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Competition drives trait evolution and character displacement between *Mimulus* species along an environmental gradient

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Closely related species may evolve to coexist stably in sympatry through niche differentiation driven by in situ competition, a process termed character displacement. Alternatively, past evolution in allopatry may have already sufficiently reduced niche overlap to permit establishment in sympatry, a process called ecological sorting. The relative importance of each process to niche differentiation is contentious even though they are not mutually exclusive and are both mediated via multivariate trait evolution. We explore how competition has impacted niche differentiation in two monkeyflowers, *Mimulus alsinoides* and *M. guttatus*, which often co-occur. Through field observations, common gardens, and competition experiments, we demonstrate that *M. alsinoides* is restricted to marginal habitats in sympatry and that the impacts of character displacement on niche differentiation are complex. Competition with *M. guttatus* alters selection gradients and has favored taller *M. alsinoides* with earlier seasonal flowering at low elevation and floral shape divergence at high elevation. However, no trait exhibits the pattern typically associated with character displacement, higher divergence between species in sympatry than allopatry. Thus, although character displacement was unlikely the process driving initial divergence along niche axes necessary for coexistence, we conclude that competition in sympatry has likely driven trait evolution along additional niche axes.

KEY WORDS: character displacement, coexistence, ecological sorting, interspecific competition, *Mimulus alsinoides* (chickweed monkeyflower), *Mimulus guttatus* (common monkeyflower), niche partitioning, phenology, photoperiodism.

For competing species to stably coexist in sympatry, they must occupy sufficiently different niches that partition the habitat and its associated resources, allowing all taxa to achieve positive growth rates at low densities (Gause 1934; MacArthur 1970; Chesson 2000; Chase and Leibold 2003). Although the niches of distantly related species are often divergent enough to permit coexistence, the survival and reproduction of recently diverged species are more likely to remain contingent on similar sets of predators, mutualists, and abiotic requirements (Losos 2008). Yet, congeners often belong to the same communities (Webb 2000). Consequently, their niches must be sufficiently differentiated to permit stable coexistence, or the species must disperse frequently enough to recolonize the same sites repeatedly (Levine and Rees 2002).

Two different mechanisms have been proposed to explain niche differentiation of closely related species in sympatry (Connell 1980). First, species may have evolved preexisting trait differences in allopatry that allow coexistence upon secondary contact, a process termed ecological sorting (or ecological filtering; MacArthur 1970; Janzen 1985; Ackerly 2003). Ecological sorting does not require adaptive trait evolution or niche differentiation of either species in sympatry; species have already evolved different advantages in allopatry that permit habitat partitioning following secondary contact. If ecological sorting is the primary mechanism of niche divergence, there should be similar divergence between trait distributions both in sympatry and in geographically proximate allopatric populations (Fig. 1, Scenario I).
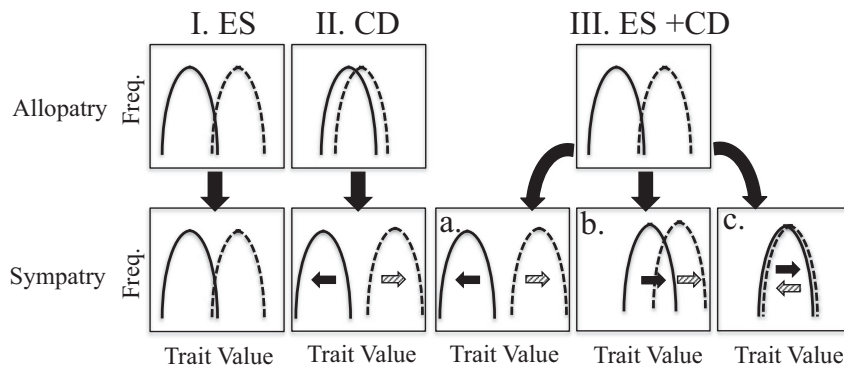
Figure 1. Predicted trait distribution outcomes in sympatry and allopatry of mechanisms leading to niche differentiation. Arrows indicate the direction of selection for each species in sympatry relative to allopatry. If ecological sorting (I. ES) drives niche differentiation, trait distributions for traits associated with essential niche axes for coexistence are predicted to be nonoverlapping both in sympatry and allopatry as no evolution has occurred. Character displacement, competition-mediated trait evolution, can create multiple different patterns of trait evolution in sympatry relative to allopatry. Divergent character displacement (II. CD) leads to greater trait divergence in sympatry than allopatry, and can create niche differentiation along essential niche axes for coexistence. Character displacement can lead to alternative patterns if ecological sorting or divergent character displacement has already created niche divergence between species and the species still compete for resources not essential for coexistence (III. ES + CD). In this scenario, trait evolution can be in any direction (a., b., or c.), but must occur consistently across multiple populations to demonstrate the role of competition. Traits involved in stabilizing niche differences in scenario III will still reflect patterns associated with either ecological sorting (scenario I) or character displacement (scenario II).

Second, interspecific competition between co-occurring species may lead to trait evolution in situ at a particular location following secondary contact, a process known as character displacement (Lack 1947; Crozier 1974; Schluter 2000). Character displacement can result in three different patterns of trait evolution in sympatry termed divergent character displacement, parallel character displacement, and mutually convergent character displacement (Abrams 1986; Fig. 1, Scenario II and III). The discussion of character displacement in the literature has almost exclusively focused on processes that generate patterns of divergent character displacement, where there is increased trait divergence between competing species in sympatry relative to divergence between allopatric populations (Fig. 1, Scenario II; Lack 1947; Brown and Wilson 1956; Pfennig and Pfennig 2009). Divergent character displacement can occur with or without previous niche differentiation through ecological sorting (Scenarios II and IIIa). Because several processes may produce this pattern (Grant 1972; den Boer 1986; Dayan and Simberloff 2005), the prevalence of divergent character displacement in natural populations has been highly contentious (Schluter 2000; Dayan and Simberloff 2005; Stuart and Losos 2013). However, examples in *Plethodon* salamanders (Adams and Rohlf 2000), *Phlox* (Levin 1985), Darwin's finches (Grant and Grant 2006, 2010), sticklebacks (Schluter and McPhail 1992), and spadefoot toads (Pfennig and Murphy 2000; Pfennig and Martin 2009, 2010) that meet explicitly defined criteria (Schluter and McPhail 1992; Losos 2000; Beans 2014) or that directly demonstrate selection for character

displacement in nature indicate divergent character displacement is a prominent means of niche differentiation.

That character displacement can also produce alternative types of shifts in trait distributions in sympatry relative to allopatry has been less appreciated (Grant 1972; Abrams 1986, 1987; Fox and Vasseur 2008). When divergent character displacement or ecological sorting results in effective resource partitioning along one or more niche axes necessary for stable coexistence, divergent character displacement need not necessarily occur along other niche axes where species still compete for additional resources not required for niche stabilization (Grant 1972; Abrams 1986, 1987; Fox and Vasseur 2008). Instead, competition can produce patterns of parallel character displacement, where trait means shift in the same direction in both species in sympatry relative to allopatry (Abrams 1986; Fig. 1, Scenario IIIa) or mutually convergent character displacement, where trait means evolve to be more similar in sympatry than allopatry (Abrams 1986; Fig. 1, Scenario IIIb). How competition can lead to these additional patterns is less clear, but is likely a consequence of species competing, often asymmetrically, along multiple niche axes for resources that affect relative fitness differences between species and/or niche stabilization. For instance, although two plant species have diverged to use two different microhabitats and associated resources within a population, they may still compete for same pollinator(s), and this competition may drive selection that leads to parallel character displacement or mutually convergent character displacement in traits promoting pollinator attraction. Additionally, the strength of selection by abiotic selective forces may be greater than and

opposite to the strength of selection by competition on trait evolution, driving parallel trait evolution in both species (Hille Ris Lambers et al. 2012). Determining which niche axes are related to niche stabilization for coexistence and which contribute to additional aspects of interspecific competition depends on the order of trait divergence, the relative strength of selection exerted by competition on particular traits, and the nature of available standing genetic variation.

In this framework, character displacement and ecological sorting are not mutually exclusive mechanisms of niche evolution. Even when niche differentiation has initially occurred in allopatry (i.e., ecological sorting), further competition between species in situ may drive ongoing evolutionary changes in phenotype and niche breadth (Fig. 1, Scenario III). Distinguishing between divergent character displacement alone (Fig. 1, Scenario II) and ecological sorting plus character displacement (Fig. 1, Scenario III) requires assessment of divergence between traits in allopatric populations; if only divergent character displacement has occurred, trait distributions should be largely overlapping in allopatry.

Assessment of the contributions of character displacement or ecological sorting to niche evolution via comparisons of sympatric and allopatric populations is complicated by spatial heterogeneity across landscapes (Goldberg and Lande 2006; Adams and Collyer 2007). Sympatric and allopatric populations of a species may differ not only in the presence of a competitor, but also in other abiotic or biotic factors. One solution is using replication among natural populations as a statistical framework for assessing how a competing species impacts a focal species (Adams and Collyer 2007). Spatial variation in additional relevant ecological factors can be accounted for by sampling paired sympatric and allopatric populations across an ecological gradient and including the gradient as a covariate in models that detect trait evolution in sympatry. Indeed, this approach has been applied successfully in tests of character displacement (Adams and Collyer 2007; Adams 2010). An added benefit of this approach is the ability to assess whether the same traits are associated with niche differentiation across environmental contexts (Rundle et al. 2003; Rice et al. 2009). For instance, harsher conditions could present additional constraints that prevent further differentiation along particular niche axes or that cause differentiation to occur through evolutionary responses by different traits in different regions.

Plant taxa are excellent systems for investigating the evolution of niche differentiation because plants are sessile, facilitating common garden studies and manipulative experiments (Beans 2014). We focus here on two *Mimulus* species that often co-occur in the Pacific Northwest. *Mimulus guttatus*, the common monkeyflower, is a model species for evolutionary and ecological genetics that occurs from northern Mexico to central Alaska (Vickery 1978; Wu et al. 2008). Across this range, *M. guttatus* exhibits extensive morphological, phenological, and physiological

diversity (Friedman et al. 2015; Kooyers et al. 2015; Kooyers et al., 2017). In the Pacific Northwest, annual populations of *M. guttatus* often co-occur with *M. alsinoides*, the chickweed monkeyflower, amidst inland seeps on rock walls and thin-soiled meadows with seasonally ephemeral water supplies. Both taxa inhabit a similar elevation range, spanning from locations just inland from the coast (∼0 m) to subalpine elevations (>2000 m) in this region. Although several other plant species live on these rock walls, *M. guttatus* and *M. alsinoides* localize to a relatively unique subset of the habitat with few other abundant competitors (Kooyers, pers. obs.). These two species are often interspersed in the same moss patches on rock walls, and seep water may disperse seeds and seedlings of both species into patches where mature plants of only one of the species is established. Thus, the species are hypothesized to compete directly in their growth habitats, and by proximity they also share the same community of potential pollinators. Even though populations of both taxa in this region consist of small, yellow monkeyflowers, these two species belong to sister sections of the genus *Mimulus* (Beardsley et al. 2004; Fig. 2A and B; now *Erythranthe*) and are isolated by significant reproductive barriers. In contrast to self-compatible but primarily outcrossing *M. guttatus* and although its flowers are always chasmogamous, *M. alsinoides* is thought to be a primarily selfing species. *Mimulus alsinoides* also has a more rapid life cycle than *M. guttatus* (Kooyers, pers. obs.). We hypothesize these two species compete where they co-occur as they occupy nearly all of a limited habitat, have similar abiotic requirements, overlap in growth season, and have similar floral displays.

Here, we examine the roles of character displacement and ecological sorting in niche differentiation of *M. alsinoides* and *M. guttatus* populations occurring along an elevation gradient. Working with geographically paired sympatric and allopatric samples, we perform field surveys, common garden experiments, and a competition experiment to evaluate four questions. First, are the niches of *M. guttatus* and *M. alsinoides* differentiated in sympatry? Species with recently differentiated niches are predicted to occupy spatially or temporally distinct portions of sympatric habitat and, if competition is associated with niche differentiation, this niche space will likely be narrower than that observed for allopatric populations.

Second, are the phenotypic differences between allopatric and sympatric populations genetically based, and what do comparisons of these distributions reveal about whether niche differentiation has occurred via divergent character displacement, ecological sorting, or ecological sorting followed by one or several forms of character displacement? If divergent character displacement drives primary niche differentiation, divergence of associated traits between *M. alsinoides* and *M. guttatus* should be greater for sympatric than for allopatric populations when all plants are grown in a common garden. If ecological sorting drives
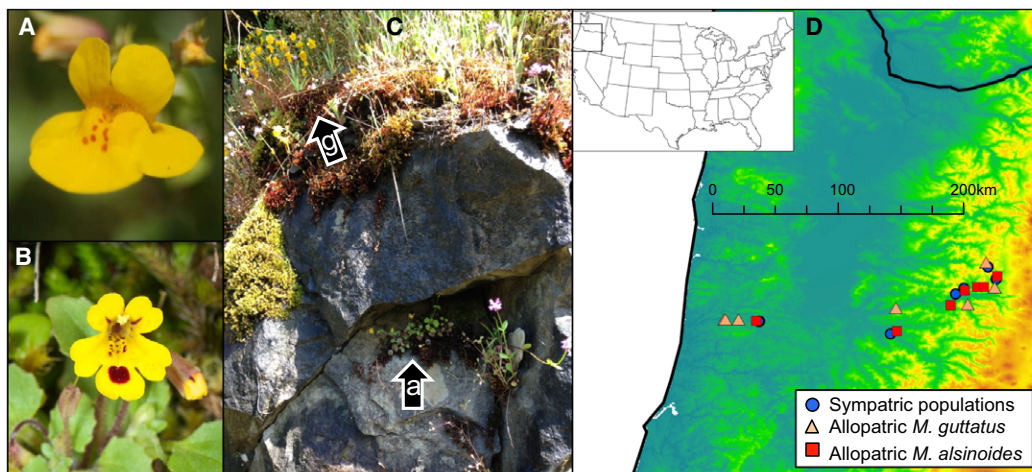
Figure 2. *Mimulus guttatus* (A) and *M. alsiniodes* flowers (B), rock wall habitat at low elevation sympatric site (C), and sampling locations in central Oregon (D). In panel C, "g" arrow marks the location of *M. guttatus* and "a" arrow marks the location of *M. alsinoides*; picture taken in April 2015. In panel (D), color gradient corresponds to elevation with greens corresponding to lower elevation and red/brown corresponding to higher elevations. Photographs used with permission by L. Sackett (A) and J. Davis (B).

niche differentiation, phenotypic divergence between allopatric populations of *M. guttatus* and *M. alsinoides* should be as great as phenotypic divergence between sympatric populations of *M. guttatus* and *M. alsinoides* in a common garden. We expect to find more complex patterns if both processes come into play and character displacement has driven evolution along niche axes not essential for stable coexistence (Fig. 1, Scenarios II and III).

Third, do patterns of competition-mediated trait evolution differ across the elevation gradient or between species? How phenotypes evolve in response to selection that promotes niche evolution may differ depending on environmental context, and one species may be more strongly impacted by competition than the other. Common garden experiments of replicated sympatric and allopatric population pairs can also assess these subtleties in trait evolution. Finally, can competition between these species act directly as a selection pressure driving trait evolution? If so, the selection gradients acting on affected traits should shift in competition experiments between treatments with interspecific competition versus without interspecific competition. Our results suggest that competition does indeed drive evolution in situ, although the magnitude and direction of competition-mediated selection depend on the individual species and environmental context of the population.

Methods

SAMPLING AND HABITAT VARIABILITY OF NATURAL POPULATIONS

We collected seed from four to 12 maternal lines from each of 14 *M. alsinoides* populations and 12 *M. guttatus* populations along an elevation gradient in the central Cascade Mountains, and recorded

Global Positioning System (GPS) coordinates, elevation, and aspect at each location (Fig. 1; see Table S1). Populations were chosen to compose sets of at least one allopatric *M. alsinoides* population, at least one allopatric *M. guttatus* population, and at least one sympatric population that were geographically proximate along the elevation gradient. Six population sets were sampled in total. Allopatric populations were located >200 m from the nearest neighboring population of the other species.

To describe climatic variation among sites, we extracted 19 bioclim variables at 1 km resolution from the BioClim dataset (Hijmans et al. 2005) as well as annual aridity index and annual potential evapotranspiration at 30 arc-sec resolution from the Consultative Group on International Agricultural Research-Consortium for Spatial Information (CGIAR-CSI) data portal (Zomer et al. 2008). These 21 variables were *z*-score transformed and used in a principal component analysis conducted with the prcomp call in R version 2.13.2 (R Foundation for Statistical Computation, Vienna, Austria). PC1 from this analysis (i.e., climate PC1) represents 54.4% of total variance and is highly associated with elevation ($r^2 = 0.9$), with higher values strongly associated with lower temperature and greater precipitation (Table S2).

Because our preliminary observations strongly suggested that niche divergence between *M. alsinoides* and *M. guttatus* in sympatry occurred via relegation of *M. alsinoides* to more marginal, barer rock surfaces, we recorded soil depth measures for 15–25 individuals from every *M. alsinoides* population and for all sympatric *M. guttatus* populations. We used a linear mixed model (LMM) approach implemented using the *lme4* package version 1.1-10 (Bates et al. 2014) to evaluate whether *M. alsinoides* occupies different habitats in sympatry versus allopatry, modeling community (sympatry or allopatry) as a

fixed effect and population as a random effect. Similarly, to test whether *M. guttatus* and *M. alsinoides* occupy distinct habitats in sympatry, we modeled species as a fixed effect and population as a random effect. Statistical significance of the fixed effect was determined by ANOVA using a Wald chi-square test with one degree of freedom implemented in the *car* R package version 2.1-1 (Fox et al. 2013).

EXAMINING GENETICALLY-BASED PATTERNS OF INTRA- AND INTERSPECIFIC PHENOTYPIC DIVERGENCE

We conducted two common garden experiments using the paired allopatric and sympatric population sets described above to evaluate whether patterns of genetically based trait evolution were caused by character displacement, ecological sorting, or combination of the two. The first common garden experiment included two sympatric and seven allopatric *M. guttatus* populations (collections, methods, and results reported in Kooyers et al. 2015). The second common garden experiment was conducted with the seed collections described above, including two *M. guttatus* populations in common with the first experiment. We planted three individuals from four to 24 maternal lines (mean: 11.9) from 13 *M. alsinoides* populations, and from 10 to 12 maternal lines (mean: 11.5) from four sympatric *M. guttatus* populations. Planting and growth conditions are reported in the Supporting Information.

Because both of these annual taxa inhabit soils that experience summer droughts and one hypothesis is that *M. alsinoides* is better adapted to habitat that dries out earlier in the season (i.e., thinner soils) than *M. guttatus*, we measured several phenological and leaf traits related to water use efficiency. We surveyed plants daily for budding and flowering. At flowering, we measured plant height, stem diameter, flowering node, leaf number, branch number, and several leaf traits including dry weight, leaf area, specific leaf area (SLA), succulence, and relative water content (see Supporting Information). Because both species share the same community of potential pollinators, we measured several floral traits at first flower, including corolla length, width, and height; corolla tube length and width; and peduncle length. These traits were used in separate principal component analyses for *M. alsinoides* and *M. guttatus* (Table S2). For both species, floral PC1 and floral PC2 reflect overall floral size and floral shape, respectively. Higher values of floral PC2 indicate longer peduncles, longer tube lengths, and longer flower tubes, but narrower corolla widths and heights. Once all *M. alsinoides* individuals had fully senesced, we counted flower number and measured plant height for each plant. Postsenescence measures were not scored for *M. guttatus* because this species does not fully senesce when adequately watered. We also performed a separate set of growth chamber experiments to examine patterns of variation in critical photoperiod (i.e., the day length necessary to induce flowering)

for these obligate long day species (Friedman and Willis 2013; Kooyers et al. 2015; see Supporting Information, Table S3).

To test for patterns consistent with character displacement causing niche divergence along axes that foster coexistence (i.e., Fig. 1), we used two methods designed to detect greater trait differentiation in sympatry versus allopatry. We used an LMM approach implemented using the *lme4* R package to detect the effects of species identity, community type (allopatric or sympatric), and their interaction on each trait. Climate PC1 was included in the model as a covariate and population, maternal line (nested within population), and common garden were included as random variables. Several variables—SLA, relative water content, leaf area—were log transformed to improve model fit. Statistical significance of each fixed effect was determined by ANOVA as above. A significant interaction between species identity and community type suggests selection mediated by interspecific competition, possibly caused by character displacement (Adams 2004).

We also directly tested for the traditional pattern associated with character displacement by calculating the difference between species trait means in sympatry (D_{sym}) and in allopatry (D_{allo}) as well as the difference between these differences ($D_{sym-allo}$). Divergent character displacement results in greater divergence in sympatry than allopatry (positive $D_{sym-allo}$). To test whether this divergence was greater than expected by chance, we used the residual randomization procedure described in Adams and Collyer (2006). Briefly, this involved creating a reduced LMM that was identical to the above model, but lacked the interaction term between species and community. Predicted marginal means for each species identity:community level from this model were extracted using the *lsmeans* R package version 2.21-1 (Lenth 2016). Observed model residuals were added to these means, and observed $D_{sym-allo}$ was calculated. We then randomized the residuals from the reduced model, added them to the predicted marginal means, and calculated expected $D_{sym-allo}$. This procedure was repeated 10,000 times to create a distribution of expected $D_{sym-allo}$. We define statistically significant character displacement as when $D_{sym-allo(obs)}$ is contained with the highest 2.5% of the distribution of $D_{sym-allo(exp)}$. To test whether patterns of character displacement were consistent across the elevation gradient, we separately performed a similar analysis for each set of geographically paired sympatric and allopatric populations using population means to calculate D_{sym}, D_{allo}, and $D_{sym-allo}$ (Supporting Information).

To detect alternative patterns of character displacement, we used a similar LMM approach where we evaluated the factors explaining trait distributions in each individual species. Traits were treated as response variables; community type and climate PC1 were treated as fixed variables; and population, maternal line (nested within population), and flat were treated as random variables. In models for *M. guttatus*, common garden was also treated as a random variable. All interaction terms were included.

Significance of fixed factors was by determined ANOVA as described above. If in situ competition is driving trait evolution, we expect a difference in trait means between sympatric and allopatric populations of a species, corresponding either to a significant community effect or to a significant community × climate PC1 interaction.

We also used the common garden data to ask whether patterns of character displacement or competition-mediated trait evolution differ across the elevation gradient or between species. Significant community × climate interactions indicate the strength of competitive interactions may vary across the gradient. We assessed asymmetries in the impact of interspecific competition on trait evolution by asking whether community or community × climate PC1 effects were significant in either or both species.

FIELD OBSERVATIONS

To explore whether patterns of trait variation identified in the common garden experiments were consistent with variation in natural populations, we revisited each *M. alsinoides* population twice and took select morphological and phenological measurements chosen based on our greenhouse results. We first visited each site early in the growing season (April 2015), marked 15–25 *M. alsinoides* per site, and recorded flowering status and number of open flowers for each plant. Where sympatric, we also marked and scored 10–22 *M. guttatus* individuals. We returned in the middle/end of the growing season (late June 2015) and measured peduncle length of the first flower, node of flowering, plant height, and number of flowers for each marked plant. LMMs were used to assess the effects of community and environment on each trait, where community (sympatric/allopatric) and elevation were treated as fixed variables and population as a random variable.

EXAMINING PATTERNS OF PHENOTYPIC SELECTION IN AN INTERSPECIFIC COMPETITION EXPERIMENT

We performed a greenhouse competition experiment to determine whether competition could promote similar patterns of trait divergence to those seen in nature. We manipulated interspecific competition (referred to below as "competition"; three levels), soil depth (two levels), and shade (two levels) in a factorial design, replicating each experimental combination four times (3 × 2 × 2 × 4 = 48 total trays). We chose soil depth and shade treatments specifically because our preliminary observations suggested that *M. alsinoides* occupied more rapidly drying, barer rock habitat than *M. guttatus*, and because we observed *M. alsinoides* under rocks and in rock crevices, areas *M. guttatus* rarely occupies, in some sympatric populations. Each tray was filled with either 1 or 3.5 cm of soil (soil depth treatment), and we planted either 50 seeds from *M. alsinoides*, 50 seeds from *M. guttatus*, or 50 *M. alsinoides* + 50 *M. guttatus* seeds per tray (competition treatment). We altered density between competition treatments, as a

preliminary experiment suggested that germination of *M. alsinoides* was low and potentially lower in competition treatment. Indeed, germination was low for *M. alsinoides*, but differences in local density of plants did not affect fitness for either species ($P > 0.05$; Fig. S1). After germination, we divided trays equally into shade or nonshade treatments and randomized pots within treatments. Shade treatment consisted of a shade enclosure and necessitated the use of a split plot design (Fig. S2). We surveyed flowering daily, and recorded species, plant height, flowering node, and peduncle length at first flower. Following senescence, we scored flower number and plant height for *M. alsinoides*. Phenological differences in shattering among individuals prevented the use of total seed count as a fitness measure. However, flower number is closely correlated with total seed count in *M. alsinoides* ($n = 57$, $r^2 = 0.82$) and thus serves as a good fitness proxy.

To detect whether competition, soil depth, shade treatment, or interactions among these factors affected fitness, we calculated separate LMMs for *M. alsinoides* and *M. guttatus*. Total flower number was used as the response variable; competition, soil depth, and shade treatments were treated as fixed variables; and tray was treated as a random variable. All interactions between fixed variables were included. Significance was assessed with ANOVA as described above. We investigated how competition, soil depth, and shade drive responses to selection by calculating directional selection gradients as a partial regression of each trait on fitness within each of the eight treatments (Lande and Arnold 1983). Unstandardized selection gradient coefficients were obtained by least squares regression using the lm() call in R with relative fitness as the response variable (calculated using flower number) and flowering time, plant height, peduncle length, and node as predictor variables. We report the gradients and their associated errors standardized by trait SDs. To test whether standardized selection gradients for each trait were more different than expected between different treatments, we conducted a permutation analysis. Trait values were randomized within each treatment, and selection gradients were recalculated as above. We then took the difference between selection gradients for each treatment, and repeated this procedure 10,000 times to create an expected distribution. The expected distribution was compared to our observed difference between selection gradients for the corresponding treatments with observed values greater than 95% of the expected distribution, indicating that selection gradients are significantly different between treatments.

Results

DIVERGENT HABITAT USE IN SYMPATRY INDICATIVE OF NICHE DIFFERENTIATION

We first assessed whether habitats where *M. guttatus* and *M. alsinoides* are sympatric differ from habitats where the

Table 1. Summary of soil depth measurements for sympatric and allopatric populations of *Mimulus guttatus* and *M. alsinoides*.

Geographic pair	Population	Species	N	Soil depth mean (cm)	Soil depth SD (cm)	Soil depth max (cm)
Group A	WCC1	*guttatus*	18	4.11	3.44	11
	WCC1	*alsinoides*	25	1.82	1.92	8.1
	WCC3	*alsinoides*	25	2.80	2.90	10.3
Group B	LPD	*guttatus*	22	5.08	3.03	11.4
	LPD	*alsinoides*	23	4.67	2.70	9.2
	PEN	*alsinoides*	25	3.02	3.69	19.2
Group C	BRR	*guttatus*	15	3.43	2.08	8.7
	BRR	*alsinoides*	25	0.62	0.78	2.5
	QCR	*alsinoides*	25	2.94	1.94	7.2
Group D	TBC	*guttatus*	17	6.18	4.40	19.2
	TBC	*alsinoides*	26	4.82	5.39	14.9
	HJA	*alsinoides*	25	3.03	3.76	19.2
Group E	TBR	*guttatus*	10	9.01	5.23	19.2
	TBR	*alsinoides*	25	1.80	2.04	8.6
	LOC	*alsinoides*	25	3.93	5.75	19.2
	LOC2	*alsinoides*	25	3.21	2.92	9.8
Group F	BR3	*guttatus*	10	4.39	4.01	10.7
	BR3	*alsinoides*	15	3.45	2.89	10
	VCM	*alsinoides*	25	3.20	2.77	12.2

Within each geographic grouping, dark gray row = sympatric *M. guttatus*; light gray row = sympatric *M. alsinoides*; white row = allopatric *M. alsinoides*.

species are allopatric by comparing abiotic variables between geographically paired sites. Across the elevation gradient, sympatric/allopatric population pairs had similar climate PC1 scores (Fig. S3), soil depths, and aspects. Although additional unmeasured factors cannot be excluded, the general uniformity of the rock wall habitats in this region and the similarity of allopatric and sympatric populations for these critical environmental influences on the timing of the growing season and resource availability indicate sympatric and allopatric habitats are unlikely to systematically differ.

Within sympatric populations, however, we found that *M. guttatus* occupied areas with greater soil depth than *M. alsinoides* ($\chi^2 = 13.9$, $P < 0.001$; Fig. S4). *Mimulus alsinoides* also occupied a different habitat range in sympatric populations versus allopatric populations. Although the mean soil depth occupied by *M. alsinoides* does not differ between sympatric and allopatric populations, plants from all six allopatric populations occupied deeper soils than plants from their sympatric pairs (Table 1; Fig. S5). Permutation tests that randomized the individual soil depths across sympatric and allopatric populations and tested whether maximum soil depth is higher in allopatric populations than in paired sympatric populations indicated that chance creates higher maximum soil depths in every allopatric population less than 1.5% of the time (code available upon request). Thus, our results suggest that although sympatric and allopatric rock walls have largely similar abiotic conditions including soil depth, *M.*

guttatus appears to limit *M. alsinoides* to marginal microhabitats with shallower, more drought prone soil in sympatry (Fig. 2C).

EXTENSIVE EVIDENCE FOR CHARACTER DISPLACEMENT ALONG ADDITIONAL NICHE AXES AND ECOLOGICAL SORTING

To determine whether *M. alsinoides* or *M. guttatus* exhibited patterns of trait evolution consistent with ecological sorting of species differences evolved in allopatry, character displacement, or a combination, we measured diverse morphological, phenological, and physiological traits in common garden experiments conducted in the greenhouse and growth chambers. We found little support for traditional divergent character displacement within either species. That is, trait divergence between species in sympatric populations did not significantly exceed trait divergence between species in allopatric populations after controlling for the environmental gradient (Adams and Collyer 2006, Tables 2 and S4). Moreover, when the full dataset was parsed into geographically paired populations, we found a pattern consistent with character displacement only for floral PC2 at low elevation ($D_{sym-allo} = 0.91$, $P = 0.015$; Table S5). Nonetheless, several findings indicated that in situ competition may have driven phenotypic evolution in sympatric populations. For many traits—including plant height, corolla width, corolla length, peduncle length, and floral PC1—there were marginal or significant species × community interaction terms in the full model (Tables 2 and S4) or

Table 2. Results summary of LMM testing for evidence of character displacement.

Trait	Observed trait divergence			Model incorporating elevation gradient		
	D_{sym}	D_{allo}	$D_{sym-allo}$	Species χ^2 (P)	Community χ^2 (P)	Species:community χ^2 (P)
Corolla length	5.78	6.84	−1.06	**116.3 (<0.001)**	1 (0.325)	*2.883 (0.09)*
Corolla width	9.7	10.61	−0.91	**242.6 (<0.001)**	0.1 (0.728)	*3.584 (0.058)*
Peduncle length	5.8	10.47	−4.67	**37.5 (<0.001)**	**4.4 (0.037)**	**5.746 (0.017)**
Tube width	3.66	3.83	−0.17	**175.2 (<0.001)**	0.2 (0.697)	0.736 (0.391)
Tube length	3.52	3.84	−0.31	**69.9 (<0.001)**	0.3 (0.613)	0.519 (0.471)
Plant height	50.74	62.25	−11.52	**82.8 (<0.001)**	1.1 (0.3)	2.2 (0.138)
Flowering time	2.32	0.78	1.54	0.3 (0.597)	0.1 (0.788)	0.789 (0.374)
Node	0.69	0.46	0.23	**6.8 (0.009)**	0 (0.843)	1.021 (0.312)
Floral PC1	2.92	3.41	−0.49	**165.5 (<0.001)**	1 (0.312)	**4.243 (0.039)**
Floral PC2	−0.23	0.29	−0.52	1.2 (0.279)	**4.4 (0.035)**	*3.601 (0.058)*
Dry mass	0.0089	0.0112	−0.0023	**37.9 (<0.001)**	0.1 (0.813)	0.672 (0.412)

Positive $D_{sym} − D_{allo}$ values are indicative of patterns associated with divergence character displacement. Species (*Mimulus alsinoides* vs. *M. guttatus*), community (allopatric vs. sympatric), and species:community interaction effects come from LMM incorporating a residual randomization procedure similar to Adams and Collyer (2007). This method tests for significant species:community interactions (i.e., character displacement) while controlling for trait variation due to position along an environmental gradient. Bold values indicate statistical significance at *P* < 0.05 and italics indicate marginal significance at *P* < 0.1.

significantly greater similarity in sympatry relative to allopatry (Table S5).

Thus, although we did not find general support for character displacement accentuating trait divergence in sympatry, our findings do suggest trait evolution due to competition has likely occurred. To further explore all potential outcomes of trait evolution due to in situ competition along the elevation gradient, we conducted separate evaluations for each species. Several traits exhibited genetically based patterns of variation that appeared consistent with competition-mediated selection in both species. For instance, in *M. alsinoides*, differences in developmental rate and timing between sympatric and allopatric populations were enhanced at low elevation. Although plant height at flowering did not differ between sympatric and allopatric populations when considered range-wide, at low elevation, plants sympatric with *M. guttatus* were taller at flowering on average and achieved taller maximum heights than plants allopatric to *M. guttatus* (Fig. 3; sympatric: 34.3 ± 6.8 mm, allopatric: 23.4 ± 2.6 mm; $t = 2.6$, degrees of freedom [df] = 101, $P = 0.01$). In addition, we found a significant interaction effect of competitor presence and climate PC1 on flowering node where sympatric populations flowered at a higher node than allopatric populations specifically at low elevation ($\chi^2 = 3.9$, $P = 0.05$). We found a similar but marginal trend for the interaction of competitor presence and climate PC1 on critical photoperiod in *M. alsinoides* (Fig. S6; Table S7; $\chi^2 = 2.5$, $P = 0.11$). Sympatric populations of *M. alsinoides* required shorter day lengths to flower at lower elevations; however, allopatric populations had no discernible relationship between critical photoperiod and elevation (Fig. S6). In contrast to these phenological traits, differences in floral morphology were

significant range wide and particularly dramatic for high elevation populations (Fig. 3). Sympatric populations had longer peduncles as well as longer and narrower flowers than allopatric populations (Table 3; Fig. S7 and Table S6; peduncle length $\chi^2 = 8.4$, $P = 0.004$; floral PC2: $\chi^2 = 11.6$, $P = 0.001$). Including elevation rather than climate PC1 as the environmental factor in the model yielded largely similar results (Table S6). Together, these results indicate that competition with *M. guttatus* has caused trait evolution in *M. alsinoides* on developmental and floral traits in different parts of the elevation gradient.

Mimulus guttatus also exhibited patterns of trait variation along the environmental gradient that appear consistent with various types of character displacement (Table 3). We found a significant interaction effect of climate PC1 and the presence/absence of *M. alsinoides* for several traits including plant height at flowering (Fig. 3D; $\chi^2 = 4.2$, $P = 0.04$), peduncle length (Fig. 3E; $\chi^2 = 4.47$, $P = 0.03$), and floral PC2 (Fig. 3F; $\chi^2 = 5.77$, $P = 0.02$). At low elevations, sympatric *M. guttatus* populations were taller with longer peduncles and longer, narrower flowers than allopatric populations, whereas at high elevations sympatric populations were shorter with smaller peduncles and shorter, broader flowers than in allopatric populations. However, when the highest elevation allopatric population (HAC) was excluded from the analyses, the interaction terms for both plant height ($P = 0.06$) and floral PC2 ($P = 0.12$) were only marginally significant. Although we found no evidence of competition-mediated selection on critical photoperiod, *M. guttatus* populations always required longer day lengths to flower on average than *M. alsinoides*, suggesting that the seasonal timing of reproduction for *M. alsinoides* commences earlier in the year than for *M. guttatus*. Thus,
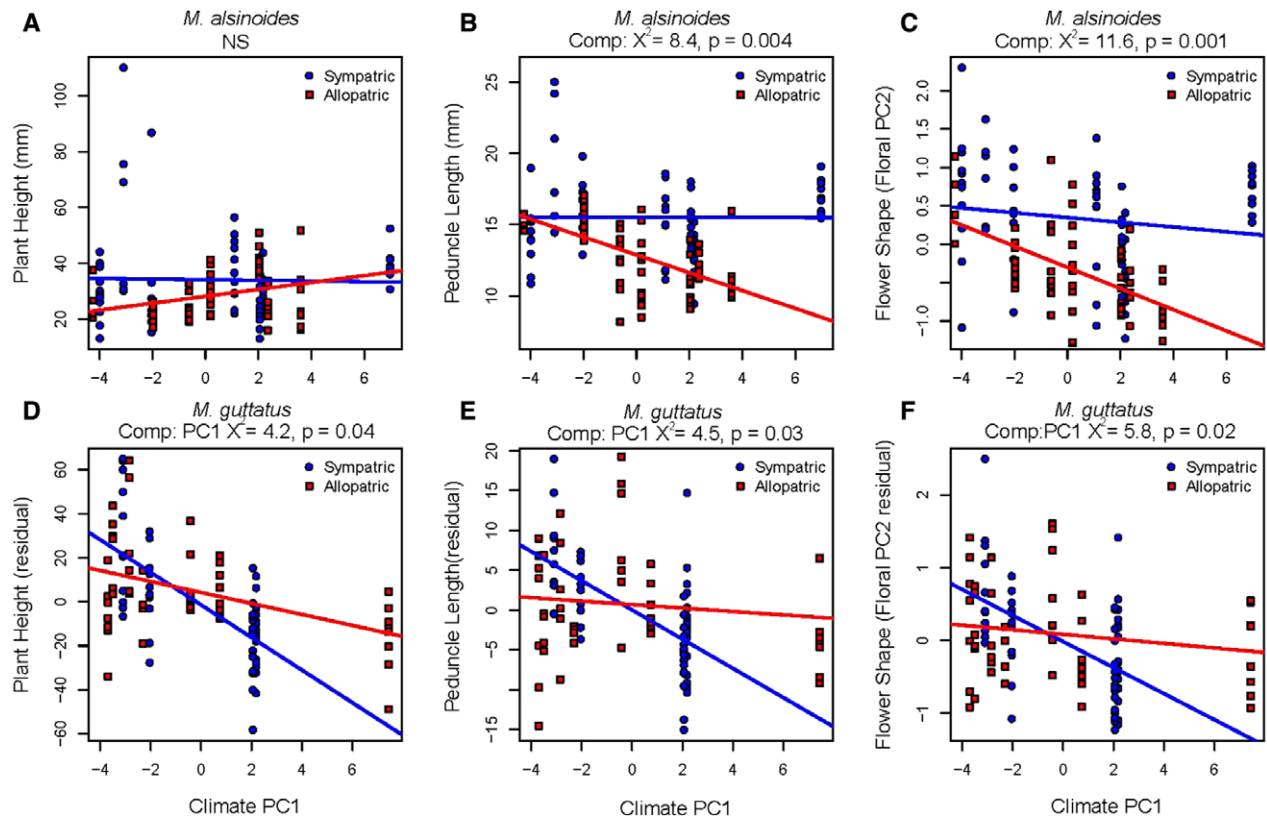
Figure 3. Species-specific models demonstrate how community and environment influence trait variation. Regressions of sympatric and allopatric *Mimulus alsinoides* population means for plant height (A), peduncle length (B), and floral PC2 (C) against climate PC1 of the population where individuals were collected. Regression of sympatric and allopatric *Mimulus guttatus* population means for residuals of plant height (D), peduncle length (E), and floral PC2 (F). Wald chi squares and *P* values for each graph were calculated via the general linear mixed models described in the text, not from the raw regression of population means on climate PC1.

consistent with competition-mediated selection, a number of traits in both species exhibited different patterns between sympatric and allopatric populations. In low elevation populations, both species have evolved to be taller at flowering in sympatry relative to allopatry reflecting parallel character displacement. In high elevation populations, peduncle length and floral shape were under selection in both species, with longer narrower flowers in sympatry in *M. alsinoides* and shorter broader flowers in sympatry in *M. guttatus*, indicating mutually convergent character displacement.

To assess whether the patterns of trait variation we observed in common garden experiments are comparable to patterns in the field, we surveyed morphological and phenological variation of natural populations. Consistent with the common garden results, the peduncles of *M. alsinoides* were longer for sympatric populations than for allopatric populations (Fig. S8; $\chi^2 = 4.2$, $P = 0.04$). We also found a marginal interaction effect of climate PC1 and presence/absence of *M. guttatus* on the plant height of *M. alsinoides* where plants were taller in sympatric populations than in allopatric populations at low elevation (Table S8; $\chi^2 = 3.3$, $P = 0.08$). Consistent with the marginal difference in critical

photoperiod between the two species, we observed that *M. alsinoides* flowers earlier in the growing season than *M. guttatus* in the field (Fig. S9). Taking number of flowers as a proxy for the duration since first flowering, we found that *M. guttatus* plants had fewer flowers than *M. alsinoides* plants in sympatric populations (Table S8; Species: $\chi^2 = 56.8$, $P < 0.0001$). No phenological or fitness differences between sympatric and allopatric *M. alsinoides* populations were observed.

COMPETITION ALTERS SELECTION ON TRAITS IN *M. alsinoides* IN COMPETITION EXPERIMENTS

We tested whether competition between *M. guttatus* and *M. alsinoides* could impact the relative fitness of either species and the direction or magnitude of selection on individual traits by conducting a greenhouse experiment that manipulated competition, shade, and soil depth. Shallower soils, greater shade, and greater competition all reduced the fitness of *M. alsinoides* relative to the control treatment (Fig. 4A; Table S9; competition: $\chi^2 = 60.1$, $P < 0.0001$; soil depth: $\chi^2 = 88.3$, $P < 0.0001$, shade: $\chi^2 = 32.6$, $P < 0.0001$). Several interactions between treatments

Table 3. Competition-mediated selection LMM results for *Mimulus alsinoides* and *M. guttatus*.

Species	Response variable	Climate PC1		Community		Climate PC1: community	
		χ^2	*P*	χ^2	*P*	χ^2	*P*
M. alsinoides	Corolla length	< 0.01	0.951	0.84	0.361	0.05	0.823
M. alsinoides	Corolla width	1.01	0.316	0.19	0.665	0.38	0.537
M. alsinoides	Tube width	0.50	0.478	0.19	0.665	0.94	0.331
M. alsinoides	Tube length	0.14	0.703	0.26	0.610	0.21	0.644
M. alsinoides	Corolla height	0.06	0.803	0.27	0.607	0.27	0.605
M. alsinoides	Peduncle length	**4.74**	**0.029**	**8.40**	**0.004**	*2.83*	*0.092*
M. alsinoides	Plant height (at flowering)	0.55	0.457	1.88	0.170	0.44	0.509
M. alsinoides	Stem diameter	1.15	0.283	0.50	0.481	1.83	0.177
M. alsinoides	Number of leaves	0.32	0.574	2.05	0.152	< 0.01	0.982
M. alsinoides	Number of branches	1.60	0.205	1.89	0.169	0.13	0.716
M. alsinoides	Flowering node	3.32	0.068	0.11	0.744	**3.89**	**0.048**
M. alsinoides	Budding time	0.01	0.942	0.02	0.894	0.81	0.368
M. alsinoides	Flowering time	< 0.01	0.972	0.02	0.882	0.89	0.345
M. alsinoides	Leaf area	0.01	0.939	0.07	0.796	1.17	0.280
M. alsinoides	Floral PC1	0.02	0.885	0.54	0.464	0.02	0.875
M. alsinoides	Floral PC2	**6.72**	**0.010**	**11.62**	**0.001**	*2.89*	*0.089*
M. alsinoides	Number of flowers	0.85	0.357	0.51	0.476	0.17	0.685
M. alsinoides	End plant height	**4.11**	**0.043**	2.44	0.118	1.01	0.316
M. alsinoides	SLA	*2.96*	*0.085*	0.33	0.568	0.13	0.722
M. alsinoides	Relative water content	1.73	0.188	0.12	0.728	0.12	0.734
M. alsinoides	Succulence	1.35	0.245	0.28	0.595	0.01	0.937
M. guttatus	Corolla length	2.19	0.139	0.45	0.500	*3.20*	*0.074*
M. guttatus	Corolla width	0.10	0.753	*3.48*	*0.062*	< 0.01	0.985
M. guttatus	Tube width	0.50	0.480	0.34	0.560	0.27	0.604
M. guttatus	Tube length	*2.97*	*0.085*	0.90	0.343	1.85	0.174
M. guttatus	Peduncle length	0.49	0.483	0.19	0.662	**4.47**	**0.035**
M. guttatus	Plant height (at flowering)	*3.28*	*0.070*	0.02	0.885	**4.20**	**0.041**
M. guttatus	Stem diameter	0.67	0.412	1.63	0.202	*2.88*	*0.089*
M. guttatus	Number of branches	**9.03**	**0.003**	0.33	0.563	*2.86*	*0.091*
M. guttatus	Flowering node	**4.89**	**0.027**	1.69	0.193	< 0.01	0.967
M. guttatus	Flowering time	**7.72**	**0.005**	1.60	0.206	< 0.01	0.987
M. guttatus	Leaf area	1.33	0.249	0.18	0.673	0.61	0.435
M. guttatus	Floral PC1	0.81	0.368	1.04	0.307	1.29	0.255
M. guttatus	Floral PC2	1.07	0.301	0.05	0.817	**5.77**	**0.016**
M. guttatus	SLA	0.33	0.567	1.00	0.318	2.16	0.142

χ^2 values are Wald chi-square values from LMMs testing for competition-mediated selection as described in the text. Bold values indicate statistical significance given 1 df at *P* = 0.05, whereas italics indicate marginally significant results.

also significantly impacted fitness (comp:light: $\chi^2 = 6.9$, $P = 0.009$; comp:soil: $\chi^2 = 17.1$, $P < 0.0001$; light:soil: $\chi^2 = 8.4$, $P = 0.004$). Neither interspecific competition from *M. alsinoides* nor intraspecific competition from other *M. guttatus* affected the fitness of *M. guttatus* (Fig. 4B and Fig. S5). Rather, only soil depth negatively impacted floral production by *M. guttatus* ($\chi^2 = 163.7$, $P < 0.0001$).

If interspecific competition drives in situ trait evolution, as the common garden findings discussed above suggest, manipulat-

ing interspecific competition should result in major differences in selection gradients between the competition and no competition treatments. Indeed, the magnitude and direction of selection gradients for flowering time and plant height changed substantially across treatments for *M. alsinoides* (Fig. 5A–C), although we observed only minimal changes in the magnitude of selection for *M. guttatus* (Fig. 5D and Table S10). Under control conditions, selection favored bushy *M. alsinoides* individuals that flowered later and that were shorter at flowering. In contrast, we found
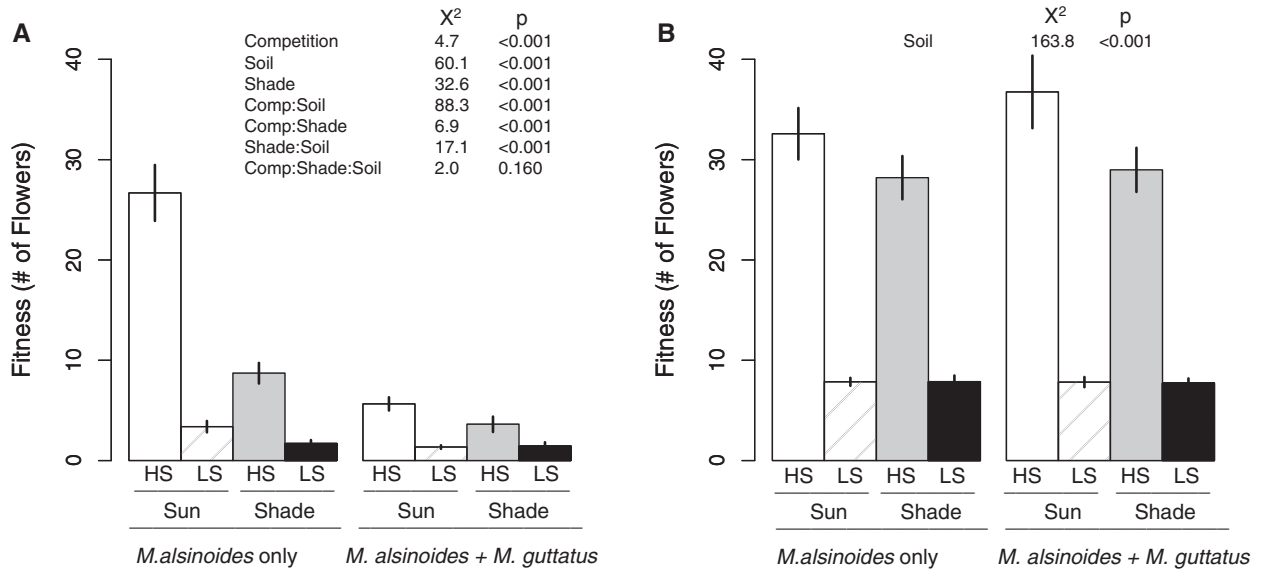
Figure 4. Relative fitness (number of flowers) changes across treatments for *Mimulus alsinoides* (A) and *M. guttatus* (B) in a greenhouse competition experiment. Competition treatment (one species or both species), shade treatment (sun vs. shade), and soil depth treatment (HS, high soil; LS, low soil) are shown on the *x*-axis. White bars indicate the HS:sun treatment, striped bars LS:sun, gray bars HS:shade, and black bars LS:shade. Wald chi-square and *P* values are from general linear models described in the main text. Error bars on bar plots represent SEs.

that the competition with *M. guttatus* favored *M. alsinoides* individuals that flowered earlier and that were taller at flowering (Fig. 5A and B and Tables S10 and S11). These findings were consistent with patterns of phenotypic divergence seen between low elevation allopatric and sympatric *M. alsinoides* populations in the common garden experiment. Selection gradients for flowering time and plant height in the shallow soil and shade treatments were intermediate to the effect of competition, suggesting that these factors impose selection acting in a similar direction. We detected selection favoring longer peduncle lengths in multiple treatments (Fig. 5C), but differences in direction and magnitude did not indicate an unambiguous influence of competition relative to other factors on this trait. Together, although we find little evidence that competition with *M. alsinoides* impacts how selection acts on *M. guttatus*, possibly because our experiments did not simulate a terminal drought, our results do strongly suggest that competition with *M. guttatus* for resources and space modifies how selection acts on flowering time and plant height in *M. alsinoides*.

Discussion

In this study, we address several basic questions regarding the role of competition in causing trait evolution and niche differentiation in monkeyflowers. We find clear evidence of niche differentiation between *M. alsinoides* and *M. guttatus*, where *M. alsinoides* is relegated in sympatric populations to occupy a narrower habitat distribution primarily consisting of areas of thin soil on rock walls

that dry out rapidly. Although we find little evidence that divergent character displacement is responsible for this habitat partitioning or initial niche divergence, we find that in situ competition does drive trait evolution in *M. alsinoides* creating alternative patterns of character displacement (Fig. 1, Scenario III). The findings of our competition experiment also confirm that competition with *M. guttatus* affects the overall fitness and phenotypic selection gradients of *M. alsinoides* in a manner consistent with our observational data. In this system, competition drives trait evolution asymmetrically, where competition with *M. guttatus* drives trait evolution in *M. alsinoides*, but not necessarily in the other direction. Further, different traits are affected by competition in different parts of the elevation gradient. Developmental and phenological traits appear most impacted by competition in low elevation populations, and floral shape traits appear most impacted by competition in high elevation populations. In general, our results suggest the role of interspecific competition in trait evolution may extend along multiple niche axes, and lead to patterns of phenotypic variation more complex than the one most frequently associated with character displacement.

NO EVIDENCE FOR DIVERGENT CHARACTER DISPLACEMENT CONTRIBUTING TO SPECIES COEXISTENCE

Although both species occupy similar rock wall habitats in allopatry, we find that *M. guttatus* and *M. alsinoides* inhabit distinct edaphic contexts in sympatric populations, suggesting niche
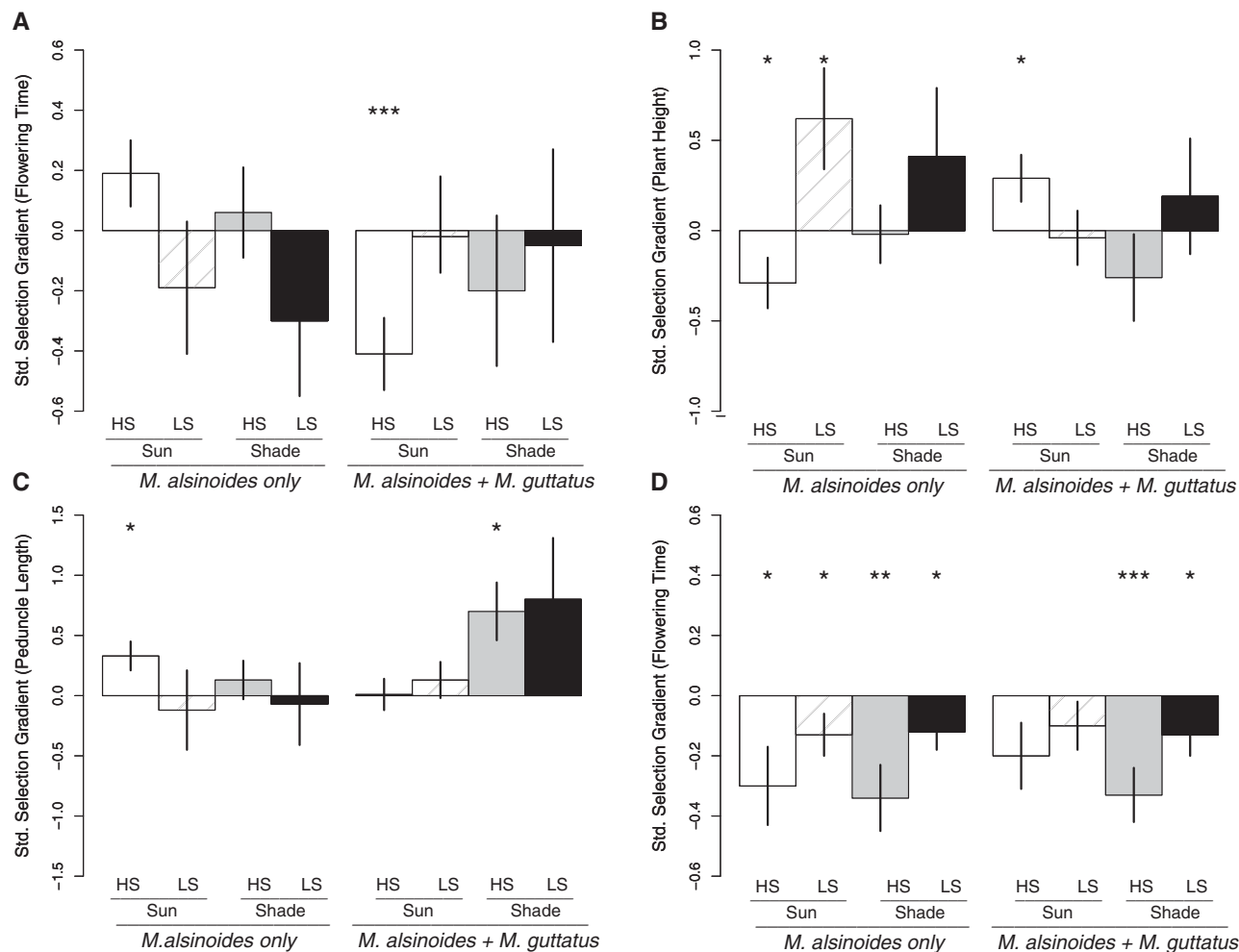
Figure 5. In a greenhouse competition experiment, experimental treatments impact the direction and magnitude of standardized selection gradients for flowering time (A), plant height (B), and peduncle length (C) in *M. alsinoides* as well as for flowering time in *M. guttatus* (D). Competition treatment (one or both species), shade treatment (sun vs. shade), and soil depth treatment (HS, high soil; LS, low soil) are shown on the *x*-axis. White bars indicate the HS:sun treatment, striped bars LS:sun, gray bars HS:shade, and black bars LS:shade. Asterisks denote a statistical deviation from no selection where * indicated 0.05 > *P* > 0.01, **0.01 > *P* > 0.001, and ***P* < 0.001. Error bars on bar plots represent standardized SEs. Permutation test results indicate that the selection gradients were significantly different between the control and competition treatment for both flowering time (*P* = 0.002) and plant height (*P* = 0.006) in *M. alsinoides*. For full permutation test results see Table S11.

differentiation via habitat partitioning has occurred (Table 1). In sympatry, *M. alsinoides* occurs primarily in areas with low soil or moss depth, directly on rock or under overhanging rocks, whereas *M. guttatus* is found in areas with deeper soil (Fig. S4). Consistent with these findings, our greenhouse competition experiments demonstrated that *M. guttatus* has greater fitness than *M. alsinoides* when competing in high soil depth or no shade habitats (Fig. 4). In contrast, *M. alsinoides* did not have greater fitness in any treatment than *M. guttatus*, though our soil depth treatment was not as extreme as often observed in natural populations (i.e., a bare rock face) nor did we simulate differences in seasonal water availability to determine if earlier terminal droughts favor *M. alsinoides*. In other field experiments, survival of *M. guttatus*

is extremely compromised in rapidly drying soil (Peterson et al. 2013). Nonetheless, the combined results of these experiments provide reasonable support that niche differentiation allows *M. alsinoides* and *M. guttatus* to coexist in sympatry.

We find little evidence that divergent character displacement has fostered divergence and coexistence, as no trait exhibited higher divergence in sympatry than in allopatry throughout the range (Fig. 1, Scenario II). We do acknowledge that we only were able to measure subset of potentially important traits, some of which may have provided important evidence for divergent character displacement. The sole trait that displayed solid evidence of divergent character displacement (floral PC2) is unlikely to contribute to observed divergence in microhabitat and

exhibited this pattern only for low elevation population pairs. High elevation populations showed the opposite pattern: higher divergence in allopatry than in sympatry (Table S5). Consequently, we infer that initial niche differentiation in sympatry was likely a consequence of ecological sorting based upon existing species differences rather than divergent character displacement. Our findings demonstrate the importance of studying niche evolution in replicated fashion along an environmental gradient, as examining only low elevation or high elevation populations would have led to overestimating or underestimating the role of specific traits in niche evolution.

COMPETITION-MEDIATED SELECTION DRIVES TRAIT EVOLUTION IN *M. alsinoides*

Although ecological sorting appears to be better supported as a mechanism for the initial niche differentiation, character displacement appears to have impacted the reproductive interactions and resource use patterns of both species. Our common garden experiment revealed genetically based patterns of phenotypic divergence among *M. alsinoides* populations along the environmental gradient that differed depending on sympatry with *M. guttatus*. At low elevation, sympatric *M. alsinoides* populations were taller, flowered at a later node, and had a marginally earlier critical photoperiod than allopatric populations. Sympatric *M. alsinoides* populations also had longer mean peduncle lengths and narrower mean flower shapes than allopatric populations (Fig. 3). These differences are most pronounced in high elevation *M. alsinoides* populations, and trait means and maxima for peduncle length and floral PC2 were higher in every sympatric population than in each geographically paired allopatric population. Both floral and developmental patterns in common garden experiments are consistent with morphological patterns in field populations (Figs. S6 and S7 and Table S8), suggesting this variation persists without being obscured by plasticity in native environments.

Because we found no environmental differences between allopatric and sympatric sites other than the presence of the congener, we infer that interspecific competition is likely to be the primary driver of these patterns, although we cannot exclude the possibility of selection caused by any unanticipated, unobserved factors that may also systematically differ between allopatric and sympatric sites. For instance, if sympatric populations differed in an unmeasured way from allopatric populations, environmental filtering may allow only *M. guttatus* or *M. alsinoides* individuals that are preadapted to the unmeasured factor to colonize the site, potentially causing greater phenotypic similarity in sympatry than allopatry (Mayfield and Levine 2010). Moreover, selection due to an unobserved factor that differs between sympatric and allopatric sites could overwhelm selection due to competition and cause trait evolution in both species. We view these possibilities as unlikely, however, given our replication of paired sympatric and allopatric sites. In addition, our greenhouse competition experiments demonstrated that the presence of *M. guttatus* drastically reduces the fitness of *M. alsinoides* and has a major influence on selection gradients for flowering time and plant height (Figs. 4 and 5).

Our common garden data also revealed an influence of competition-mediated selection on *M. guttatus*. Phenotypic differences between allopatric and sympatric populations in plant height at flowering, peduncle length, and floral PC2 were dependent on the climate/elevation of the population (Fig. 3). However, experimental competition with *M. alsinoides* did not alter the selection gradients for plant height, and more generally, experimental competition with *M. alsinoides* had no impact on relative fitness in *M. guttatus*. Several reasons could exist for this contrast. There were large differences in the germination rate for *M. guttatus* versus *M. alsinoides* in this experiment, and the density of *M. alsinoides* may have been insufficient to alter selection gradients. It is also possible that differences in all three traits could be involved in competition with *M. alsinoides* for reproductive resources (i.e., pollinators), which the competition experiment did not incorporate, or in the timing of reproductive allocation relative to terminal drought, which our experiment did not impose.

One striking pattern that emerges from our data is that the impacts of competition-mediated selection change along the elevation gradient. Phenotypic differences between allopatric and sympatric populations in plant height at flowering, peduncle length, and floral PC2 were dependent on the climate/elevation of the population (Fig. 3). In low elevation populations, competition appears to favor taller plants in both species and earlier flowering in *M. alsinoides*. At high elevation, selection favors longer, narrower *M. alsinoides* flowers and shorter, wider *M. guttatus* flowers. These environment-dependent patterns of divergence could reflect alternative, but equivalent, evolutionary solutions to a common selective pressure, or they may indicate that the selection pressure due to competition varies in magnitude or relative importance (compared to abiotic or other biotic factors) based on the environmental context of a population. We view the latter scenario as most likely, given the phenotypes involved and the clinal patterns of variation. The environmental context of competition is also important in sticklebacks, where the presence/absence of predation influences competitive dynamics between ecotypes (Rundle et al. 2003), and in Darwin's finches, where competition only occurs in drought years (Grant and Grant 2006). However, in these cases, only the magnitude of selection imposed by competition is changing, whereas our results suggest that the direction and phenotypic targets of competition-mediated selection may also vary with the environment.

Our observation that competition-mediated selection likely affects different traits in different parts of the elevation gradient is somewhat complicated by the assumptions used to infer

competition as the driving selective pressure. We intentionally surveyed multiple pairs of sympatric and allopatric populations along an elevation gradient with the expectation that sympatric populations would share similar patterns of trait divergence compared to allopatric populations if in situ competition was driving trait evolution. Parsing our dataset into low, middle, and high elevation populations effectively reduces our sample size to two populations pairs in each group and could either cause false inference or missed inferences of competition-mediated selection. However, we only did this as a post hoc test and only tested these hypotheses after looking at the overall clinal trends in our full LMMs. We also have attempted to remedy this limitation by pursuing multiple lines of inquiry—common garden experiment, field observations, and a competition experiment—that all point to similar conclusions.

POTENTIAL EXPLANATIONS FOR ADAPTIVE TRAIT EVOLUTION

Although our results implicate competition between these two *Mimulus* species is likely a key driver of phenotypic evolution, why the evolved differences could be adaptive and how the impact of competition is mediated and interacts with other selective pressures require further investigation. One explanation for the differences in plant height and developmental timing between sympatric and allopatric *M. alsinoides* populations at low elevation could be competition for sunlight. Our experimental finding that both competition with *M. guttatus* and shade treatment favor taller and more rapidly developing *M. alsinoides* plants is consistent with a crowding effect, where *M. alsinoides* must grow quickly to maintain access to sunlight unfettered by the taller, more robust *M. guttatus* (Harley and Bertness 1996). Crowding could also explain differences in developmental rate, seasonal phenology, and marginal differences in critical photoperiod, all of which could lead to earlier annual floral initiation and allow *M. alsinoides* more time for reproduction before *M. guttatus* effectively shades it out. Alternatively, adaptation to life on a more marginal, rapidly drying microhabitat—bare or thinly moss-covered rock wall—at sympatric sites may have driven the evolved differences in plant height and reproductive timing. In sympatry, *M. alsinoides* does not occupy resource-rich areas at the bases of rock walls at sympatric sites, but it does occupy these areas at allopatric sites (Table 1). Notably, in the competition experiment, the shallower soil treatment selected for taller and earlier flowering *M. alsinoides*. Future manipulative experiments in field settings will be required to distinguish whether adaptation to crowding or to resource-poor microhabitats has been the primary driver of the patterns of phenotypic variation among allopatric and sympatric populations.

Several mechanisms may explain how competition selects for longer peduncle lengths and longer, narrower floral shapes in high elevation sympatric populations. Divergence in floral shape could be driven by competition for pollinators (Levin 1985; Bradshaw and Schemske 2003; Grossenbacher and Whittall 2011; Norton et al. 2015). We consider this possibility unlikely for several reasons. First, pollinators are more likely to prefer showy displays of tall, wide corollas unlike the narrow corollas found in sympatric *M. alsinoides* populations. Second, the difference in mean peduncle length between sympatric and allopatric populations is ∼5 mm, an increment we consider unlikely to confer greater visibility to a pollinator. Finally, although the importance of outcrossing for *M. alsinoides* is unknown and its flowers are chasmogamous, *M. alsinoides* may primarily reproduce by selfing in nature. An alternative explanation is that differences in floral shape evolve to prevent fertilization with heterospecific pollen (Galen and Gregory 1989). Longer styles are predicted to effectively reduce heterospecific pollen load (Arceo-Gómez and Ashman 2014). Moreover, species similar to *M. alsinoides*—self-compatible, less abundant, and with wet stigmas—are the most likely to suffer detrimental impacts from heterospecific pollen transfer (Ashman and Arceo-Gomez 2013). Future experiments distinguishing these alternative hypotheses will help reveal whether the phenotypic evolution observed is driven by an arms race for pollinators or a mechanism for niche divergence that allows more efficient reproduction in both species.

Conclusions

A critical conclusion of our studies is the suggestion that empirical studies of ecological sorting and character displacement require experimental designs that allow for nuanced exploration of patterns over environmental gradients and along multiple niche axes in natural populations. Here, we find no evidence for divergent character displacement, and thus we would have inferred that competition between species plays little role in trait evolution, niche differentiation, or coexistence of our focal, closely related congeners had we not compared replicate sympatric/allopatric population pairs in each species. Rather, a more prevalent form of character displacement may be competition-driven changes that occur when niche differentiation necessary to allow coexistence in sympatry has already been achieved through divergence in allopatry, and traits involved in additional competitive interactions are affected instead. Although theoretical work has demonstrated that competition can generate alternative patterns of character displacement (Abrams 1986, 1987; Fox and Vasseur 2008), additional theoretical and empirical studies that examine what parameter spaces lead to various outcomes of trait evolution in sympatry following initial niche differentiation are needed. Developing this extended framework of character displacement may provide new insight into the mechanisms by which closely related species coexist and how species interactions evolve.

DATA ARCHIVING
The doi for our data is doi:10.5061/dryad.176f9.

LITERATURE CITED

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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. Population sampling, climatic information, trait means from common garden, and trait means from field observations.

Table S2. Summaries of climate and floral PCA analyses.

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Table S5. Divergence between sympatric and allopatric populations for each pair of geographically clustered populations.

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Table S9. Summary of relative fitness modeling results from the greenhouse competition experiment.

Table S10. Summary of selection analysis from the greenhouse competition experiment.

Table S11. Permutation test results assessing the statistical significance between selection coefficients for different treatments from greenhouse competition experiment

Figure S1. Plant fitness was not impacted by nearby plant density within each tray. To get plant density, each tray was divided into nine sections and the number of individuals within each section was counted. To get a measure of plant fitness independent of shade and soil depth treatments, we generated residuals from a linear mixed model with flower number as the dependent variable and shade and soil treatments modeled as random variables. If plant fitness was associated with local plant density, we predict a significant correlation between LMM residuals and plant density. Neither *M. guttatus* fitness (A) or *M. alsinoides* fitness (B) was associated with plant density (*M. guttatus*: $r^2 = 0.002$, $p = 0.64$, *M. alsinoides*: $r^2 = 0.004$, $p = 0.38$).

Figure S2. Photograph of the split plot factorial design used in the greenhouse competition experiments

Figure S3. Scatterplot of climate PC1 vs. climate PC2 with points for each sampled population. Loading values indicate that climate PC1 is strongly associated with temperature and precipitation, and climate PC2 is associated with temperature and precipitation seasonality.

Figure S4. Comparison of the distribution of plant soil depths in sympatric populations of *M. alsinoides* (red bars) and *M. guttatus* (blue bars). Paired bars correspond to the populations groups (A-F) described in Table 1.

Figure S5. Comparison of the distribution of plant soil depths in M. alsinoides populations with *M. guttatus* (blue filled bars) and without *M. guttatus* (red filled bars). Paired bars correspond to the populations groups (A-F) described in Table 1.

Figure S6. Regression of mean ppd50 from *M. guttatus* and *M. alsinoides* populations against elevation. Linear models were the best fitting models for allopatric and sympatric *M. guttatus* and *M. alsinoides*, while a logarithmic model best fit sympatric *M. alsinoides* populations.

Figure S7. Boxplots comparing peduncle length (A) and floral PC2 (B) between sympatric and allopatric populations of *M. alsinoides*. Points represent line means.

Figure S8. Mean peduncle length differs between plants measured in sympatric vs. allopatric field populations. The whiskers of the boxplot are the minima and maxima of the data, box lower and upper limits are quartiles and the heavy line is the sample median. Each dot is one plant. $X^2 = 4.2$, $p = 0.04$.

Figure S9. Percentage of plants flowering in each population early in the 2015 growth season (April). Red circles in scatterplot represent sympatric *M. alsinoides* populations, red squares represent allopatric *M. alsinoides* populations, and blue circles represent sympatric *M. guttatus* populations. Flowering status was not measured from the highest elevation sympatric population (BR3) as the location was covered in snow. Number of samples per populations ranged from 10–26 individuals.