

Deren Eaton 2010 DDIG

Summary: A pervasive question in ecology and evolution is the extent to which species interactions influence rates of adaptation and diversification. In this context, negative diversity-dependent processes such as the saturation of available niche-space have dominated the literature, while species interactions that can positively affect diversification have received little attention. An example of one such interaction is that of reproductive interference, which occurs when sympatric species negatively affect one another's reproductive success. Variation in this type of biotic-interaction across a species range can cause populations to experience divergent selection on reproductive traits, leading to adaptations that increase isolation and potentially lead to speciation.

The angiosperm genus *Pedicularis* presents an ideal system in which to test for, and examine the evolutionary consequences of, reproductive interference. Co-occurrence is common among species in this lineage, particularly within its center of diversity in the Chinese Himalaya, where nearly 270 species are endemic. Here, a highly seasonal climate, combined with a paucity of pollinator types, requires that many species flower synchronously and share pollinators; raising the potential for interspecific pollination. Accordingly, *Pedicularis* exhibit exceptional interspecific variation in floral traits that affect precision in the location of pollen-placement; a mechanism that can reduce interspecific pollination when using shared pollinators. Even small differences to these highly precise floral morphologies can potentially reduce pollen-flow between species, as well as between populations.

Many species of *Pedicularis* exhibit intraspecific variation in floral traits across populations. The objective of this project is to examine this variation with respect to patterns of co-occurrence with other *Pedicularis* species. Using molecular genetic markers to infer measurements of gene-flow between populations, a model-based approach will be employed to compare the effects of geography and floral similarity in explaining gene-flow between populations. Furthermore, these gene-flow data will be used to correct for statistical auto-correlation among populations, which allows fitting of adaptive models to population floral phenotypes to estimate selection imposed by local biotic communities (sympatric congeners). More simply, the first analysis tests whether floral differences are associated with patterns of gene-flow, and the second tests whether these floral differences have arisen through selection or drift. The proposed study will be replicated across three species of *Pedicularis* to investigate whether biotic interactions are a general cause of species divergence in this lineage. Field collection for one species is already complete, funding from the DDIG would support an additional expedition to collect data for two more species, as well as support extraction and genotyping of individuals in the lab.

Intellectual Merit: Although reproductive interference has been heavily studied in an ecological context, its long term evolutionary consequences remain poorly understood, including its potential effect in explaining patterns of trait evolution and diversification within hyper-diverse lineages where many close relatives co-occur. The greatest contribution of this research comes from increasing our understanding of processes that can generate diversity, particularly for flowering plants within the unique Hengduan Mountains biodiversity hotspot. Here, plant diversity and endemism far exceeds the expectation based on its temperate climate and latitude; interspecific reproductive interactions driven by constraints on out-crossing strategies may be one potential explanation for its exceptional species diversity. Identifying factors promoting diversification is a major goal in ecology and evolution research as it relates to conservation, because only by understanding the processes that generate diversity can we design strategies to protect future diversity.

Broader Impacts: Broader impacts of this research include collaboration with researchers from two Chinese Institutions, and funding for a Chinese student to assist in the field expedition. Results will be broadly disseminated to the public through resources at the Field Museum of Natural History in Chicago. Here, the research will be part of an interactive display at the DNA discovery center, where visitors can learn the basics of DNA and how it relates to evolutionary research, including learning about scientists working in the lab through information and photos of their research. Also, the CoPI will participate in "talk to a scientist hour" in the Pritzker laboratory at the museum, where lab work is conducted in an open area that is visible to visitors. Both molecular and biodiversity data will be made available on-line.

DISSERTATION RESEARCH:
COMPARATIVE STUDIES IN REPRODUCTIVE INTERFERENCE: LINKING
FLORAL ADAPTATIONS AND SPECIES DIVERGENCE

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Comparative studies in reproductive interference: linking floral adaptations and species divergence

Variation in the strength or identity of interspecific interactions across a species geographic range can cause populations, or species, to experience divergent selection (Thompson 2005). If such interactions directly interfere with reproductive success (i.e., cause reproductive interference), selection to reduce their negative effect can promote the divergence of reproductive traits (Waser 1978, Armbruster *et al.* 1994, Hoskin & Higgle 2010); a phenomenon known as reproductive character displacement (Grant 1972, Schlüter 2000). This type of species interaction occurs in flowering plants when species sharing pollinators suffer reduced fitness from interspecific pollen flow, pollen loss, or reduced visitation (Waser 1978). While this process has been widely demonstrated in experimental studies (Morales & Traveset 2008), its prevalence in natural communities, and its evolutionary consequences, remain poorly understood. Indeed, the extent to which species interactions influence rates of adaptation and diversification is a central question in ecology and evolution (Schemske 2009); yet, in this context, the role of reproductive interactions has received little attention, despite its potential for generating reproductive variation and even isolation (Rice & Pfennig 2010).

The angiosperm genus *Pedicularis* provides an ideal system in which to test for reproductive interference, and to investigate its evolutionary consequences. Co-occurrence is common among species in this lineage, particularly within its center of diversity in the Hengduan Mountains of China, where nearly 270 species are endemic (Yang *et al.* 1998). Here, a highly seasonal climate, combined with a paucity of pollinator types, requires that many species flower synchronously and share pollinators; raising the potential for interspecific pollination. Furthermore, the concentration of diversity in this region causes individual species to co-occur with a mosaic of sympatric congeners across their geographic range, such that the intensity of reproductive interference is expected to vary across communities. This variation can be investigated in the context of selection on reproductive traits to test for adaptive character displacement both between and within species (Hoskin & Higgle 2010, Rice & Pfennig 2010). In this way, I will address the question of whether reproductive interactions affect rates of adaptation and diversification in *Pedicularis* by focusing on two simpler questions: Do species interactions promote interspecific divergence of floral traits through character displacement? And, does variation in species interactions across communities drive the divergence of floral traits within species, potentially leading to reproductive isolation?

My dissertation research addresses these questions in three distinct phases: (i) First, I test for evidence of reproductive interference by examining the phenotypic and phylogenetic structure of diverse communities of *Pedicularis*; (ii) second, I examine the relationship between species co-occurrence and rates of floral trait evolution and diversification on a molecular phylogeny, and test for associations between floral traits that increase pollination precision, and rates of speciation; and (iii) third, I examine intraspecific floral variation with respect to species co-occurrence and use comparative models of gene-flow and selection to test for adaptive character displacement. The first phase is now complete; results from my study show a pattern consistent with the hypothesis that reproductive interference is pervasive among *Pedicularis* (see *previous results*). The second phase, involving phylogenetic comparative analyses, is in progress and all required data are either available or already being generated. Therefore, I focus this proposal on the third phase of my dissertation. Here, I examine species exhibiting intraspecific floral variation to test the role of species interactions in generating floral divergence, and to investigate its consequences on reproductive isolation.

***Pedicularis* and the Hengduan Mountains**

The Hengduan Mountains region of south-central China represents one of the few biodiversity hotspots located outside of the tropics (Boufford & van Dijk 2000). As a latitudinal anomaly, high levels of species richness and endemism belie expectations based on its temperate-montane seasonal climate. The Hengduan flora has a high proportion of species pollinated by bumble bees, and observational studies have revealed a

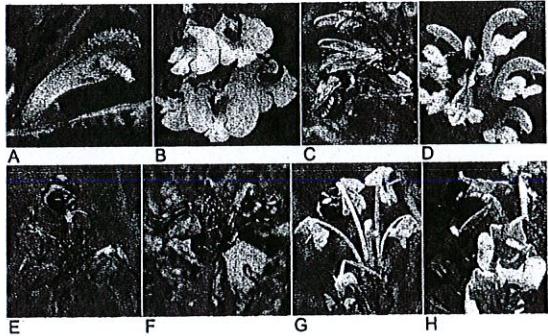


Figure 1: Floral diversity and pollination in *Pedicularis*. Bumble bees forage for nectar upright (C, D), but adopt more variable body positions while foraging for pollen (E–H), which requires manipulating the galea (fused dorsal petals enclosing the anthers and style). In species of *Pedicularis* without nectar (B, E–H) the galea is beaked, its length and curvature influencing where the protruding stigma contacts the insect's body. From Eaton *et al.* (*in review*).

high degree of pollinator sharing and low floral visitation rates (Macior *et al.* 2001), suggesting that pollinator services are a limited resource. Synchronous flowering within communities is common, as the short summer season constrains the timing of reproduction.

Consistent with the expectation that these factors combine to promote reproductive interference, flowers of *Pedicularis*, one of the most diverse lineages in this region, exhibit conspicuous interspecific variation in traits that can functionally reduce interspecific pollination. The most important structure in this regard is the galea, the hood-like dorsal corolla lip enclosing the anthers and style, at the apex of which the stigma protrudes (Figure 1). Variation in the shape and size of the galea affects the location of pollen deposition and pick-up on bumble bees. In species that produce nectar, the galea is generally unmodified, and nectar-foraging bumble bees tend to visit flowers upright, such that pollen transfer occurs on their dorsal side; by contrast, species providing only pollen as a reward tend to have a galea that is elongated into a beak (Macior & Tang 1997, Macior *et al.* 2001). Bumble bees forage for pollen by manipulating the galea, requiring a lateral or inverted body position, and the position of pollen deposition and stigmatic contact is more variable, depending on the length and curvature of the beak (Macior & Tang 1997, Huang & Fenster 2007, Yang *et al.* 2007). This allows *Pedicularis* species to partition shared generalist pollinators through specialization in the location of pollen placement. Because *Pedicularis* species do not exhibit species-specific associations with individual bumble bee species, this mechanism for partitioning pollen placement appears important for reducing reproductive interference arising from the use of shared generalist pollinators. In being highly precise, however, small floral differences between populations may greatly affect pollen-flow between them.

Project outline: Within-species tests for character displacement

Many species of *Pedicularis* exhibit intraspecific floral variation, often in the form of populations segregated in floral color, size, and/or shape. This variation reflects the broader lability in floral evolution observed between species, and suggests that intraspecific floral divergence may be a first-step in species divergence. Due to rapid species turn-over across communities (high beta diversity), individual species overlap with dozens of other *Pedicularis* across their range, creating an ideal system in which to test how variation in species interactions influences floral divergence through the process of character displacement. While phenotypic differences among populations can arise from different selection pressures across communities, gene-flow between them is expected to prevent local adaptations (Savolainen *et al.* 2007). Detecting reproductive character displacement, and its effect on the genetic isolation of populations, therefore requires determining the balance between gene-flow and selection within communities, and identifying the local factors selecting on floral phenotypes.

I propose a two-part study designed for this purpose: (i) First, I will infer patterns of gene-flow among populations from genetic data using a model-based approach to test competing hypotheses about how spatial and phenotypic variation affect patterns of neutral genetic variation. (ii) Second, using these gene-flow data to correct for statistical auto-correlation among populations, I will fit adaptive and stochastic models to pop-

ulation mean phenotypes to test for evidence of selection from local biotic or abiotic variables. More simply, the first analysis tests whether floral differences are associated with patterns of gene-flow, and the second analysis asks whether these floral differences have arisen due to selection or drift. These tests will be replicated across three different species of *Pedicularis* (Figure 2) exhibiting intraspecific variation in different combinations of floral traits to test whether a relationship between genetic and floral divergence is consistent across species, and to identify major factors contributing to population divergences.

Project Management Plan

Three focal species, *Pedicularis cranolopha*, *P. batangensis*, and *P. semitorta*, were chosen for this study on the basis that each is geographically widespread, occurs in an area that is politically stable and for which collection permits are routinely awarded, and exhibits intraspecific variation in different combinations of floral traits (Figure 2).

I have experience conducting fieldwork in the Hengduan Mountains, having traveled there in both 2009 and 2010. In the summer of 2010, I completed field sampling for one of the species involved in this study (*P. cranolopha*), and will begin molecular analysis in December 2010. Preliminary data showing the geographic distribution of floral variation in this species are presented below (*Preliminary data* section). An expedition is planned for July 2011 to collect samples for the remaining two species. This will require approximately one month of field work, with a goal of sampling at least ten populations per species. The primary cost for field work is transportation. For this, a local Chinese driver with an off-road vehicle will be hired for safe navigation, a standard practice in this region. In addition, a Chinese undergraduate student from the Kunming Institute of Botany will be hired as a field assistant to aid in data collection. The trip will be planned based on localities from previously collected herbarium specimens of the two focal species. Funding from the DDIG would support a stipend for the field assistant, as well as the cost of a driver, vehicle, gas, and hotels. Lab work (described below) on the additional two species will begin in October 2011, and continue throughout 2012, to generate and sequence genetic markers from sampled populations. A timeline for the proposed study is shown in Figure 3.

The data collection protocol used in 2010 to sample populations of *P. cranolopha* within Yunnan and Sichuan Provinces is described here, and will be the same for the other two species. Eight floral traits were measured on fifteen individuals in each site, and tissue samples were collected for genetic analyses. Measured floral traits included (1) corolla-tube length, (2) corolla width, (3) corolla-lip length, (4) corolla-lip width, (5) galea length, (6) galea angle, (7) pollen diameter, and (8) a binary measurement of whether the galea is forked versus non-forked. Because the two species yet to be sampled exhibit color variation, a reflectance spectrophotometer will also be used to measure color on populations in the field. At each collection locality environmental data were recorded representing potential biotic and abiotic selective factors. Biotic factors included number and identity of sympatric synchronously flowering species of

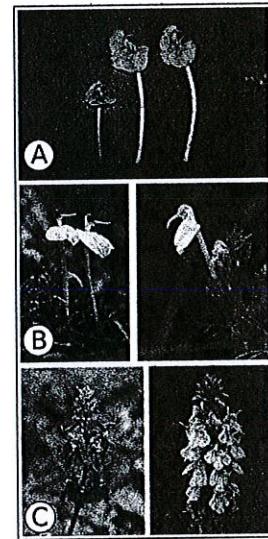


Figure 2: Flowers from different populations of (A) *P. cranolopha*, (B) *P. batangensis* and (C) *P. semitorta* exhibiting intraspecific variation in shape, size or color.

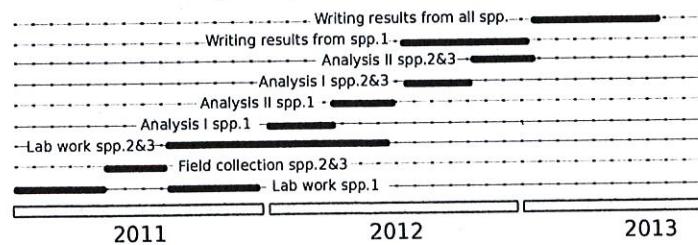


Figure 3: Project timeline

Pedicularis, and measurements of their floral phenotypes. Abiotic factors included elevation and slope.

Nuclear microsatellite markers will be developed for each of the three species. These markers are commonly used in population-level studies due to their high rate of mutation and have been used previously in studies of *Pedicularis* (Huang *et al.* 2008; 2010). Funding from my advisor, in addition to a grant awarded to me by the Pritzker lab at the Field Museum of Natural History in Chicago, will support the cost of identifying primers for 8-12 microsatellite markers for each of the three species. Funding from the DDIG would support DNA extractions and genotyping of approximately 600 individuals from the three species using these primers. All laboratory work will be conducted in the Pritzker lab.

The data from field and laboratory work will yield matrices representing geographic, morphological and genetic distances between populations, as well as biotic and abiotic variables within sites. Below, I outline two analyses that will use these data to test the role of species interactions in generating intraspecific floral variation through character displacement, and to investigate the consequences of this variation on reproductive isolation.

Analysis I: The relationship between floral variation and gene-flow

Hypothesis: If intraspecific floral variation reduces gene-flow between populations with divergent phenotypes, this should be apparent through shared neutral genetic variation in populations with similar phenotypes, and greater isolation of populations with divergent phenotypes

Genetic sampling across a species geographic range is unlikely to reflect true population structure (Beerli & Palczewski 2010). As a result, different geographic population samples may represent a single randomly mating population, or several populations with varying degrees of isolation. By examining genetic similarity among these populations, the factors that affect gene-flow between them can be investigated. With respect to the question of whether species interactions promote speciation in *Pedicularis* by causing floral divergences between populations, it is of interest to ask whether populations with divergent floral traits exchange fewer genes than those with similar floral morphologies, and to test this hypothesis against others including geographic distance and major biogeographic barriers to gene-flow.

I will investigate this question using the program MIGRATE (Beerli & Palczewski 2010), which uses Bayesian inference to compare alternative models of population structure by estimating population parameters from coalescent simulations on multi-locus genetic data, for which nuclear microsatellite markers will be used. I will test a number of alternative models representing different evolutionary hypotheses (Figure 4), and compare their relative support as measured in Bayes factors, which allows comparison of both nested and non-nested models. These models include (i) panmictic migration (Figure 4A), in which all populations exchange genes freely, (ii) stepping-stone models in which migration decreases with geographic distance (Figure 4B), (iii) models of local adaptation in which migration is reduced between populations in different selective (biotic or abiotic) environments (Figure 4C), and (iv) floral isolation models, in which migration is reduced between populations divergent in floral morphology. Within the floral isolation framework individual hypotheses concerning floral traits can also be tested, such as whether gene-flow is directional between populations varying in some quantitative floral trait (Figure 4D).

As the number of populations in the analysis increases, the number of possible models does also, likely beyond the point where testing all models is possible. The approach I will employ is to test specific scenarios reflecting evolutionary hypotheses of interest. These models will then also be tested against, and informed by, models in which populations are structured by group assignments using the program STRUCTURE (Pritchard *et al.* 2000), which uses cluster analysis to group samples by estimating admixture; an approach

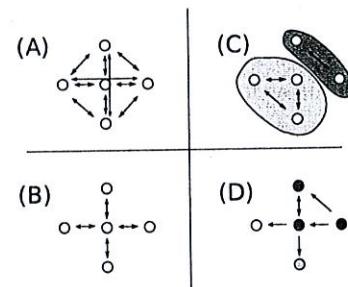


Figure 4: Migration models describing gene-flow among populations or population clusters. Described in text.

more similar to classic Fst measurements (Hudson *et al.* 1992, Beerli & Palczewski 2010), although it does not allow directionality in gene-flow. This analysis can also be used to reduce the number of populations to distinct population clusters, to test more broad-scale patterns of gene-flow, which may aid in detecting major barriers

The comparative framework of this analysis allows testing of numerous alternative variables (e.g., geography, floral similarity) as factors affecting interpopulation gene-flow, as well their combined effects. Support for floral isolation-type models would suggest that intraspecific floral variation is associated with reduced gene-flow. While this result doesn't elucidate whether floral differences cause a reduction in gene-flow or reduced gene-flow allows floral differences to arise, the relationship would suggest floral divergence is involved in genetic isolation of populations, and therefore potentially involved in speciation of *Pedicularis*.

Analysis II: Adaptive models to test floral character displacement

Hypothesis: If within-species variation arises from local character displacement across a geographic mosaic of species interactions, then population phenotypes should co-vary with differences in the biotic community.

The geographic mosaic theory of coevolution (Thompson 2005) posits that variation within species results from differences in selection across the landscape. Accordingly, a common goal when investigating within-species variation is to detect correlated differences between phenotype and local environmental (biotic or abiotic) variables. A potentially confounding factor in such analyses is the fact that geographically proximate populations can exhibit similar phenotypes for two reasons: (i) parallel adaptations to similar environments, or (ii) because common ancestry or gene-flow causes them to be similar. Due to this homogenizing effect of gene-flow, populations should not be treated as independent data points in analyses (Felsenstein 1985; 2002), and properly accounting for such effects may actually elucidate signals of local adaptation that are otherwise obscured.

Analogous to the use of a phylogeny to account for auto-correlation during interspecies comparisons (Felsenstein 1985, Hansen 1997), migration matrices (representing gene-flow) can account for auto-correlation when comparing populations, creating “within-species contrasts” – statistically-independent values for comparing population phenotypes (Felsenstein 2002). Only recently, with the increased availability of multi-locus datasets, and advent of efficient programs for coalescent simulation (Beerli & Felsenstein 2001, Hey & Nielsen 2004, Beerli & Palczewski 2010), has estimation of migration matrices needed for this type of analysis become feasible.

Using the best-fitting migration matrices estimated in MIGRATE from *Analysis I*, population phenotypes will be modeled according to Lande (1976), as values that are homogenized by gene-flow with other populations and by drift, but which are drawn towards some local phenotypic optima by selection. This model allows biotic and/or abiotic variables measured within sampling sites to be fit as local phenotypic optima towards which phenotypes are selected. The rate of adaptation towards these optima is then estimated by comparing observed phenotypes to expected phenotypic values based on gene-flow among populations and drift (Figure 5; Felsenstein 2002).

For example, in a two-population model, if the populations are experiencing symmetric gene-flow they will have an expected phenotype intermediate

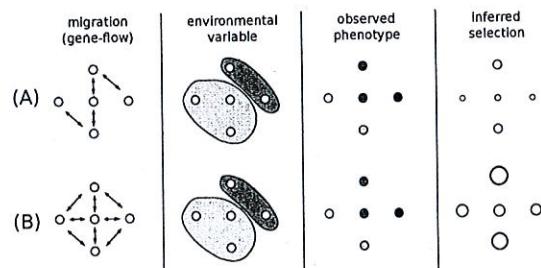


Figure 5: Two models of migration depict how different patterns of gene-flow influence the inferred level of selection from the same phenotypic data. If population phenotypes do not deviate from expected values, and do not co-vary with environmental variables, selection will be low (A). However, if phenotypic values are highly divergent between populations despite gene-flow, and co-vary with local environment, then selection will be high (B).

to their observed values, with a variance scaled by the parameter estimated for drift. Deviation of observed phenotypes from their expected values will therefore represent either selection overcoming gene-flow, or drift. The greater the deviation, the less power drift has to explain phenotypic differences, and the more power selection has to explain them. Selection will only improve model fit, however, if the local phenotypic optima towards which selection is pulling co-varies with deviations in phenotypes.

Local environmental variables measured in the field will be used to represent phenotypic optima towards which phenotypes are selected. Examples include the presence/absence, mean phenotypic distances, or multivariate measures of floral morphospace filled by sympatric congeners, as well as local species richness or mean phylogenetic distance to sympatric congeners. Maximum likelihood will be used to estimate the rate of adaptation towards local optima (Felsenstein 2002), and models will be compared using the Akaike information criterion (AIC). The best fit adaptive model will also be compared to a model in which the 'rate of adaptation' parameter is set to zero, to ask whether selection significantly improves model fit over a pure-drift model.

If the best fitting model is one in which local phenotypic optima are represented by biotic variables within sites (e.g., species composition), this would support the hypothesis that a geographic mosaic of interspecific interactions causes divergent selection on floral phenotypes. Furthermore, the amount of intraspecific floral variation explained by species interactions (or other measured variables) can be quantified using this model.

Preliminary data

Patterns of geographic and morphological variation among 25 populations of *P. cranolopha* show that the distribution of floral variation among populations is only weakly structured with respect to geographic proximity (Figure 6). Here, I highlight one conspicuous trait observed segregating among populations: the forked versus non-forked phenotype in the galea, presumed to affect the location of pollen deposition and stigmatic contact on bumble bees. This trait is most frequent on the eastern part of the species range (Figure 6A), however, populations exhibiting this trait do not appear to be more similar to each other with respect to variation in the other six quantitative floral traits measured, as shown in a principal components analysis performed on these traits (Figure 6B). The first two PC axes explain 49 and 35 percent of the variance in floral phenotypes, respectively; reflecting variation in corolla size and shape on the first axis, and galea length and curvature on the second. Across its sampled range *P. cranolopha* was observed to co-occur with 27 other synchronously flowering species of *Pedicularis* (mean = 3.48, s.d. = 1.53, max. = 9). The presence of large floral differences between geographically proximate populations suggests that local adaptation may be rapid, potentially aided by isolation caused by floral differences or geographic barriers. Genetic analyses will be important for understanding how these morphological and geographic patterns relate to geographic barriers and patterns of gene-flow among populations.

Previous results: broad-scale evidence for reproductive interference

As mentioned above, the first phase of my dissertation involves testing for broad-scale evidence of reproductive interference within communities of sympatric *Pedicularis* (Eaton *et al.*; *in review*). This was done

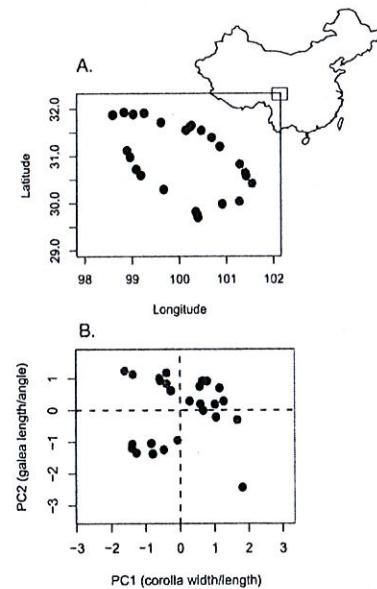


Figure 6: Geographic (A) and morphological (B) patterns of floral variation across 25 populations of *P. cranolopha*. Black vs. red circles represent populations having a split vs. non-split galea beak, respectively.

by examining phenotypic and phylogenetic patterns in the structure of local communities (Webb 2000), from data compiled in records of species co-occurrence from a biodiversity inventories database, as well as species mean floral trait measurements, and a reconstructed 116 species molecular phylogeny. In this way, we tested two distinct predictions: first, if disparity in floral traits reduces competitive interactions between species (e.g., if floral isolation mitigates reproductive interference arising from interspecific pollination), then assembly of *Pedicularis* communities should be disassortative with respect to floral similarity (Sargent & Ackerly 2008); and second, if such interactions have repeatedly driven character displacement, floral traits should exhibit homoplasy, the phylogenetic signature of labile evolution.

Our results support both of these predictions. The phenotypic and phylogenetic structure of 236 communities, containing 2-9 species, is shown in Figure 7. Phylogenetic structure was not significantly different from the null expectation of zero, consistent with the expectation if species assemble with respect to highly labile traits (Cavender-Bares *et al.* 2004), which floral traits were observed to be. Phenotypic structure, in contrast, was significantly greater than zero (two-tailed Wilcoxon-test, median = 0.44, $P = 1.7e^{-5}$), indicating that co-occurring species have more dissimilar floral traits than expected by chance. Furthermore, communities were shown to become increasingly phenotypically overdispersed with increasing local species richness ($r_s = 0.23$, $P = 3.0e^{-4}$).

In the absence of reliable estimates of both ancestral states and ancestral patterns of species co-occurrence, phylogenetic analyses are unable to explicitly test for character displacement. They can, however, reveal patterns of trait evolution, such as homoplasy, that suggest historical selection driving species divergences. This was the case for this study, where we test for ecological evidence consistent with a process (reproductive interference) that could give rise to a pattern of trait evolution consistent with character displacement, and we find evidence of both pattern and process. These results motivated the present proposal to directly test whether floral variation among populations exhibits the signature of adaptive character displacement using comparative models of gene-flow and species co-occurrence.

Intellectual merit

The extent to which species interactions influence rates of adaptation and diversification is a central question in ecology and evolution. In this context, the role of reproductive interactions has received little attention, despite its theoretical potential for generating reproductive variation and isolation. This study employs novel methods that have yet to be applied to the study reproductive character displacement. This is true both of the coalescent simulation framework used to infer the migration matrix among populations, as well as in using the Felsenstein (2002) model of within-species independent contrasts to test for selection on floral phenotypes while accounting for gene-flow. By combining the population-level research proposed here with species and clade-level studies of diversification conducted in a phylogenetic comparative framework, my dissertation research aims to broadly understand how reproductive interference among a diverse lineage of interacting species can affect diversification.

To this point, most studies examining the influence of species interactions on community assembly or patterns of diversification have overlooked reproductive traits, focusing instead only on ecophysiological traits relating to niche occupancy. Reproductive interference and reproductive isolation are distinct from these types of species interactions, both in the way in which selection can act of these traits, as well as in

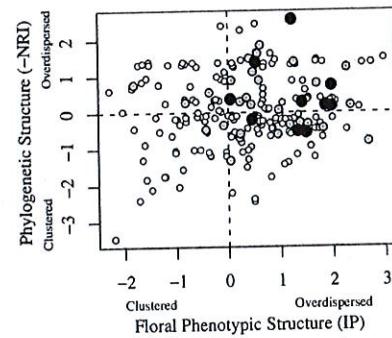


Figure 7: The phylogenetic and floral structure of 236 *Pedicularis* communities plotted in units of standard deviation from their expected values under a null model of random community assembly. Local species richness is indicated by shading of circles: white ($n = 2-4$), gray ($n = 5-7$) or black ($n = 8-9$). Figure from Eaton *et al.*; (*in review*).

the assumption of when they become limiting (i.e., saturation of niche space). For this reason, reproductive interactions may give rise to clade-level patterns that are inconsistent with current theory relating ecological and evolutionary phenomena. This is particularly true within studies of adaptive radiations, where diversification is often linked to the filling of open niche spaces. However, diverse lineages exemplifying adaptive radiation often compose many close relatives occurring in sympatry, precisely where interspecific reproductive interactions may be most influential. My research will help to identify the signature of reproductive interactions in species and population level patterns of divergence.

This research has broad implications for understanding patterns of biodiversity, particularly for flowering plants within the unique Hengduan Mountains biodiversity hotspot. Here, plant diversity and endemism far exceeds the expectation based on its temperate climate and latitude. Interspecific reproductive interactions driven by constraints on out-crossing strategies may be one potential explanation for the exceptional diversity in this region. *Pedicularis* is a ideal system for understanding these processes due to its exceptional species richness, floral diversity and species co-occurrence. Identifying factors promoting diversification is a major goal in ecology and evolution research as it relates to conservation. Only by understanding the processes that generate diversity can we design strategies to protect future biodiversity.

Broader impacts

My dissertation involves international collaboration with Chinese scientists conducting work in a politically and ecologically sensitive area. Through my work here I have met a great number of Chinese scientists, and have formed direct collaborations with professors from two institutions, Wuhan University and the Kunming Institute of Botany. In previous field expeditions I have worked alongside Chinese graduate students, and I plan to provide a stipend for a Chinese student to join my expedition described in this proposal as a field assistant. Forging US-Chinese scientific collaborations will be increasingly important in the future.

As a resident graduate student at the Field Museum of Natural History in Chicago, a large number of resources are available for public outreach. For example, a primary outlet for my work will be the “our scientist map” interactive feature at the Field Museum’s “DNA Discovery Center”. This exhibit introduces museum visitors to the basics on DNA and how it is involved in molecular ecology and systematics. Projects for individual researchers conducting work in the lab are highlighted and visitors can scroll through to see pictures of the field work and lab work involved in the described research. Furthermore, I will participate in “Talk to the Scientist Hour” in the DNA discovery center, which takes place weekdays from 11-12 where scientists host a question and answer session in the lab, and conduct laboratory work in a space visible to visitors. Annually, more than 200,000 K-12 students visit the museum on field trips, giving us the opportunity to make these students aware of the importance of ecology and evolutionary biology, and also to show them how fun the research can be.

I enjoy participating in outreach to encourage student involvement in science. Last year I participated in the Science and Engineering Research and Teaching Synthesis (SERTS) Program with Northwestern University, in Chicago. Here, early undergraduate students from Northwestern were paired with graduate students from the University of Chicago working at the Field Museum to learn about our research, and to talk about issues pertaining to the environment. I led students through the Field Museum for two days and discussed with them issues like global warming, population control, pollution, conservation, and volunteering. I will be participating in the program again this year. In addition, as a member of the Botanical Society of America I participate in *PlantingScience*, a program in which I mentor two groups of middle- to high-school students each year, through an on-line website, and guide them in conducting plant biology related research. This experience has been rewarding in the past, in which students have been very active in using the message board to discuss not only the project they are working on, but also to learn about my research and what it means to be a professional scientist.

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Budget justification

(1) Salaries and Wages = \$500

Field technician = \$500

(2) Fringe Benefits = 0

(3) Consultant Costs = 0

(4) Other Services = \$2 550

Driver = \$2550 (\$85/day x 30 days, includes cost of gas)

(5) Equipment = 0

(6) Supplies = \$10 450

Field work:

A visa is required to enter China:	\$140
Hotel cost (approx. \$45/day) for three people	\$1350
Shipping plant specimens from China to USA:	\$200

Itemized budget for lab supplies:

Qiagen DNeasy tissue kit:	\$1800
Agarose, Seakem:	\$560
1 kb Ladder:	\$220
Taq Gold, PE Biosystems:	\$2200
Other PCR reagents (water, dNTPs, BSA):	\$240
POP-7 polymer:	\$1520
373 10X running buffer:	\$440
LIZ-500 internal ladder:	\$1300
Labeled primers, Integrated DNA Technologies:	\$500

(7) Travel = \$1500

Roundtrip airfare Chicago, IL to Kunming, China: \$1500

(8) Computer Costs = 0

(9) Publication Costs = 0

(10) Subawards = 0

(11) Animal Costs = 0

(12) Other and misc. expenses = 0

Total requested = \$15 000

Context for Improvement

The proposed study on intraspecific floral divergence and isolation in *Pedicularis* is a critical part of my dissertation research examining how reproductive interactions among co-occurring species affects patterns of floral trait evolution and diversification. Here, I briefly outline my advisor's research, my own dissertation research, and how the proposed study relates to and will improve my dissertation.

R. Ree (advisor) – *Phylogenetic systematics and biogeography of the Hengduan Flora*

Phylogenetic and macroevolutionary inference should be integrated with biodiversity informatics to more accurately characterize, and understand, biodiversity hotspots. Dr. Ree's research is oriented towards this purpose both globally as well as regionally, with a particular focus on the Hengduan Mountains biodiversity hotspot in south-central China, where he has conducted research for over a decade. Here, Dr. Ree conducts plant systematics research, including phylogenetic study of the genus *Pedicularis*. In addition, he is involved in the development and use of phylogenetic comparative methods for inferring historical biogeography.

D. Eaton – Dissertation: *Diversity, disparity and sympatry: floral evolution in Pedicularis*

While my advisor's research aims to characterize patterns of diversification within biodiversity hotspots, my research focuses on the mechanisms or processes driving trait evolution and species divergence. Specifically, I am interested in how biotic interactions influence rates of adaptation and diversification, with a focus on the genus *Pedicularis*. To this effect, I am employing methods from community ecology, phylogenetic comparative studies, and population genetics. I have described these as being the three phases of my dissertation research: (i) the first was aimed at detecting an ecological signal of reproductive interference by examining patterns of community assembly with respect to floral similarity; (ii) the second involves broad-scale tests for associations between rates of speciation or extinction and the evolution of floral traits that influence pollination precision, as inferred on a molecular phylogeny; (iii) the third phase of my research was described in this proposal, and involves the examination of species exhibiting intraspecific floral variation to test whether this variation has arisen through character displacement from a geographic mosaic of species interactions.

D. Eaton – DDIG proposal: *Comparative studies in reproductive interference: linking floral adaptations and species divergence*

Funding from the NSF DDIG would support an additional field season as well as laboratory work for the third phase of my dissertation. This will allow me to sample two additional species, so that my study can be replicated across a total of three species. Such replication is integral for identifying processes that are broadly capable of explaining diversification in this lineage, and therefore would be expected to be similar across different taxa. My advisor currently does not have funding available to support my research, and his pending grant applications are directed at phylogenetic systematics and historical biogeography. In its similarity, our work is complimentary, and together can be used to better understand plant diversity in this unique region. However, species interactions and biogeography are distinct processes, and I am pursuing questions that are of my own design and interests.

