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Author(s): James M. Sobel

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# Ecogeographic Isolation and Speciation in the Genus *Mimulus*

James M. Sobel\*

Department of Plant Biology and Ecology, Evolutionary Biology and Behavior Program, Michigan State University, East Lansing, Michigan 48824

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**ABSTRACT:** Despite a long history of examining the geographic context of speciation, differences in geographic range have rarely been considered a legitimate isolating mechanism. This likely results from the complex relationship between historical and ecological processes in determining the spatial distribution of species. Ecogeographic isolation is the proportion of geographic isolation that results from genetically based ecological differences between taxa and should therefore be measured as an isolating mechanism under the biological species concept. In this study, species distribution modeling was used to evaluate the potential ranges of 12 recently diverged pairs of species in the genus *Mimulus*. Variation in the distribution models showed that these species differ significantly in the niches they occupy. These differences result in substantial ecogeographic isolation, with an average strength of 0.67, revealing that, on average, *Mimulus* species exhibit only 33% overlap in the extent of suitable habitat with their closest relatives. Because prezygotic barriers act early in the life cycle of organisms, this strong barrier has the potential to contribute greatly to the total isolation experienced between diverging species. Therefore, ecogeographic isolation appears to play an important role in *Mimulus*, and estimating the strength of this barrier is essential to our general understanding of speciation.

**Keywords:** habitat isolation, speciation, biological species concept, reproductive isolation, ecogeographic isolation, *Mimulus*.

## Introduction

One of the most debated topics in speciation biology is the relative impact of various forms of reproductive isolation (Coyne and Orr 2004). Despite the fact that a complete inventory of potential reproductive barriers has been recognized since the modern synthesis (Dobzhansky 1937; Mayr 1942), some forms of reproductive isolation have been overlooked in this debate. Although the role of geographic separation in limiting gene flow has been approached from a population genetics perspective (Wright 1943; Rousset 1997; Crispo et al. 2006), very few direct

estimates of the overall impact of spatial isolation on gene flow between taxa have been attempted (but see Kirkpatrick and Ravigne 2002; Ramsey et al. 2003; Kay 2006). In the sequence of barriers that can act to separate species, those that prevent hybridization have the first opportunity to limit gene flow (Coyne and Orr 1989; Ramsey et al. 2003). Therefore, geographic isolation can have a disproportionate impact on the total reproductive isolation experienced between taxa, providing ample motivation for its investigation.

David Starr Jordan provided some of the earliest insights into the geography of speciation, formulating what would later become known as Jordan's rule: "Given any species, in any region, the nearest related species is not to be found in the same region nor in a remote region, but in a neighboring district separated from the first by a barrier of some sort or at least by a belt of country, the breadth of which gives the effect of a barrier" (Jordan and Kellogg 1907, p. 120; also see Jordan 1908). Indeed, much of the speciation research in the past several decades has focused on whether Jordan's "rule" is correct (Mayr 1947; Feder et al. 2005; Bolnick and Fitzpatrick 2007). While previous studies of habitat isolation have considered how adaptation to a particular environment can affect the spatial distribution of taxa (e.g., Lynch 1978), few workers have considered how the biology of organisms is involved in creating or maintaining allopatry at a broad geographic scale (Wiens 2004; Sobel et al. 2010; Harrison 2012).

Historically, several authors have pointed out the need to consider geographic isolation as a barrier per se. For example, Stebbins notes that closely related species "are usually separated from their relatives by ecological barriers as well as by geographic ones" (Stebbins 1950, p. 197). Dobzhansky noted that "the occupation of separate areas by two species may be due not only to the fact that they have developed there, but also to the presence of physiological characteristics that make each species attached to the environment" (Dobzhansky 1937, p. 231). These views suggest a revision to Jordan's rule that might read as follows: the most closely related species are spatially proximi-

\* Present address: Department of Biological Sciences, Binghamton University, State University of New York, Binghamton, NY 13902; e-mail: [jsobel@binghamton.edu](mailto:jsobel@binghamton.edu).

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mate and are sometimes separated by a physical barrier, but also exhibit ecological divergence that enhances the fidelity of each to its home range.

The biological species concept (BSC) identifies reproductive barriers as biological differences between groups of organisms that actually or potentially limit gene flow (Mayr 1942). Geographic ranges are unique among potential barriers because they are the outcome of the interaction between historical and ecological forces (Endler 1982; Thorpe et al. 2008). Historical factors such as vicariance events may contribute to allopatric distributions; however, the resulting geographic isolation does not result from biological differences between taxa and is therefore excluded as an isolating mechanism from the BSC. Nevertheless, populations and taxa separated only by historical factors experience impediments to gene flow and can therefore diverge via stochastic processes such as genetic drift. This form of spatial separation is referred to as “effective geographic isolation” (Sobel et al. 2010). In contrast, genetic differences for traits related to ecological factors such as physiological tolerances can result in spatial distributions that mechanistically alter the probability of encountering con- and heterospecific mates. Thus, “ecogeographic isolation” is defined as the reduction in encounter rates that occurs because of broad-scale spatial separation resulting from intrinsic biological differences between taxa (Schemske 2000; Ramsey et al. 2003; Sobel et al. 2010).

Reciprocal transplants can reveal geographically structured fitness variation in nature (e.g., Angert and Schemske 2005; Geber and Eckhart 2005) and provide the ultimate test of whether variation in geographic ranges is the product of intrinsic biological differences between taxa. The labor involved in reciprocal transplants precludes its adoption as a general method in comparative biology, and transplants for many species may be unethical, unlawful, or impossible. However, species distribution modeling has emerged as another approach for examining the distribution of suitable habitat across the available landscape (e.g., Peterson 2003; Kozak et al. 2008), and this method can provide predictions about potential biological differences between taxa. Well-constructed species distribution models may make it possible to predict the outcome of transplant studies (e.g., Cunningham et al. 2009), allowing for an evaluation of the geographic impact of niche differences. Therefore, species distribution models can generate hypotheses of ecogeographic isolation, with the geographic extent of shared and unshared suitable habitat providing a measure of how variation in ecological requirements affects potential gene flow between taxa.

In this study, species distribution modeling was applied to a diverse group of western North American wildflowers in the genus *Mimulus*. Because barriers continue to evolve

after speciation is complete, it is at the earliest stages of divergence that measures of reproductive isolation are most informative. Therefore, distribution models were developed for 12 pairs of taxa that represent the most recent speciation events across the genus. The overlap in niche models was used to answer the following questions: (1) Are the modeled distributions of closely related species consistently different? (2) How do niche differences between species translate into broad-scale geographic discontinuities? (3) How strong is the ecogeographic isolation that results? and (4) Which environmental features are associated with ecogeographic isolation?

## Methods

### *Selection of Species Pairs*

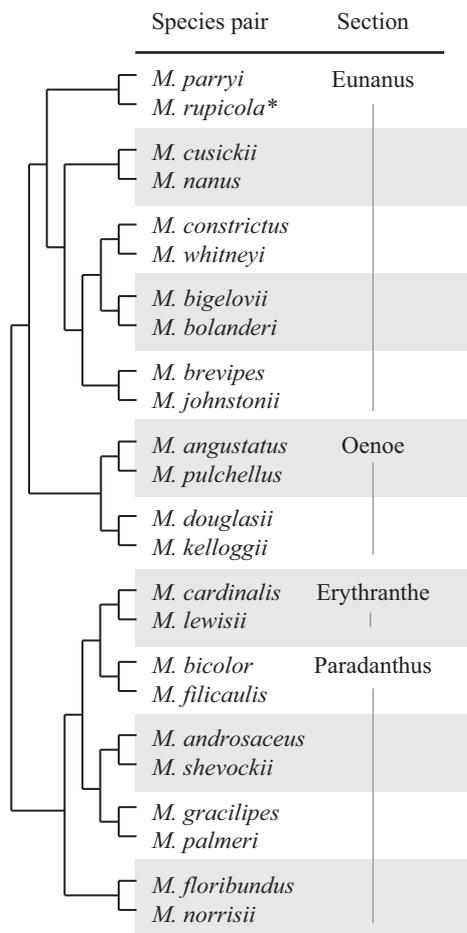
The genus *Mimulus* consists of at least 120 species of wildflowers, roughly 75% of which are found in western North America (Grant 1924; Beardsley et al. 2004). A rich history of genetic work, coupled with developing genomic resources, has elevated the genus to an emerging model system, especially in studies of adaptation and speciation (Wu et al. 2008). The phylogenetic data presented in Beardsley et al. (2004) was used to select 12 pairs of species that represent the most recent speciation events from across the genus (fig. 1). In all cases, species pairs are more closely related to each other than to any other species in the study, ensuring phylogenetic independence of the reproductive isolation measured (Felsenstein 1985).

### *Collection of Georeferenced Population Data*

Georeferenced locality data were collected for each species from the Jepson online interchange for California floristics (Rosatti 2003). From a combination of cartographic resources, including Google Earth (<http://earth.google.com>) and National Geographic TOPO! software (National Geographic TOPO! Maps, San Francisco; <http://maps.nationalgeographic.com>), latitude and longitude were added to samples lacking these data, resulting in more than 2,000 georeferenced accessions at an average of 84 populations per species (ranging from 6 to 323 populations per species; table 1). These compiled georeferenced collection records and all other data presented are deposited in the Dryad Digital Repository: <http://doi.org/10.5061/dryad.j92k8> (Sobel 2014).

### *Species Distribution Modeling*

Species distribution modeling was performed on all 24 focal species with Maxent (Phillips et al. 2006). Environmental variables consisted of eight climatological layers



**Figure 1:** The 12 pairs of *Mimulus* species used in this study. A generalized cladogram shows evolutionary relationships among pairs, adopted from Beardsley et al. (2004). Sectional assignments from traditional morphological taxonomy are indicated (Grant 1924). *Mimulus rupicola*, marked by an asterisk, was previously assigned to section *Oenoe*.

from the WORLDCLIM data set (Hijmans et al. 2005) and geological data (table 2), and the equal training sensitivity and specificity (ETSS) threshold was used to establish binary species distribution models. This cutoff represents the threshold at which predictions of which sites are suitable or unsuitable have an equal probability of being correct, given the data, and is therefore preferable to the use of arbitrary options (Liu et al. 2005; Freeman and Moisen 2008). Maxent compares the distributions of environmental variables at sites occupied by a focal species to the distributions at randomly drawn background points. A constant probability of detecting the presence of a given species within suitable habitat is assumed; therefore, care must be taken to avoid drawing background points from regions where the species is absent for reasons other than

its ecological tolerances. For example, dispersal limitation may affect the extent to which a species occupies suitable habitat, and inclusion of regions that have been untested by colonization may result in erroneous prediction of suitable habitat (Elith et al. 2011). This is especially crucial in applying species distribution models to estimates of ecogeographic isolation, because models that underpredict the extent of suitable habitat could inflate estimates of ecogeographic separation.

In choosing an appropriate study area, problems can arise when the defined extent is either too limited or too expansive (Thuiller et al. 2004; VanDerWal et al. 2009). When regions surrounding collection records are too small, the variation in environmental variables used to construct distribution models may be too narrow, leading to difficulty in projecting the model onto regions outside the immediate vicinity of collection records. When study

**Table 1:** Ecogeographic reproductive isolation in pairs of *Mimulus* species

Species	N	Ecogeographic isolation		
		Minimum	Estimate	Maximum
<i>M. parryi</i>	8	.07	.77	.93
<i>M. rupicola</i>	11	.14	.52	.91
<i>M. cusickii</i>	7	.00	.79	.81
<i>M. nanus</i>	67	.92	.95	.97
<i>M. constrictus</i>	71	.45	.58	.71
<i>M. whitneyi</i>	66	.39	.52	.62
<i>M. bigelovii</i>	155	.99	1.00	1.00
<i>M. bolanderi</i>	84	.98	.99	1.00
<i>M. brevipes</i>	214	.81	.82	.83
<i>M. johnstonii</i>	56	.63	.65	.68
<i>M. angustatus</i>	37	.63	.63	.76
<i>M. pulchellus</i>	32	.52	.63	.81
<i>M. douglasii</i>	82	.50	.61	.71
<i>M. kelloggii</i>	107	.17	.24	.35
<i>M. cardinalis</i>	231	.91	.96	.99
<i>M. lewisii</i>	84	.92	.93	.93
<i>M. bicolor</i>	133	.64	.66	.72
<i>M. filicaulis</i>	17	.14	.39	.56
<i>M. androsaceus</i>	29	.85	.88	.94
<i>M. shevockii</i>	11	.07	.19	.30
<i>M. gracilipes</i>	8	.00	.08	.18
<i>M. palmeri</i>	93	.91	.92	.96
<i>M. floribundus</i>	323	.91	.91	.91
<i>M. norrisii</i>	6	.46	.53	.76
Average (SD)	8.5 (81.2)	.54 (.35)	.67 (.26)	.76 (.23)

Note: *N* indicates the number of populations used in generating species distribution models. The “Estimate” column provides the extent of ecogeographic isolation experienced by each species with regard to its sister pair, using the degree of overlap in suitable habitat that results from the mean equal training sensitivity and specificity (ETSS) threshold. The minimum and maximum values were obtained by similarly calculating isolation from the lower and upper 95% confidence interval limits on ETSS threshold, respectively.

**Table 2:** Environmental layers used in distribution modeling and correlation coefficients between continuous variables

Layer	Description	Correlation coefficients						
		Bio1	Bio4	Bio5	Bio6	Bio12	Bio15	Bio16
Bio1	Annual mean temperature							
Bio4	Temperature seasonality	.327						
Bio5	Maximum temperature of warmest month	.885	.684					
Bio6	Minimum temperature of coldest month	.869	-.148	.567				
Bio12	Annual mean precipitation	-.548	-.509	-.599	-.25			
Bio15	Precipitation seasonality	.031	-.662	-.208	.391	.418		
Bio16	Precipitation of wettest quarter	-.492	-.551	-.572	-.172	.894	.496	
Bio17	Precipitation of driest quarter	-.836	-.054	-.658	-.824	.556	-.312	.473
Geology	Primary and secondary geologic parent material							

areas are too large, test statistics can be artificially inflated, and models become overly simplified (VanDerWal et al. 2009). Ideally, the study area in which distribution modeling is performed should be of intermediate size surrounding collection records, representing an area that focal taxa likely have had the opportunity to disperse into and test for habitat suitability.

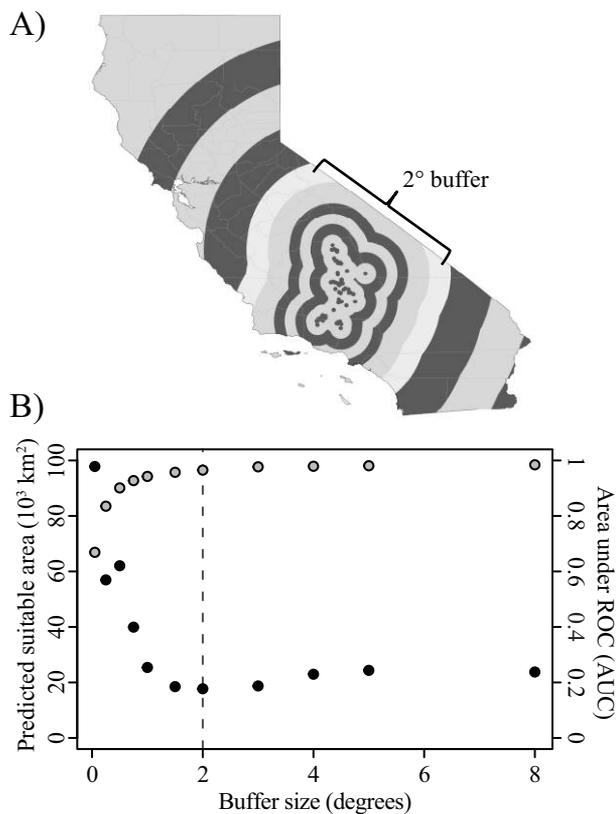
In order to determine appropriate distribution modeling extents in this study, *Mimulus constrictus* was selected haphazardly as a focal species. Buffers with diameters of 0.05°, 0.025°, 0.5°, 0.75°, 1°, 1.5°, 2°, 3°, 4°, 5°, and 8° longitude (fig. 2A) surrounding each accession were extracted from the nine environmental layers (table 2) with Hawth's Analysis Tools for ArcGIS (Beyer 2004). Because these buffers were drawn on the nonprojected latitude/longitude format of the WORLDCLIM data set, each buffer is slightly elliptical. However, to correct for variation in the spatial extent of degrees longitude across latitude, a bias layer was incorporated into the distribution modeling that allows Maxent to weight pixels appropriately across these spatial extents (see the appendix, available online). The area under the receiver operator curve (AUC) is a threshold-independent indicator of species distribution model performance that ranges from 1 if a model perfectly predicts suitable habitat to 0.5 when it is equivalent to a random draw (Phillips et al. 2006). This statistic is prone to artificial inflation in presence-only distribution modeling (Lobo et al. 2008), especially as spatial extents increase (VanDerWal et al. 2009). However, the prediction can be an informative statistic in comparing the outcome of several related distribution models.

In *M. constrictus*, the AUC increases rapidly over the smallest buffer sizes, indicating improvement in model performance as buffers expand beyond the immediate neighborhood of collection records (fig. 2B). The extent of predicted suitable area declines rapidly as buffer size increases, reaching a local minimum that coincides with the plateau in AUC values at approximately 2°. Beyond this range AUC continues to rise, most likely as an artifact

of the presence-only method employed. Predicted suitable area also increases beyond this range. This may occur initially as background points are selected from large areas of mostly unsuitable habitat, expanding the area of predicted suitable habitat. As buffers expand to their largest extent, potentially suitable habitat outside the dispersal capability of the species may be treated as unsuitable, resulting in a plateau in the predicted range. Most relevant to this study, the estimated strength of ecogeographic isolation (see description of calculation below) for this species stabilizes at values of buffers above 1.5° (fig. S1; figs. S1–S6 available online in a supplementary PDF). Therefore, a buffer size of 2° was selected to roughly approximate the extent of dispersal limitation in *Mimulus* species, and all subsequent niche models used this spatial buffer zone around collection records (see appendix).

#### *Comparing Distributions of Recently Diverged Species*

For each species pair, differences in habitat were compared by extracting climatic and geologic data from population locations. One-way MANOVA (Wilks's  $\lambda$ ) was conducted in R (R Development Core Team 2010), with species identity as the dependent variable and the continuous environmental data as independent. To determine which variables were responsible for significant MANOVA results, post hoc Mann-Whitney *U*-tests were performed on each continuous environmental variable in JMP 8.0 (SAS Institute, Cary, NC). Contingency table analyses were also performed on the categorical geology variable in JMP 8.0. To further identify the climatic variables most responsible for species differences, discriminant function analyses (DFAs) were performed in SPSS, version 20 (IBM 2011). The relative impact of each variable was indicated by the function structure matrix, which represents the correlations between variables and the standardized canonical discriminant functions. In addition, to aid in visualization of the environmental differences between species, princi-



**Figure 2:** Local buffering of environmental variables for use in species distribution modeling. *A*, Range of buffer sizes examined surrounding collection records of the focal species *Mimulus constrictus*. The 2° buffer size selected for modeling is shown by a bracket. *B*, Response of predicted suitable area (km<sup>2</sup>) and an indicator of model quality (area under receiver operator curve [ROC (AUC)]) to variation in buffer size used. Suitable area (black circles) decreases to a local minimum at 2°. AUC (gray circles) increases with buffer size used, approaching an asymptotic maximum at 2°.

pal-components analysis (PCA) was performed on environmental variable correlation coefficients in JMP 8.0.

In order to directly compare species distribution models (SDMs), the niche identity feature within the software package ENMTools (<http://enmtools.blogspot.com>) was used to calculate the test statistic *I* from the equation

$$I(p_1, p_2) = 1 - \frac{1}{2}H(p_1, p_2) \quad (1)$$

(Warren et al. 2008), where *p*<sub>1</sub> and *p*<sub>2</sub> refer to the SDM probability distributions for species 1 and 2 of a paired comparison and *H* is the Hellinger distance between the distributions (Warren et al. 2008). To assess statistical significance, actual values of *I* were compared to a null distribution generated from 100 randomly permuted SDM comparisons for each species pair.

### Estimating Ecogeographic Isolation

Ecogeographic isolation was calculated by examining the overlap in suitable ranges between species pairs. Species distribution models were projected onto the combined geographic area of the spatial buffers used for both members of each pair. Ecogeographic reproductive isolation was therefore calculated for each species as

$$RI_{ecogeo} = 1 - \frac{S}{S + A}, \quad (2)$$

where *S* represents the shared geographic area of suitable habitat for a species pair and *A* represents the unshared allopatric extent of suitable habitat for a focal species. This equation produces a unitless metric that estimates ecogeographic isolation as 0 when a species occupies no unique suitable geographic area (complete sympatry) and 1 when the entire extent of suitable habitat is unshared (complete allopatry; see Sobel and Chen 2014). Error estimates were produced for ecogeographic isolation by constructing 95% confidence intervals on the ETSS threshold values used to produce binary suitable/unsuitable range maps. For each focal species, ecogeographic isolation was calculated over this entire interval while the alternate species constant was held at its mean approximation. The lowest and highest reproductive-isolation values are reported to represent the range of values across the ETSS 95% confidence interval.

Because species may often experience reproductive isolation asymmetrically with regard to potential gene flow (e.g., Tiffin et al. 2001; Bolnick et al. 2008), measures of ecogeographic isolation are presented as individual values for each species. Species pairs with asymmetry in the extent of suitable habitat may therefore have very different values for ecogeographic isolation, even when there is substantial overlap. For example, species 1 and 2 may have highly unequal ranges, such that the entire extent of suitable habitat for species 2 is contained within the suitable habitat predicted for species 1. Therefore, from the point of view of species 2, there is no allopatry, and ecogeographic isolation should be 0. Alternatively, if substantial suitable habitat exists for species 1 outside of this area of overlap, much of the reproduction in species 1 could occur with very little potential gene flow from species 2. Therefore, rather than reporting a combined metric, measures of ecogeographic isolation are presented as an individual value for each species in reference to the extent of potential gene flow that could be experienced with the alternate species.

## Results

### Species Distribution Models

Maxent performed well in producing distribution models, with an average AUC across species of 0.92 (range: 0.84–

**Table 3:** Results of MANOVA and discriminant function analysis on climatic variables

<i>Mimulus</i> species pair	Wilks's $\lambda$	Approximate $F$	Populations correct (%) <sup>a</sup>	Most discriminating variable
<i>M. parryi</i> / <i>M. rupicola</i>	.038	$F_{8,20} = 40.92^{***}$	100, 100	Maximum temperature in warmest month
<i>M. cusickii</i> / <i>M. nanus</i>	.552	$F_{8,77} = 7.10^{***}$	71.4, 91.7	Precipitation seasonality
<i>M. constrictus</i> / <i>M. whitneyi</i>	.493	$F_{8,141} = 17.24^{***}$	85.3, 85.3	Minimum temperature in coldest month
<i>M. bigelovii</i> / <i>M. bolanderi</i>	.106	$F_{8,250} = 256.7^{***}$	98.2, 98.9	Precipitation in wettest quarter
<i>M. brevipes</i> / <i>M. johnstonii</i>	.390	$F_{8,283} = 53.90^{***}$	92.4, 90.3	Annual mean temperature
<i>M. angustatus</i> / <i>M. pulchellus</i>	.341	$F_{8,73} = 15.97^{***}$	86.8, 100	Minimum temperature in coldest month
<i>M. douglasii</i> / <i>M. kelloggii</i>	.636	$F_{8,190} = 13.07^{***}$	71.4, 83.3	Annual mean precipitation
<i>M. cardinalis</i> / <i>M. lewisii</i>	.296	$F_{8,317} = 92.18^{***}$	92.3, 94.0	Annual mean temperature
<i>M. bicolor</i> / <i>M. filicaulis</i>	.792	$F_{8,160} = 5.024^{***}$	75.7, 95.5	Precipitation seasonality
<i>M. androsaceus</i> / <i>M. shevockii</i>	.488	$F_{8,56} = 6.417^{***}$	78.6, 93.8	Annual mean precipitation
<i>M. gracilipes</i> / <i>M. palmeri</i>	.414	$F_{8,105} = 17.32^{***}$	100, 99.0	Annual mean precipitation
<i>M. floribundus</i> / <i>M. norrisii</i>	.933	$F_{8,334} = 2.935^{**}$	81.8, 85.7	Temperature seasonality

Note: The variable with the largest magnitude in the discriminant function structure matrix is given in the last column; see table S2, available online, for the full matrix.

<sup>a</sup> The two values, from the discriminant function analysis, refer to the species in the order listed in the "Species pair" column.

\*\*  $P < .01$ .

\*\*\*  $P < .001$ .

0.99). At the equal training sensitivity and specificity (ETSS) threshold used for producing binary range maps, binomial probabilities indicate that models perform better at predicting known occurrences than a random expectation in all cases ( $P < 10^{-11}$ ), and 95% confidence intervals on this threshold were much wider for species with limited numbers of collection records (table S1; tables S1, S2 available online in a supplementary PDF). Jackknifing indicated that precipitation and geology variables were the most informative in building individual species models (fig. S2).

#### *Habitat Differentiation between Species Pairs*

In all 12 species pairs examined, MANOVA revealed significantly different climatic conditions between species ( $P < .01$ ; table 3), and niche identity tests (Warren et al. 2008) largely corroborated these results (fig. S3). DFA was successful at assigning populations to the correct species on the basis of climatological conditions (mean: 89.4% correct species classification; table 3). Figure 3 provides two-dimensional PCA plots to illustrate the differences in environmental features experienced by two species pairs. *Mimulus constrictus* and *M. whitneyi* inhabit distinct altitudinal ranges; therefore, the PCA shows that populations of these species occupy a largely distinct set of environmental conditions (fig. 3A). Post hoc analysis reveals that seven of the eight individual climatic variables are significantly different for this comparison (fig. 3C). The most discriminating variable for this species pair is minimum temperature of the coldest month, which exhibits the largest magnitude of difference between the two species (fig. 3C). Approximately half of the species pairs examined are

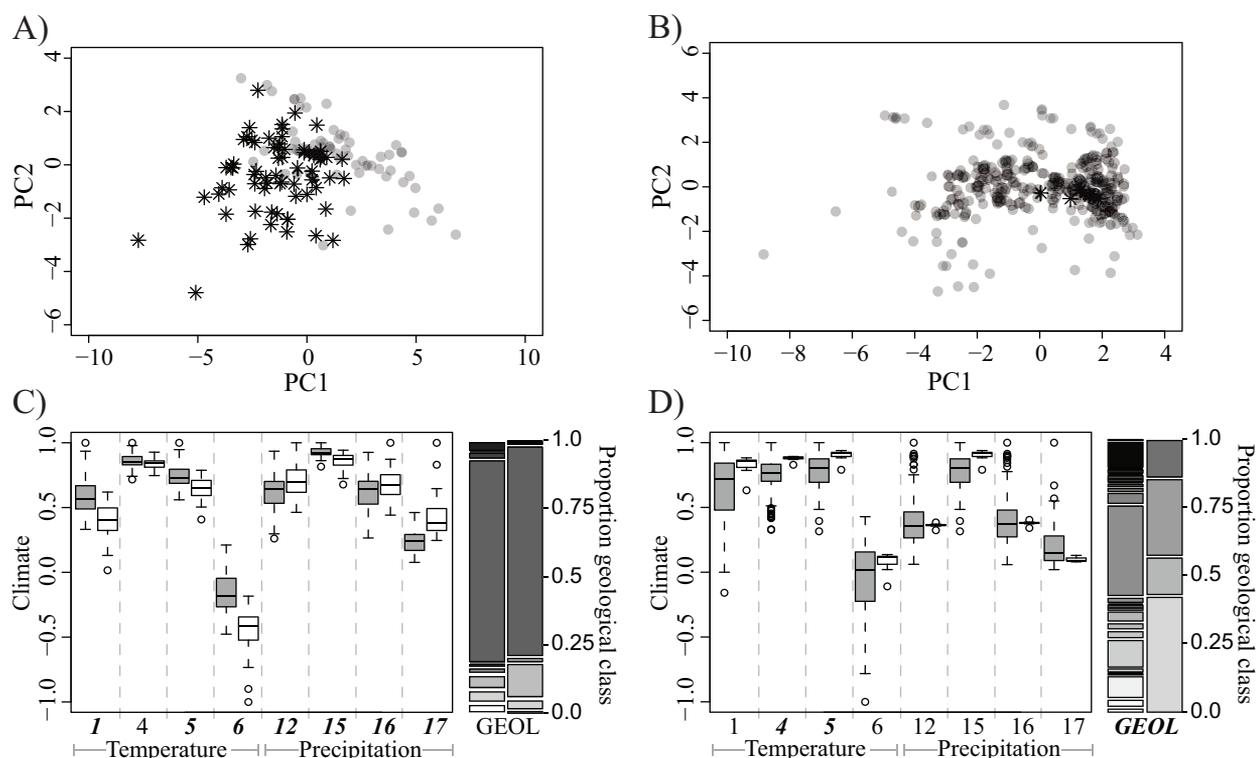
similar, with many climatic variables exhibiting significant differences between species (figs. S4, S5).

In contrast, *M. floribundus* and *M. norrisii* exhibit a pattern in which the variation present in the smaller-ranged *M. norrisii* is largely nested within the wider-ranged *M. floribundus* (fig. 3B). While the MANOVA demonstrates an overall significant difference between species ( $P < .001$ ; table 3), post hoc analysis exhibits statistical differences between only two climatic variables: temperature seasonality and maximum temperature in the warmest month (fig. 3D; table S2).

These species pairs also differ substantially in how geology contributes to their distribution. For example, in *M. constrictus* and *M. whitneyi*, geology is an important component in building each niche model (fig. S2); however, no significant difference in geology between the species was detected ( $P = .104$ ). Indeed, *M. constrictus* and *M. whitneyi* largely occur on the same basic geologic material, with 75% of *M. constrictus* and 78% of *M. whitneyi* populations occurring in regions dominated by granodiorite (fig. 3C). Conversely, the *M. floribundus* and *M. norrisii* species pair show significant differences in geology occupied (fig. 3D), and indeed, *M. norrisii* is a specialist on limestone substrates.

#### *The Strength of Ecogeographic Isolation*

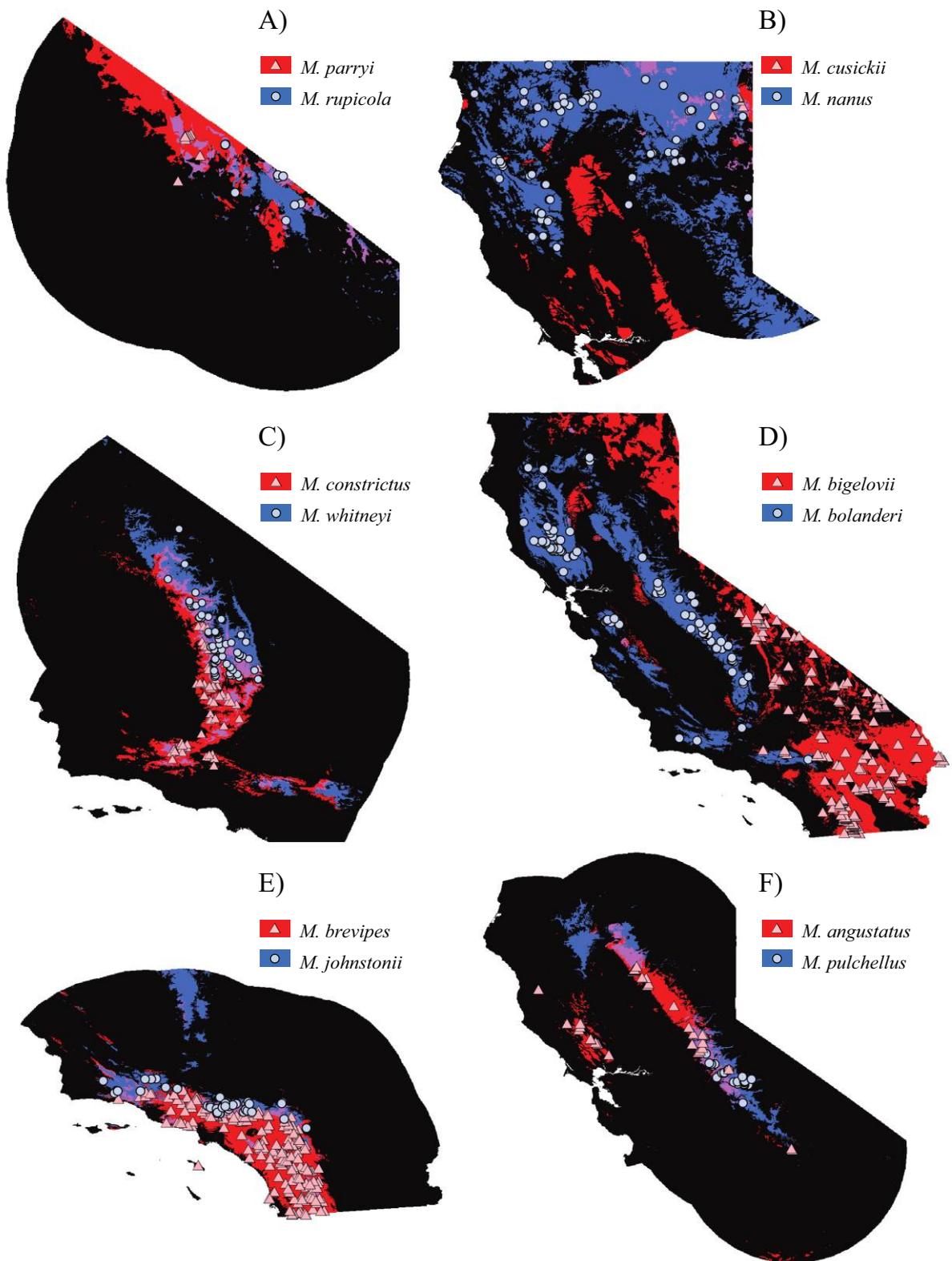
The geographic consequence of niche differences gives an estimate of the degree of ecogeographic isolation between species. Figure 4 provides overlapping species distribution models for each pair of recently diverged species to illustrate this isolation. The amount of suitable habitat that is



**Figure 3:** Environmental variation in two example species pairs in *Mimulus*. *A, B*, Visualization of climatic differences by principal-component analysis (PCA). Two-dimensional PCA plots are plotted with the first principal component (PC1) on the X-axis and the second component (PC2) on the Y-axis. *A*, *Mimulus constrictus* (gray circles) and *M. whitneyi* (asterisks) inhabit relatively discrete habitats associated with elevational differences, resulting in occupation of distinct climatic variable space. *B*, Alternatively, the PCA plot suggests that *M. norrisii* (asterisks) experiences a subset of the climatic conditions that *M. floribundus* (gray circles) faces. *C, D*, Quantile boxplots demonstrating variation between species in environmental variables. Each climatic variable is labeled by its corresponding WORLDCLIM number (table 2). Variables with numbers in bold italics are those that exhibit significant differences between species by Mann-Whitney *U*-test ( $P < .05$ ). Side-by-side gray and open boxes correspond to the respective members of a species pair. Climatic variables were standardized to the absolute value of the most extreme value, resulting in a metric that varied between  $-1$  and  $1$ . Contingency plots are given for geology (GEOL), and significant difference between species is again indicated by bold italics ( $P < .05$ ). Each grayscale shade indicates a different geologic class, and the size of each box represents the proportion of populations assigned. *C*, *Mimulus constrictus* (gray boxes) and *M. whitneyi* (white boxes) display extensive variation in climatic conditions occupied, with seven of the eight variables exhibiting significant differences by post hoc tests. *D*, *Mimulus floribundus* populations (gray boxes) span a much larger range of climatic conditions than do *M. norrisii* populations (white boxes), and the variation present in *M. norrisii* is largely nested within *M. floribundus*. However, geological conditions are significantly different, with *M. floribundus* inhabiting more geological classes than the limestone endemic *M. norrisii*.

unshared (red or blue) represents the extent of geographic area that could be occupied by each species, given the corresponding distribution model. In this portion of the range, the potential for gene flow is essentially zero. The amount of shared suitable space is shown in purple and represents the area where gene flow is possible between taxa. While additional barriers are present in many of these taxa (J. M. Sobel, unpublished data), the strength of ecogeographic isolation was calculated independently by assuming random mating within these shared suitable regions. The strength of isolation resulting from the mean threshold value among replicate niche models was generally strong across the species pairs considered (table 1; mean  $RI_{\text{ecogeo}} = 0.67 \pm 0.26$ , range = 0.08–1.0).

As values of niche model ETSS thresholds are increased, potential suitable habitat area contracts, while smaller threshold values result in expansion of projected suitable range. This results in a complex relationship between the threshold value and ecogeographic isolation. In the species pair *M. angustatus*/*M. pulchellus*, for example, comparing the mean niche models of the two species results in moderately strong ecogeographic isolation of 0.63 (fig. 5A). As the threshold varies across its 95% confidence interval, overlap in suitable ranges fluctuates (fig. 5B, 5C), resulting in a range of ecogeographic isolation estimates from 0.63 to 0.76 (fig. 5D). This relationship is variable across all species pairs studied (fig. S6), resulting in a range of calculated ecogeographic isolation values, with especially



**Figure 4:** Overlapping species distribution models, showing the extent of ecogeographic isolation between species pairs of *Mimulus*. For each species pair, one member of the pair is represented by unique suitable habitat in red, with pink triangles indicating collection records. Populations of the alternate species pair are shown with light blue circles, and unique suitable habitat is shown in dark blue. Shared suitable habitat between species is shown in purple. Ecogeographic isolation is calculated from the relative proportions of shared and unique suitable habitat across the shared geographic range.

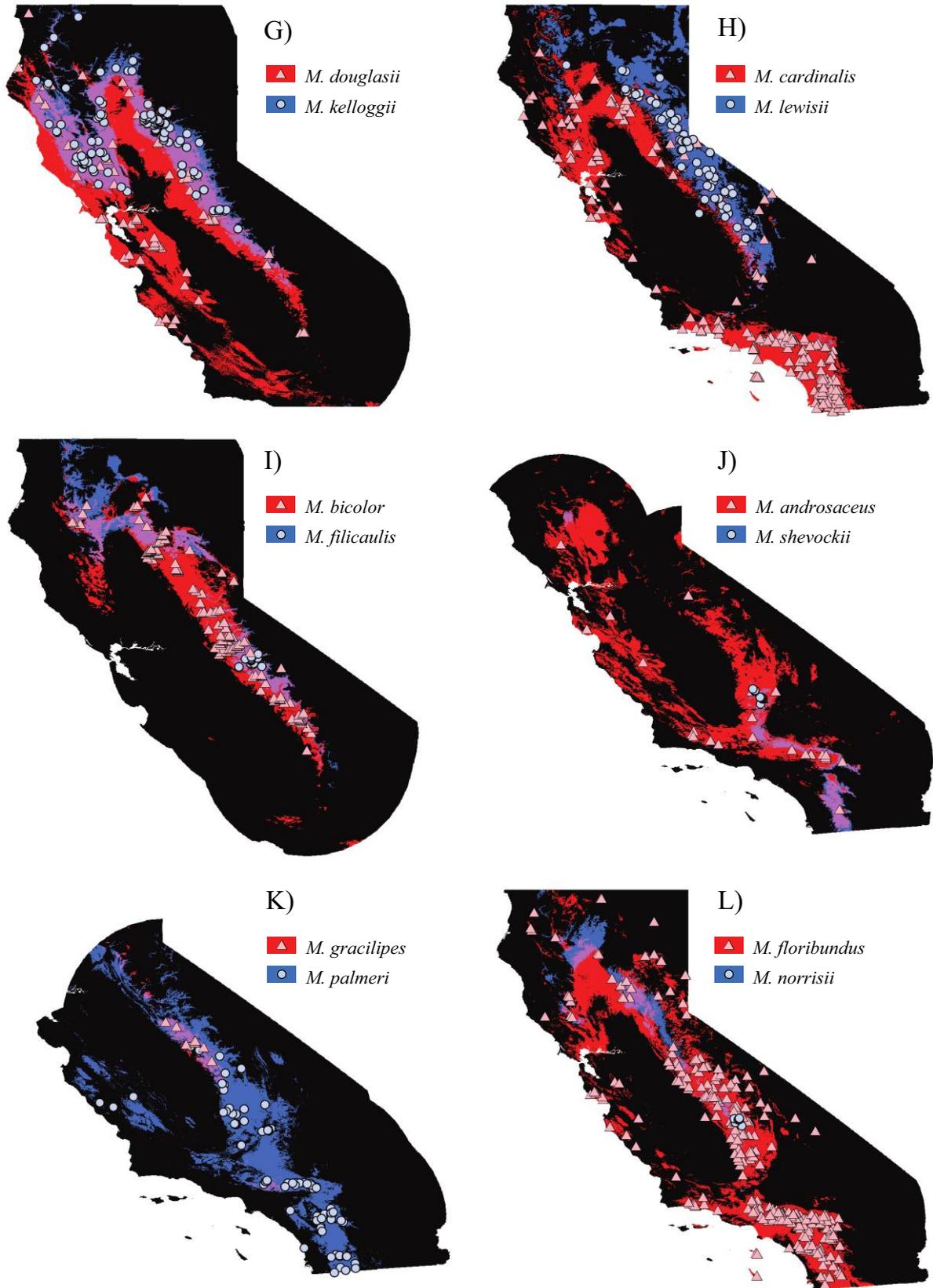
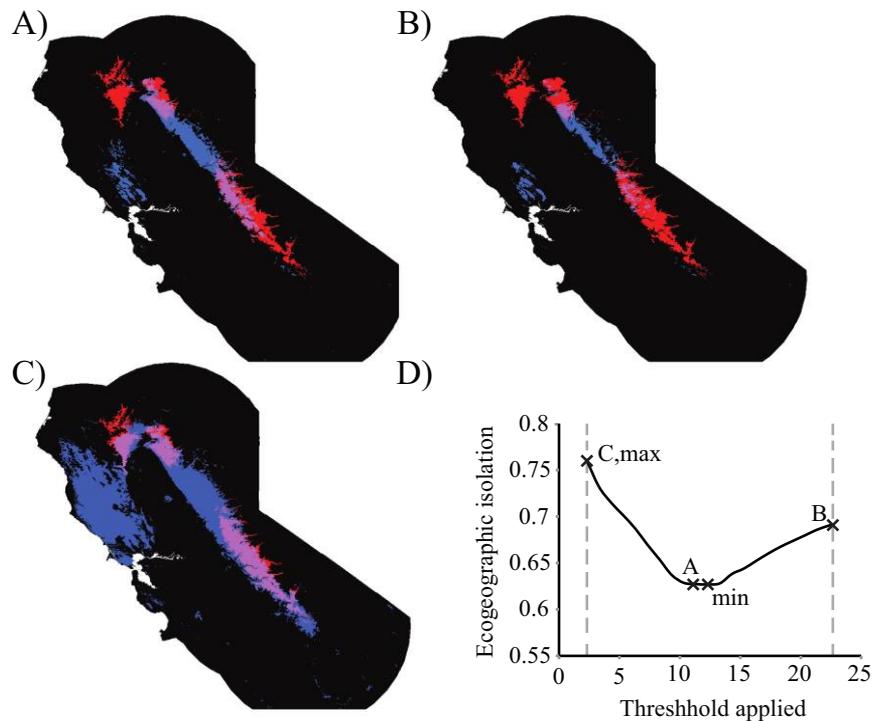


Figure 4: (Continued)



**Figure 5:** Variation in the extent of suitable habitat and ecogeographic isolation throughout the range of the 95% confidence interval in the equal training sensitivity and specificity (ETSS) threshold. In A–C, predicted suitable habitat for the focal species *Mimulus angustatus* is shown in blue, while that of its closest relative *Mimulus pulchellus* is shown in red. Overlap between the two species is shown in purple. A, Geographic extent of suitable habitat in *M. angustatus* and *M. pulchellus*, using the mean ETSS from 100 bootstrap replicates. B, At the upper 95% confidence interval limit for the ETSS threshold in *M. angustatus*, a smaller relative extent of shared suitable habitat with *M. pulchellus* leads to an increase in ecogeographic isolation, compared to the mean estimate. C, At the lower 95% confidence limit for ETSS, the spatial extent of *M. angustatus* is considerably larger, increasing the amount of unique suitable area substantially. D, Relationship between ecogeographic isolation and ETSS threshold values across the entire 95% confidence interval. Vertical dashed lines indicate the lower and upper limits. Capital letters indicate the estimates that correspond to the distribution models shown in A–C.

broad ranges in species with limited populations available (table 1).

### Discussion

#### *Ecogeographic Isolation Is an Important Feature of Speciation*

Ecogeographic isolation is generally strong across the 12 species pairs included in this study (table 1), and Stebbins's (1950) assertion that sister species are often separated by ecological as well as geographic factors is well supported in *Mimulus*. Because reproductive barriers act sequentially throughout the life history of organisms, early-acting barriers have the highest potential to impede gene flow during speciation. Using the sequential multiplicative series developed by Coyne and Orr (1989) and expanded by Ramsey et al. (2003), if ecogeographic isolation is 0.5 or greater, no later-acting barrier can exceed its relative impact on

total gene flow. While various definitions of importance may rely on either the individual or the relative strength of reproductive barriers (Hendry 2009; Sobel et al. 2010), this threshold represents a significant impact on gene flow at this stage. The average strength of ecogeographic isolation found here for *Mimulus* is  $0.67 \pm 0.26$  (table 1), and the mean value drops below 0.5 in only 5 of the 24 possible species comparisons. In some cases, species with low strengths of ecogeographic isolation exhibit additional prezygotic barriers that may allow for co-occurrence in shared habitat. For example, the estimated strength of ecogeographic isolation for *M. kelloggii* from *M. douglasii* is relatively weak, at a value of 0.24. However, *M. douglasii* self-fertilizes readily, and therefore the potential for interbreeding is likely low. Indeed, several examples of floral divergence in areas of sympatry appear to be associated with self-fertilization in the genus (Grossenbacher and Whittall 2011), and this feature may be important in facilitating coexistence in some cases.

Previous work in speciation has not typically measured geographic isolation as a barrier per se, but two lines of evidence suggest that strong ecogeographic isolation found here in *Mimulus* would be common in other groups. First, the phylogenetic signal of geographic overlap often exhibits the least overlap at the closest genetic distances between species (e.g., Barraclough and Vogler 2000; Fitzpatrick and Turelli 2006). While the lability of geographic ranges creates uncertainty for conclusively demonstrating an allopatric mode of speciation for any one species pair (Losos and Glor 2003), these comparative data reveal that many sister species exhibit at least partial allopatry at or near the time of speciation. In addition, in a review of seven recent reproductive isolation studies in plants, Schemske (2010) estimated the average relative contribution of geographic isolation to the total reproductive isolation between species at  $\sim 0.75$ . This value far exceeded the magnitude of isolation for the remaining barriers considered, suggesting that this mechanism is a driving force in plant speciation.

#### *Ecogeographic Isolation Results from a Variety of Factors in Mimulus*

The manner in which *Mimulus* species pairs differed in environmental conditions was quite idiosyncratic. While precipitation variables were the most important in constructing niche models for individual species (fig. S2), these were not any more likely than temperature variables to be significantly different between closely related species (figs. 3, S4). Few generalities emerged from the DFA, with seven different variables exhibiting the highest magnitude in the discriminant function structure matrix (table 3). Despite the heterogeneity in the impact of individual variables, elevational divergence and edaphic specialization emerged as two common features generating niche differences between species.

#### *Elevational Divergence*

Many of the climatic variables used in this study are highly correlated with each other (table 2), largely because of covariation with elevation. Therefore, species pairs that occupy different elevations also tend to experience differences in many of the climatological variables examined. For example, *Mimulus constrictus* is a midelevation species of the southern Sierra Nevada ( $\sim 200$ – $2,000$  m). Its close relative *M. whitneyi* is typically found at much higher elevations ( $\sim 1,500$ – $3,200$  m). As a result, these two species experience different conditions for almost every variable used in this analysis (fig. 3C). However, strong correlations between environmental variables (table 2) necessitate drawing conclusions with caution, and direct measure-

ments of targets and agents of selection will be necessary to characterize divergence. At least three additional *Mimulus* species pairs also differ in average elevation, leading to extensive niche divergence, including *M. angustatus*/*M. pulchellus*, *M. johnstonii*/*M. brevipes*, and *M. cardinalis*/*M. lewisii*.

#### *Edaphic Specialization*

Much of the previous work on edaphic specialization in plants has focused on relatively abrupt discontinuities in soil chemistry, such as serpentine endemism (Macnair and Gardner 1998; Brady et al. 2005) or community structure in regions with calcareous versus siliceous bedrock (Gigon 1987; Michalet et al. 2002). Indeed, there are known serpentine endemics in the genus *Mimulus* (e.g., *M. nudatus*), and a number of other species occur both on and off this distinct soil type (e.g., *M. guttatus*, *M. douglasii*). Additional work in *Mimulus* has suggested the potential importance of other edaphic types in generating diversity, such as adaptation to copper mine tailings (Macnair 1983). In this study, *M. norrisii* and *M. rupicola* are limestone endemics, and both exhibited significant differences in geology compared to their closest relative (figs. 3D, S4A). In addition to these two species pairs, there were also significant effects of geology in 9 of the 12 species pairs studied.

#### *Correspondence between Distribution Models and Biological Differences*

While suggestive of biological differences between taxa, results from species distribution models should be interpreted with caution (Guisan and Thuiller 2005). There are many potential sources of error in niche modeling (Wiens et al. 2009), and as expected, species with limited numbers of georeferenced populations exhibit high levels of uncertainty, with wide ranges in potential isolation (table 1; fig. S6). In addition, especially for species with limited geographic ranges, it is difficult to assess whether restricted ranges result from intrinsic biological differences between species or the combined effects of stochastic historical events and/or dispersal limitation (Allouche et al. 2008). This is an important consideration, because ecogeographic isolation results from the former but not the latter.

For example, a recently formed species with a small geographic range may indicate very strict environmental tolerances. However, the geographic range could also be actively expanding or contracting. Species distribution modeling uses the range of environmental conditions over which a species has been sampled and predicts the location of similar environmental conditions across the landscape. Therefore, these models provide information about both

where a species currently lives and where it could potentially live, giving our best estimation of ecogeographic isolation. However, this estimate relies on the assumption that the range of environmental conditions a species can occupy is well characterized by sampling known locations, and at best it provides an indirect glimpse into the physiological tolerances of species. Direct experimentation is therefore highly desirable to substantiate the hypotheses generated via species distribution modeling when possible.

Perhaps the most powerful tool for validating models is reciprocal-transplant studies; therefore, it is informative to compare previous transplant studies to the estimates of ecogeographic isolation obtained here. For example, *M. cardinalis* and *M. lewisii* inhabit distinct elevational distributions in the Sierra Nevada, with *M. lewisii* at high elevations and *M. cardinalis* at lower elevations (Hiesey et al. 1971). Reciprocal-transplant experiments demonstrate strong, geographically structured fitness variation between these species (Angert and Schemske 2005), with nearly complete selection against the foreign species. Using a gridded resampling technique to estimate co-occurrence, Ramsey et al. (2003) reported that ecogeographic isolation between these species was 0.587 but acknowledged this as a potentially large underestimate. The estimates of ecogeographic isolation for *M. lewisii* and *M. cardinalis* with regard to each other are 0.93 and 0.96, respectively, providing a value that is much more congruent with the reciprocal-transplant data.

However, ecogeographic isolation is inherently spatial, as it can be defined as the deviation from random mating that occurs between taxa because of broad-scale spatial separation of suitable habitat. Therefore, while reciprocal transplants can reveal the suitability of specific habitats, the approach does not typically provide much spatial information about where these habitats can be found in nature. In the *Mimulus* example provided above, *M. lewisii* has very low survival in the middle of *M. cardinalis* habitat, but that does not reveal where suitable habitat for *M. lewisii* ends and unsuitable habitat begins across the landscape. Both of these species live in riparian areas, and moving *M. lewisii* a few meters away from a constant water source would undoubtedly have a negative impact on fitness equivalent to or larger than moving it hundreds of miles away into *M. cardinalis* habitat. Therefore, in order to assess the impact of habitat-mediated fitness variation on gene flow, the spatial distribution of suitable habitat must be considered. Ultimately, combining distribution modeling with extensive transplant or growth chamber studies (e.g., Angert et al. 2011) that span suitable, marginal, and unsuitable conditions may provide the best opportunity to connect biological differences between taxa to variation in geographic ranges. However, the effort required to perform these experiments at what could be

staggering ranges of sites or environmental conditions will likely preclude their adoption as a general method of measuring ecogeographic isolation for more than a handful of species pairs. Therefore, overlap in species distribution models will likely provide the most attainable estimates of ecogeographic isolation, despite the drawbacks and caveats. Given the potential importance of this barrier, obtaining these estimates via distribution modeling is highly preferable to omitting the barrier as a potential mechanism of speciation.

#### *Ecogeographic Isolation and Immigrant Inviability*

Reciprocal transplants involving species or ecologically distinct populations commonly reveal fitness variation in nature (Linhart and Grant 1996; Kawecki and Ebert 2004; Nosil et al. 2005). Transplant experiments are probably rarely performed in instances where previous data are not predictive of this result (Harrison 2012); however, these data suggest that habitat-based fitness variation commonly structures the geographic ranges of taxa (Sexton et al. 2009). In addition to indicating the potential for ecogeographic isolation, significant fitness variation between taxa in transplants has also been taken as an indication of isolation via “immigrant inviability” (Nosil et al. 2005). However, for immigrant inviability to play a role in reproductive isolation, taxa from alternate habitats must first disperse into each other’s range. Therefore, the spatial arrangement of alternative suitable habitat and the average dispersal distance of the taxa under consideration determine the impact of immigrant inviability on patterns of gene flow (Mallet et al. 2009). Ecogeographic isolation and immigrant inviability may often result from the same traits, but they can be considered distinct because the former reduces the probability of encounter between taxa, while the latter may affect gene flow if and when encounters occur.

In the case of the *Mimulus* species presented here, the coarseness of the niche models used to estimate ecogeographic isolation (~1 km<sup>2</sup>; see fig. 4) likely subsumes the vast majority of dispersal events in these species. For example, dispersal distances in water-dispersed populations of *M. guttatus* have been estimated to average 275 m (Waser et al. 1982); this dispersal mechanism is also used by the species *M. cardinalis* and *M. lewisii*. Gravity/wind-dispersed populations of *M. guttatus* have a much smaller average dispersal range (0.25–4.75 m; Vickery et al. 1986), and the remaining species presented here share dispersal characteristics with these populations. Therefore, at the resolution of the distribution models presented, dispersal rates between alternate suitable habitats between species are likely negligible. However, while the estimates of habitat-based reproductive isolation presented are at a broad

ecogeographic scale, microspatial habitat isolation may also exist. For example, within the areas predicted to be suitable for both members of a species pair (purple regions in fig. 4), habitat-based spatial structuring presumably leads to additional reproductive isolation. Within these areas of potential sympatry, measures of microspatial isolation would require incorporation of dispersal dynamics and immigrant inviability to be effective.

### Conclusions

Closely related species in the genus *Mimulus* exhibit substantial variation in the geographic ranges they occupy. Species distribution modeling reveals variation in broad-scale distributions of suitable habitat in nature, providing an estimate of how habitat-based fitness variation translates into altered probabilities of hetero- and conspecific encounter rates across the distribution of focal taxa. From these models, it is revealed that ecogeographic isolation plays an important role in *Mimulus* speciation, and the traits involved in adaptation to habitat warrant future scrutiny as agents of divergence.

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Two species of *Mimulus* that experience strong ecogeographic isolation. *Mimulus angustatus* (left) inhabits lower elevations of the northern Sierra Nevada and Coast Range of California, while *Mimulus pulchellus* (right) is found at middle elevations of the central Sierra Nevada. Photo credit: James Sobel.