

Floral diversity and community structure in *Pedicularis* (Orobanchaceae)

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Abstract. A pervasive hypothesis at the interface of ecology and evolution is that biotic interactions contribute to regional biodiversity by accelerating adaptation and speciation. We investigated this question in the context of closely related, bumble bee-pollinated plants (*Pedicularis* spp.) in the Hengduan Mountains of south-central China, where they exhibit spectacular levels of richness, endemism, and floral diversity. Because these species co-occur frequently, flower synchronously, and share pollinators during the brief reproductive season, we predict that pollinator-mediated interactions may influence their community assembly and evolutionary diversification. If disparity in floral traits reduces competitive interactions between species, as would happen if floral isolation mitigates reproductive interference caused by heterospecific pollen flow, then species with dissimilar flowers should co-occur more often, yielding greater floral diversity at local scales than expected by chance. Moreover, if such interactions have repeatedly driven character displacement, then floral traits should exhibit homoplasy, the phylogenetic signature of labile evolution. We present evidence supporting these predictions, and find that local species richness is best explained by a model including both floral diversity and phylogenetic distance. Our results suggest that a dynamic mosaic of pollinator-mediated interactions among *Pedicularis* in the Hengduan region promotes ecological sorting through recurrent selection against reproductive interference, causing rapid species turnover at local scales, and accelerating the rate of floral divergence among species. Together these processes may have contributed to the remarkable accumulation of florally diverse species of *Pedicularis* endemic to the Hengduan Mountains biodiversity hotspot.

Key words: biodiversity hotspot; coexistence; floral isolation; Hengduan Mountains; phylogenetic ecology; reproductive character displacement; reproductive interference.

INTRODUCTION

The hypothesis that biotic interactions play a role in shaping biodiversity gradients has a long history (Wallace 1878, Schemske 2009). For flowering plants, a more specific but similarly venerable theory is that negative interactions between species that share pollinators may influence community structure (Robertson 1895). In this paper, we investigate these ideas, and possible links between them, in the context of closely related species that co-occur extensively, share pollinators, and exhibit high floral diversity and species richness in a temperate hotspot of biodiversity. We are especially interested in the concept of reproductive interference; more precisely, of heterospecific pollinator-mediated reproductive interference, defined here as the negative fitness effects of pollen flow between species by shared pollinators. Such effects may be pre- or post-zygotic,

and bear directly on reproductive success through effects such as reduced fertilization due to stigma clogging or pollen tube competition, reduced male fitness due to pollen discounting (reduction in the amount of pollen available for conspecific fertilization), or the production of inviable hybrids. Reproductive interference is thus a negative ecological interaction that may potentially drive local adaptation and influence community assembly, but to date has received little empirical study. Our motivation in this study stems from theoretical predictions of how pollinator-mediated reproductive interference might shape the structure and diversity of natural plant communities (Sargent and Ackerly 2008).

These predictions require consideration of both phenotype and phylogeny. If differences in floral traits reduce the transfer of heterospecific pollen (floral isolation; reviewed in Kay and Sargent 2009), then flowers of co-occurring species experiencing reproductive interference should be more phenotypically differentiated than expected by chance (Armbruster et al. 1994). In other words, the distribution of floral traits in communities should exhibit greater variance (“overdispersion” sensu Cavender-Bares et al. 2004) than

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expected by random assembly from a regional species pool.

A related question is whether reproductive interference structures the phylogenetic relatedness of co-occurring species. If floral traits evolve slowly relative to the tempo of lineage proliferation, such that close relatives tend to be similar, then reproductive interference may limit their co-occurrence. This would lead to a more even phylogenetic distribution of species within communities, such that distant relatives co-occur more often than expected by chance. On the other hand, if reproductive interference accelerates floral evolution by recurrent selection for character displacement (Armbruster et al. 1994), homoplasy will cause close relatives to appear less similar by descent, and common ancestry should exert a lesser effect on community structure. The relatedness of co-occurring species would then exhibit more stochastic patterns.

In this study we tested these hypotheses in the spectacularly diverse genus *Pedicularis* (Orobanchaceae). Phenotypic and phylogenetic predictions of reproductive interference are analyzed in a community context, using an extensive data set of co-occurrence records, floral traits, and estimates of molecular phylogeny. Our analysis is restricted in scope both taxonomically (to species of *Pedicularis* only) and geographically (to the Hengduan Mountains region of China; see *Methods and materials: Study system*) in order to focus on the ecological and evolutionary dynamics of closely related species that co-occur and share pollinators extensively. If reproductive interference is pervasive, any floral trait that reduces the rate of heterospecific pollen flow is a candidate for selection, whether it reduces the frequency of inconstant foraging (e.g., corolla shape or color traits that comprise pollinators' visual search patterns; Spaethe et al. 2001), or reduces the likelihood of foreign pollen transfer in the event of a switch (e.g., traits affecting mechanical placement of pollen and stigmatic contact; see *Methods and materials: Study system*). Selection on traits could occur at the level of ecological sorting (community assembly) or at the level of character displacement (trait evolution). The main predictions were (1) that co-occurring species sharing pollinators should exhibit higher floral diversity (trait disparity) than expected by chance, and (2) to the extent that floral traits are phylogenetically conserved, community assembly should favor distant relatives.

MATERIALS AND METHODS

Study system

The geographic setting of our investigation is a temperate hotspot of biodiversity, the Hengduan Mountains region of south-central China, encompassing the highlands of western Sichuan, northern Yunnan, eastern Xizang (Tibet), and southern Qinghai. This region is a latitudinal anomaly, with richness (over 11 000 species of vascular plants in ca. 500 000 km²) and endemism

that approach tropical levels (Boufford and Van Dijk 2000). *Pedicularis* is a clade of hemiparasitic, mostly perennial herbs that is one of the most diverse lineages in the Hengduan flora, having ~270 endemic species (Yang et al. 1998). In the Hengduan region, species of *Pedicularis* frequently co-occur in alpine meadows and reproduce synchronously during the brief temperate-alpine summer, flowering primarily in June through August, and fruiting primarily in July through September. Their bilabiate, zygomorphic flowers display striking interspecific variation in shape and color, especially in the shape of the galea, the constricted, hoodlike upper lip of the corolla that encloses the anthers and presents the stigma (Fig. 1).

A number of studies have examined pollination of *Pedicularis* in the Hengduan region, showing that services are provided almost exclusively by bumble bees (*Bombus* spp.) and that the plants are mainly obligate outcrossers (Macior and Tang 1997, Macior et al. 2001, Wang and Li 2005, Yang et al. 2005, 2007, Tang and Xie 2006, Huang and Fenster 2007, Tang et al. 2007a, b). The region is a hotspot for bumble bees, having over 50 native species that represent more than one-fifth of global diversity (Williams 1998, Williams et al. 2009). Whereas little information is currently available on their precise geographic ranges and habitat preferences, their behavior as pollinators of *Pedicularis* is relatively well understood. Pollen is transferred either nototribically (on the dorsal side), with the bee in an upright position while foraging for nectar at the base of the corolla, or sternotribically (on the ventral side), with the bee more or less dorsoventrally inverted while foraging for pollen by grasping and manipulating the galea (Fig. 1). In this way, foraging behavior, and thus the position of pollen deposition and stigma contact on the insect's body, vary with reward type, with important functional consequences that are reflected in galea shape. In nectar-producing species of *Pedicularis*, the galea is relatively simple, and bees foraging for nectar tend to receive pollen on their dorsal side; by contrast, species providing only pollen as a reward often have a galea that is elongated into a beak. The beak can be short or long, up to ~15 mm, and straight or variously curved, coiled, or twisted (Li 1949, 1951, Yang et al. 1998). This translates to corresponding variation in where the stigma contacts the pollinator, e.g., a curved beak may place the stigma on the lateral or dorsal side of the abdomen or thorax, while a short, straight beak is more likely to place the stigma on the ventral side (Macior and Tang 1997, Macior et al. 2001, Wang and Li 2005, Tang and Xie 2006, Huang and Fenster 2007, Yang et al. 2007). Species that differ in beak length and curvature are thus less likely to exchange heterospecific pollen from shared pollinators. The combination of ethological and mechanical complexity represented by flowers of nectarless species is hypothesized to reflect adaptations for floral isolation (Macior and Tang 1997,

Macior et al. 2001, Ree 2005, Huang and Fenster 2007, Yang et al. 2007).

Another floral trait that is conspicuously variable is the length of the corolla tube, which in nectar-producing species is always short, less than ~2 cm, but in nectarless species can be very long, exceeding 12 cm. Pollinator observations of long-tubed species show no apparent effect of the tube on bumble bee behavior (Macior and Tang 1997, Macior et al. 2001, Wang and Li 2005, Huang and Fenster 2007, Williams et al. 2009). These studies show that long tubes certainly do not represent an adaptation for pollination by long-tongued, nectar-feeding lepidopteran pollinators, as both the reward and the insect visitors are lacking. Moreover, there is no evidence that they mimic such species. While the functional significance of long tubes is not yet known, a speculative, untested idea is that style length is coevolved with pollen tube length (Ree 2005). Long styles thus potentially serve an adaptive purpose as a prezygotic barrier preventing smaller or slower growing foreign pollen tubes from reaching the ovules.

Where multiple species of *Pedicularis* co-occur and flower synchronously, the potential for heterospecific pollen flow by shared pollinators is abundantly supported. Field studies in the Hengduan region have shown that a single species of *Pedicularis* can receive visits by multiple species of bumble bee, and a single species of bee may visit multiple species of *Pedicularis* (Macior and Tang 1997, Macior et al. 2001, Wang and Li 2005, Yang et al. 2007). Bumble bee pollinators of *Pedicularis* are generalists, and are not specialized with respect to plant species, floral type (e.g., long- or short-tubed), or reward type (nectar or pollen). At the scale of individual foraging, the composition of corbiculate pollen loads reveal moderate but incomplete constancy in the visitation patterns of single bees, and pollinator switching between species of *Pedicularis* has been directly observed in single foraging bouts (Wang and Li 2005, Yang et al. 2007). The hypothesis that co-occurring species of *Pedicularis* in the Hengduan region may experience pollinator-mediated reproductive interference thus appears to be empirically grounded and worthy of investigation.

Geographic co-occurrence

We compiled presence-absence data on co-occurrence of synchronously flowering species of *Pedicularis* from collection localities in the Hengduan Mountains region of Yunnan, Sichuan, Qinghai, and Xizang, China (25.63°–36.59° N and 91.27°–102.67° E; Supplement 1). The primary sources were a specimen database representing several biodiversity inventory expeditions to the region conducted from 1997 to 2007 (D. E. Boufford et al., *unpublished data [available online]*,⁶ and an additional expedition to Yunnan and Sichuan in 2007 specifically

targeting *Pedicularis*. One or more of the authors participated in most of the expeditions. All observations were made in summer, June through August, and included only species observed to flower synchronously in sympatry. Sample sites were all <1 km², within the potential flight range of bumble bee pollinators. We tested for spatial autocorrelation in species composition among sites by calculating the rank correlation between pairwise Euclidean geographic distances and the Sorenson's index of similarity. A Mantel test (Sokal and Rohlf 1994) was performed to compare the observed correlation coefficient to the expectation from 9999 permutations of the geographic distance matrix.

Phylogeny reconstruction

Phylogenetic relationships for 97 of the 116 species of *Pedicularis* in the co-occurrence data set were reconstructed from DNA sequences of one nuclear marker (ITS, the internal transcribed spacer region of the nrDNA tandem repeat) and two chloroplast markers (*matK*, including partial sequence of the *trnK* intron, and the *rps16* intron). For rooting purposes, we included sequences from nine outgroup species representing six related genera in Orobanchaceae, but pruned them from trees for subsequent analyses. The data set included a total of 294 sequences, of which 47 were previously published (Ree 2005), and 247 were generated for this study. For new sequences, genomic DNA was extracted from silica-dried or air-dried leaf tissue of voucher specimens using DNEasy plant extraction kits (Qiagen, Valencia, California, USA). PCR amplification and direct sequencing of markers generally followed protocols used previously for *Pedicularis* (Ree 2005), using published primers for ITS (White et al. 1990), *matK* (Ree 2005), and *rps16* intron (Oxelman et al. 1997). Information on voucher specimens and sequences are provided in Supplement 2.

Sequences were aligned using MUSCLE (Edgar 2004), with minimal subsequent adjustment by visual inspection. Final alignment lengths were 737 bp (ITS), 1204 bp (*rps16*), and 1336 bp (*matK*). Alignments were first analyzed individually to confirm general congruence in phylogenetic topology, then concatenated and the maximum likelihood phylogeny was estimated using RAxML 7.04 (Stamatakis 2006), running 200 replicate analyses with random starting trees, and using the GTR+Γ nucleotide substitution model with parameters partitioned by marker. This tree was then used as the starting point in a Bayesian analysis estimating relative divergence times using a relaxed molecular clock, the uncorrelated lognormal rates model, implemented in BEAST (Drummond and Rambaut 2007). The Markov chain Monte Carlo analysis ran for 10 million generations, sampling the tree and parameters every 10 000 generations. We extracted a posterior sample of 500 trees from the latter half of the chain and used these for estimating phylogenetic parameters in all subsequent analyses.

⁶ <http://hengduan.huh.harvard.edu/fieldnotes>

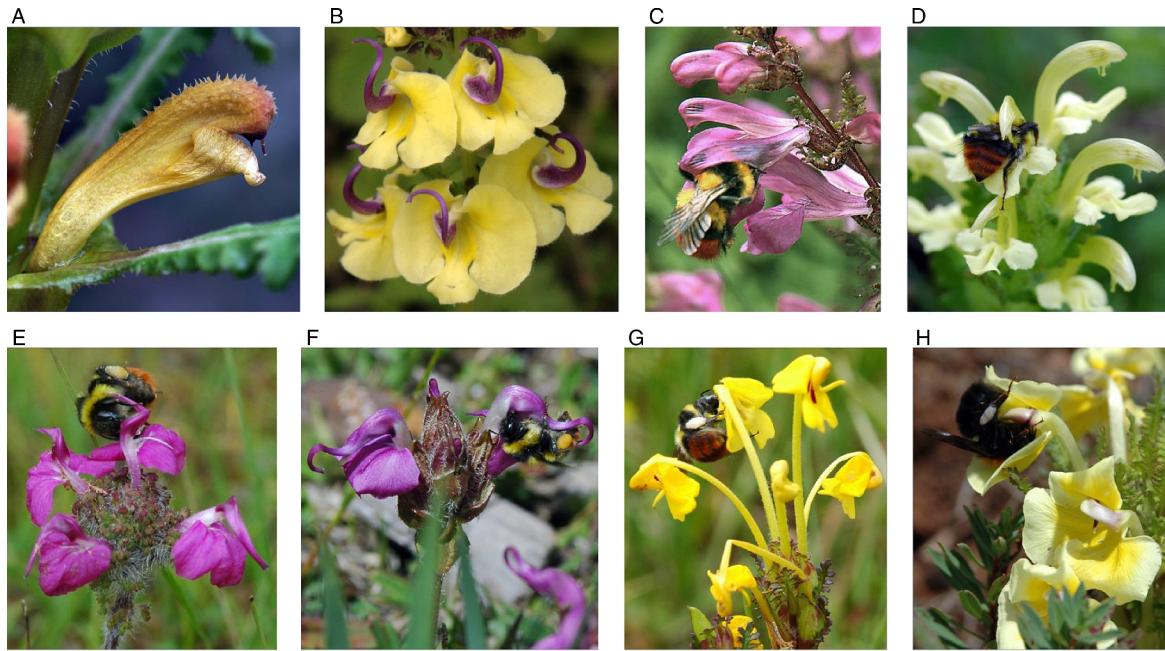


FIG. 1. Floral diversity and pollination in *Pedicularis*: (A) *P. rex*, (B) *P. torta*, (C) *P. pseudomelampyrriflora*, (D) *P. lutescens*, (E) *P. confertiflora*, (F) *P. integrifolia*, (G) *P. longiflora*, and (H) *P. tricolor*. Bumble bees forage for nectar upright (C, D) but adopt more variable body positions while foraging for pollen (E–H), which requires manipulating the galea (fused dorsal petals enclosing the anthers and style). In species of *Pedicularis* without nectar (B, E–H), the galea is beaked, its length and curvature influencing where the protruding stigma contacts the insect's body. Photo credits: A, D. Eaton; B, C, R. Ree; D–H, S.-Q. Huang.

Floral traits

Data on six floral traits were recorded for each species: (1) galea length (mm), (2) corolla tube length (log-transformed, originally measured in mm), (3) nectar production (present, absent), (4) orientation of bee during foraging (nototribic, sternotribic), (5) location of stigmatic contact (dorsal thorax, dorsal abdomen, ventral thorax, ventral abdomen, lateral thorax, hind leg), and (6) corolla color. Data for galea length and corolla tube length were taken from published species descriptions (Yang et al. 1998). Nectar, bee orientation, and location of stigmatic contact were recorded from field observations, or in some cases inferred from floral morphology (for nectar, presence/absence of nectaries; for bee orientation and pollen location, degree of curvature and/or twisting of the galea). Corolla color was recorded at the population level to account for intraspecific variation across sites. In general, the corolla of *Pedicularis* is yellow, purplish red to pink (“red”), or white, either uniformly or in combination. We tested different coding schemes that yielded similar results, and used here eight discrete states for all combinations of primary (dominant) and secondary corolla colors: red-red, red-white, red-yellow, yellow-yellow, yellow-white, yellow-red, white-white, white-red.

Phylogenetic analyses were conducted to measure evolutionary conservatism and homoplasy in the floral-trait data, both separately as individual traits and as a multivariate ordination that accounted for trait correla-

tions. The latter was constructed using principal components analysis for mixed data (Hill and Smith 1976), a method which uses a modification of correspondence analysis and standard PCA to find correlations between continuous and discrete variables. This method is relatively robust to coding scheme (Hill and Smith 1976), which we confirmed for our data by testing several alternatives. To account for phylogenetic uncertainty, each analysis was repeated over the posterior sample of ultrametric trees saved from the phylogeny reconstruction step.

For the continuous traits (galea length, corolla tube length, and PC axes 1 and 2), we measured phylogenetic signal using the *K* statistic of Blomberg et al. (2003), which measures the tendency of related species to be more similar ($K > 1$) or less similar ($K < 1$) than expected under a null model of evolution (Brownian motion). The significance of trait *K* values were tested by comparison to two null models: trait values were simulated across a sample of trees by Brownian motion, or states were randomly permuted across the tips of the tree. In both cases, observed *K* values were tested for a deviation from the null distribution. For the discrete traits (nectar production, flower color, bee orientation, and stigma contact position), we measured homoplasy as the number of state changes implied on the phylogeny under parsimony and likelihood criteria. The latter were obtained directly from the BEAST analysis, which included the discrete trait data and an *Mk* transition

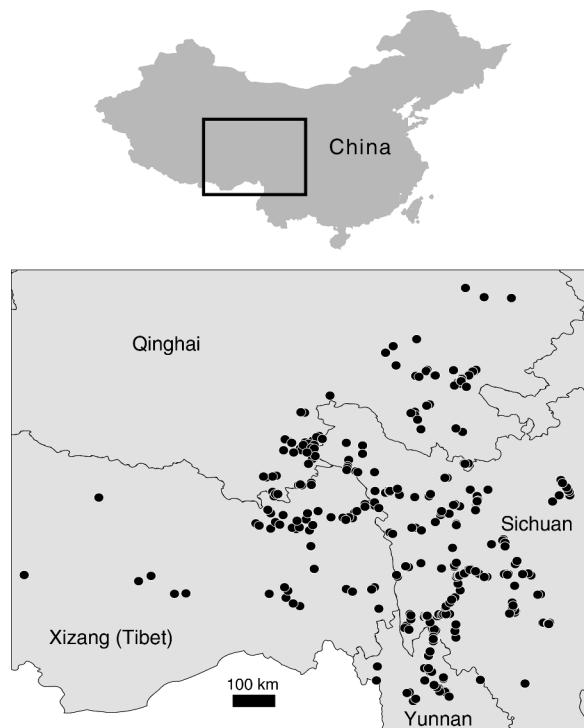


FIG. 2. Geographic distribution of sample localities within the Hengduan Mountains, China, for which two or more species of *Pedicularis* were observed flowering synchronously.

model for each trait, where k is the number of states (Lewis 2001). The analysis sampled ancestral states for each trait on each tree visited in the Markov chain. We summarized these results for each trait as the frequency of ancestor–descendant nodes that differed in state.

Phylogenetic community structure

For the 210 communities in which all species of *Pedicularis* were represented in the phylogeny, we measured two indices of phylogenetic structure, the net relatedness index (NRI) and nearest taxon index (NTI) (Webb et al. 2002) as implemented in Phylocom (Webb et al. 2008). These compare the relatedness of species within a community to a null model of random assembly from the regional species pool. We derived the null distribution for this model using Phylocom's independent swap algorithm, with 10 000 swaps for each of 10 000 iterations. This model creates random species assemblages by resampling from the regional species pool (97 species), retaining both the species richness within communities and the relative frequency of occurrence of species within assemblages.

Phenotypic community structure

A two-dimensional multivariate morphospace was created from the first two PC axes and used to quantify floral diversity at each site. We measured two different metrics of floral diversity (Appendix A: Fig. A1), and

report here on one: the average pairwise distances between species. Analyses using an alternative measure, the convex hull of floral morphospace, gave similar results. In addition, we measured the within-site diversity of each of six traits individually as either the variance (for quantitative and binary traits) or number of overlapping states (for multistate characters) observed. For the multivariate and individual trait data sets, we calculated corresponding values of an index (IP and Z , respectively) that measures the number of standard deviations the observed floral diversity deviates from its expected value, given a null model of random community assembly from the regional species pool (Cornwell et al. 2008, Pausas and Verdú 2008), where positive values indicate greater disparity than expected.

The null distribution for phenotypic structure was analogous to that used for phylogenetic structure, and was generated using the same independent swap algorithm. The regional species pool contained all 116 observed species. To account for intraspecific variation in flower color, a resampling procedure was used in which the color state of each species in a null community was sampled from a list of all color states observed in that species across the regional pool; thus, retaining the relative frequency of intraspecific color variation. All community metrics, IP, Z , and NRI, are standardized for the number of species at a site (richness). In each case we used a Wilcoxon signed-rank test for deviation from the expected value of zero. Floral diversity measurements were made using a modified version of the TraitHull Python script (Cornwell et al. 2008) and the ade4 library (Dray and Dufour 2007) in R (R Development Core Team 2008).

Community richness

We used generalized linear models implemented in R to individually and jointly test floral diversity and phylogenetic distance as predictors of species richness (the number of co-occurring species of *Pedicularis*, log-transformed). The best model was selected using the Akaike Information Criterion corrected for finite sample sizes (AIC_c). The index of dispersion (Z) for each of the six individual floral traits was tested for an association with local richness using Spearman's rank correlation coefficient.

RESULTS

Geographic co-occurrence

The co-occurrence data set included 116 species of *Pedicularis* and 236 sites in the Hengduan region where at least two species were recorded as synchronously flowering (site richness = 3.43 ± 1.60 [mean \pm SD], maximum site richness = 9; Fig. 2). Geographic proximity had no effect on the similarity of species composition across sites ($r_s = -0.03$; Mantel test, $P = 0.975$), indicating that the data are not spatially autocorrelated.

TABLE 1. Spearman's rank correlation coefficients between six floral traits and their first two principal components axes.

Trait	PC1	PC2
Quantitative		
Galea length (mm)	0.87	-0.10
Corolla tube length†	0.69	0.44
Binary		
Bee orientation (up/down)	0.80	-0.29
Nectar (presence/absence)	-0.83	0.29
Multistate		
Flower color (eight states)	0.27	0.62
Stigma contact position (six states)	0.43	-0.40
Eigenvalue		
Variance explained (%)	3.78	1.69
	21.01	9.40

Note: All correlations are significant at $P < 0.005$.

† Corolla tube length was originally measured in millimeters, and log-transformed.

Phylogenetic signal and homoplasy in floral traits

The first two PC axes of the multivariate ordination explained 21% and 9% of the variance in floral traits, respectively (Table 1). The first PC axis was strongly correlated with the presence/absence of nectar, the length of the galea, and the orientation of bumble bees during visitation, the latter a functional trait reflecting galea angle and curvature. The second PC axis was mainly correlated with corolla color (Appendix B: Fig. B1).

The phylogenetic distribution of floral-trait data (six individual traits and two PC axes) are shown in Fig. 3. For continuous characters, all values of K were significant, whether compared to the permutation or simulation null model ($P < 0.05$; Table 2). This signal was consistently significantly less than one, meaning that all continuous traits exhibit greater variance (less signal) on the phylogeny than expected under a Brownian motion model of trait evolution. This is corroborated by the simulation null model, where observed K values were significantly lower than expected (simulation mean $K = 1.00$, $SD = 0.36$, $P < 0.05$). With respect to the permutation null model, K values were significantly greater than expected ($P < 0.05$), meaning that, although floral traits exhibit high variance, they still retain some phylogenetic signal. For all discrete traits,

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FIG. 3. Rooted molecular phylogeny of 97 species of *Pedicularis* surveyed in communities. Solid circles at nodes represent posterior probability support: ≥ 95 , black; > 75 to < 95 , gray; ≤ 75 , white. Species mean floral-trait values are shown at the tips as squares: (A) presence (black) vs. absence (white) of nectar; (B) nototribic (black) vs. sternotribic (white) bee visitation; (C) galea length; (D) corolla tube length [for columns (C) and (D), the darker the gray, the longer the length]; (E) (simplified) location of pollen placement [dorsal (white), ventral (black), others (gray)]; (F) primary corolla color (red, yellow, or white); (G) Score on PC1; (H) Score on PC2; for PC scores, size indicates magnitude, with white showing negative, and black showing positive.

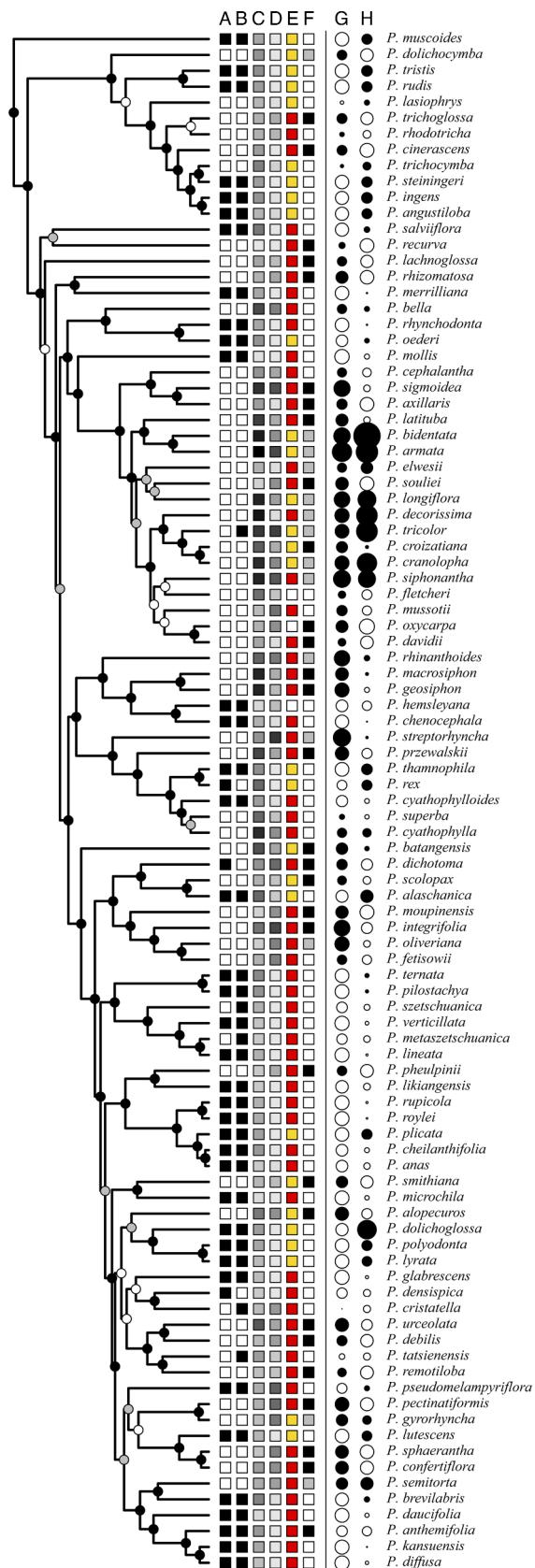


TABLE 2. Phylogenetic signal in continuously valued floral-trait data reported as the mean \pm SD of 500 trees sampled from the posterior distribution.

Trait	<i>K</i>	<i>P</i> _{sim}	<i>P</i> _{rand}
log(corrula tube length)	0.46 \pm 0.05	0.001 \pm 0.000	0.001 \pm 0.000
Beak length	0.49 \pm 0.04	0.003 \pm 0.000	0.001 \pm 0.000
PC1	0.51 \pm 0.06	0.006 \pm 0.000	0.001 \pm 0.000
PC2	0.31 \pm 0.05	0.001 \pm 0.000	0.009 \pm 0.035

Note: Significance is assessed relative to two null models: Brownian motion simulated along the branches of the tree (*P*_{sim}), and permutation of states at the tips of the tree (*P*_{rand}).

the number of state changes implied by parsimony and likelihood exceed the theoretical minimum (i.e., the number of observed states) by factors ranging from 5 to 12 (Table 3).

Community structure

When analyzed separately, all six floral traits showed greater variance within communities than expected (Table 4). Among these, presence/absence of nectar is the most pronounced, for which greater variance represents a more even distribution of nectar producing and nectarless species (positive *Z*). Similarly, the number of overlapping pollen-placement character states was significantly less than expected (negative *Z*), indicating that co-occurring species tend to place pollen on different positions of bumble bees. Only the number of overlapping flower color states was not significantly different from the null expectation (Wilcox test, *P* > 0.05).

With respect to occupation of the two-dimensional floral morphospace, communities of *Pedicularis* are significantly evenly dispersed (median IP = 0.43; two-tailed Wilcoxon test, *P* = 2.9×10^{-5} ; *n* = 236), indicating that the flowers of co-occurring species are more dissimilar than expected. By contrast, indices of phylogenetic community structure did not deviate significantly from zero, reported as the 95% confidence interval (CI) across the posterior sample of trees, median NRI = (-0.15 to -0.13); two-tailed Wilcoxon test, *P* = (0.09–0.12); median NTI = (-0.09 to -0.07); two-tailed Wilcoxon test, *P* = (0.84–0.87), *n* = 210, meaning that patterns of relatedness within sites cannot be distinguished from random assembly.

Community richness

The index measures of dispersion (*Z*) in two of six floral traits (nectar and bee orientation) showed a significant negative correlation with species richness (Table 4), such that these characters exhibited decreasing variance as richness increases, indicating a greater occurrence of nectarless, sternotribically visited taxa in species-rich communities. Tests for multivariate floral diversity (IP) and phylogenetic distance (NRI) as individual and joint predictors of species richness across communities reveals support for a joint model, reported

as the 95% CI across the posterior sample of trees: multiple regression, *r*² = (0.058–0.059), *P* = (0.002–0.003), *n* = 210, showing that both factors have explanatory power (Fig. 4). However, support for the joint model (IP + NRI) is not significantly better ($\Delta\text{AIC}_c > 2$) than floral diversity alone (Table 5); removing NRI changes AIC_c , on average, by only 0.3, meaning that its contribution is very small relative to that of floral diversity.

IP is standardized for species richness, so the null hypothesis is that floral diversity and richness are uncorrelated across sites. However, they are in fact positively correlated (*r*_S = 0.22, *P* = 7.3×10^{-4} , *n* = 236), although the exclusion of communities composing only two species shows this pattern to be partly driven by species poor sites (*r*_S = 0.15, *P* = 0.059, *n* = 158).

DISCUSSION

Trait dispersion and community assembly

The clearest and strongest result from our study of *Pedicularis* in the Hengduan region is that, across a large phylogenetic and geographic sample at the center of their diversity (236 sites, 116 species), co-occurring species exhibit significantly greater variance, or dissimilarity, in floral traits than expected by chance. To our knowledge, such a pattern has only been empirically demonstrated twice previously, the first being in communities of species of trigger plants (*Stylidium* spp.) in western Australia (25 sites, 31 species; Armbruster et al. 1994), and the second in bat-pollinated flowers of *Burmeistera* spp. in Ecuador (18 sites, 19 species; Muchhal and Potts 2007). Several parallels between *Pedicularis*, *Stylidium*, and *Burmeistera* are notable in this context. Each is species rich, has a relatively small geographic center of diversity, and contains many species that frequently co-occur and flower synchronously. Moreover, each is characterized by a unique suite of floral traits that vary conspicuously between species, and are functionally important in positioning the contact of reproductive organs with the bodies of pollinators. In *Pedicularis*, this is exemplified by the elaboration of the galea into a variety of beaked forms that requires correspondingly variable sternotribic behavior; in *Stylidium*, it is interspecific variation in the “trigger” mechanism that explosively and precisely propels the column and stigma to contact the pollinator (Armbruster et al. 1994); and in *Burmeistera* it is inter- and intraspecific variation in exertion of the

TABLE 3. Transitions in discrete floral traits implied by parsimony (MP) and Bayesian (ML) ancestral-state reconstruction, reported as the mean \pm SD of 500 trees sampled from the posterior distribution.

Trait	No. states	MP	ML
Flower color	8	40 \pm 1.00	49.28 \pm 2.13
Bee orientation	2	23 \pm 0.55	26.30 \pm 1.00
Nectar	2	24 \pm 0.51	28.28 \pm 1.44
Sigma contact position	6	46 \pm 0.79	55.31 \pm 4.63

TABLE 4. Phenotypic dispersion of individual floral traits within communities.

Trait	Metric	Median Z	SE	Wilcox P	r_s	Corr. P
Corolla tube length	var	0.032	0.109	0.001	0.030	0.644
Galea length	var	0.143	0.097	0.001	0.014	0.829
Bee orientation	var	1.104	0.114	9.6×10^{-9}	-0.266	3.4×10^{-5}
Nectar	var	1.678	0.108	2.7×10^{-16}	-0.271	2.4×10^{-5}
Flower color	n overlap	-0.295	0.068	0.535	-0.027	0.684
Stigma contact position	n overlap	-0.587	0.060	1.2×10^{-9}	-0.038	0.557

Notes: An index value (Z) indicates the difference in variance (var), or number of overlapping discrete states (n overlap), between observed and null communities in units of standard deviations. Distributions of Z are tested for a significant deviation from zero by a Wilcoxon-signed rank test. Spearman's correlation coefficients (r_s) measure the association between Z and species richness. The P value shown is for the Spearman's rank correlation coefficient (r_s).

staminal column (Muchhala and Potts 2007). These shared characteristics favor the idea of a common cause underlying the nonrandom patterns of floral community structure respectively observed in these clades.

The pattern of increased phenotypic dispersion, or high variance, of pollination traits in communities is consistent with the prediction that pollinator-mediated reproductive interference limits the floral similarity of co-occurring species. That is to say, if such species share pollinators and have similar flowers, they will experience reduced fitness from heterospecific pollen flow, and selection ("sorting") at the level of community assembly will thus favor dissimilar species. Negative effects of interspecific pollination on plant reproduction are well documented in the literature, from the standpoint of empirical experiments (e.g., Campbell and Motten 1985, Murcia and Feinsinger 1996, Flanagan et al. 2009), as well as theoretical models and simulation studies (e.g., Montgomery 2009, Muchhala et al. 2010). The significant pattern observed here also corroborates the substantial body of independent evidence that links flower form to reproductive function in *Pedicularis*. Numerous studies of pollination ecology in natural populations of *Pedicularis* in the Hengduan region have shown that floral traits, such as the type of pollinator reward (nectar vs. pollen) and the shape of the galea (absent/present, short/long, straight/curved) influence pollinator behavior and the position of stigma contact (Macior and Tang 1997, Macior et al. 2001, Wang and Li 2005, Tang and Xie 2006, Huang and Fenster 2007, Tang et al. 2007a, b, Yang et al. 2007). For *Pedicularis*, experimental confirmation that differences in floral traits contribute to reduced heterospecific pollen transfer and increased reproductive isolation in co-occurring species has been shown for only a single pair of species, *P. longiflora* and *P. rhinanthoides* (Yang et al. 2007). The pattern that emerges from our large geographic and phylogenetic data set strongly suggests that these limited experimental results apply generally to *Pedicularis* across the region.

Compared to individual traits, the multivariate floral morphospace appears to provide greater opportunity for functional divergence, and is less prone to saturation as the species richness of a community increases. Each trait individually shows a pattern of high variance within

communities, supporting their independent importance in partitioning shared pollinators. Correlation among traits could also cause this pattern, for example, if only one trait is functionally important but several others covary with it; however, the relatively low eigenvalues of our PCA analysis (Table 1) refute this notion, as little correlation is observed among traits. Individual traits all show either nonsignificant or negative correlations of disparity with richness (Table 4), while IP, the multivariate measure of floral disparity, is positively correlated (Table 5). Thus, while traits individually support the reproductive interference hypothesis, our multivariate analysis additionally suggests that diverse combinations of traits becomes increasingly important for the ability of species to occupy unique portions of floral morphospace as local richness increases.

Trait lability and phylogenetic community structure

Phylogenetic analysis of floral traits in *Pedicularis* (Tables 2 and 3) suggests that they experienced high

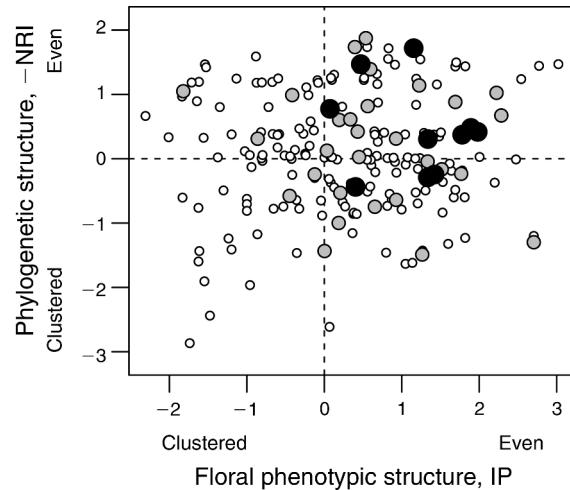


FIG. 4. Relationship between species richness and the phylogenetic and floral structure of *Pedicularis* communities (net relatedness index, NRI; and IP, multivariate trait data set index). Data on phylogenetic and floral structure are plotted in units of standard deviation from their expected values under a null model of random community assembly. Species richness is indicated by the shading of circles: white ($n = 2-4$), gray ($n = 5-7$), or black ($n = 8-9$).

TABLE 5. Regression and sample size-corrected Akaike information criterion (AIC) fit of phenotypic and phylogenetic structure to log-transformed richness within local communities of *Pedicularis*, reported as the mean \pm SD from the posterior sample of trees.

Model	r^2	P	AIC_c	ΔAIC_c
IP + NRI	$0.06 \pm 4.3 \times 10^{-3}$	$2.6 \times 10^{-3} \pm 1.3 \times 10^{-3}$	216.06 ± 0.7	0.00
IP	0.05	1.43×10^{-3}	216.41	0.26 ± 0.98
NRI	$0.02 \pm 8.3 \times 10^{-3}$	0.11 ± 0.09	223.31 ± 1.7	7.34 ± 0.73

Notes: ΔAIC_c was measured relative to the joint model. Abbreviations are: NRI, net relatedness index; IP, multivariate trait data set index.

rates of evolution as species proliferated in the Hengduan region. This inference is supported by the result that values of K are consistently and significantly less than one, meaning that species are less similar to each other than expected if the traits had evolved by Brownian motion on the phylogeny. Of course, $K \ll 1$ does not, in itself, necessarily indicate evolutionary lability, as it has been shown via simulation that constrained evolution at depressed rates can also degrade phylogenetic signal (Revell et al. 2008). However, there is no evidence that the regional sample of *Pedicularis* studied here has experienced such constraints on floral evolution relative to the genus as a whole. On the contrary, floral diversity of *Pedicularis* in the Hengduan region is conspicuously higher than any other geographic area (e.g., long-tubed species are almost entirely endemic here). This pattern suggests a regionally elevated, rather than constrained, rate of floral evolution.

Interpreting phylogenetic patterns as evidence for “lability” or “conservatism” in trait evolution requires acknowledging the null expectation against which observations are compared. In our analyses of K , the null models are provided by Brownian motion. We justify this on the grounds that species of *Pedicularis* are, relatively speaking, closely related and recently diverged, and we presume that floral traits have high heritability across species in this context. From this point of view, Brownian motion appears to be a reasonable null model for trait variation. By contrast, an alternative null is randomly distributed trait values at the tips of the phylogeny. This is the approach taken by Cavender-Bares and Reich (2012) in their study of phylogenetic patterns in community responses to fire. They interpret values of K that are significantly less than expected from random permutations as phylogenetic conservatism, even though the values are also less than one (and less than our values for floral traits). This view is justified by the fact that their species pool is a sparse sample across all vascular plants. At that scale, it seems more reasonable to expect that trait values or species subsamples will be independent of phylogeny (i.e., exhibit no phylogenetic signal at all) than to expect similarity dictated by Brownian motion.

For discrete characters, the question of how much variation to expect given the phylogeny is less clear, and no statistical measure of phylogenetic signal analogous to K has been devised (Blomberg et al. 2003). Our

perspective is that, for closely related species, a reasonable null expectation is that each character state has arisen only once on the phylogeny: in other words, the amount of expected change is equal to the number of states observed. This is the rationale for comparing the number of implied changes in discrete floral traits to the number of states (Table 3). In all cases, the inferred number of changes is much greater than the null, the lack of a test statistic notwithstanding. It is also worth remembering that the discrete trait data observed here are representative of the full extent of floral variation across *Pedicularis*, countering any claim that our species pool is evolutionarily constrained.

We acknowledge that the apparently high rate of floral evolution in *Pedicularis* may have nonadaptive causes, e.g., recurrent drift in allopatry, or pleiotropy. While it is impossible to know with certainty which processes were most important in generating the pattern of labile evolution, evidence presented here for widespread assembly of florally dissimilar communities implicates reproductive interference as a selective agent of trait divergence.

The inference of labile trait evolution is relevant to the question of phylogenetic community structure. We found little evidence that phylogenetic relatedness in and of itself exerts a strong influence on the composition of *Pedicularis* communities. This is entirely consistent with the idea that if reproductive interference favors species with dissimilar flowers, and if common ancestry is a poor predictor of floral similarity, then phylogenetic relatedness within communities is less likely to deviate from random expectation. In this context, lack of phylogenetic community structure may actually constitute positive evidence for the reproductive interference hypothesis. Hypothetically, if floral traits were phylogenetically conserved, then assembly of florally diverse communities would favor distant relatives; i.e., communities would show evenness in phylogenetic relatedness and phenotypic diversity. But if this were the case, it would be harder to support reproductive interference as an explanation for high phenotypic dispersion, because any other phylogenetically conserved trait unrelated to pollination could potentially explain the pattern of phylogenetic evenness.

The inference of labile evolution in floral traits does not necessarily imply that the proximate cause of elevated floral diversity in communities of *Pedicularis* is local adaptation (i.e., the result of in situ evolutionary

divergence). Rather, it seems more likely that it is primarily the result of ecological sorting, i.e., reproductive interference causing the failure of species to persist in sympatry. This assumes that geographic ranges and the composition of communities can generally evolve (via dispersal and local extinction) at a faster rate than floral traits. In this view, the importance of pervasive character displacement between species at the community level (reflecting ecological processes) is that it may cause character displacement within species at the population level (reflecting evolutionary processes). This will increase floral diversity in the regional species pool, and thereby enhance opportunities for ecological selection. The current data set lacks detailed information on intraspecific variation in floral traits (with the exception of flower color, which was scored at the population level), but we predict that if such data were available, they would reveal evidence of local adaptation and character displacement across species' ranges.

The observed patterns (strong signals of homoplasy and community dispersion of floral traits, and a lack of clear phylogenetic community structure) beg the question of alternative explanations that do not involve the functional significance of floral traits in pollination. If an ecological trait unrelated to pollination was the actual cause of high variance of floral traits in communities, it would have to covary strongly with the phylogenetically labile patterns displayed by the floral traits; if it did not, floral traits would appear randomly distributed, or less likely clustered in communities. A variety of factors (e.g., non-floral traits, climate, elevation, host-parasite interactions, and co-occurrence of unrelated flowering plants) may influence the assembly of *Pedicularis* communities, but it is difficult to explain how they would systematically result in elevated floral diversity across a broad range of species and geographic localities.

The general difficulty of attributing cause to pattern highlights the need for experimental studies that directly test hypotheses related to pollinator-mediated reproductive interference in *Pedicularis*. Reproductive interference has been experimentally demonstrated in other plants (Morales and Traveset 2008) and in animals (Grönig and Hochkirch 2008), typically in the form of population fitness or trait responses to the presence of competitors. Direct measurements of this kind are important for understanding the limits of species distributions (Kishi et al. 2009), and for identifying traits important in species interactions (Takakura and Fujii 2010). However, they are less useful for determining the extent to which reproductive interference has exerted an effect at larger geographic and phylogenetic scales. Few studies to date have examined the role of floral-trait diversity in community assembly (Armbruster et al. 1994, Muchhala and Potts 2007). To our knowledge, only one has done so in a phylogenetic context (McEwen and Vamosi 2010), at a much coarser level of phylogenetic resolution. Our study stands apart

from these in its scope, i.e., focused and extensive sampling of a large radiation of species in their center of diversity. This approach allowed us to test specific predictions about the role of reproductive interference in structuring communities, and link phylogenetic and ecological patterns of floral-trait dispersion to evolutionary and biogeographic processes.

Facilitation, competition, and coexistence

Previous studies of co-occurring, closely related plants have demonstrated the importance of facilitation in attracting pollinators and increasing reproductive success (e.g., Schemske 1981, Moeller 2004). *Pedicularis* appears to be no exception: a positive relationship between population density and floral visitation has been recorded in natural mixed communities (Williams and Batzli 1982, Yang et al. 2007). Low visitation rates are associated with autogamy in one Hengduan species, *P. dunniana* (Sun et al. 2005), but where species of *Pedicularis* co-occur, there is little evidence for pollinator services being limiting; indeed, stigma clogging and interference between self and outcross pollen was found in natural populations of *Pedicularis siphonantha* and *P. longiflora* (Yang et al. 2005).

If facilitation is pervasive in the alpine habitats of the Hengduan Mountains where species of *Pedicularis* co-occur most frequently, a logical consequence is an increased likelihood of reproductive interference and increased selection for floral isolation (as opposed to other potential isolating mechanisms, such as divergence in phenology). The diversity of floral forms in *Pedicularis* may thus reflect a combination of mechanical and ethological factors allowing co-occurring species to share rather than compete for pollinator services. For example, the greater complexity and handling time required for sternotribic (ventral-facing) foraging on nectarless, beaked species relative to nototribic (dorsal-facing) nectar foraging is associated with increased species fidelity by individual bumble bees (Macior and Tang 1997, Macior et al. 2001, Tang and Xie 2006, Yang et al. 2007).

That floral diversity and phylogenetic distance are joint predictors of species richness in communities (Table 5) is a novel result, consistent with the hypothesis that the potential for negative interactions increases with the number of co-occurring species. If floral diversity is a response to reproductive interference, then greater floral diversity should allow more species to coexist. At the highest levels of species richness, there is a small but non-negligible signature of phylogenetic evenness, i.e., the nonrandom assembly of distantly related species (Fig. 4). This could reflect low levels of phylogenetic conservatism in floral traits, or competitive exclusion of close relatives for reasons other than pollination.

Reproductive interference and lineage diversification

The patterns presented thus far represent independent lines of indirect evidence that floral evolution in

Pedicularis has been accelerated by recurrent selection to reduce reproductive interference in communities of *Pedicularis*. We speculate that the influence of these interactions extends beyond character displacement and community structure, and includes the process of speciation. A common pattern in our data is that a single species of *Pedicularis* will co-occur with several other species, in various combinations, across its range. Collectively, this represents a complex geographic mosaic of species interactions (Thompson 2005). For an individual species, we expect the strength and direction of selection imposed by reproductive interactions to be variable across its range, depending on the composition of local communities. This has two potential consequences. First, the direction of local adaptation and hence floral-trait evolution would be correspondingly variable, and will accelerate the reproductive isolation of populations and divergence of new species (Doebeli and Dieckmann 2000). Second, it would cause local species turnover, if populations that fail to adapt suffer increased rates of local extinction, and allow geographically proximate species with more optimal complements of floral traits to expand their ranges and successfully replace those that were extirpated. Over evolutionary timescales, particularly during climatic fluctuations from the late Miocene through to the Pleistocene, geographic ranges and communities of *Pedicularis* in the Hengduan Mountains were likely to be extremely dynamic, with a combination of pollinator-mediated interactions and abiotic processes contributing to its diversification.

The idea that reciprocal interactions may occur between local adaptation (to reduce reproductive interference) and rates of lineage diversification is one that deserves further study. The case of *Pedicularis* suggests the possibility of a positive feedback loop, in which character displacement accelerates speciation, and the regional accumulation of species accelerates character displacement. Armbruster and Muchhal (2009) proposed similar ideas in the context of floral specialization (partitioning of the pollinator fauna) and speciation. They concluded that high species diversity can select for floral diversity when sympatry drives character displacement, but found little evidence that floral specialization promotes speciation. In *Pedicularis*, the response has apparently not been to partition pollinators, but rather to partition the behavior of common pollinators to increase conspecific pollen flow. Intraspecific studies are needed to ascertain whether speciation in *Pedicularis* is accelerated by floral divergence.

The Hengduan Mountains region is the center of diversity of many plant lineages, such as *Rhododendron* (Ericaceae), *Gentiana* (Gentianaceae), *Saussurea* (Asteraceae), and *Aconitum* and *Delphinium* (Ranunculaceae), to name a few (Boufford and Van Dijk 2000). Like *Pedicularis*, these lineages are pollinated by bumble bees (Williams et al. 2009), and congeners commonly co-

occur at local scales (Boufford et al., available online, see footnote 6). This raises the question of whether pollinator-mediated reproductive interference is implicated generally in the diversification of Hengduan plants, or if *Pedicularis* is a unique case. No other lineage in the region seems to exhibit the same combination of conspicuous floral diversity (in traits that influence pollinator behavior) and synchronous flowering as *Pedicularis*, suggesting that the effects of reproductive interference, if any, are manifested in other ways. However, quantitative data are lacking, and comparative studies of pollination ecology, reproductive biology, and phylogenetic community structure in these lineages are needed. Of course, reproductive interference is only one of many potential causes of high net diversification in the Hengduan region. The mountainous terrain itself may enhance opportunities for speciation, via geographic isolation, divergent selection along steep environmental gradients, and habitat specialization. *Pedicularis* is as likely as any other lineage to be influenced by these processes; however, none other than reproductive interference can adequately explain the patterns of floral diversity and community structure presented here.

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SUPPLEMENTAL MATERIAL

Appendix A

A schematic diagram showing measurements of floral disparity within communities (*Ecological Archives* E093-185-A1).

Appendix B

Representation of six floral traits distributed across samples within the two-dimensional morphospace created by the first two PC axes (*Ecological Archives* E093-185-A2).

Supplement 1

Site and specimen data collected during biodiversity inventories of the Hengduan Mountains region (*Ecological Archives* E093-185-S1).

Supplement 2

Genbank accession numbers of DNA sequences used in phylogeny reconstruction. Voucher information is provided for new sequences generated for this study (*Ecological Archives* E093-185-S2).

Supplement 3

Mean values for six floral traits measured on 117 species of *Pedicularis* (*Ecological Archives* E093-185-S3).