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NICHE AND RANGE SIZE PATTERNS SUGGEST THAT SPECIATION BEGINS IN SMALL, ECOLOGICALLY DIVERGED POPULATIONS IN NORTH AMERICAN MONKEYFLOWERS (*MIMULUS SPP.*)

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Closely related species (e.g., sister taxa) often occupy very different ecological niches and can exhibit large differences in geographic distributions despite their shared evolutionary history. Budding speciation is one process that may partially explain how differences in niche and distribution characteristics may rapidly evolve. Budding speciation is the process through which new species form as initially small colonizing populations that acquire reproductive isolation. This mode of species formation predicts that, at the time of speciation, sister species should have highly asymmetrical distributions. We tested this hypothesis in North American monkeyflowers, a diverse clade with a robust phylogeny, using data on geographical ranges, climate, and plant community attributes. We found that recently diverged sister pairs have highly asymmetrical ranges and niche breadths, relative to older sister pairs. Additionally, we found that sister species occupy distinct environmental niche positions, and that 80% of sister species have completely or partially overlapping distributions (i.e., are broadly sympatric). Together, these results suggest that budding speciation has occurred frequently in *Mimulus*, that it has likely taken place both inside the range and on the range periphery, and that observed divergences in habitat and resource use could be associated with speciation in small populations.

KEY WORDS: **Climate niche, isolation, *Mimulus*, natural selection, phylogenetic, reproductive, sister pairs.**

Closely related species often vary widely in their niche characteristics and geographic ranges (e.g., Warren et al. 2008; Evans et al. 2009; Dormann et al. 2010; Nakazato et al. 2010), contrary to the expectation that that they should be most similar (Darwin 1859; Peterson et al. 1999; Wiens 2004; Wiens and Graham 2005; Waldron 2007). Clues as to how such rapid niche divergence and disparity in distributions arise might be found in the history of speciation. For instance, such differences may arise during periods of geographic separation and subsequent reproductive isolation (Mayr 1942, 1947) and/or adaptation to different ecological conditions (Darwin 1859)—more recently referred to as "ecological speciation" (Schluter 2009; Sobel et al. 2010).

Many studies have focused on distinguishing modes of speciation that dictate the initial degree of geographic range overlap and potential gene flow during speciation (i.e., distinguishing allopatric, parapatric, and sympatric speciation; e.g., Barraclaugh and Vogler 2000; Fitzpatrick and Turelli 2006; Kisel and Barraclough 2010). However, relatively few studies have focused on understanding differences in the size of ecological niches and geographic ranges between closely related species. Mayr (1954) proposed "budding speciation" as a speciation mode

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by which an initially small colonizing population becomes reproductively isolated from the larger-ranged species. Alternatively, vicariant speciation describes scenarios in which a geographic barrier bisects a species' range, generating species pairs with variable asymmetries in range size, depending on the evenness of bisections (Mayr 1982; Barraclough and Vogler 2000; Losos and Glor 2003). In the former case, the resulting "budded" species will initially occupy a very small range (Lynch 1989; Barraclough and Vogler 2000; Fitzpatrick and Turelli 2006), and potentially a small realized niche due to correlations between niche breadth and range size (Brown 1984; Slatyer et al. 2013). In this vein, initially extreme differences in range size and realized niche breadths between close relatives are a unique prediction of budding speciation and could partially explain the wide variation in niche characteristics we see today among close relatives.

Budding speciation was proposed as a peripheral or internal range process (reviewed in Coyne and Orr 2004, p. 105). In the range periphery model, originally termed peripatric speciation by Mayr (1954) reduced gene flow between a large-ranged species and an allopatric population on its range periphery facilitates reproductive isolation. The range periphery model is thus considered a type of allopatric speciation (Losos and Glor 2003). For example, the island endemic *Drosophila sechellia* likely formed via a founder event and subsequent specialization on a fruit that is toxic to its widespread relatives from which it is nearly completely reproductively isolated (Lachaise et al. 1986; Jones 2005). In another example, a wildflower in California, *Clarkia lingulata*, likely formed when the ancestral species advanced northwards following climatic warming—leaving behind a small "rear-edge" population (sensu Hampe and Petit 2005) that subsequently became differentiated (Lewis 1973; Gottlieb 2004).

Alternatively to the range periphery model of budding speciation, when a small population colonizes a novel habitat nested within the ancestral range (i.e., is broadly geographically sympatric), habitat-mediated reproductive isolation and strong selection against immigrants can reduce gene flow and facilitate speciation (Mayr 1982; Barton and Charlesworth 1984; Dawson and Hamner 2005). In this vein, the geographically nested model may be more likely in highly heterogeneous landscapes and, if strong selection is necessary to maintain reproductive isolation, it follows that geographically nested sister species may have highly diverged niches to maintain reproduction isolation. For example, the California wildflower, *Layia discoidea* (Baldwin 2005), is locally adapted to a rare soil type to which it is narrowly restricted, and is nested within the range of its widespread, generalist sister species, *L. glandulosa* (Baldwin 2005).

The geographically nested model of budding speciation has not received much attention in the literature and has so far been discussed in the context of peripatric speciation. It was first proposed in the discussion of Mayr (1982), then again by Barton and Charlesworth (1984); Dawson and Hamner (2005), and discussed in relation to *Layia discoidea* (Baldwin 2005). Coyne and Orr (2004, p. 105) state that this model is unlikely to occur because "it is hard to imagine that a central population could remain geographically isolated long enough to become a new species."

The origin of reproductive isolation in small populations is a historically contentious issue (Mayr 1982; Barton and Charlesworth 1984; Coyne 1992, 1994). Isolation was initially thought to be driven by extreme founder effects and subsequent genetic drift (Mayr 1954); however, it is now widely believed that adaptation should underlie speciation in small populations inhabiting novel environments (Barton and Charlesworth 1984; Coyne 1992, 1994; Baldwin 2005; Dawson and Hamner 2005). If natural selection facilitates speciation, then newly formed small-ranged species should occupy a distinct realized niche when compared to their large-ranged sisters.

Current clade-wide evidence for budding speciation is equivocal, and based largely on examination of the predicted negative relationship between range size asymmetry and time since speciation. Examining several bird, fish, and insect clades, Barraclough and Vogler (2000) found greater range size asymmetry between younger nodes—a pattern consistent with budding speciation. In contrast, Fitzpatrick and Turelli (2006) did not detect a significant relationship between range size asymmetry and node age in five mammalian clades, suggesting that either budding speciation is rare or that fluctuations in range size eliminate its signal, especially at deeper nodes. Restricting analyses to recent speciation events (i.e., sister species comparisons) with differing relative divergence times may mitigate this concern. The few such studies that have relied on sister species comparisons have yielded mixed results. In Malagasy frogs, range sizes of sister species (both members) increased with time since speciation and range size asymmetry increased, suggesting budding speciation is not common in this group (Wollenberg et al. 2011). In contrast, in marine snails (Claremont et al. 2012), hermit crabs (Malay et al. 2010), and water beetles (Toussaint et al. 2013) most sister pairs have highly asymmetrical ranges suggestive of budding speciation followed by conservative changes in range. In summary, we are still at an early stage of understanding how common budding speciation may be in nature—studies vary in how range size asymmetry is calculated, in the phylogenetic depth examined, and are so far restricted to animal clades. We have yet to understand whether other taxonomic groups, such as plants, bear a footprint of budding speciation.

Here, we test for clade-wide evidence of budding speciation in the plant genus *Mimulus* in North America, we examine where such speciation might occur in species ranges, and we assess whether niche divergence is common. We address the following questions: (1) Do closely related species have more similar ecological niches and distributions than distantly related species?

Table 1. Phylogenetic signal (Blomberg's *K*) for range size and the first two axes of niche position and breadth across 900 North American *Mimulus* phylogenies sampled from the Bayesian posterior distribution of trees.

Niche attribute	Observed *K* value	*P*-Value H0: $K = 0$	*P*-Value H0: $K = 1$
Range size ln	0.04	0.761	<0.001
Niche position PC1	0.10	0.135	<0.001
Niche position PC2	0.10	0.152	<0.001
Niche breadth ln PC1	0.06	0.580	<0.001
Niche breadth ln PC2	0.06	0.490	<0.001

***K* = 1 is consistent with a Brownian motion model of trait evolution, whereas *K* = 0 is consistent with no phylogenetic signal. We report mean *K* and mean *P* values under two null hypotheses across all phylogenies.**

(2) Does the primary prediction of budding speciation hold, that is, is there greater range asymmetry for younger compared to older sister pairs? (3) Do sister species occupy distinct realized niches? (4) Based on relative age and range overlap, does budding speciation appear more likely to originate from range peripheries ("allopatric") or geographically nested areas ("sympatric")?

Methods

STUDY SYSTEM AND PHYLOGENY

The plant genus *Mimulus* contains about 120 described species and inhabits a wide variety of environments (Wu et al. 2008). Although it has a worldwide distribution, approximately 75% of the species are restricted to western North America. Species in this diverse genus display large differences in habitat affinity, range size, life history, pollinator guilds (Beardsley et al. 2004), and ability for autonomous self-pollination (Grossenbacher and Whittall 2011). Although different subclades of *Mimulus* vary in ploidy across the genus (Beardsley et al. 2004), most sister species (all but two pairs in the present study) have identical ploidy levels. Fruits are many-seeded, and dispersal of the tiny wingless seeds is largely passive. Nevertheless, long-distance seed dispersal (i.e., hundreds of meters) by water and animals can occur (Waser et al. 1982; Vickery et al. 1986).

We simultaneously estimated the phylogenetic relationships and relative divergence times among *Mimulus* species in a Bayesian framework in BEAST version 1.6.2 (Drummond et al. 2012) using the nuclear ribosomal internal and external transcribed spacer regions (ITS and ETS) and chloroplast *trn*L-F region of Beardsley et al. (2004) with near-complete taxon sampling for North America ($N = 114$ species). See Supporting Information for detailed description of methods and associated files.

CHARACTERIZING GEOGRAPHIC RANGES AND ENVIRONMENTAL NICHES USING OCCURRENCE RECORDS

We obtained North American occurrence records from the Global Biodiversity Information Facility (http://www.gbif.org) for 68 species and filtered for quality excluding records with missing coordinates, coordinates failing to match the locality description, coordinate error greater than 1 km, and taxonomic misidentifications (verified by the authors and other regional botanists). When within species' occurrence localities were within 1 km of one another, localities were deleted at random to restrict observations to one record per environmental grid cell. The average number of occurrences per species was 100 ($\pm$SE = 30.0, maximum = 1760, minimum = 6). Range size for each species was calculated from the area (km^2) inside minimum convex polygons that contained all occurrence records using ArcGIS 9.2.

To estimate the environmental niche of each species, we obtained 19 variables representing aspects of temperature and precipitation (http://www.worldclim.org/); five variables representing elevation, aspect, slope, and hydrologic regimes (http://eros.usgs.gov/products/elevation/gtopo30/hydro/namerica.html); and three variables representing vegetation cover (www.landcover.org). See Table S1 for a description of each variable. All data were projected into an Albers equal area projection and resampled to a 1 km $\times$ 1 km grid cell size.

Realized niche position and niche breadth of each species was estimated by circumscribing each species' occurrence-based niche relative to all possible niche space across North America using the "PCA-env" ordination technique (Broennimann et al. 2012). Here, the dimensions of the environmental space for North America were reduced to the first and second axes from a principal components analysis (PCA). The PCA of 23 environmental variables was constructed using 10,000 random points sampled from the background environmental space, that is, North America. We then created a grid with 100 $\times$ 100 PCA unit grid cells and used the species presence data to project the density of each species into environmental space using a kernel density function (Broennimann et al. 2012). Niche position and niche breadth for each species is estimated as the mean and variance, respectively, of the PCA axes 1 and 2 scores.

DO CLOSELY RELATED SPECIES HAVE SIMILAR DISTRIBUTIONS AND ECOLOGICAL NICHES COMPARED TO MORE DISTANTLY RELATED SPECIES?

To address this question, we estimated the degree of phylogenetic signal, or dependence of species attribute values on phylogenetic

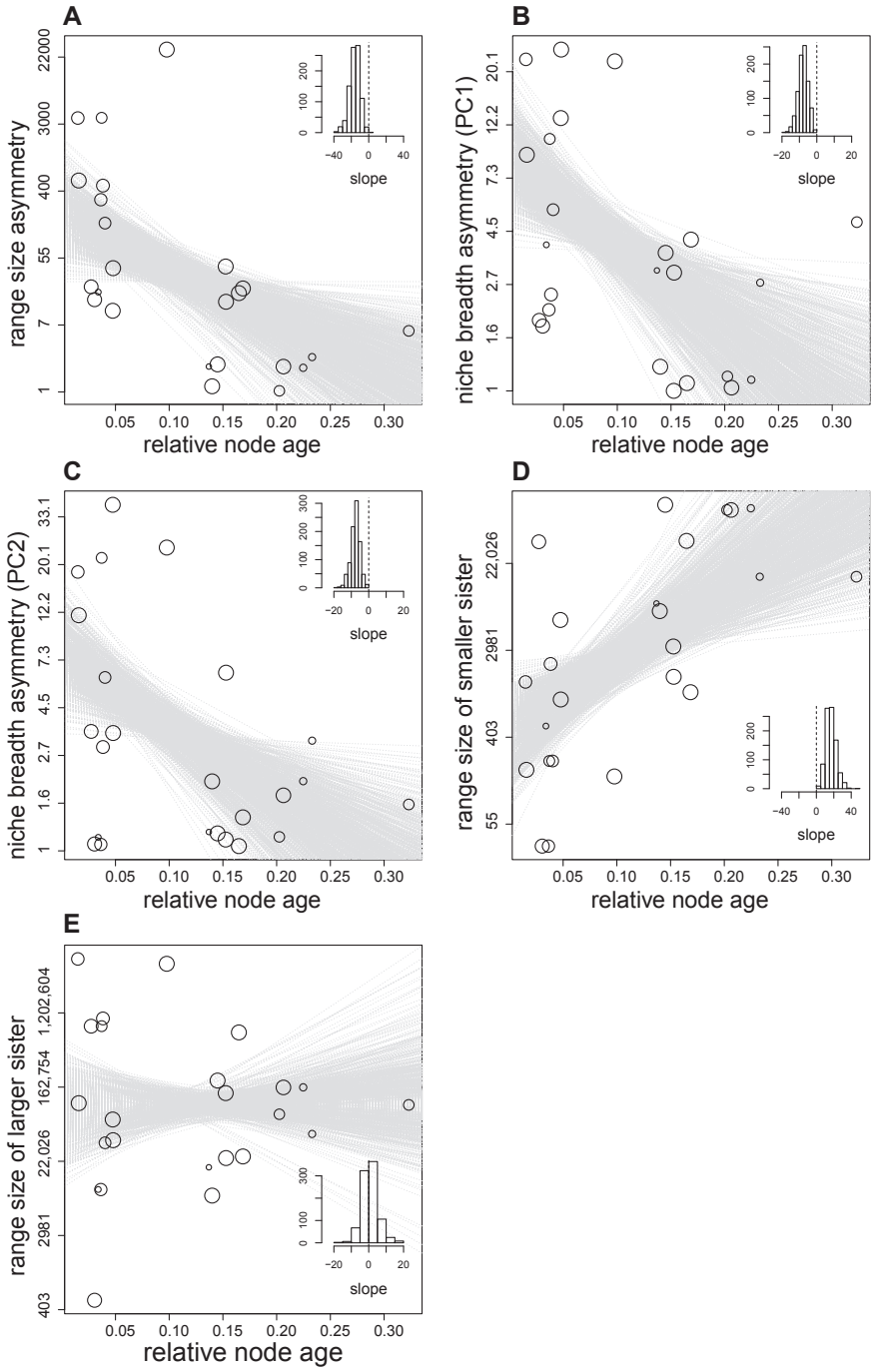

Figure 1. ***Mimulus*** **sister pair asymmetry in range size (A), asymmetry in niche breadth (B and C), and range size of the smaller and larger ranged sister (D and E), and as a function of relative node age (time since speciation). Asymmetry is measured as the log of the larger divided by the smaller ranged sister species. *Y*-axis is natural logarithmic scale (back-transformed kilometer square). The size of each open circle represents the proportion of phylogenetic trees (from a sample of 900 trees from the Bayesian posterior distribution) containing that sister pair. Gray dashed lines represent linear regression lines from each of the 900 sister pair datasets; histograms in the upper or lower right corner are of the 900 slopes.**

relatedness, using Blomberg's K (Blomberg et al. 2003) for the following attributes: ln range size, niche position, and ln niche breadth (niche position and breadth were described by environmental PC1 and PC2, see above). Blomberg's K ranges from 0 to infinity; higher values indicate a greater degree of phylogenetic signal. Values of $K = 1$ are expected for data that follow a Brownian motion model of character evolution, whereas 0 represents no phylogenetic signal. We performed simulations to test

Table 2. Tests of range size asymmetry, relative age, and absolute ecological divergence (Niche position PC1 and PC2) between nested and nonnested sister pairs.

	ln range size asymmetry			Relative age			Niche position PC1			Niche position PC2		
Definition of nested vs. nonnested	Mean nested	Mean non-nested	t	Mean nested	Mean non-nested	t	Mean nested	Mean non-nested	t	Mean nested	Mean non-nested	t
1.0 ($N = 9$) vs. <1.0 ($N = 15$)	5.56	2.23	3.14^{**}	0.05	0.15	-3.74^{***}	0.61	0.81	−0.75	0.62	0.84	−0.82
<0.95 ($N = 13$) vs. <0.95 ($N = 11$)	4.58	2.18	2.40^{*}	0.10	0.13	−1.07	0.73	0.74	−0.02	0.68	0.85	−0.54
>0.85 ($N = 14$) vs. <0.85 ($N = 10$)	4.46	2.11	2.40^{*}	0.10	0.13	−0.83	0.73	0.75	−0.09	0.69	0.87	−0.56
>0.7 ($N = 14$) vs. <0.7 ($N = 10$)	4.46	2.11	2.40^{*}	0.10	0.13	−0.83	0.73	0.75	−0.09	0.69	0.87	−0.56
>0.4 ($N = 14$) vs. <0.4 ($N = 10$)	4.46	2.11	2.40^{*}	0.10	0.13	−0.83	0.73	0.75	−0.09	0.69	0.87	−0.56
0 ($N = 5$) vs. <0 ($N = 19$)	3.69	2.68	0.89	0.12	0.10	0.322	0.76	0.64	0.54	0.65	1.19	−0.93

Nested versus nonnested sister pair categories were defined in six ways according to the degree of nestedness (ranging from 0 to 1 and calculated as the ln area of geographic range overlap/the ln area of the smaller ranged sister). Welch two-sample, two-tailed *t*-test statistics are listed.
*****P < 0.001, **P < 0.01, *P < 0.05.**

the null hypothesis of $K = 1$ by comparing the observed value of K with 1000 K values measured on characters simulated under perfect Brownian motion (i.e., $K = 1$). We used a two-tailed test of significance, recording the fraction, f, of simulated datasets with smaller K values than the observed data and calculating P values as $2 \times$ minimum (f, $1 - f$). Because K for all attributes was significantly less than 1, we also tested the null hypothesis of no phylogenetic signal, that is, $K = 0$, using the R phytools package. Phylogenetic uncertainty was taken into account by performing these tests on a sample of trees from the posterior distribution ($N = 900$) pruned to just the 68 species in North America for which we were able to obtain occurrence records.

DOES THE PRIMARY PREDICTION OF PERIPATRIC SPECIATION HOLD FOR MIMULUS, THAT IS, IS THERE GREATER RANGE SIZE AND NICHE BREADTH ASYMMETRY FOR YOUNGER COMPARED TO OLDER SISTER PAIRS?

To address this question, we restricted our analysis to sister species pairs. Range size asymmetry was calculated as the area of the larger range divided by the area of the smaller ranged species (Fitzpatrick and Turrelli 2006). Niche breadth asymmetry for PC1 and PC2 was calculated in the same manner (niche breadth of the larger ranged species divided by the niche breadth of the smaller ranged species). We excluded the species from Mimulus section Erythranthe because this section is poorly resolved with the three-gene dataset used in this study (Beardsley et al. 2003). We note however that this exclusion does not change the qualitative relationship between relative divergence time and niche attribute divergence (not shown).

This analysis was a two-step process. First, we extracted all sister species pairs from the phylogeny, their corresponding relative divergence times, range size and niche values when available, and calculated range size and niche breadth asymmetries as described above. Second, we performed standard linear regressions separately on each of five attributes by relative divergence time (range size asymmetry, niche breadth asymmetry for PC1 and PC2, and the area of the smaller ranged and larger ranged species separately), recording the observed slope. All attributes were log transformed prior to analysis. Significance was determined using a permutation test in which we randomly reassigned range size and niche breadth parameters from across the phylogeny to sister species and repeated steps 1 and 2 to generate null distributions for model slope and intercept ($N = 1000$). P values were estimated as the fraction of randomized datasets with smaller slopes than the observed data. To account for phylogenetic uncertainty, we repeated these tests using a sample of 900 trees from the Bayesian posterior distribution. These analyses were

performed in R version 2.14.1 (see supplemental material for code).

DO SISTER SPECIES OCCUPY DISTINCT REALIZED NICHES?

To test whether sister species occupy distinct realized niches, we used multivariate analysis of variance (MANOVA; JMP Version 10.0.0, SAS Institute, 2012) to test for environmental differences between localities occupied by sister species. Environmental variables used in the environmental niche overlap analysis were analyzed simultaneously for each set of sister taxa. Variables were log or square-root transformed when necessary to improve normality, or rank transformed (Conover and Iman 1981), when other transformations failed to improve model fit. We used discriminant analysis to identify the three best discriminating variables for each sister-species pair.

WHICH GEOGRAPHIC MODE OF BUDDING SPECIATION APPEARS MOST LIKELY, PERIPHERAL (ALLOPATRIC) OR NESTED (SYMPATRIC)?

To discriminate between two models of budding speciation (allopatric speciation on the range periphery vs. geographically nested, sympatric speciation), we compared range size asymmetry and relative node age for sister pairs that have a nested versus nonnested distribution (Welch's two-sample *t*-test, two-tailed). If the range periphery model is common, sister pairs with nonnested distributions should be younger and have greater range asymmetry than nested pairs. In contrast, if the geographically nested, sympatric model is more prevalent, then nested pairs should be youngest and have the greatest range asymmetry. Additionally, we tested whether ecological divergence (absolute difference in niche position on PC1 and PC2) was greater for sister species that are nested and broadly sympatric (consistent with predictions to maintain reproductive isolation) than sister species that originated peripherally.

The degree of nestedness ranged from 0 to 1 and was calculated as the area of geographic range overlap/the area of the smaller ranged sister. To determine whether our results were sensitive to the definition of "nested," we defined sister pairs as nested versus nonnested in six ways according to the degree of nestedness: 1.0 versus <1.0, >0.95 versus <0.95, >0.85 versus <0.85, >0.7 versus <0.7, >0.4 versus <0.4, and 0 versus >0.

Results

PRINCIPLE COMPONENT ANALYSIS OF ENVIRONMENTAL VARIABLES

Environmental PC1 and PC2 axes accounted for 30.1 and 24.3% of the habitat variation in the study region, respectively (Fig. S1). PC1 captured variation in precipitation and vegetation cover—high values were associated with high precipitation and tree cover, and low values were associated with high diurnal temperature range and bare ground (Table S1; Fig. S1). Orthogonally, PC2 described variation related to temperature seasonality—high values of axis 2 were associated with temperature range and temperature seasonality (SD × 100), and low values were associated with high minimum temperature in the coldest month, high average temperature in the warmest quarter, and high mean temperature in the coldest quarter.

Closely related species do not have more similar distributions and ecological niches compared to more distantly related species. Phylogenetic signal was less than expected under a Brownian motion model of trait evolution for all species' attributes in this study, including range size, niche breadth, and niche position (in all cases $K < 1$, $P = 0.001$). In fact, none of the attributes exhibited significant phylogenetic signal, and so we cannot reject the null hypothesis that $K = 0$. Table 1 summarizes across a sample of 900 trees from the posterior distribution reporting the mean observed K and the mean P-value for the two null hypotheses (K is different from 1, and K is different from 0).

The primary prediction of budding speciation holds for Mimulus—there is greater range size and niche breadth asymmetry for younger compared to older sister pairs. Asymmetry in both range size and niche breadth decreased with relative age of sister species, a pattern robust to phylogenic uncertainty (mean P-value across phylogenies ≤ 0.051 for range size asymmetry, and $P < 0.050$ for niche breadth asymmetry; Figs. 1A–C and 2). On average, range size asymmetry was 284:1 at speciation (mean intercept of the regression across all phylogenies). Additionally, by assessing the smallest ranged member of each sister pair in isolation, we found that the sister with the smaller of the two range sizes increased in range size with increasing time since speciation (this pattern was consistent across phylogenies; mean P-value across phylogenies < 0.050; Fig. 1D). In contrast, range size of the sister with the larger of the two range sizes did not co-vary with time since speciation (this pattern was consistent across phylogenies; mean P-value across phylogenies $= 0.528$; Fig. 1E).

Sister species tend to occupy distinct realized niches. All sister species that could be analyzed occupied different environments, the exception being *Mimulus ampliatus–Mimulus hymenophyllis, Mimulus jungermanioide–Mimulus washingtonensis, Mimulus patulus–M. ampliatus, and M. patulus-hymenophyllus* sister pairs, in which limited ranges left us with too few degrees of freedom to perform the MANOVAs (Table S2). Variables contributing most to species-level environmental differences varied, but several were relatively prominent (present in the top-three list of discriminating

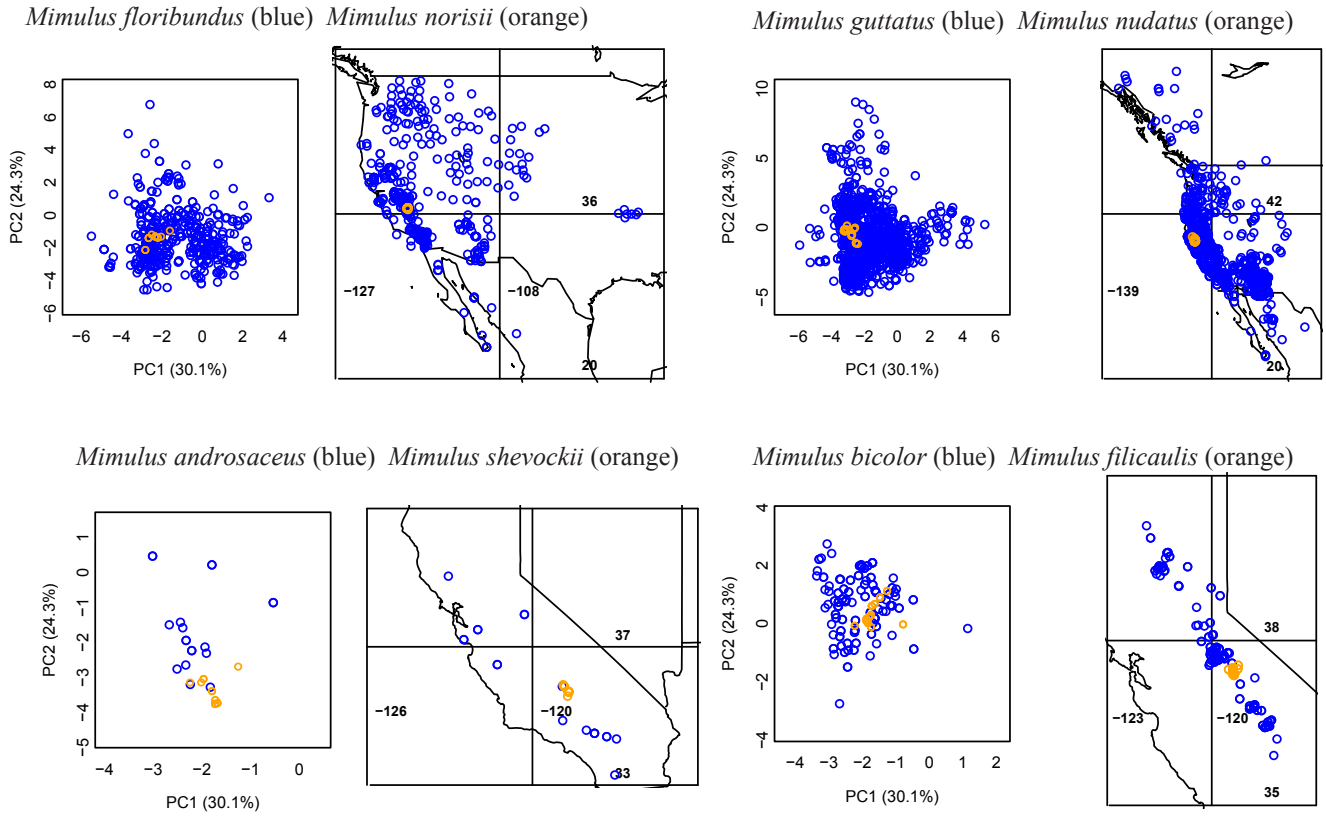

Figure 2. **Four of the most asymmetrical sister pairs sampled in the posterior distribution of trees. Left panels: sister pair position in environmental PC space. Right panels: sister pair position in geographic space.**

variables for >21% of sister pairs): minimum temperature of coldest month, precipitation of driest quarter, precipitation of wettest month, mean temperature of coldest month, and precipitation of coldest quarter.

No single geographic mode of budding speciation, peripheral (allopatric) or nested (sympatric), predominates. Of the 24 potential sister species pairs, 38% were completely nested species distributions (nestedness = 1.0), 42% were partially nested, and 21% were completely nonnested. For sister pairs that had a nested versus nonnested distribution, range size asymmetry and relative node age differed depending on the definition of "nested" (defined in six ways according to the degree of nestedness: 1, >0.95, >0.85, >0.7, >0.4, or >0), whereas ecological divergence did not differ. Nested pairs had significantly greater range asymmetry compared to nonnested sister pairs when "nested" was defined as >0.4 (Table 2). Nested pairs were significantly younger than nonnested sister pairs only when nested was defined as 1.0 (Table 2). Finally, nested sister pairs did not significantly vary in their degree of ecological divergence compared to nonnested sister pairs; this result was qualitatively similar for both niche position on PC1 and PC2, and under all definitions of "nested" (Table 2).

Discussion

We find that the most recently formed sister species of North American monkeyflowers (*Mimulus*) differ most in their geographic range size, that sister species differ in their realized environmental niche, and that most sister species have partial to wholly nested distributions (i.e., are broadly sympatric). These patterns suggest that speciation often begins in small, ecologically diverged populations, that is, ecological speciation between a small population and a larger population, or set of populations, and are inconsistent with the expectation of environmental niche conservatism (Peterson et al. 1999; Wiens 2004; Wiens and Graham 2005). Moreover, these "budding" speciation events may occur nested within the range, peripherally near range limits, or beyond the range of the large-ranged sister taxon. Thus within a single clade, speciation may occur sympatrically or allopatrically according to the classic, geographic definitions of speciation. These results suggest that the nature of species' current distributions (e.g., range size, niche breadth, and niche position) is influenced not only by contemporary ecological factors, but also by evolutionary history, that is, the conditions under which speciation occurred. We discuss the evolutionary, biogeographic, and conservation implications of these findings.

How common is budding speciation across the tree of life? It appears to be a repeated mode of speciation in *Mimulus* and a recent study has found it to be potentially common in additional plant clades inhabiting the California Floristic Province (Anacker and Strauss 2014). However, previous studies assessing budding speciation across a wide range of taxa have found mixed results (Barraclaugh and Vougler 2000; Fitzpatrick and Turelli 2006; Malay et al. 2010, Wollenberg et al. 2011; Claremont et al. 2012; Rovito et al. 2012). There are several possible reasons for this discrepancy. First, most previous studies have not focused on sister taxa (but see Malay et al. 2010; Wollenberg et al. 2011), possibly obscuring the relevant signature between closest relatives (see intro). Second, budding speciation might be more common in certain taxonomic groups that are underrepresented in previous studies, such as those with low dispersal rates or in sessile organisms with highly locally adapted populations. Third, budding speciation might be more common in certain regions or ecological contexts, such as complex, heterogeneous landscapes. Finally, previous studies have mostly looked for budding in peripheral populations (peripatric speciation in the traditional sense, Mayr 1954), and thus have overlooked potential budding in geographically nested or central locations.

The finding that the majority (80%) of sister pairs have partial to completely overlapping distributions leads to the larger question: how would internal populations remain reproductively isolated long enough to form new species? This has been the classic problem of sympatric speciation (Coyne and Orr 2004, p. 105), yet speciation research has recently witnessed rapid changes, offering some new interpretations and solutions (Harrison 2012). Part of this shift has occurred through a reemphasis on gene flow rather than geography alone (e.g., "speciation-with-gene-flow;" reviewed in Smadja and Butlin 2011), and speciation with gene flow has been increasingly acknowledged as possible, a significant shift from when it was once strongly opposed by Mayr (Harrison 2012; Santini et al. 2012). Further, drift was once a major mechanism in Mayr's allopatric speciation model, but attention has been drawn more recently to the role of divergence under selection making speciation in sympatry seem more feasible (Santini et al. 2012).

Examples of what would classically be considered sympatric speciation continue to emerge and are largely attributed to ecological speciation in the presence of gene flow. There are many mechanisms by which nearby populations may diverge, including environment-driven nonrandom mating (Bird et al. 2012). Genetic isolation by environment or ecology (IBE) has been found to be more common in nature than isolation by distance (Sexton et al. in press) and a general signal of IBE has been invoked as evidence for widespread ecological speciation (Shafer and Wolf 2013). For example, Moyle et al. (2012) found postzygotic mating barriers between soil ecotypes of the plant *Collinsia sparsiflora* at small spatial scales (100 to 1000 m). A recent study on Lord Howe Island suggests that sympatric speciation (through ecological divergence with gene flow) has been common in some plant groups there (Papadopulos et al. 2011). Additionally, Bolnick and Fitzpatrick (2007) list a variety of taxa that have satisfied sympatric models, most of which display some level of ecological divergence.

It is known that ecological divergence in the presence of gene flow is common, but we often do not know the details of how divergence initially took place. Speciation with gene flow may occur in many contexts, and geographic (spatially overlapping) and population genetic definitions (interbreeding) may not always agree, leading to different conclusions on whether sympatry is met or not and calling into question the use of this term (Bird et al. 2012). Kisel and Barraclough (2010) found that speciation is more likely to occur within larger geographic range areas and with lower gene flow, but when speciation-with-gene-flow does occur, it is considered to be more likely when (1) few traits are responsible for speciation and (2) there are strong trait associations (Smadja and Butlin 2011). Despite new terminology to depict speciation processes, many of the underlying principles, namely that ecology often has divergent effects with various underlying genetic causes and consequences, remain the same as in classic Dobzhansky and Mayr literature (Harrison 2012).

The geographically nested *Mimulus* sister species represent a broad geographic definition of sympatry, but likely inhabit a continuum of local overlap and potential gene flow. The topographically and ecologically complex landscape of North America likely results in barriers to dispersal, selection against migrant genotypes, or habitat-mediated reproductive isolation, all of which may cause differentiation and speciation in *Mimulus*. Here, we found that *Mimulus* sister pairs occur in different realized "macro"-niches (at the 1 km scale), indicating there is potential to reduce gene flow along climate gradients. However, "micro"-niche divergence (i.e., local-scale divergence of resource use) such as local adaptation to rare substrates and microclimates (unmeasured in the present study) likely acts to reduce gene flow and increase reproductive isolation.

If adaptation to local-scale environments has also caused increased reproductive isolation (e.g., see McNeilly and Antonovics 1968; Savolainen et al. 2006), this could facilitate speciation, particularly in geographically nested populations. In fact, for two of the youngest, geographically nested sister pairs, the small-ranged member is restricted to rare substrates—*M. nudatus* only occurs on serpentine soil; *M. norrisii* is only on limestone outcrops. Similarly, *M. filicifolilus*, *M. lacinatus*, and *M. nasutus* (not included in the present study because of missing genetic data) are likely offshoots of the large-ranged *M. guttatus* and also fit a model of geographically nested budding speciation (Fenster and Ritland 1994; Sweigart and Willis 2003; Modliszewski and Willis 2012;

Sexton et al. 2013). All three putative offshoot species occupy different local niches from *M. guttatus*, and *M. laciniatus* and *M. nasutus* have been shown to have greater drought avoidance, achieved through faster reproductive development and increased selfing (Hall and Willis 2006; Martin and Willis 2007; Peterson et al. 2013). These traits in turn contribute to reproductive isolation from locally occurring *M. guttatus* (Martin and Willis 2007; Peterson et al. 2013). Although autogamous species such as these are sometimes considered to be evolutionary "dead ends" (Stebbins 1957; Takebayashi and Morrell 2001), they illustrate how speciation may occur in close proximity under traditionally "sympatric" conditions.

A caveat to consider is that increased gene flow via hybridization in sympatric regions has depressed the genetic distance, making nested sister pairs appear younger than they actually are. For example, increased postspeciation hybridization could underlie the result that sister pairs with completely nested distributions (nestedness = 1.0) were significantly younger than nonnested sister pairs (nestedness 0 to 0.99). Two lines of reasoning can largely exclude this possibility. First, only one of nine completely nested sister pairs in this study is known to hybridize in nature despite extensive field and greenhouse studies (Vickery 1956, 1966; Grossenbacher, pers. obs.; Schoenig, pers. comm.) Second, the low degree of discordance among gene regions from different genomes (in only 1 of 115 taxa; Beardsley 2004) is consistent with minimal hybridization after speciation.

Concluding Remarks

Examining species ranges and ecology in a phylogenetic context can give clues to the evolution of, and variation in, distribution, and the realized ecological niche. We found that closest relatives in the clade *Mimulus* have extreme differences in range size and aspects of their realized ecological niche. This pattern is consistent with budding speciation, a process that could mask or eliminate effects of niche conservatism, even at a broad environmental scale. In *Mimulus*, budding speciation may often occur within, near, or outside the range of the ancestral species and this observation highlights the importance of population-level variation.

Budding speciation could potentially be a common phenomenon, yet recently evolved species might be particularly vulnerable to extinction given their small initial geographic range sizes (Collen et al. 2006; Walker et al. 2006; Pigot et al. 2012). Small-ranged sister species may go extinct if their populations stay small or may be reabsorbed into their large-ranged sister species (Rosenblum et al. 2012). Nevertheless, wherever there are small, ecologically novel populations, there is the potential for niche evolution and new speciation events, thus reinforcing populations as highly important conservation units. Measuring environmental tolerances of and testing for signatures of population expansion/contraction (e.g., using population genomics) in young, small-ranged species are important future avenues for understanding the processes by which species begin, disperse, and differentiate.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. List of 27 environmental variables used to estimate environmental niches of each species.
Table S2. MANOVA analyses of environmental variables for each potential pair of sister taxa.
Figure S1. Principle component axis 1 and 2 loading scores.
Figure S2. Sister pair position in environmental PC space (left panel) and geographic space (right panel) for all sister pairs from the 900 trees sampled from the posterior distribution of trees.