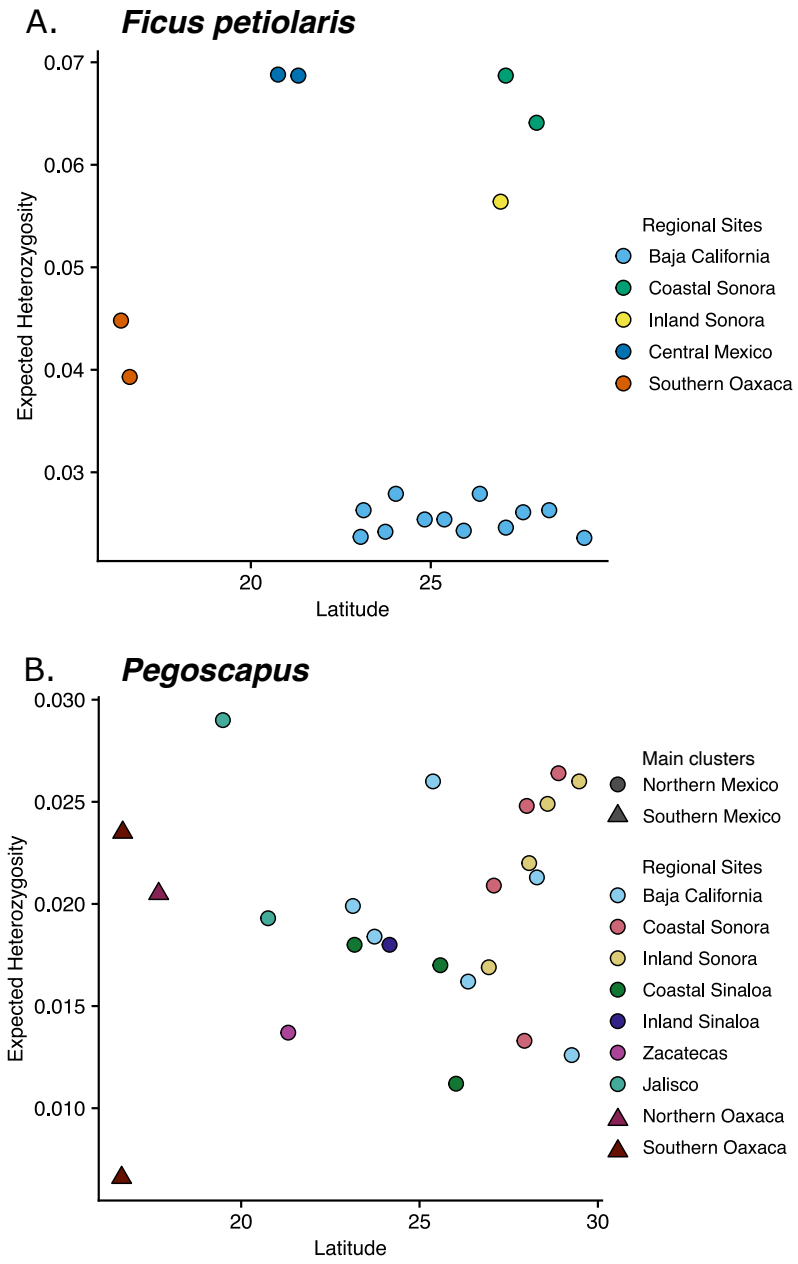

Figure 5: Expected heterozygosity of host fig and pollinator populations as a function of latitude. (A) In *F. petiolaris*, Baja California and Oaxaca populations, which are peninsular and located at the southern limits of the species range, respectively, had similarly low genetic diversity. In contrast, Coastal Sonora, Inland Sonora, and Central Mexico had substantially higher genetic diversity. (B) In *Pegoscapus sp.*, there were no discernable regional differences in genetic diversity and expected heterozygosity varied widely among populations within each region and between regions. Main clusters distinguishes between sites from inferred genetic clusters located north and south of the Trans-Mexico Volcanic Belt.

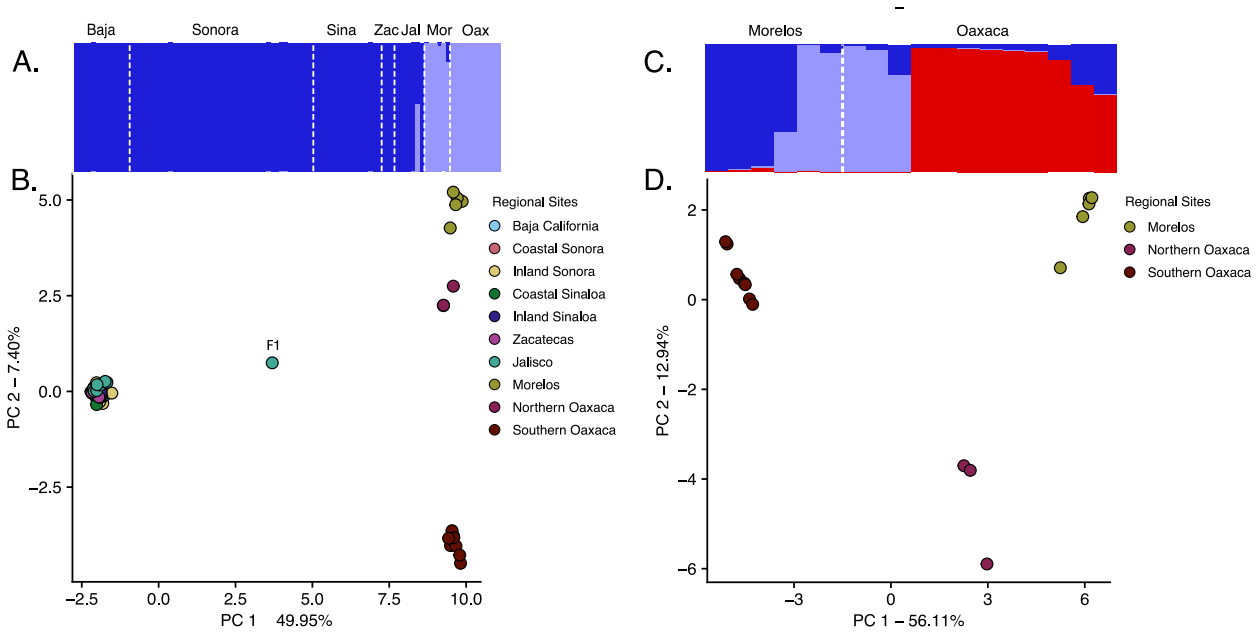

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Figure 6: Population genetic structure of *Pegoscapus sp.* individuals, which are colored according to the region in which they were sampled.(A) Two genetic clusters ($K = 2$) were inferred by STRUCTURE, with individuals from Morelos and Oaxaca, in the south, forming one cluster and all more northern samples forming the other. One individual from Jalisco had equal admixture from these two genetic clusters. (B) In a principal component analysis (PCA), axis PC 1 separated individuals into two major genetic clusters, northern and central Mexico versus southern Mexico, and explained nearly 50% of variation among samples. The second PCA axis separated the southern samples into three clusters, Morelos, Northern Oaxaca, and Southern Oaxaca, for four genetic clusters total. As in the STRUCTURE analysis, the PCA identifies an apparently admixed individual from Jalisco, subsequently shown using *snapclust* to be a F1 hybrid between northern-central Mexico and southern Mexico clusters. (C) STRUCTURE was subsequently run on each of the two previously inferred clusters (northern + central Mexico; southern Mexico), but only southern Mexico showed further subdivision, with $K = 3$ clusters corresponding to Morelos, Northern Oaxaca, and Southern Oaxaca. Consistent the PCA analysis, the STRUCTURE analyses revealed $K = 4$ distinct genetic clusters total. (D) PCA analysis of samples from southern Mexico further differentiated genetic clusters from Morelos, Northern Oaxaca, and Southern Oaxaca. Pairwise analyses using Wright's F_{st} subsequently showed the four clusters identified by both STRUCTURE and PCA analyses to be significantly genetically differentiated from each other (Table S4).

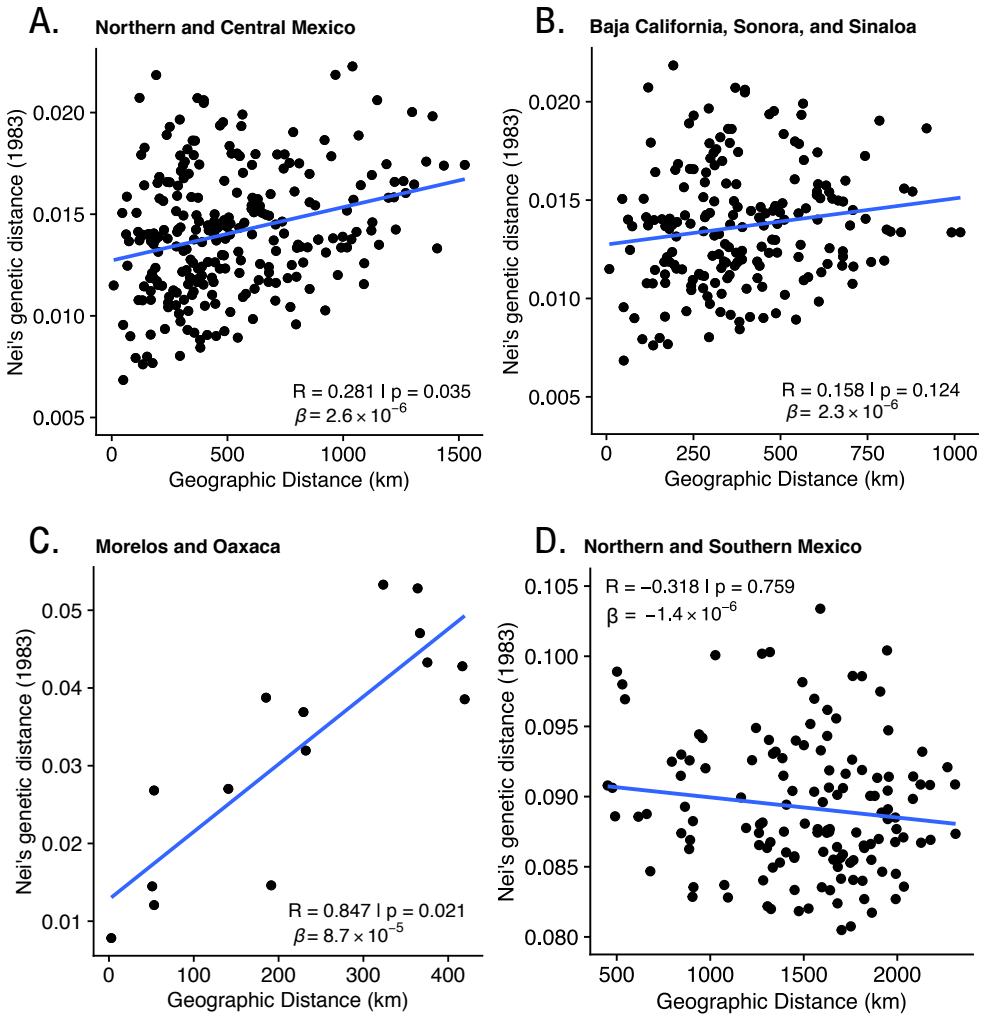

Figure 7: Isolation by distance (IBD) analysis of landscape-level genetic connectivity regressing population-pairwise Nei's genetic distance versus geographic distance for *Pegoscapus sp.* pollinator. Each plot shows the best fit regression line, correlation, Mantel p-value, and slope. Weak but significant IBD was detected across the inferred phylogroup comprising Baja California and northern and central mainland Mexico (A), whereas a nested analysis of Baja, Sonora, and Sinaloa samples and spanning the Gulf of California was not significant (B). In southern Mexico (C), strong and significant IBD was detected across samples from Morelos, northern Oaxaca, and southern Oaxaca, despite their having been identified as distinct phylogroups by STRUCTURE and PCA analyses. (D)Analysis of IBD between *Pegoscapus sp.* populations located north and south of the Trans-Mexican Volcanic Belt, a hypothetical source of vicariance (Figure 1D). IBD between these two regions was not significant, indicating that this area of active volcanos and high plateaus is a strong barrier to dispersal and gene flow in this species.

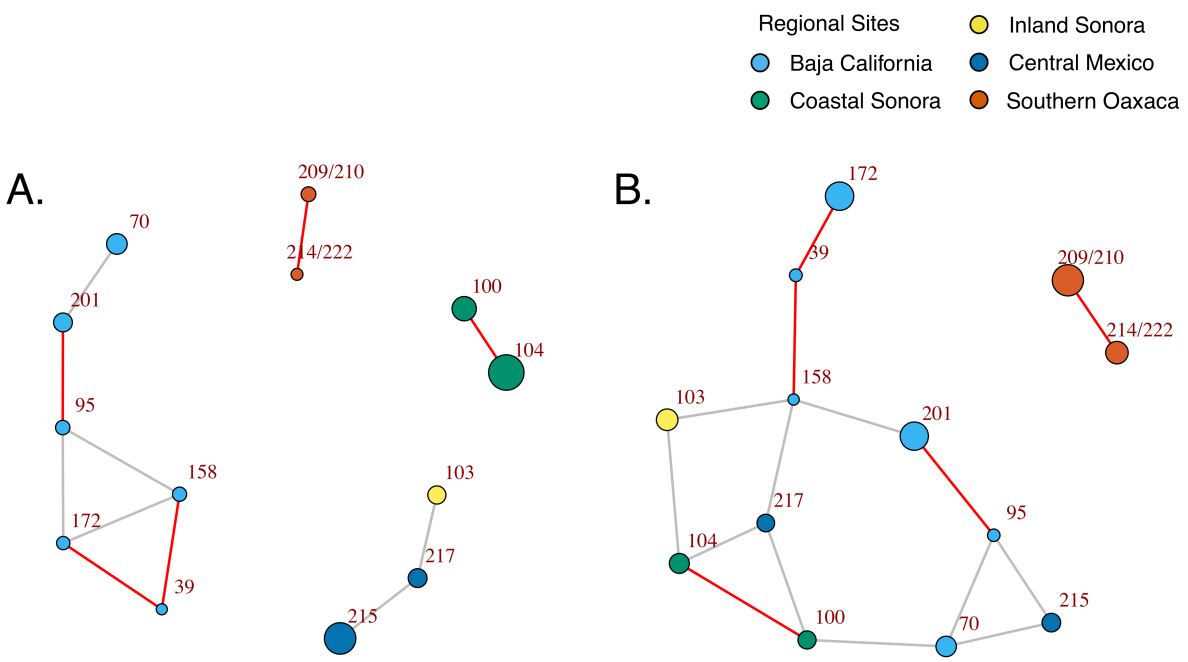

Figure 8: Population graphs representing the genetic relationships among *F. petiolaris* (A) and *Pegoscapus* (B) regional sites. Each node represent sampling sites, and the color of each node corresponds to the regional site where the sampling site is located. Node sizes reflect within-population genetic variability, whereas the edge lengths represent the among-population component of genetic variation due to the connecting nodes. A significant number (permutation test p=0.0048, edge correlation=0.2696, reps=10,000) of edges (colored in red) were shared than expected by chance between *F. petiolaris* and *Pegoscapus* population graphs. All shared edges are between neighboring sites, except those involving site 39. Site 39 is the southernmost Baja site yet it shares edges with the two most northerly Baja sites, 172 and 158. These results imply a broad difference in genetic structure between *F. petiolaris* and *Pegoscapus* with localized similarities in population genetic connectivity.

10 Supplemental Figures

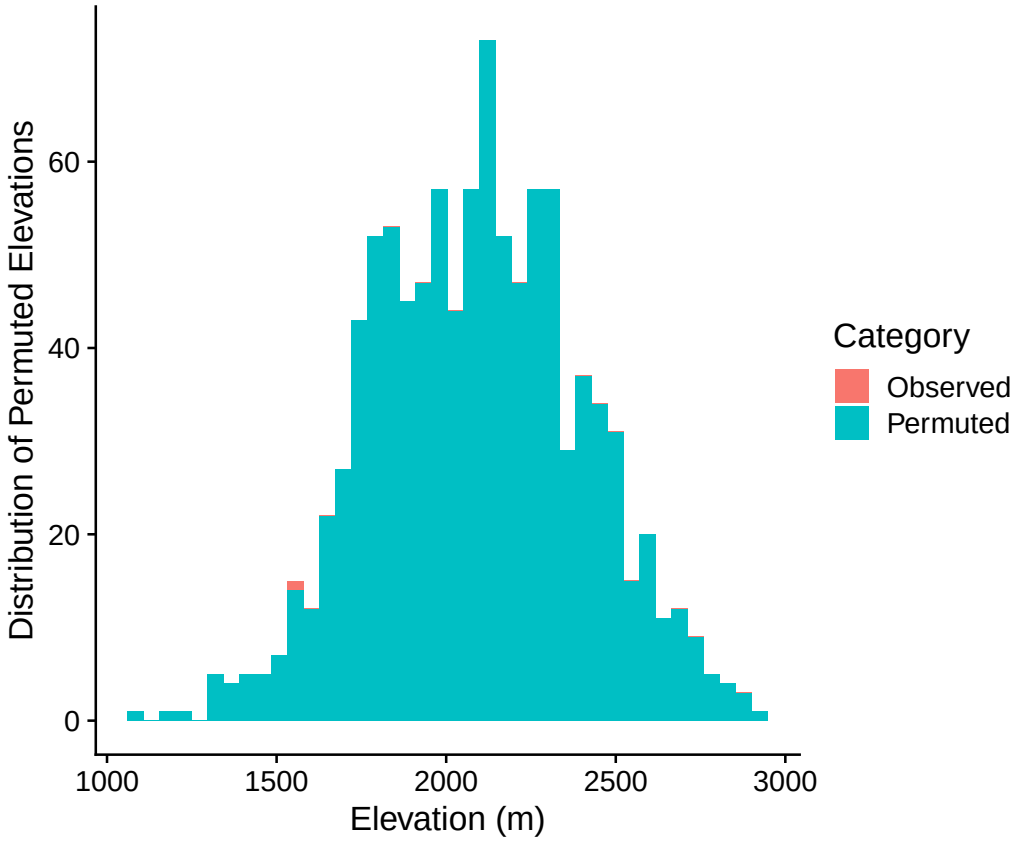

Figure S1: Distribution of Permutated Elevation ($p = 0.037$, $mean_{obs} = 1559.69$ m, 999 permutations) . The data shows that our observed values consistently fall towards the lower end of the elevation spectrum. This trend remains even when accounting for the geographical distribution of populations and the graph's structure.

Region	Sample Site	Trees Sampled	Wasps Sampled	Latitude	Longitude	Elevation Range (m)
Baja California	39	7	2	23.13187	-109.75406	188
	70	23	2	23.73801	-109.81612	3089
	95	17	2	26.36298	-111.80387	263
	96	23	2	24.02943	-110.13009	750
	112	27		27.56675	-113.07373	864
	113	19		27.08696	-112.51638	644
	158	20	2	29.26645	-114.02414	899
	172	19	2	28.29053	-113.11295	697
	179	22		25.91582	-111.34837	19
	201	21	2	25.37728	-111.31542	288
	204	6		24.83127	-110.80179	384
	205	7		23.05125	-110.09175	9
Coastal Sonora	36		5	28.89523	-112.00658	192
	100		5	28.00606	-111.04223	84
	100T	6	2	27.94121	-111.08463	33
	104	6	9	27.08341	-109.68225	52
Inland Sonora	103	4	6	26.94181	-108.88808	254
	106		5	29.47434	-110.25687	423
	107		5	28.58991	-109.55771	224
	108		6	28.07264	-109.32043	224
Coastal Sinaloa	226		5	23.17888	-106.42636	101
	230		5	25.58337	-109.11327	5
	231		3	26.02424	-109.03569	98
Inland Sinaloa	228		1	23.61378	-106.33288	146
	229		2	24.16040	-106.74877	188
Zacatecas	217	6	3	21.31893	-103.14842	1271
Jalisco	215	6	2	20.75497	-103.32047	1414
	218		6	19.48764	-103.46098	1055
Morelos	219		5	18.67604	-98.77137	1368
	220		1	18.91518	-99.20843	1457
Northern Oaxaca	221		3	17.68726	-97.94190	1341
Southern Oaxaca	209		2	16.64853	-96.07933	784
	210	3		16.39097	-95.38333	201
	214	5	1	16.63315	-96.05944	805
	222		6	16.67925	-96.55636	1746

Table 1: *F. petiolaris* and *Pegoscapus* sp. sample site information and the number of trees ($n = 247$) and wasps ($n = 102$) sampled per site for phylogeographic analysis.

F. petiolaris	Baja California	Coastal Sonora	Inland Sonora	Central Mexico	Southern Oaxaca
Baja California	*NA*	**0.2803 (0.2282 - 0.3255)**	**0.5904 (0.5272 - 0.6455)**	**0.5564 (0.4941 - 0.6072)**	**0.694 (0.6412 - 0.7344)**
Coastal Sonora		*NA*	**0.1622 (0.1254 - 0.2047)**	**0.1246 (0.0981 - 0.1512)**	**0.4049 (0.3582 - 0.4456)**
Inland Sonora			*NA*	**0.0703 (0.0405 - 0.1039)**	**0.4312 (0.3794 - 0.482)**
Central Mexico				*NA*	**0.3215 (0.2725 - 0.3653)**
Southern Oaxaca					*NA*

Table S1: Estimates of Wright F_{st} between pairs of inferred phylogroups of *F. petiolaris*. Values in parentheses are bootstrapped 95% confidence intervals and significant positive F_{st} estimates are denoted in bold.

F. petiolaris								
Regional Sites	**Sample Site**	**Sampled Trees**	**N**	**n_ests**	**Fis**	**ll**	**hl**	**Hs**
Baja California	39	7	7	75	0.0781	0.0257	0.2622	0.0263
	70	23	17	107	0.0449	0.0196	0.1836	0.0242
	95	17	16	119	0.0588	0.0468	0.2123	0.0279
	96	23	14	115	0.0869	0.0533	0.2209	0.0279
	112	27	20	135	0.0442	0.0300	0.1748	0.0261
	113	19	15	117	0.0613	0.025	0.2329	0.0246
	158	20	16	107	0.0549	0.0373	0.1892	0.0236
	172	19	16	119	0.0262	-0.0111	0.1253	0.0263
	179	22	19	121	0.0447	0.0469	0.2014	0.0243
	201	21	19	114	0.0436	-0.0100	0.1507	0.0254
	204	6	6	75	0.0641	0.0231	0.2636	0.0254
	205	7	7	71	0.0477	-0.0208	0.2104	0.0237
Sonora	100T	6	5	147	0.0351	-0.0020	0.1603	0.0641
	103	4	4	109	0.0341	-0.0209	0.197	0.0564
	104	6	6	148	-0.0209	-0.0987	0.0687	0.0687
Central Mexico	215	6	6	155	0.0016	-0.0581	0.0814	0.0688
	217	6	6	163	0.0663	0.0284	0.1949	0.0687
Southern Oaxaca	210	3	3	68	0.1177	-0.0047	0.3350	0.0448
	214	5	5	74	-0.0691	-0.1883	0.0950	0.0393

Table S2: Estimates of inbreeding coefficient (F_{IS}) of *F. petiolaris* regional sites. Columns indicate the Regional sites, Sampling site, the number of sampled *F. petiolaris* individuals per site (Sampled Trees), the number of *F. petiolaris* samples after filtering (N), the number of loci used to calculate F_{IS} (n_ests), The point estimate of F_{IS}, the lower confidence limit of F_{IS} estimate (ll), the upper confidence limit of F_{IS} estimate (ul), and expected heterozygosity (HS).

F. petiolaris PHRAPL Results							
Rank	AIC	dAIC	wAIC	Parameters	Migration ($m_{1\to2}$)	Migration ($m_{2\to1}$)	Coalesce ($t_{1.2}$)
1	15576.996	0	0.568	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	4.64	4.64	7.690
2	15577.738	0.742	0.392	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	4.64	4.64	0.300
3	15582.284	5.288	0.040	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	4.64	4.64	7.720
4	15597.388	20.392	2.12E−5	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	4.64	4.64	2.131
5	15599.090	22.094	9.05E−6	$m_{1.2}$, $t_{1.2}$	4.64	4.64	0.300
6	15604.526	27.530	5.97E−7	$m_{1.2}$	4.64	4.64	NA
7	15604.567	27.571	5.85E−7	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	4.64	4.64	1.415
8	5609.904	32.908	4.06E−8	$m_{1.2}$, $t_{1.2}$	4.64	4.64	0.353
9	15613.998	37.002	5.24E−9	$m_{1.2}$, $t_{1.2}$	4.64	4.64	9.611
10	15668.925	91.929	6.20E−21	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	2.150	4.639	1.110

Table S3: The top ten ranked *Ficus petiolaris* demographic models from PHRAPL. Columns indicate values of the model rank, Akiake Information Criterion (AIC), difference in AIC from the top-ranked model (dAIC), AIC weights (wAIC), and the vector of demographic parameters (parameter vector, with northern Mexico denoted as 1 and southern Mexico as 2) corresponding to migration from population 1 to 2 ($m_{1\to2}$), migration from population 2 to 1 ($m_{1\to2}$), single migration parameter representing asymmetric or symmetric migration ($m_{1.2}$), and a coalescent event of population 1 and 2 ($t_{1.2}$). The last three columns correspond to the estimate migration parameter value for migration from population 1 to 2 ($m_{1\to2}$), migration parameter value for migration from population 2 to 1 ($m_{1\to2}$), and coalescent event between population ($t_{1.2}$). Migration rates are given in units of 4Nm and time parameters are given in units of 4N.

Pegoscapus	Baja California	Coastal Sonora	Inland Sonora	Coastal Sinaloa	Inland Sinaloa	Zacatecas	Jalisco	Morelos	Northern Oaxaca	Southern Oaxaca
Baja California	*NA*	0.0043 (-0.0034 - 0.0115)	**0.0266 (0.0125 - 0.0427)**	**0.0282 (0.0062 - 0.0536)**	0.0159 (-0.0711 - 0.1082)	0.0263 (-0.0539 - 0.1098)	**0.0508 (0.0063 - 0.1098)**	**0.7339 (0.6755 - 0.7813)**	**0.7408 (0.6855 - 0.7842)**	**0.7624 (0.7181 - 0.7997)**
Coastal Sonora		*NA*	**0.0147 (0.002 - 0.0296)**	0.0066 (-0.0046 - 0.018)	0.0357 (-0.0408 - 0.1113)	0.0544 (-0.0455 - 0.157)	**0.0498 (0.015 - 0.0917)**	**0.7554 (0.7046 - 0.7991)**	**0.7566 (0.7071 - 0.797)**	**0.7718 (0.7285 - 0.8083)**
Inland Sonora			*NA*	0.0119 (-1e-04 - 0.0252)	-0.0127 (-0.0706 - 0.046)	0.0605 (-0.0364 - 0.1594)	**0.0342 (0.0081 - 0.0622)**	**0.7443 (0.6894 - 0.7893)**	**0.7459 (0.6927 - 0.7861)**	**0.7625 (0.7179 - 0.8009)**
Coastal Sinaloa				*NA*	0.0721 (-0.0052 - 0.1486)	**0.134 (0.0196 - 0.2359)**	**0.0642 (0.0304 - 0.0979)**	**0.8094 (0.7616 - 0.8492)**	**0.8056 (0.7602 - 0.8438)**	**0.8064 (0.7684 - 0.8393)**
Inland Sinaloa					*NA*	**0.1597 (0.0274 - 0.2986)**	-0.0132 (-0.0751 - 0.0617)	**0.8017 (0.747 - 0.8514)**	**0.7813 (0.728 - 0.8329)**	**0.792 (0.7464 - 0.8323)**
Zacatecas						*NA*	0.0449 (-0.0372 - 0.1371)	**0.8092 (0.7509 - 0.8587)**	**0.7977 (0.7475 - 0.8433)**	**0.7971 (0.7509 - 0.8366)**
Jalisco							*NA*	**0.7157 (0.6547 - 0.7687)**	**0.7267 (0.6712 - 0.7764)**	**0.7624 (0.7166 - 0.8007)**
Morelos								*NA*	0.0577 (-0.0809 - 0.1999)	**0.5779 (0.4671 - 0.6636)**
Northern Oaxaca									*NA*	**0.5582 (0.4558 - 0.6488)**
Southern Oaxaca										*NA*

Table S4: Estimates of Wright's F_{st} between pairs of regional samples of *Pegoscapus sp.* pollinator, including inferred phylogroups from Morelos, Northern Oaxaca, and Southern Oaxaca. Values in parentheses are bootstrapped 95% confidence intervals and significant positive F_{st} estimates are denoted in bold.

Pegoscapus								
Regional Site	**Sampling Site**	**Sampled Wasps**	**N**	**n_ests**	**Fis**	**ll**	**hl**	**Hs**
Baja California	39	2	2	27	0.5185	0.3111	0.8039	0.0199
	70	2	2	29	0.2069	0	0.5476	0.0184
	95	2	2	23	0.2609	-0.1155	0.5791	0.0162
	96	2	0	NA	NA	NA	NA	NA
	158	2	2	21	0.0952	-0.0801	0.5	0.0126
	172	2	2	31	0.1935	-0.1668	0.4002	0.0213
	201	2	2	34	0.4412	0.1702	0.6668	0.026
Coastal Sonora	36	5	5	77	0.495	0.5368	0.7457	0.0264
	100	5	4	62	0.5244	0.5667	0.7755	0.0248
	104	9	9	104	0.3773	0.4084	0.6067	0.0209
	100T	2	2	21	0.4762	0.4242	0.8276	0.0133
Inland Sonora	103	6	6	51	0.553	0.4687	0.7753	0.0169
	106	5	5	62	0.7505	0.7023	0.9013	0.026
	107	5	4	57	0.5002	0.5229	0.7624	0.0249
	108	6	6	76	0.4051	0.432	0.6675	0.022
Coastal Sinaloa	226	5	5	55	0.4082	0.4336	0.6767	0.018
	230	5	5	51	0.4793	0.4487	0.7584	0.017
	231	3	3	23	0.2826	0.1723	0.613	0.0112
Inland Sinaloa	228	1	0	NA	NA	NA	NA	NA
	229	2	2	26	0.2692	-0.0345	0.5882	0.018
Zacatecas	217	3	3	29	0.2931	0.2222	0.6192	0.0137
Jalisco	215	2	2	28	0.3571	0.1111	0.6452	0.0193
	218	6	4	68	0.3618	0.3401	0.6481	0.029
Morelos	219	5	0	NA	NA	NA	NA	NA
	220	1	0	NA	NA	NA	NA	NA
Northern Oaxaca	221	3	3	38	0.5263	0.4527	0.7934	0.0205
Southern Oaxaca	209	2	2	8	0.5	-0.2512	0.9476	0.0066
	214	1	0	NA	NA	NA	NA	NA
	222	6	6	77	0.4148	0.4352	0.6813	0.0235

Table S5: Estimates of inbreeding coefficient (F_{IS}) of *Pegoscapus* regional sites. Columns indicate the Regional sites, Sampling site, the number of sampled *Pegoscapus* individuals per site (Sampled Wasps), the number of *Pegoscapus* samples after filtering (N, individuals with less than 50% phased data), the number of loci used to calculate F_{IS} (n_ests), The point estimate of F_{IS}, the lower confidence limit of F_{IS} estimate (ll), the upper confidence limit of F_{IS} estimate (ul), and expected heterozygosity (HS).

Pegoscapus PHRAPL Results							
Rank	AIC	dAIC	wAIC	Parameters	Migration ($m_{1\to2}$)	Migration ($m_{2\to1}$)	Coalesce ($t_{1.2}$)
1	25265.173	0	0.849	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	0.46	1	4.07
2	25269.128	3.954	0.117	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	0.220	1.000	4.069
3	25271.699	6.526	0.032	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	1	1	2.12
4	25288.654	23.481	6.77E−6	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	0.46	2.149	7.808
5	25290.092	24.919	3.30E−6	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	0.220	2.149	2.120
6	25294.179	29.006	4.27E−7	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	1	1	7.809
7	25306.558	41.385	8.76E−10	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	0.455	1.073	1.299
8	25312.562	47.389	4.35E−11	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	0.459	2.149	2.119
9	25314.244	49.071	1.88E−11	$m_{1\to2}$, $m_{2\to1}$, $t_{1.2}$	0.458	2.144	3.829
10	25319.975	54.802	1.07E−12	$m_{1\to2}$, $m_{2\to1}$	0.22	2.15	NA

Table S6: The top ten ranked *Pegoscapus sp.* demographic models from PHRAPL. Columns headings are as in Table S3.