Adaptation to lower latitudes and lower elevations precedes the evolution of hummingbird pollination in western North American *Penstemon*

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Hummingbird adaptation in *Penstemon* involves ecological shifts

ABSTRACT

Premise: A switch in pollinator can occur when a plant lineage enters a new habitat where the ancestral pollinator is less common and a novel pollinator is more common. Since pollinator communities vary according to environmental tolerances and availability of resources, there may be consistent associations between pollination mode and specific regions and habitats. Such associations can be studied in lineages that have experienced multiple pollinator transitions, representing evolutionary replicates.

Methods: Our study focused on a large clade of *Penstemon* wildflower species in western North America that has repeatedly evolved hummingbird-adapted flowers from ancestral bee-adapted flowers. For each species, we estimated geographic ranges from occurrence data and inferred environmental niches from climate, topographical, and soil data. Using a phylogenetic comparative approach, we investigated whether hummingbird-adapted species occupy distinct geographic regions or habitats relative to bee-adapted species.

Results: Hummingbird-adapted species occur at lower latitudes and lower elevations than bee-adapted species, resulting in a difference in their environmental niche. Bee-adapted species sister to hummingbird-adapted species are also found in relatively low elevations and latitudes, similar to their hummingbird-adapted sister species, suggesting ecogeographic shifts precede pollinator divergence. Sister species pairs – regardless of whether they differ in pollinator – show relatively little geographic range overlap.

Conclusions: Adaptation to a novel pollinator may often occur in geographic and ecological isolation from ancestral populations. The ability of a given lineage to adapt to novel pollinators may critically depend on its ability to colonize regions and habitats associated with novel pollinator communities.

KEY WORDS

Ecogeographic isolation, geographic range, niche divergence, *Penstemon*, phylogenetic comparative methods, pollinator shifts, speciation

INTRODUCTION

A key source of diversity within angiosperm lineages is adaptation to different pollen vectors (Grant and Grant, 1965; Stebbins, 1970; Grant, 1994b; Kay and Sargent, 2009; Van der Niet et al., 2014). Since different types of animal pollinators vary in their sensory system, morphology, and pollinating behavior, many plant species have specialized on particular animal pollinators through the evolution of adaptive combinations of floral traits (pollination syndromes; Faegri and Van der Pijl, 1979; Fenster et al., 2004). Evolutionary transitions in pollinator result in closely related species that differ in both pollinator and in floral syndrome. The prevalence of such events should depend not only on the capacity of plant lineages to evolve novel floral traits but also on ecological circumstances that favor a switch in pollinator. Pollinator communities vary across the landscape according to the environmental tolerances of pollinators and the availability of their preferred resources. Therefore, we might expect that pollinator switches occur when a lineage enters a new habitat or environment where either the ancestral pollinator is less common or a new, more effective pollinator is more common (Stebbins, 1970; Thomson and Wilson, 2008).

This hypothesized allopatric scenario leads to the following predictions. First, transitions to a particular novel pollinator should be consistently associated with particular regions or habitats. Second, the ability of a plant lineage to colonize such habitats should be a prerequisite for pollinator transitions. Finally, closely related species (i.e., sister species) that differ in pollinator should have relatively little overlap in their geographic ranges and their habitat preferences. This allopatric scenario is consistent with classic examples of pollinator isolation involving species pairs that differ not only in pollinator but also in geographic range and habitat features (e.g., Hodges and Arnold, 1994; Ramsey et al., 2003; Sobel and Streisfeld, 2015). An alternative scenario is that shifts in pollinator occur in sympatry via disruptive selection, enabling the coexistence of incipient species competing for the same community of pollinators. If this scenario is common, we would predict little association between pollinator adaptation and particular regions and habitats, and sister species pairs that differ in pollinator should have overlapping ranges and occur in similar habitats. This sympatric scenario may be unlikely. Pollinator transitions often involve complex change in multiple floral traits. Therefore, a switch in pollinator should require genetic isolation from ancestral populations since gene flow would counteract local adaptation in pollination syndrome. The geographic and environmental context of pollinator shifts are best studied in lineages that have experienced repeated evolutionary switches in pollinator. In such lineages, we can use natural evolutionary replication in a comparative approach to study whether pollinator transitions occur in specific geographic regions and habitats.

Here we investigated the geographic and environmental correlates of hummingbird pollination in the genus *Penstemon*, a large North American wildflower genus that has experienced repeated transitions from ancestral bee to derived hummingbird pollination. We estimated geographic ranges and environmental niches for species within a large *Penstemon* clade that includes at least 10 origins of specialized hummingbird pollination. Using a phylogenetic framework and analysis of sister species pairs, we examined whether hummingbird-adapted species show consistent differences in geographic location or environmental niche relative to bee-adapted species. We found that hummingbird-adapted species occur at consistently lower elevations and at lower latitudes than bee-adapted species. Our results suggest these habitat shifts may precede the speciation event associated with pollinator divergence. Finally, we found that pairs of sister species in *Penstemon* generally show little overlap in geographic range, and show similar patterns of niche overlap, regardless of whether shifts in pollinator have occurred.

MATERIALS AND METHODS

Study system – *Penstemon* is a genus of nearly 300 species that shows substantial ecological and floral diversity (Wolfe et al., 2006; Wilson et al., 2007; Stone and Wolfe, 2021). Most species are pollinated by bees or wasps and present a bee pollination syndrome: flowers are short, wide, and bluish-purple with included anthers, a landing platform formed by the lower petal lobes, and producing small amounts of nectar. At least 30 *Penstemon* species are adapted to be pollinated primarily by hummingbirds and display a hummingbird pollination syndrome: flowers are bright red, narrowly tubular, with exserted anthers, lacking a landing platform, and producing large amounts of nectar (Wilson et al., 2006). Transitions from ancestral bee syndrome to hummingbird syndrome have occurred in at least 17 lineages (Wilson et al., 2007; Wessinger et al., 2019; Wolfe et al., 2021). This evolutionary replication makes *Penstemon* an exceptional system to examine the geographic and environmental context of hummingbird pollination.

***Phylogeny and identification of sister pairs* –** We previously inferred a maximum likelihood phylogeny for a set of 120 *Penstemon* species (Wessinger et al., 2019) using genome-wide multiplexed shotgun genotyping data (MSG; Andolfatto et al., 2011). This analysis identified a monophyletic clade within *Penstemon* that comprises members of *Penstemon* sect. *Coerulei*, sect. *Gentianoides*, subg. *Habroanthus*, and sect. *Spectabiles*. This previous study sampled 86 of the estimated 99 species in this clade (Freeman, 2019), and includes 12 hummingbird syndrome species that represent at least 10 origins of this pollination strategy. We focused on this clade for the present study (Appendix S1; see Supplemental Data with this article).

In order to maximize the number of variable markers within the focal set of species and improve our confidence in species relationships, we re-analyzed the consensus loci identified in our prior analysis (Wessinger et al., 2019). We generated a concatenated sequence alignment for loci present in at least 15 of the 86 species using ipyrad v0.7.22 (Eaton and Overcast, 2020). We then used IQ-TREE v1.6.2 (Nguyen et al., 2014) to perform 10 independent maximum likelihood phylogenetic analyses under a GTR + Γ model of nucleotide substitution, with 1000 ultrafast bootstrap approximations (Hoang et al., 2017), and otherwise default settings. We used the highest-likelihood tree of the 10 replicates for downstream analyses. We rooted this tree based on consistent relationships observed in prior analyses (Wessinger et al., 2016; Wessinger et al., 2019). We then used BEAST2 v2.5.1 (Bouckaert et al., 2014) implemented on CIPRES (Miller et al., 2010) to estimate relative divergence times on this topology. We included all sites in a single partition, used a Γ site model, a GTR substitution model, a relaxed normal distribution clock model, and a Yule model prior on diversification rate. We performed four MCMC chains, each consisting of 50,000,000 generations, sampling every 50,000 generations. We discarded the first 20% of our chains as burnin and then combined replicate runs. We ensured that the combined sample reached stationarity using Tracer v1.7.1 (Rambaut et al., 2018). Finally, we used TreeAnnotator within BEAST2 to export a Maximum Clade Credibility (MCC) tree with median node heights for downstream analyses.

We identified all 30 independent pairs of sister species using the extract_sisters function of the R package *diverge* (Anderson and Weir, 2020). We assigned these species pairs to one of three categories: 'bee-bee' (both species are bee syndrome; n = 21), 'bee-hummingbird' (one species of each type; n = 7), or 'hummingbird-hummingbird' (both species are hummingbird syndrome; n = 2). Assignments of pollination mode follow Wilson et al. (2007) (see Appendix S1), who based syndrome assignments on ordination of multi-trait phenotypes (flower color, width and length of floral tube, length of stamen filaments and style, and degree of lower lip reflexion) and associations with pollinator visitation (Wilson et al., 2004).

***Occurrence data* –** We collected occurrence records for each species, primarily from the Southwest Environmental Information Network (SEINet; https://swbiodiversity.org/seinet), a portal for digitized herbarium records. SEINet maintains notes of individual observations that can include information on whether plants were grown as ornamentals on roadsides or in gardens. We removed occurrences outside the known range of each species, according to the Flora of North America (Freeman, 2019) and the Biota of North America Program (BONAP; http://www.bonap.org/). We cleaned our dataset to correct misspelled or outdated specific epithets and to exclude any record that included the words "seeded", "sown", "planted", or "botanic garden". Eight rare and/or protected species in our dataset (*P. alamosensis*, *P. gibbensii*, *P. havardii*, *P. haydenii*, *P. navajoa*, *P. neomexicanus*, *P. penlandii*, and *P. petiolatus*) lacked locality information in SEINet, therefore we collected occurrence records for these species from the Global Biodiversity Information Facility (GBIF; http://www.gbif.org). For all species, we removed occurrences lacking subdegree resolution and then removed duplicate occurrences. Our final occurrence dataset included 19,016 records, averaging 221.1 per species (Appendix S1).

***Geographic range and range overlap* –** We estimated geographic ranges for each species from occurrence records using the following approach (Anacker and Strauss, 2014; Boucher et al., 2016; Li et al., 2018). We created 10 km buffers around each occurrence point after reprojecting the points to Alber's equal area projection. We merged these buffers into a single polygon using the st_union function in the R package *sf* (Pebesma, 2018). We calculated the area of the unified range using the st_area function in the *sf* package and recorded the minimum, median, and maximum latitude. We extracted elevational data at each occurrence record using the getData and extract functions implemented in the R package *raster* (Hijmans and van Etten, 2012), which obtains and aggregates elevational data at 90 m resolution from a hole-filled Shuttle Radar Topography Mission (SRTM) elevation model (https://srtm.csi.cgiar.org). We then calculated minimum, median, and maximum elevation for each species.

For each identified sister species pair, we calculated geographic range overlap as the ratio of the area of intersection divided by the union of the two ranges (Phillimore et al., 2008; Li et al., 2018). We found the area of range intersection and the union of species ranges using the st_intersection and st_union functions in the *sf* package.

***Environmental niche estimation and niche divergence* –** For each occurrence record, we obtained four climatic variables (annual mean temperature, temperature seasonality, annual precipitation, precipitation seasonality) from the WORLDCLIM dataset (https://www.worldclim.org/), one topographical variable (slope) derived from the elevation data we obtained from the *raster* package in R, and three edaphic variables (percent clay in soil, soil organic carbon, and soil pH) from the Soilgrids database (https://files.isric.org/soilgrids/). This set of variables captures major axes of environmental variation experienced by the ecologically diverse species within our focal species (e.g., Wolfe et al., 2021) . All raster data was reprojected into an Albers equal area projection and resampled to 1 km x 1 km grid cell resolution.

We estimated environmental niches for each species relative to the total niche space occupied by all sampled species using the PCA-env method (Broennimann et al., 2012) implemented in the R package *ecospat* (Di Cola et al., 2017). We first extracted raster data from all grid cells occupied by any species in our dataset. We used these data to construct a PCA, retaining the first two axes to describe environmental niche space. We then created a grid of 100 x 100 PCA cells and projected each species' occurrence records into this environmental niche space using the ecospat.grid.clim.dyn function in *ecospat*. We estimated niche position and niche breadth for each species as the median values and variances, respectively, of projected PC1 and PC2 values (Grossenbacher et al., 2014).

For each sister species pair, we calculated niche distance as the Euclidean distance between median position in PC space. We also calculated niche overlap as the Schoener's D metric of niche divergence where 0 denotes complete divergence and 1 denotes complete overlap (Schoener, 1970). We calculated Schoener's D and its significance relative to a simulated null distribution of D values using the ecospat.niche.equivalency.test function implemented in *ecospat*. We excluded two species pairs from niche overlap calculations, *P. penlandii* + *P. caryi* and *P. eximius* + *P. cedrosensis*, because each pair involves a species that occupied less than five unique raster cells.

***Associations between ecogeographic attributes* –** We identified associations between geographic and environmental niche attributes across species using phylogenetic generalized least squares (PGLS). We used the gls function implemented in the R package *nlme* (Pinheiro et al., 2017) to construct separate linear models to test for a significant effect of a given geographic variable on a given environmental niche variable. We specified a correlation structure based on Pagel's lambda (Pagel, 1994) using the corPagel function implemented in the R package *ape* (Paradis et al., 2004) to account for phylogenetic structure.

We tested for associations between median latitude and median PC1, median elevation and median PC1, median latitude and median PC2, and median elevation and median PC2.

***Associations of geographic range or environmental niche with pollination mode* –** We tested whether aspects of geographic range or environmental niche depend on pollination mode using a phylogenetic ANOVA approach that accounts for correlations due to shared ancestry. We used the phylANOVA function implemented in the R package *phytools* (Revell, 2012) to separately examine an association of pollinator (bee-adapted vs. hummingbird-adapted) with each of the following: natural logarithm of species range size, median latitude, latitudinal range, median elevation, elevational range, median PC1 value, variance in PC1 values, median PC2 value, and variance in PC2 values. *P. penlandii* lacked sufficient occurrence points to calculate variance on PC1 or PC2 and was excluded from all analyses involving these attributes.

We additionally investigated whether bee-adapted species that are sister to hummingbird-adapted species differed in specific geographic and environmental attributes relative to bee-adapted species that are not sister to hummingbird-adapted species. This addresses the hypothesis that shifts to a novel habitat associated with hummingbird pollination might precede the speciation event involving pollinator divergence. We scored all bee-adapted species as either sister or non-sister to hummingbird-adapted species and performed a phylogenetic ANOVA to test for differences in geographic and environmental variables.

***Geographic range and environmental niche divergence of sister species pairs* –** Within bee-hummingbird sister species pairs, we tested whether hummingbird-adapted species showed consistent differences in geographic or environmental niche variables compared to their bee-adapted sister species using Wilcoxon signed rank tests implemented in the *stats* package.

We tested whether niche distance significantly differs for bee-hummingbird sister species pairs compared with bee-bee species pairs using one-way ANOVAs with sister species pair type as the explanatory variable using the R function aov from the *stats* package. Since measures of geographic range overlap and niche overlap are proportions that vary from 0 to 1, we tested for significant differences between sister pair types in these measures using a beta-regression implemented in the *betareg* package (Cribari-Neto and Zeileis, 2010). We excluded hummingbird-hummingbird sister pairs from our species pair analyses since there were only two sister pairs of this type.

RESULTS

***Hummingbird-adapted penstemons occur at lower latitudes and lower elevations than bee-adapted penstemons* –** Our updated phylogeny, based on 3088 MSG loci (277,819 bp), shows relatively strong nodal support within the focal clade of *Penstemon* species, particularly for sister species pairs (Appendix S2; see Supplemental Data with this article). From geographic ranges estimated from occurrence records, we found that species differ substantially in median latitude, median elevation, and in their position in environmental niche space (Fig. 1). The first two axes of our environmental PCA explain a total of 64.2% of the variance in environmental niche across species. PC1 primarily captured variation in soil pH, soil organic carbon, and annual precipitation, whereas PC2 primarily captured variation in temperature seasonality, annual mean temperature, and precipitation seasonality.

In our sample of 86 *Penstemon* species, our phylogenetic ANOVA identified significant associations of pollination mode with geographic and environmental variables. Hummingbird-adapted species occur at significantly lower latitudes and lower elevations than bee-adapted species (latitude: $F = 31.4$, $p = 0.001$; elevation: $F = 10.3$, $p = 0.006$; Fig. 1A). Hummingbird-adapted species also occupy environmental niches with lower median PC2 values ($F = 27.0$, $p = 0.001$; Fig. 1B), which means that they occur in habitats with higher mean temperature, lower temperature seasonality, and higher precipitation seasonality. Pollination mode is not significantly associated with median PC1 value ($F = 2.31$, $p =$

0.206), geographic range size ($F = 0.003$, $p = 0.953$), or environmental niche breadth estimated as variance in PC1 or PC2 value (PC1: $F = 0.078$, $p = 0.826$; PC2: $F = 1.53$, $p = 0.253$). Our PGLS identified that median PC2 value is strongly associated with both median latitude (slope = 0.233; $p < 0.0001$) and median elevation (slope = 0.0009; $p = 0.0003$) across species (Appendix S3; see Supplemental Data with this article). These correlations are a feature of the landscape, reflecting greater temperature seasonality, reduced precipitation seasonality, and reduced mean temperature with both increased latitude and increased elevation. Thus, the association between pollination mode and median PC2 value may simply be explained by the association of pollination mode with latitude and/or elevation.

***Bee-adapted relatives of hummingbird-adapted species also occupy lower latitudes and elevations* –** We found that hummingbird-adapted species do not consistently differ from their bee-adapted sister species in elevation ($p = 0.1563$, Wilcoxon signed-rank test), latitude ($p = 0.2969$, Wilcoxon signed-rank test), or PC2 value ($p = 0.4688$, Wilcoxon signed-rank test), across the seven bee-hummingbird species pairs in our sample (Fig. 2). This result is notable given the overall pattern that hummingbird-adapted species tend to occur at lower elevations and lower latitudes than bee-adapted species. It is therefore possible that ecogeographic shifts precede pollinator shifts to hummingbirds. In this scenario, we predict that bee-adapted species sister to hummingbird-adapted species are found at lower elevations and latitudes, similar to their hummingbird-adapted sister species, compared with those bee-adapted species that are not sister to hummingbird-adapted species. We found evidence for this scenario: among bee-adapted species, those that are sister to hummingbird-adapted species occur at significantly lower latitudes ($F = 3.89$, $p = 0.048$) and lower elevations ($F = 4.51$, $p = 0.028$), as well as occupy niches with significantly lower median PC2 values ($F = 6.20$, $p = 0.016$), compared with those bee-adapted species that are not sister to hummingbird-adapted species (Fig. 3).

***Patterns of geographic and habitat isolation are similar across all species pairs* –** Although we found no significant association of pollination mode with median latitude, median elevation, or median PC2 value within the sample of bee-hummingbird sister species pairs, this does not address the degree to which pairs of sister species overlap in their geographic ranges or environmental niches. We found that the overall degree of geographic range overlap of sister species was relatively low, ranging from 0 – 0.119 (median: 0.018) (Appendix S4; see Supplemental Data with this article). We found no significant differences between bee-bee species pairs and bee-hummingbird pairs in geographic range overlap of sister species ($Z = -1.566$, $p = 0.783$; see Appendix S4), suggesting a general pattern of geographic isolation accompanying speciation in *Penstemon*, regardless of whether speciation events were accompanied by pollinator shifts. We also found no overall difference between bee-bee vs. bee-hummingbird species pairs in the degree of environmental niche overlap ($Z = -0.275$, $p = 0.117$) or environmental niche distance ($F = 0.198$, $p = 0.66$) of sister species (see Appendix S4). This result suggests that degree of habitat isolation is not qualitatively different depending upon whether speciation events are accompanied by pollinator shifts.

DISCUSSION

Geography of shifts to hummingbird pollination – Relative to bees, hummingbirds can be more efficient at transporting pollen for certain plant taxa (e.g., *Penstemon*; Castellanos et al., 2003), creating an impetus for repeated shifts from bee to hummingbird pollination (Thomson and Wilson, 2008). Adaptation to hummingbird pollinators is thought to evolve in lineages that possess certain life history and morphological traits, such as perenniality, tubular corollas, and dynamic nectar replenishment (Stebbins, 1989; Thomson and Wilson, 2008), all of which characterize the genus *Penstemon*. The evolution of hummingbird pollination also requires colonizing an area where hummingbirds are common. *Penstemon* has its origins in the eastern Pacific Northwest (Wolfe et al., 2021). We found that hummingbird-adapted *Penstemon* species generally occur at lower latitudes than bee-adapted species, a pattern that matches the general distribution of hummingbird species in western North America: hummingbirds have a South American origin and hummingbird diversity increases with decreasing latitudes (Grant and Grant, 1968; McGuire et al., 2014). Greater diversity of hummingbird-adapted plants overlaps with hummingbird diversity – this diversity has built up gradually over time as range extensions brought bee-pollinated progenitor species in contact with hummingbirds (Grant and Grant, 1968; Grant, 1994a; Abrahamczyk and Renner, 2015). In turn, accumulated diversity of hummingbird-adapted plants supports greater numbers of hummingbirds, creating a positive feedback loop that has contributed to the association between hummingbirds and hummingbird-adapted plants.

In addition to occurring at lower latitudes, we found that hummingbird adaptation in *Penstemon* is associated with lower elevation habitats. This association matches constraints that likely limit hummingbirds from accessing high elevation alpine habitats. Elevation describes a steep gradient in abiotic conditions that, at least in temperate North America, may limit the presence of hummingbirds and their resources. A primary limitation thought to restrict hummingbirds from high elevation habitats is the lack of nesting sites above the treeline, whereas lower elevations correspond to forested areas that support nesting hummingbirds (Grant and Grant, 1968). We speculate that truncated warm seasons at high elevations constrain both the presence of hummingbirds and hummingbird-adapted plants that may experience increased costs of copious nectar production over long flowering periods at high elevations. We note that the observed elevational trend is opposite from the pattern often seen in the Neotropics where hummingbird pollination is associated with high elevation montane habitats (e.g., Cruden, 1972; Dellinger et al., 2021). This discrepancy suggests elevational gradients in pollinators differ across tropic vs. temperate zones, which is a phenomenon that deserves further attention in future work. In temperate North America, hummingbird pollination is also posited to be associated with deep and shady canyon habitats where bees are less active, and possibly with wetter microhabitats such as proximity to streams (Stebbins, 1989). Exploration of whether hummingbird-adapted species are consistently associated with such habitat features will require fieldwork examining microhabitat associations that cannot be easily examined using climatic database variables.

Adaptation to hummingbird habitats precede shifts to hummingbird pollination – If shifts into lower latitudes and lower elevations occur simultaneously with shifts from bee to hummingbird pollination, sister species pairs that differ in pollinator should also differ in geographic and environmental niche attributes. Yet these events likely occur in stepwise fashion, with lineages first colonizing and adapting to novel regions and habitats where hummingbirds are common, followed by adaptation to hummingbird pollination (e.g., Stebbins, 1989). We identified evidence for this stepwise scenario through our analysis of sister species pairs. Within bee-hummingbird species pairs, bee-adapted species did not significantly differ in elevation, latitude, or environmental variables compared to their hummingbird-adapted sister species because they occurred in similarly low elevations and latitudes. These bee-adapted species that are sister to hummingbird-adapted species occurred in lower elevation, lower latitude, hummingbird habitats relative to other bee-adapted species that are unrelated to hummingbird-adapted species. Overall, these results suggest that *Penstemon* lineages colonize hummingbird habitats prior to the speciation event associated with the switch to hummingbird pollination. Expansion into these new habitats then spurs a shift to hummingbird pollination.

Despite this overall pattern that switches to hummingbird pollinators involve prior colonization of new regions and habitats, we do note a trend that hummingbird-adapted species occur at somewhat lower elevations than their sister bee-adapted species in all but one sister species pair. This pattern suggests that elevational divergence between sister species may accompany pollinator shifts. This elevational divergence is consistent with patterns observed in other western North American taxa, where several hummingbird-adapted taxa occur at lower elevations than closely related insect-pollinated species (see Table 1 for prominent examples consistent with this elevational pattern).

***Speciation in Penstemon involves geographic and environmental isolation* –** Adaptation to a new pollinator is expected to occur in geographic and ecological isolation, where floral syndromes – complex multi-trait adaptation – can diverge in response to the local pollinator community in the absence of gene flow from ancestral populations. Indeed, ecogeographic isolation is a prevailing mode of speciation in other western North American plant genera (e.g., *Mimulus*; Sobel, 2014). Accordingly, we found that sister species pairs showed little overlap in geographic range. This was true not only for species pairs that experienced pollinator shifts but also species pairs that share the same type of pollinator. Moreover, patterns of environmental niche divergence and overlap were not significantly different for species pairs with different pollinators vs. those that share the same pollinator. Overall, our results are consistent with recent work suggesting that the radiation of *Penstemon* species has involved allopatric speciation accompanied by pervasive ecological niche divergence (Stone and Wolfe, 2021; Wolfe et al., 2021).

CONCLUSIONS

Our results support the concept that adaptation to a novel pollinator depends on the geographic and environmental context of populations. The ability of lineages to radiate onto novel pollinators depends on the ability to disperse into new geographic regions and to adapt to novel environmental conditions. In addition, a key ingredient for repeated evolutionary shifts in pollinator is the propensity to become locally isolated from ancestral populations, enabling multi-trait adaptive change. Fieldwork-based microhabitat studies are needed to fully understand ecological features of pollinator adaptation at a local scale that cannot be captured using the broadscale environmental variables employed in this study.

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Author Contributions

A.M.H. and C.A.W. conducted phylogenetic analyses, species distribution analyses, and environmental niche analyses. C.A.W wrote the manuscript with input from A.M.H.

Data Availability

All datasets and scripts used in this study will be archived in a Dryad repository upon acceptance.

Online Supporting Information

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

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TABLE

Table 1. Notable species pairs in other western North American taxa that have diverged in both pollinator and elevation. Note that species pairs are related but not necessarily each other's closest relatives.

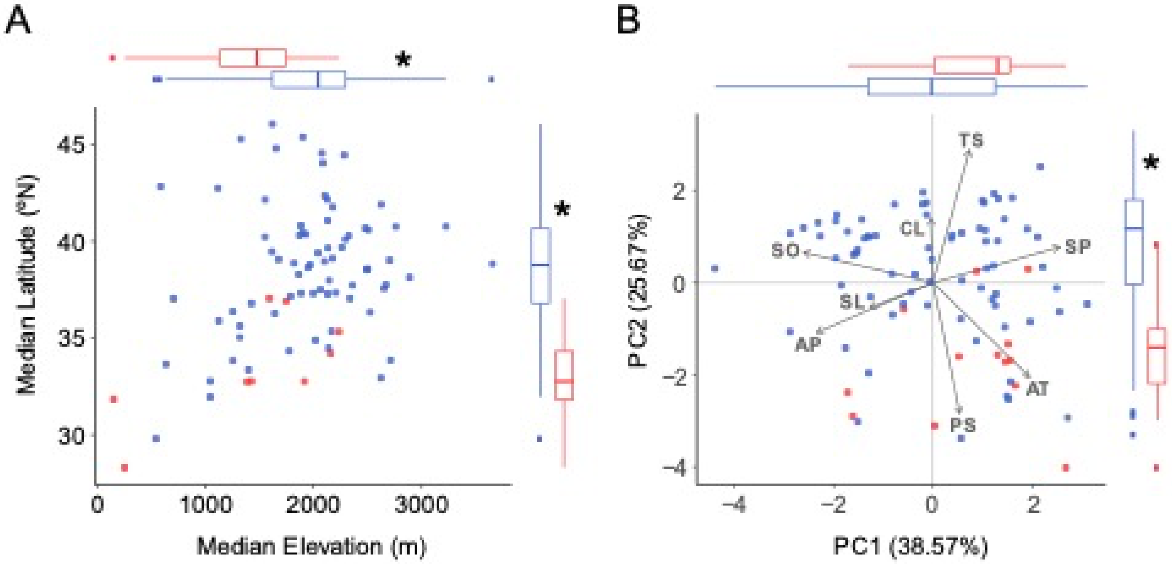
Genus	Hummingbird-adapted species at lower elevation	Insect-adapted species at higher elevation	Reference
Aquilegia	*A. formosa*	*A. pubescens*	(Hodges and Arnold, 1994)
Ipomopsis	*I. aggregata*	*I. tenuituba*	(Campbell and Waser, 2001)
Mimulus (Diplacus)	*M. aurantiacus ssp. puniceus*	*M. aurantiacus ssp. australis*	(Streisfeld and Kohn, 2007)
Mimulus (Erythranthe)	*M. cardinalis*	*M. lewisii*	(Hiesey et al., 1971)
Penstemon subg. *Dasanthera*	*P. newberryi*	*P. davidsonii*	(Kimball, 2008)
Polemonium	*P. brandegei*	*P. viscosum*	(Grant, 1989)

FIGURE LEGENDS

Figure 1. Scatterplots and boxplots showing variation between species in geographic and environmental niche attributes. (A) Variation in median elevation and latitude. (B) Variation in median PC1 and PC2 values. Blue: bee-pollinated species, red: hummingbird-pollinated species. Asterisks indicate significant difference in value for bee- vs. hummingbird-pollinated species. AT: annual mean temperature, AP: annual precipitation, CL: percent clay, PS: precipitation seasonality, SL: slope, SO: soil organic content, SP: soil pH, TS: temperature seasonality.

Figure 2. Median geographic and environmental attributes for members of bee-hummingbird sister species pairs. Each colored line is a species pair. BEE: value for bee-pollinated species, HB: value for hummingbird-pollinated species. A: median latitude, B: median elevation, C: median PC2 value. Red: *P. eximius – P. cedrosensis*, gold: *P. glaber – P. cardinalis*, green: *P. laevis – P. eatonii*, teal: *P. grandiflorus – P. murrayanus*, blue: *P. neomexicanus – P. barbatus*, purple: *P. parryi – P. superbus*, pink: *P. confusus – P. utahensis*.

Figure 3. Median geographic and environmental attributes for bee-pollinated species. N: species are not sister to a hummingbird-pollinated species, S: species are sister to a hummingbird-pollinated species. A: median latitude, B: median elevation, C: median PC2 value.



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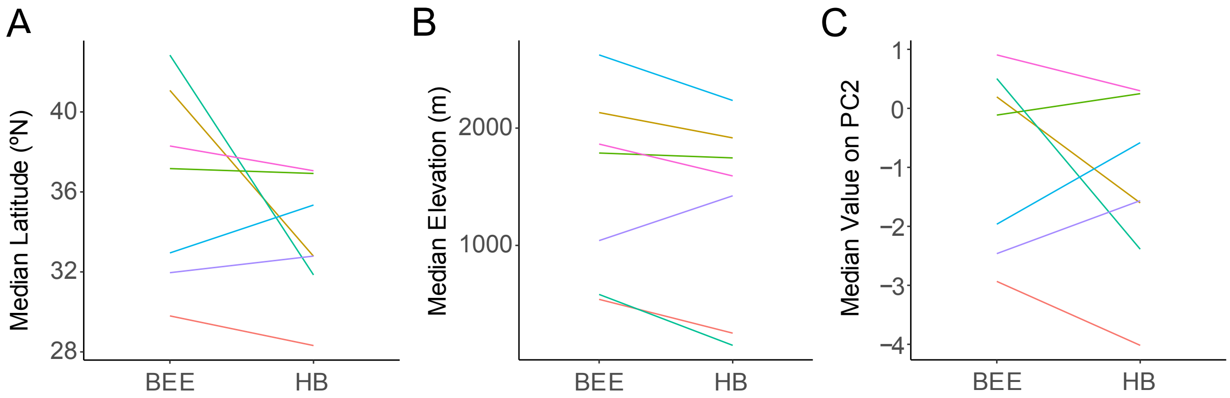

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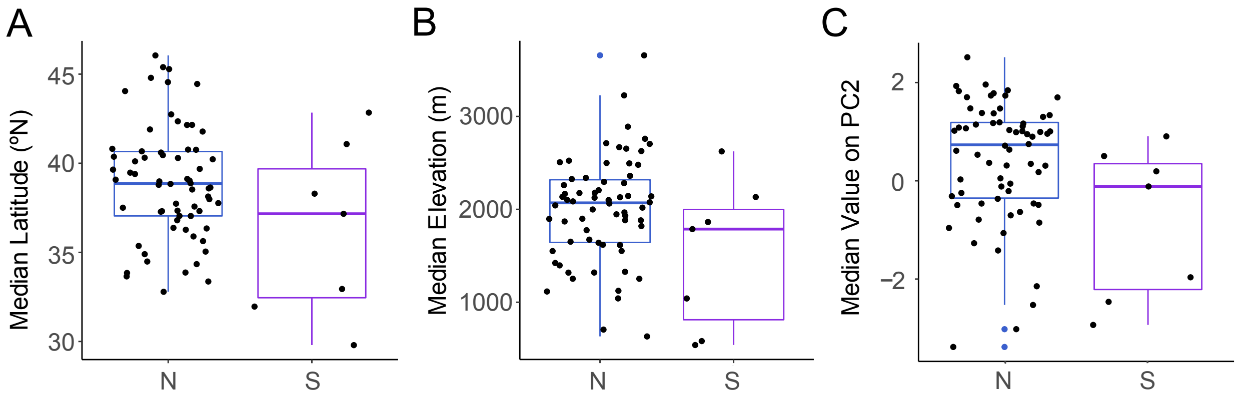

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