

Grow with the flow: a latitudinal cline in physiology is  
associated with more variable precipitation in *Mimulus*  
*cardinalis*

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Running Head: Latitudinal cline and climate in *Mimulus*

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Data will be archived on Dryad upon publication.

## 1 Abstract

2 Local adaptation is one of the most ubiquitous observations in nature: organisms perform  
3 well in their natal environment, but poorly outside it. Correlation between traits and  
4 latitude, or latitudinal clines, are among the most common pieces of evidence for local  
5 adaptation, but identifying the traits under selection and the selective agents are chal-  
6 lenging. Here, we investigated a latitudinal cline in growth and photosynthesis across 16  
7 populations of the perennial herb *Mimulus cardinalis* (Phrymaceae). Using machine learn-  
8 ing methods, we identify interannual variation in precipitation as a likely selective agent:  
9 Southern populations from more variable environments had higher photosynthetic rates  
10 and grew faster. We hypothesize that selection may favor a more annualized life history  
11 – grow now rather than save for next year – in environments where severe droughts occur  
12 more often. Thus our study provides insight into how species may adapt if Mediterranean  
13 climates become more variable due to climate change.

## 14 Introduction

15 Local adaptation within species is ubiquitous; populations generally have higher fitness in  
16 their native environment, but perform poorly outside it (Schluter, 2000; Hereford, 2009).  
17 Local adaptation also frequently leads to clines in both phenotypes and allele frequencies  
18 when selection varies over environmental gradients (Huxley, 1938; Endler, 1977). Pheno-  
19 typic differences between populations along a cline most often have a genetic basis and can  
20 be studied in a common garden (Turesson, 1922; Clausen et al., 1940; Hiesey et al., 1942).  
21 Despite a long history of studying local adaptation and clines, it remains challenging to  
22 identify exactly which traits are under selection and which differ for nonadaptive reasons.  
23 In particular, the role that physiological differences play in local adaptation is poorly un-  
24 derstood, despite the fact that physiology is frequently assumed to explain adaptation to

25 the abiotic environment. We need to understand physiological adaptations within species  
26 as a baseline for anticipating how organisms will respond to climate change. A related  
27 problem is identifying which features of the environment, abiotic factors like soil water  
28 availability or biotic interactions, cause spatially varying selective pressures.

29 When populations are locally adapted, reaction norms for fitness will cross, such that local  
30 genotypes have higher fitness than foreign genotypes and rank orders change across envi-  
31 ronments (Kawecki and Ebert, 2004). The traits that underlie local adaptation, however,  
32 need not mirror this pattern. Populations can have fixed genetic differences conferring trait  
33 values that are adaptive at home but neutral or maladaptive away. Alternatively, genotype-  
34 by-environment interactions could indicate that variation in plasticity mediates local adap-  
35 tation. We distinguish between these patterns of adaptive trait differences by referring to  
36 ‘intrinsic’ and ‘plastic’ trait variation, respectively. Adaptation involves both intrinsic and  
37 plastic trait variation. For example, intrinsic differences in photoperiod (Blackman et al.,  
38 2011) and developmental rate (Stinchcombe et al., 2004) allow organisms to properly time  
39 their life history with the local environment. Conversely, sun and shade plants do not  
40 have intrinsically higher or lower rates of carbon assimilation, but rather, genotype-by-  
41 environment interactions cause sun plants to assimilate more under high light and shade  
42 plants under low light (Givnish, 1988). In plants especially, we know little about the  
43 prevalence and adaptive significance of variation in fundamental physiological traits like  
44 photosynthesis and their impact on plant performance.

45 A basic approach to identify candidate traits underlying local adaptation is to find associ-  
46 ations between traits and environments. Either intrinsic and/or plastic variation should  
47 vary clinally along environmental gradients. Indeed, clines in ecologically important traits  
48 are widespread in nature (Endler, 1977) and often adaptive, but in most cases the selective  
49 agent is unknown. For example, in *Drosophila* numerous latitudinal clines exist for traits  
50 like thermal tolerance (Hoffmann et al., 2002), body size (Coyne and Beecham (1987) and  
51 references therein), and life history (Schmidt et al., 2005). Some *Drosophila* clines have

52 evolved multiple times (Oakeshott et al. (1982); Huey et al. (2000), see also Bradshaw and  
53 Holzapfel (2001)) or shifted in response to climate change (Umina et al., 2005), evincing  
54 climatic adaptation. Similarly, plant species exhibit latitudinal clines in traits like flowering  
55 time (Stinchcombe et al., 2004), cyanogenesis (Kooyers and Olsen, 2012), leaf morphology  
56 (Hopkins et al., 2008), and drought response (Kooyers et al., 2015) that likely relate to  
57 climatic variation.

58 Despite the fact that latitudinal clines have been studied for a long time, latitude *per se*  
59 cannot be a selective agent. Latitude may be strongly correlated with one or two key  
60 climatic variables, such as temperature, precipitation, or growing degree-days. Hence,  
61 latitude is an effective proxy for the underlying climatic driver, but we would expect a  
62 yet stronger relationship between traits and the key climatic variable(s) driving selection.  
63 Alternatively, latitude may be more strongly related to traits than any single climatic  
64 variable for at least two reasons. First, latitude may be correlated with several climatic  
65 agents of selection that are individually weak, but add up to a strong latitudinal cline.  
66 Alternatively, gene flow among neighboring populations could smooth out local climatic  
67 effects, since alleles will experience selection across populations linked by migration. We  
68 refer to this as the ‘climatic neighborhood’. For example, in mountainous regions average  
69 temperature at a given latitude varies widely, but in aggregate, a lower latitude set of  
70 populations will experience warmer climate than a higher latitude one. Thus, any particular  
71 low latitude population would be warm-adapted, even if it was located in a cooler (e.g. high  
72 elevation) site. Because many climatic factors vary latitudinally, and which climatic factors  
73 vary latitudinally changes over the earth’s surface (e.g. coastal vs. continental), dissecting  
74 the evolution of latitudinal clines across many species will help identify generalities, such  
75 as whether thermal tolerance maxima or seasonal timing is more important (Bradshaw and  
76 Holzapfel, 2008), or whether local versus regional climate shape selective pressures.

77 In this study, we investigated two major questions: 1) whether intrinsic or plastic physiolog-  
78 ical trait variation corresponds with latitude; and 2) what climatic factor(s) could plausibly

79 be responsible for latitudinal clines. Within question 2, we tested three hypotheses outlined  
80 in the previous paragraph: latitudinal clines are explained by a single dominant climatic  
81 factor, multiple climatic factors, or the climatic neighborhood experienced by nearby pop-  
82 ulation connected through gene flow. These hypotheses are not mutually exclusive since,  
83 for example, single or multiple factors in a climatic neighborhood may lead to latitudinal  
84 clines.

85 We examined these questions in *Mimulus cardinalis* because linking physiological traits  
86 to potentially complex patterns of local adaptation requires integrating multiple lines of  
87 evidence from comparative, experimental, and genomic studies under both lab and field  
88 conditions. Many classic and contemporary studies of local adaptation use species from  
89 genus *Mimulus* because of its natural history, easy propagation, and genetic/genomic re-  
90 sources (Clausen et al., 1940; Hiesey et al., 1971; Bradshaw and Schemske, 2003; Wu et al.,  
91 2008; Lowry and Willis, 2010; Wright et al., 2013). Yet, there is a conspicuous deficiency of  
92 links between local adaptation and physiological mechanisms (Angert, 2006; Angert et al.,  
93 2008; Wu et al., 2010). We measured genetic and genotype-by-environment variation in  
94 response to temperature and drought among 16 populations distributed over 10.7° of lat-  
95 itude. We found a latitudinal cline of intrinsic variation in photosynthesis and growth,  
96 but little evidence for variation in plasticity. Interannual variation in precipitation and  
97 temperature are associated with this axis of variation, suggesting that climatic variance  
98 rather than mean may be an important driver of local adaptation in *M. cardinalis*. The  
99 climatic neighborhoods around populations explained trait variation better than local cli-  
100 mate, indicating that latitudinal clines may be common because latitude integrates effects  
101 of selection on populations connected through gene flow. We place these findings in the  
102 context of life history theory and consider future directions in the Discussion.

## 103 Material and Methods

### 104 Population Selection

105 We used 16 populations from throughout the range of *M. cardinalis* (Table 1). Seeds were  
 106 collected in the field from mature, undehisced fruit left open for 2-4 weeks to dry, then  
 107 stored at room temperature.

**Table 1:** Geographic region, latitude, longitude, and elevation (mas = meters above seal level) of 16 focal populations used in this study.

Name	Region	Latitude	Longitude	Elevation (mas)
Hauser Creek	South Margin	32.657	-116.532	799
Cottonwood Creek	South Margin	32.609	-116.7	267
Sweetwater River	South Margin	32.9	-116.585	1180
Grade Road Palomar	South Margin	33.314	-116.871	1577
Whitewater Canyon	Transverse	33.994	-116.665	705
Mill Creek	Transverse	34.077	-116.873	2050
West Fork Mojave River	Transverse	34.284	-117.378	1120
North Fork Middle Tule River	South Sierras	36.201	-118.651	1314
Paradise Creek	South Sierras	36.518	-118.759	926
Redwood Creek	South Sierras	36.691	-118.91	1727
Wawona	Central Sierras	37.541	-119.649	1224
Rainbow Creek	Central Sierras	37.819	-120.007	876
Middle Yuba River	North Sierras	39.397	-121.082	455
Little Jamison Creek	North Sierras	39.743	-120.704	1603
Deep Creek	North Coast	41.668	-123.11	707
Rock Creek	North Margin	43.374	-122.957	326

### 108 Plant propagation

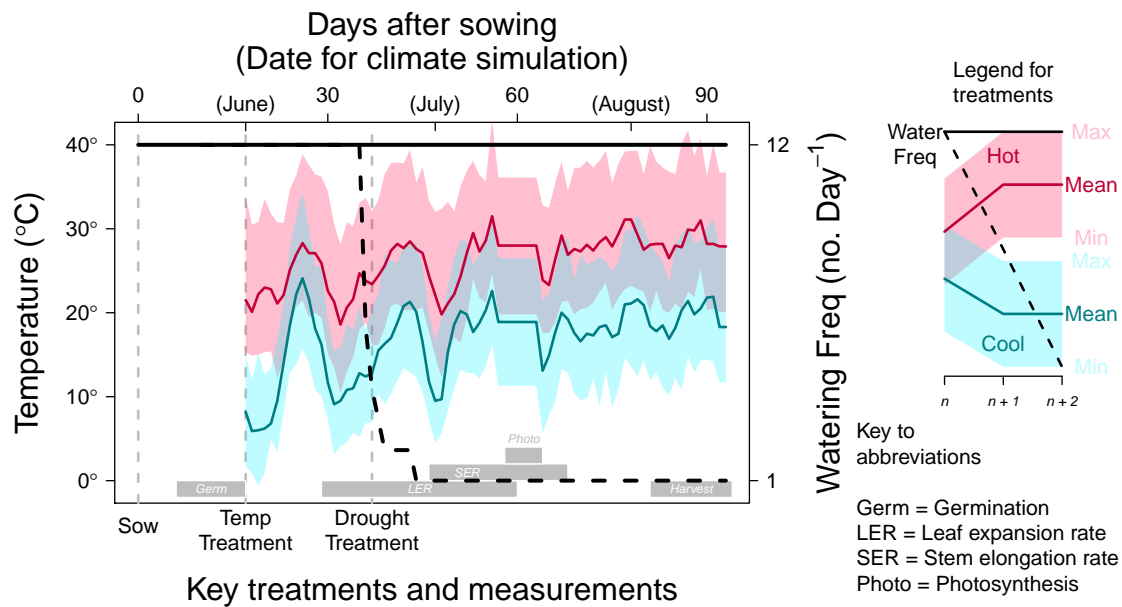
109 On 14 April, 2014, 3-5 seeds per family were sown directly on sand (Quikrete Play Sand,  
 110 Georgia, USA) watered to field capacity in RLC4 Ray Leach cone-tainers placed in RL98  
 111 98-well trays (Stuewe & Sons, Inc., Oregon, USA). We used pure sand both to facilitate

112 root-washing and because *M. cardinalis* typically grows in sandy, riparian soils (A. Angert,  
113 pers. obs.). Two jumbo-sized cotton balls at the bottom of cone-tainers prevented sand  
114 from washing out. Cone-tainers sat in medium-sized flow trays (FLOWTMD, Stuewe  
115 & Sons, Inc., Oregon, USA) to continuously bottom-water plants during germination in  
116 greenhouses at the University British Columbia campus in Vancouver, Canada (49°15'  
117 N, 123°15' W). Mistlers thoroughly wetted the top of the sand every two hours during  
118 the day. Most seeds germinated between 1 and 2 weeks, but we allowed 3 weeks before  
119 transferring seedlings to growth chambers. We recorded germination daily between one to  
120 two weeks after sowing, and every 2-3 days thereafter. On 5 May (21 days after sowing),  
121 we transferred seedlings to one of two growth chambers (Convion, Manitoba, Canada).  
122 We thinned seedlings to one plant per cone-tainer, leaving the center-most plant. 702  
123 of 768 (91.4%) had plants that could be used in the experiment. We allowed one week  
124 at constant, non stressful conditions (day: 20°C, night: 16°C) for plants to acclimate to  
125 growth chambers before starting treatments. The initial size of seedlings, measured as the  
126 length of the first true leaves, did not differ between populations, families, or treatments  
127 (Table S1).

## 128 **Temperature and drought treatments**

129 We imposed four treatments, a fully-factorial cross of two temperature levels and two  
130 watering levels. The temperature levels closely simulated an average growing season at the  
131 thermal extremes of the species range, which we designate as Hot and Cool treatments.  
132 Watering levels contrasted a perennial and seasonal stream, which we refer to as Well-  
133 watered and Drought treatments. A detailed description of treatments is provided in the  
134 Supplemental Materials and Methods and summarized in Fig 1. Because growth chambers  
135 cannot be subdivided, one chamber was assigned to the Hot treatment level and another  
136 to the Cool treatment level. Within each chamber, there were two Well-watered blocks

137 and two Drought blocks. The photosynthetically active radiation in both chambers was  
 138 approximately  $400 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ . The growth chambers did not control humidity,  
 139 but because of watering and high plant transpiration rates, the relative humidity was quite  
 140 high in both temperature levels (data not shown).



**Figure 1:** Overview of experimental treatments and timing of key trait measurements. All plants germinated within 21 days of sowing. At that time, we began temperature treatments (left axis), simulating a typical June-August weather pattern at Hot (red) and Cool (blue) sites. The bold lines track the average daily temperatures. Within each day, there was a maximum daytime temperture (top of translucent polygons) and minimum nighttime temperature (bottom of translucent polygons). The drought treatment commenced later by ramping down the frequency of bottom-watering episodes (dashed black line; right axis), while watering frequency was maintained in the control treatment (solid black line). Grey boxes on the bottom of the plot outline the period of key measurements described in the Material and Methods.

## 141 Trait measurements

142 We measured five traits in response to temperature and watering treatments (Table 2).



**Table 2:** Key traits measured in this study.

Trait	Units
Days to germination	day
Leaf expansion rate	mm day <sup>-1</sup>
Stem elongation rate	mm or cm day <sup>-1</sup>
Photosynthetic rate	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
Mortality	probability of death

143 **Days to germination** We tested for population variation in germination rate, measured  
144 as Days to Germination, using a lognormal survival model fit using the survreg function  
145 in the R package **survival** version 2.38 (Therneau, 2015). We treated Population as a fixed  
146 effect and Family as random effect using a  $\Gamma$  frailty function. Statistical significance of the  
147 Population effect was determined using analysis of deviance. Note that, unlike other traits  
148 discussed below, we did not include Block, Treatment, or Population  $\times$  Treatment inter-  
149 actions because during germination plants had not been placed into blocks and treatments  
150 had not yet been applied.

151 **Growth rate: leaf expansion and stem elongation** We measured growth rate during  
152 two phases: leaf expansion and stem elongation. We censused leaf length twice per week  
153 from 12 May – 12 June (28–59 days after sowing), resulting in 10 measurements. We ceased  
154 measuring leaf length once it appeared to asymptote and growth shifted to stem elongation.  
155 We also censused plant height on 7 occasions (twice per week) between 29 May and 20 June  
156 (45 to 67 days after sowing). Both leaf expansion and stem elongation were modeled as  
157 second-order polynomials of time with individual coefficients (separate for leaf and stem  
158 growth) using empirical Bayes' estimates from linear mixed-effects models fit with the R  
159 package **lme4** version 1.1-12 (Bates et al., 2015).

160 **Photosynthesis** During the week of 10 to 16 June (57 to 63 days after sowing), we  
 161 measured daytime photosynthetic rate on a subset of 329 plants evenly spread between  
 162 treatments and families within populations. The youngest, fully-expanded leaf acclimated  
 163 for 3 minutes to reach steady state in a 6-cm<sup>2</sup> chamber of a LI-COR 6400XT Portable Pho-  
 164 tosynthesis System (LI-COR Biosciences, Lincoln, Nebraska). We made all measurements  
 165 at ambient light (400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of photosynthetically active radiation), atmospheric  
 166 CO<sub>2</sub> (400 ppm), temperature, and moderate relative humidity. During this period, we  
 167 suspended normal day-to-day temperature fluctuations and set daytime temperatures to  
 168 its average for that period (Cool: 26.5°; Hot: 36.1° so that all plants within a temperature  
 169 level could be measured under the same conditions.

170 **Mortality** We assayed mortality during twice-weekly growth measurements. We ana-  
 171 lyzed the probability of surviving until the end of the experiment as a function of popula-  
 172 tion, treatment, and their interactions using a Generalized Linear Mixed Model (GLMM)  
 173 assuming binomially distributed errors. We included Family and Block as random effects.  
 174 We assessed significance of fixed effects using Type-II Analysis of Deviance with Wald  $\chi^2$   
 175 tests in the R package **car** (Fox and Weisberg, 2011).

## 176 **Intrinsic variation and plasticity**

177 For all traits (Table 2) except germination (see above), we tested for Population, Treatment,  
 178 and Population  $\times$  Treatment interactions. We interpreted significant Population effects to  
 179 indicate intrinsic variation and Population  $\times$  Treatment interactions to indicate variation  
 180 in plasticity. As mentioned above, we used survival and GLMM models for germination rate  
 181 and mortality, respectively. For all other traits, we used mixed model ANOVAs with Family  
 182 and Block included as random factors. We fit models using restricted maximum likelihood  
 183 in lmer, a function in the R package **lme4** (Bates et al., 2015). We determined significant

fixed effect terms using a step-wise backward elimination procedure implemented with the step function in the R package **lmerTest** version 2.0-32 (Kuznetsova et al., 2016). This package uses Satterthwaite’s approximation to calculate denominator degrees of freedom for  $F$ -tests. We also included days to germination as a covariate in growth analyses.

## Principal components of germination, growth, and photosynthesis

For each single-trait model above, we extracted the Population coefficient (factoring out Treatment and other effects). The multivariate distribution of these coefficients was then summarized using principal components analysis. The first principal component of these traits (TraitPC1) loaded positively with germination, growth, and photosynthetic rate, therefore we define this as a phenotypic axis delineating fast to slow growth.

## Identifying putative selective agents

We found that a population’s position along TraitPC1 correlated strongly with the latitude or origin (see Results) and next used Random Forest regression (Liaw and Wiener, 2002) to identify putative climatic factors underlying trait-latitude associations in *M. cardinalis*. Hypothesis 1: if a single climatic factor explained more trait variation than latitude alone, this would suggest that that factor is a selective agent underlying the latitudinal cline in our 16 focal populations. Hypothesis 2: if multiple climatic factors together explained trait variation, we interpreted this as evidence that multiple climatic factors together have generated the latitudinal cline. We hereafter refer to factors identified in this analysis as ‘Climate-TraitPC1’ variables. In addition, to help eliminate potentially spurious correlations between TraitPC1 and climate, we tested for overlap between climatic variables that best predict latitude of all *M. cardinalis* occurrence records (see detail below), not just the 16 focal populations. We refer to these climatic factors as ‘Climate-Latitude’ variables. The logic is that climatic factors associated with both TraitPC1 and latitude for all popu-

lations are more likely to be important selective agents than climatic factors that happen to correlate with TraitPC1 but do not covary with latitude throughout the *M. cardinalis* range. Therefore, we did not consider Climate-TraitPC1 variables to be candidates selective agents unless the same or very similar variable was found in the Climate-Latitude analysis. We selected Climate-Latitude and Climate-TraitPC1 variables independently using Variable Selection Using Random Forest (VSURF) algorithm in the R package **VSURF** version 1.0.3 (Genuer et al., 2016). VSURF ranks variables by their importance over regression trees in the forest. We kept only variables selected for prediction, the most stringent criterion. A visual overview of how we selected climatic variables is depicted in Fig 2.

To test the third hypothesis about climatic neighborhoods driving selection, we directly competed local with neighborhood climate to see which correlated with traits (Climate-TraitPC1 variables) and latitude (Climate-Latitude variables) better. We used the immediate collection location for local climate. For climate neighborhoods, we sampled climate at 1000 random points (at 90-m resolution) within a 62-km radius buffer around the collection collection. We chose this buffer size because neutral genetic differentiation increases slowly with geographic distance, indicating significant gene flow between nearby populations (Paul et al., In review). Significant spatial autocorrelation persisted for approximately 62 km. Since *M. cardinalis* is found exclusively in riparian areas, we only selected points along streams using the National Hydrography Dataset (United States Geological Survey, 2015). Climatic means and variances (see below) were weighted by their climatic suitability as determined using a multimodel ensemble average of ecological niche models (Angert et al., 2016).

For Climate-Latitude analyses, we compiled a representative set of 356 recent (since 2000) known *M. cardinalis* occurrences. These occurrences were thinned by 50% to correct for uneven sampling from a comprehensive set of herbarium records and an exhaustive field survey in 2010-11 (Angert et al., 2016). For both Climate-TraitPC1 analyses (16 focal populations) and Climate-Latitude (many populations), we used a 90-m digital elevation

235 model from HydroSHEDS (Lehner et al., 2006) to extract elevation. Monthly interpolated  
 236 climate layers were calculated using ClimateWNA version 5.30 (Wang et al., 2012), which  
 237 accurately downscales climate data specifically for the rugged topography of western North  
 238 America. For each occurrence, we calculated bioclimatic variables using the biovars function  
 239 in the R package **dismo** version 1.1-1 (Hijmans et al., 2016). We included 24 climate factors,  
 240 9 from ClimateWNA and 15 bioclimatic variables (Table S2). The bioclimatic variables  
 241 included all permutations of two climatic factors, temperature and precipitation, and six  
 242 temporal scales (annual average, coldest quarter, warmest quarter, wettest quarter, driest  
 243 quarter, or seasonality) as well as mean diurnal range, isothermality, annual temperature  
 244 range. For each variable, we calculated both a 30-year normal by averging annual values  
 245 between 1981 and 2010 and 30-year coefficient of variation, a standardized metric of inter-  
 246 annual climatic variation. Temperatures were converted to Kelvin to be on a ratio scale  
 247 appropriate for calculating the coefficient of variation (CV). In total, the VSURF algorithm  
 248 selected among 96 climate variables: 24 climatic factors  $\times$  2 types (30-year average and  
 249 CV)  $\times$  2 spatial scales (local and neighborhood).

## 250 **Results**

### 251 **A coordinated latitudinal cline in germination, growth, and photosynthe-** 252 **sis**

253 There are strong genetically-based trait differences in time to germination, growth, and  
 254 photosynthetic rate among populations of *M. cardinalis*, as evidenced by large and signif-  
 255 icant population effects for these traits (Table 3). A single principal component captured  
 256 71.6 % of the trait variation among populations, defining an axis of variation from fast  
 257 to slow growth (Fig 3). As we explain below, intrinsic differences between populations in  
 258 terms of plant function (photosynthesis) and performance (growth) contrasted with the

low amount of variation in plasticity. There were similar latitudinal clines for individual traits underlying PC1 (Fig S1-S4).

**Table 3:** Summary of Population, Treatment, and Population  $\times$  Treatment effects. We used different statistical modeling for the diverse traits assayed – glmer: generalized linear mixed model using the R package **lme4** (Bates et al., 2015); lmer: linear mixed model using the R package **lme4** (Bates et al., 2015); survreg: survival regression using the R package **survival** (Therneau, 2015). Note that temperature and water treatments were imposed after germination, hence are not applicable to this trait. Complete analysis of variance/deviance tables for each trait are available in the Supporting Information. Key to statistical significance: \* $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

Trait	Germination	Leaf expansion	Stem elongation	Photosynthesis	Mortality
Statistical model	survreg	lmer	lmer	lmer	glmer
Population	***	***	***	***	
Temperature	NA	***	***	**	***
Water	NA	*			***
Pop $\times$ Temp	NA			*	
Pop $\times$ Water	NA	*			
Temp $\times$ Water	NA				***
Pop $\times$ Temp $\times$ Water	NA				

## Little evidence for variation in plasticity

Genotype  $\times$  environment (G $\times$ E) interactions are also a common signature of local adaptation. We found little evidence G $\times$ E in *M. cardinalis*. There were only two statistically significant Population  $\times$  Treatment interaction (Table 3), but these were not strong compared to Population and Temperature effects. Otherwise, populations responded similarly to treatments: faster growth in the hot treatment, slower growth in the dry treatment, and high mortality in the hot, dry treatment (Table 3). Note that interactions were calculated after factoring out intrinsic trait differences, necessarily reducing statistical power to detect significant interactions relative to main effects. However, the fact that the Population and Temperature effects were often highly significant ( $P \ll 0.001$  in most cases) suggests that statistical power alone cannot explain why we failed to detect Population  $\times$  Treatment

interactions. Complete ANOVA tables are available in the Supporting Information (Table S3-S6).

## **Neighborhood climatic variability best explains latitudinal cline**

Latitudinal clines are common, but it is often difficult to ascribe this variation to a particular selective agent. To reiterate, we tested three non-mutually exclusive hypotheses about how such latitudinal clines emerge: 1) one or two climatic variable explains latitudinal trait variation; 2) latitude is a proxy for multiple climatic factors that together shape trait variation; and 3) latitude integrates selection in a broader climatic neighborhood. Based on exploratory variable selection analyses, one or two aspects of interannual climatic variation (hypothesis 1) and climatic neighborhoods (hypothesis 3) are likely more important drivers of trait divergence than average climate immediately surrounding populations. These general conclusions are based on the fact that interannual variation in climate averaged over 62-km radius climatic neighborhoods around populations correlated most strongly with trait variation and latitude of *M. cardinalis* occurrences (Fig. 4, Table S7). All 16 Climate-Latitude and 3 Climate-TraitPC1 variables were neighborhood rather than local variables (Fig. 4). The most important climatic variables related to the interannual variation rather than average climate.

Among Climate-Latitude and Climate-TraitPC1 variables, neighborhood climatic variability over 30 years (1981–2010) in either winter precipitation ( $\text{bio16}_\sigma$ ) and/or temperature ( $\text{bio11}_\sigma$ ) are the strongest candidates driving the latitudinal cline in *M. cardinalis* (see Table S2 for a key to climate variable abbreviations). Note that the coefficient of variation of a climatic factor is subscripted with  $\sigma$  whereas the mean is subscripted with  $\mu$ . More specifically, greater winter precipitation variability and lower winter temperature variability are associated with Southern latitudes and higher TraitPC1 values (Fig. 5A,B). Neighborhood interannual variation in winter precipitation ( $\text{bio16}_\sigma$ ) was the most important Climate-

297 Latitude variable (Fig. 4A). However, neighborhood  $\text{bio16}_\sigma$  did not overlap with Climate-  
 298 TraitPC1 variables (Fig. 4B). We nevertheless consider it a plausible candidate for two  
 299 reasons. First, neighborhood  $\text{bio16}_\sigma$  correlated strongly with TraitPC1 (Fig. 5A). Second,  
 300 one of the most important Climate-TraitPC1 variables (neighborhood  $\text{bio15}_\sigma$ ; Fig. 5B,C)  
 301 is very similar to  $\text{bio16}_\sigma$ . In Mediterranean climates like California, most precipitation  
 302 occurs in the wettest quarter (winter), so years with low winter precipitation also have  
 303 low precipitation seasonality. Hence, highly variable year-to-year winter precipitation at  
 304 lower latitude (Fig. 5D) is closely associated with large swings in precipitation seasonality  
 305 (Fig. 5C).

306 Interannual variation in temperature of the coldest quarter (neighborhood  $\text{bio11}_\sigma$ ) is an-  
 307 other plausible candidate because it was only variable in both Climate-Latitude and Climate-  
 308 TraitPC1 analyses (Fig. 4). Neighborhood  $\text{bio11}_\sigma$  explained more variation in TraitPC1  
 309 than latitude (latitude  $r^2 = 0.55$  vs.  $\text{bio11}_\sigma$   $r^2 = 0.6$ ; Fig. S5), whereas neighborhood  
 310  $\text{bio16}_\sigma$  did slightly worse ( $\text{bio16}_\sigma$   $r^2 = 0.49$ ). Models using  $\text{bio15}_\sigma$  or  $\text{bio11}_\sigma$  to predict  
 311 TraitPC1 also had significantly lower Akaike Information Criteria (AIC) than the latitude  
 312 model (AIC of different models –  $\text{bio15}_\sigma$ : 48.5;  $\text{bio11}_\sigma$ : 52.4; latitude: 54.5). The best two-  
 313 factor model including both neighborhood  $\text{bio15}_\sigma$  and  $\text{bio11}_\sigma$  did not significantly improve  
 314 explanatory power ( $r^2 = 0.71$ , AIC= 49.2). In summary, either variation in precipita-  
 315 tion or temperature seasonality may be important selective agents, but there is no strong  
 316 evidence that they are both important. The most important Climate-TraitPC1 variable,  
 317 neighborhood variation in mean diurnal range ( $\text{bio2}_\sigma$ ; Fig. 4B) did not have any obvious  
 318 similarity to Climate-Latitude variables. Given the large number of potential associations,  
 319 we therefore think this may be a spuriously strong relationship.



## Discussion

We found evidence for one of two common signatures of local adaptation in the perennial herb *Mimulus cardinalis*. Latitudinal clines in germination rate, photosynthesis, and growth, suggest adaptive differentiation in fundamental physiological traits of the species. However, we found little evidence that populations respond differently to temperature or drought. As we discuss below, this latter result may indicate that the fundamental abiotic niche is relatively conserved. Finally, we found that neighborhood climatic variation between years may be a more important selective agent than the local average climate. In the paragraphs that follow, we tie these results into the broader threads of evolutionary theory that might help explain why intrinsic variation in physiology changes clinally, whereas plastic responses to temperature and drought are relatively similar.

Evolutionary theory indicates that the shape of fitness tradeoffs, demography, and gene flow can constrain adaptation (Levins, 1968; Ronce and Kirkpatrick, 2001) and hence the type of variation maintained within species. Specifically, adaptive variation cannot be maintained by spatially varying selection if tradeoffs are too strong, demography is strongly asymmetric, and/or maladaptive gene flow is too high. In *M. cardinalis* we found substantial genetically based variation among populations along a phenotypic axis from fast to slow growth that varied over a large spatial scale (Fig. 3). If this variation is adaptive, it suggests that the fitness tradeoff between low versus high latitude environments is not too strong nor swamped by demographic asymmetry or maladaptive gene flow. That is, alleles favoured at one latitude are not strongly selected against when they flow to another population, allowing locally adaptive genetic variation to be maintained by spatially heterogeneous selection. We also know from previous work that population size does not vary strongly with latitude. Gene flow appears to be high, but attenuates at broad spatial scales, especially between Southern ( $< 35^{\circ}\text{N}$ ) and Northern portions of the range (Paul et al., In review). Nevertheless, local gene flow from similar environments may shape how

346 selection varies with latitude. Climatic neighborhoods, defined here as the average climate  
347 in a 62-km radius around populations, correlated more strongly with trait and population  
348 latitude than local climate. This may not greatly constrain local adaptation because local  
349 and neighborhood climate values were generally similar in *M. cardinalis* populations (data  
350 not shown).

351 Another possibility we could have seen is that southern populations, which appear to  
352 experience more frequent drought years (see below), could have adapted by tolerating  
353 drought better than northern populations, thereby expanding the fundamental niche of the  
354 species as a whole. We found no evidence for this; all populations responded to drought and  
355 temperature similarly (Table 3). Plants grew faster in the Hot treatment, but there was  
356 little effect of drought on growth. Rather, the effects of drought took longer to materialize  
357 but resulted in high mortality, especially in the Hot chamber. However, there was no  
358 differential mortality among populations in this treatment. We hypothesize that evolution  
359 of the fundamental niche may be constrained by a combination of strong fitness tradeoffs,  
360 demographic asymmetry, and gene flow. Riparian habitats where *M. cardinalis* live are  
361 highly heterogeneous at small spatial scales. Plants in the stream never have to tolerate  
362 drought whereas plants only a few meters away may experience extreme drought since  
363 there is little direct precipitation during the growing season in Mediterranean climates of  
364 western North America. But alleles that confer greater drought tolerance may be quite  
365 costly in well-watered soils, and *vice versa*, leading to strong fitness tradeoffs. Such tradeoffs  
366 promote specialization to one soil type or another, thereby inhibiting the evolution of broad  
367 environmental tolerance within a population. Demography and gene flow may reinforce  
368 niche conservatism. A new mutant with increased drought tolerance that can survive at the  
369 resource-poor margin of a population will be demographically overwhelmed by the larger  
370 census populations that can be maintained in higher-resource environments. Infrequent  
371 wet years may also produce most seeds, so selection is weighted towards alleles that have  
372 high fitness in the wet environment, even if dry years are more frequent. Finally, gene

373 flow, which is generally high among *M. cardinalis* populations within the same ecoregion  
374 (Paul et al., In review), will thwart local adaptation and reinforce specialization. Thus,  
375 the spatial grain of the environment, demographic asymmetry, and gene flow may conspire  
376 to constrain local adaptation via altered fundamental niche.

377 To reiterate, these results indicate that differences in physiology and growth, but not re-  
378 sponses to temperature and drought, mediate local adaptation to climate in *M. cardinalis*.  
379 Next, we would like to understand why variation in these particular traits may be adap-  
380 tive. We argue that temporally more variable environments, as experienced by southern  
381 populations, select for a more ‘annualized’ life-history strategy.

382 Greater investment in aboveground growth, as opposed to belowground storage for future  
383 seasons, may be favoured in climates with more frequent drought years, but maladaptive  
384 in climates with more consistent precipitation. Suppose plants that grow quickly and  
385 allocate new resources to continued growth rather than storage have higher fitness over a  
386 single growing season. However, by not allocating resources to storage, these fast-growing  
387 plants begin future seasons at a deficit. Therefore, in a stable environment where winter  
388 survivorship is assured in most years, failure to store resources may reduce lifetime fitness.  
389 But the environment is not constant. For perennial herbs in Mediterranean climates, a  
390 dry winter (rainy season) can kill the rhizomes (underground stems that store nutrients  
391 for future growth) before emergence or aboveground stems before flowering. If drought  
392 years occur frequently enough, selection may favour the fast-growing strategy because  
393 there is no advantage to storage if drought kills plants before flowering. Considering life-  
394 history strategy as a continuum from no storage (annual) to lots of storage (perennial),  
395 we hypothesize that the optimal allocation to aboveground growth is more ‘annualized’ in  
396 southern climates that have greater interannual variation in precipitation.

397 The hypothesis that greater precipitation variability selects for an annualized life history  
398 is tentative, but consistent with theory, data from other species, and other observations in

399 *M. cardinalis*. Life history theory shows that less variable environments are one factor that  
 400 favours the evolution of perenniality (Stearns, 1976; Iwasa and Cohen, 1989; Friedman and  
 401 Rubin, 2015). Populations of the perennial *Plantago asiatica* show a similar latitudinal  
 402 cline in growth and allocation to storage (Sawada et al., 1994), though these authors  
 403 attribute the cline to variation in growing season length. There are also life history clines  
 404 in the closely related species *M. guttatus*, but the underlying traits and climatic drivers are  
 405 quite different. Annual *M. guttatus* flower sooner and produce fewer stolons in response  
 406 to climates with shorter seasons and more intense summer drought (Lowry and Willis,  
 407 2010; Friedman et al., 2015; Kooyers et al., 2015). In contrast, there are no truly annual  
 408 (monocarpic and semelparous) populations of *M. cardinalis*. Rather, our hypothesis states  
 409 climatic variability selects on quantitative variation in allocation to growth versus storage.  
 410 Several lines of evidence provide tentative support for this hypothesis. Preliminary surveys  
 411 suggest that northern populations not only grow slower, but also produce greater numbers  
 412 of rhizomes (C.D. Muir, unpub. data), suggesting an allocation tradeoff. Ecological niche  
 413 models also show that occurrence of southern populations is best predicted by recent climate  
 414 ( $< 5$  years), whereas northern occurrences are best predicted by climate over the previous  
 415 30 years (M. Bayly & A. Angert, unpub. data). Finally, demographic surveys of natural  
 416 populations show greater variation in the size of recruits in southern populations, suggesting  
 417 higher maximum growth rates under natural conditions (M. Bayly & A. Angert, unpub.  
 418 data). Exploratory analysis also pointed to variation in winter temperature as a potential  
 419 selective agent. Specifically, winter temperature is more variable in the north, which may  
 420 lead to less predictability in the start of the growing season or late frost. This could affect  
 421 selection on phenology, which we did not study here, but it is not obviously connected to  
 422 the latitudinal cline in physiological traits.

423 In summary, we found evidence for a coordinated latitudinal cline in germination rate,  
 424 photosynthesis, and growth, suggesting local adaptation. These differences between popu-  
 425 lations suggest different trait optima in different climates. We did not find evidence that

the relative performance of populations shifts with temperature or watering regime, suggesting relatively little variation in plasticity. Exploratory analysis implicate that more variable precipitation regimes at lower latitude drive much of the latitudinal cline, though other climatic factors could also contribute. Interestingly, the climatic neighborhood may shape selective pressures than local climate. In the future, we will use field experiments to test whether greater variation in precipitation selects for faster growth and that selection on temperature/drought responses does not vary among populations. By doing so, we aim to understand why certain physiological and developmental mechanisms, but not others, contribute to local adaptation.

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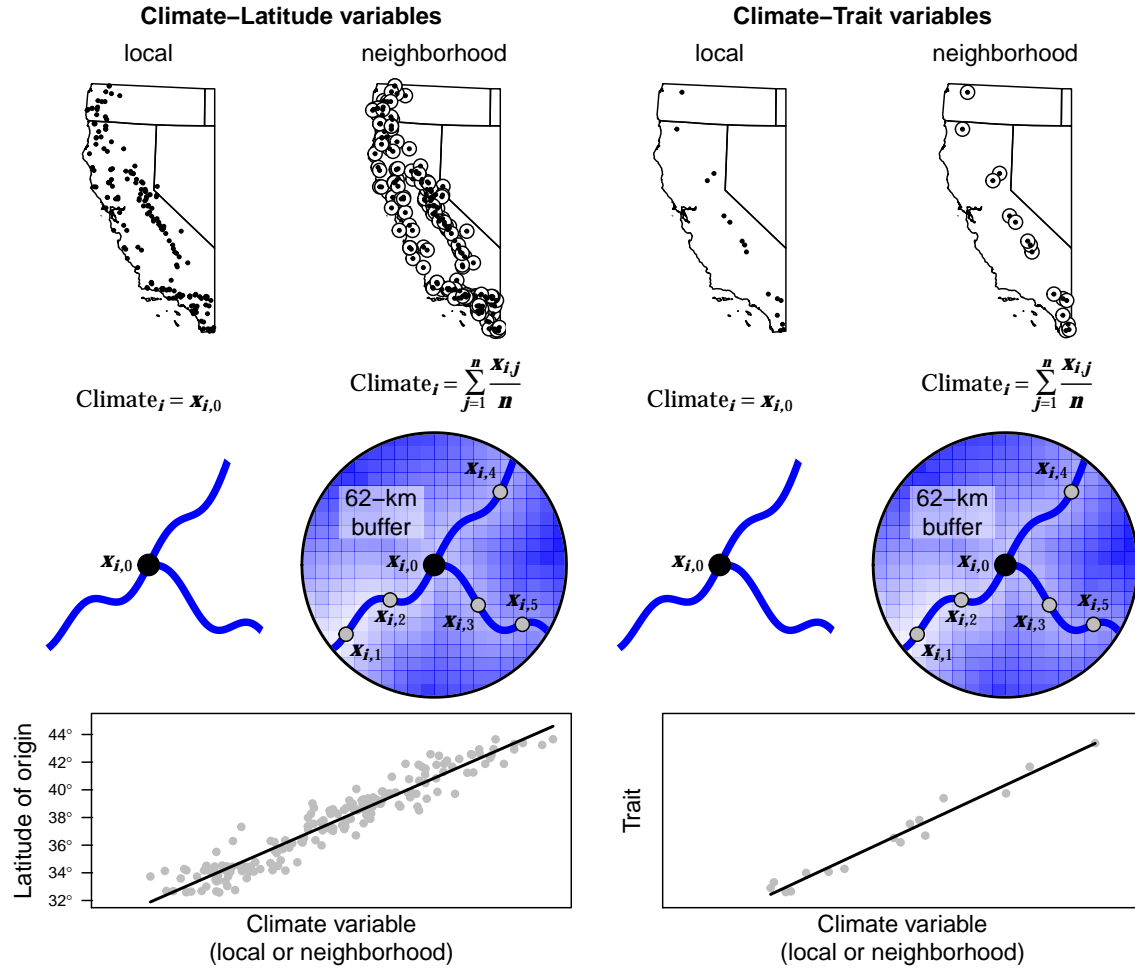
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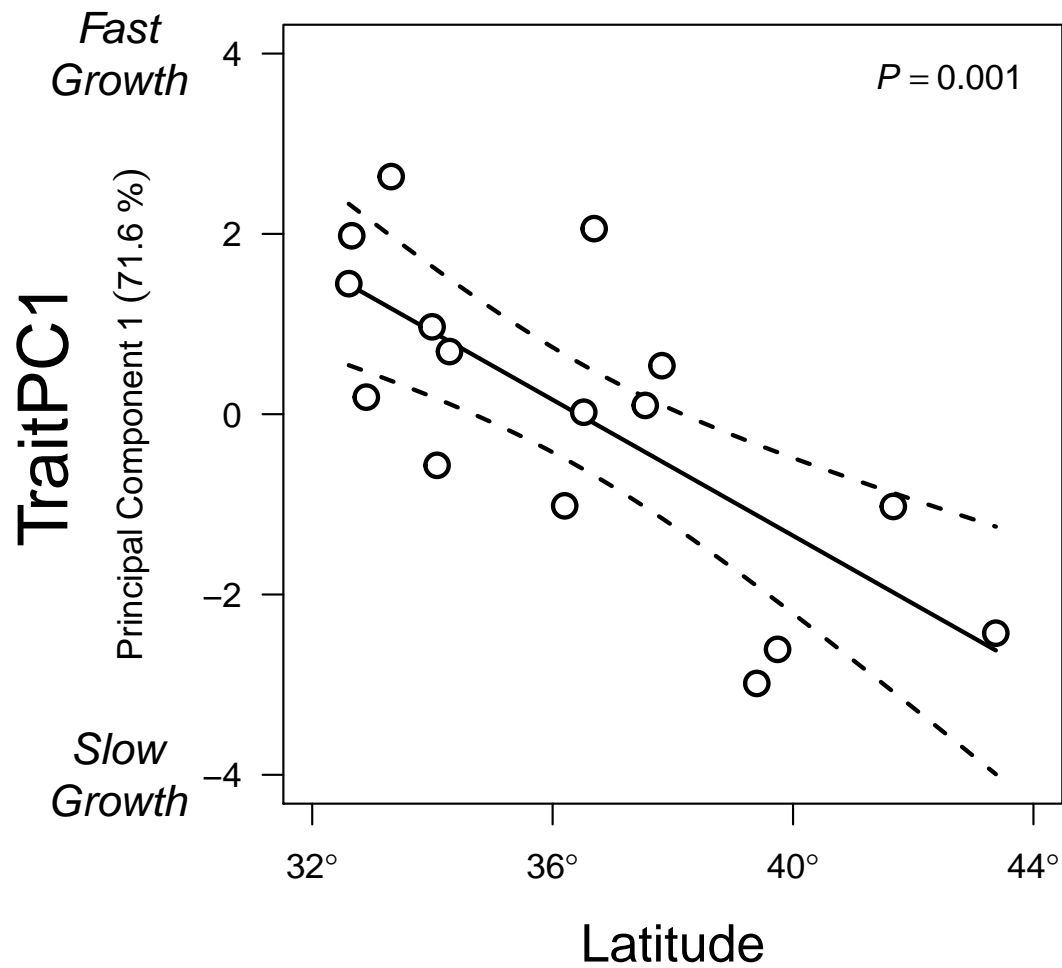
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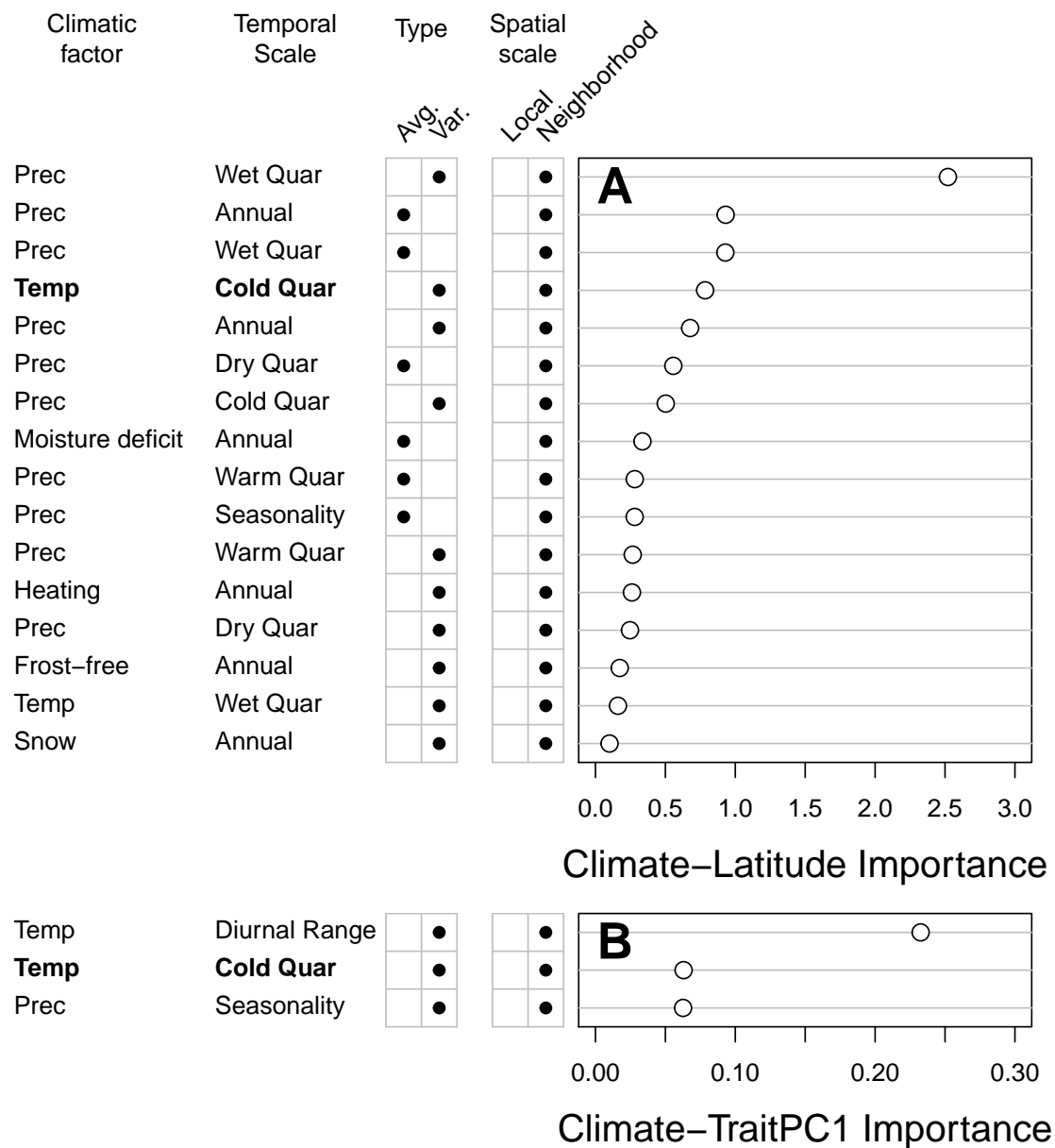
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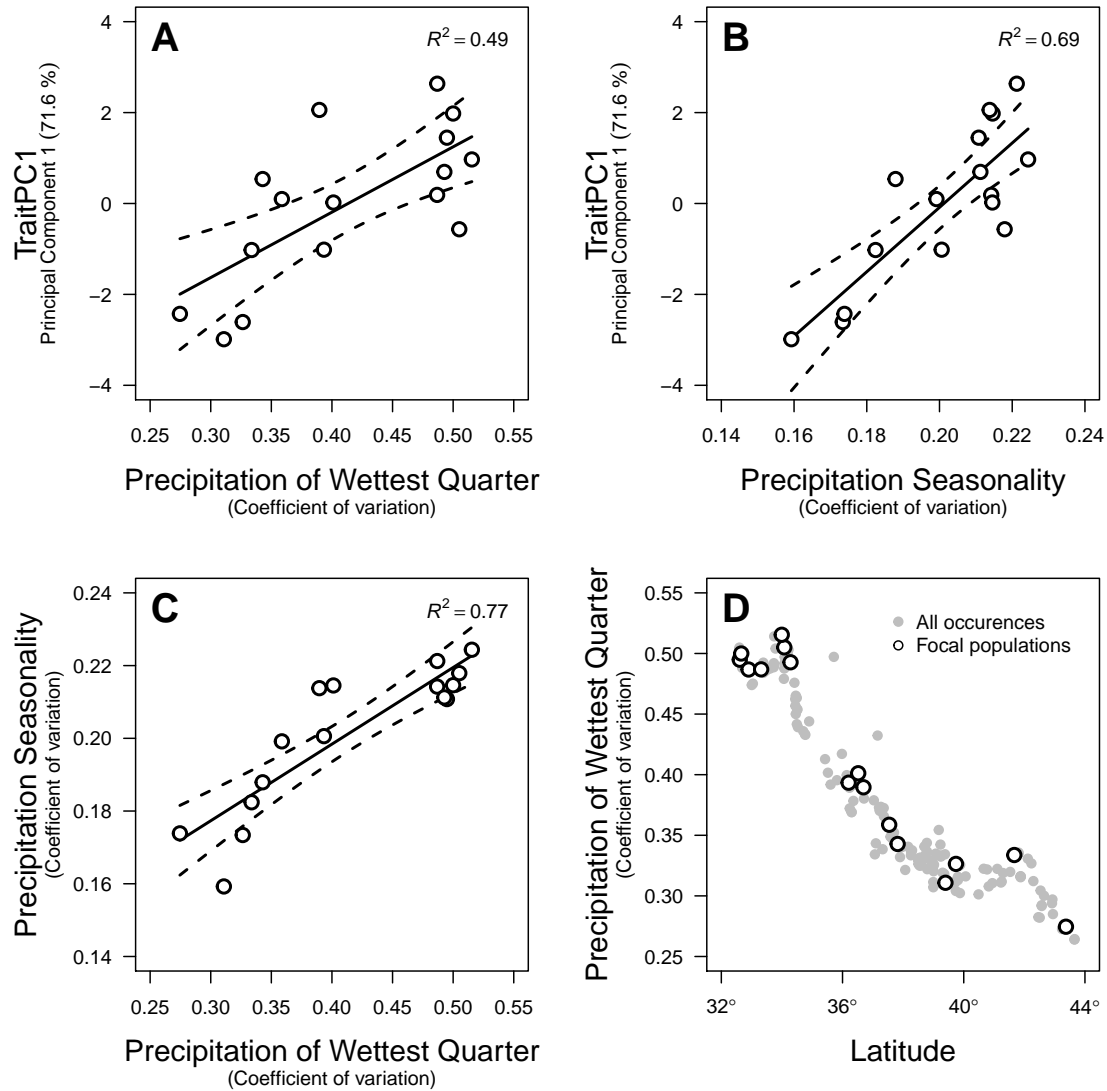
**Figure 2:** Overview of method for identifying putative climatic selective agents underlying latitudinal cline. We looked for climate variables that explained both the latitude of 356 *M. cardinalis* occurrences ('Climate-Latitude variables') and with traits ('Climate-Trait variables'). For Climate-Latitude variables we extracted climate data from recent occurrences located throughout California and Oregon, USA (shown in map). For Climate-Trait variables, we extracted climatic data for the 16 focal populations. For both analyses, we extracted local and neighborhood climate. Local climate refers to climate only from where a population was collected ( $x_{i,0}$ ). Neighborhood climate was calculated as the average over 1000 points in a 62-km radius climatic neighborhood ( $x_{i,1}, x_{i,2}, \dots$ ), but only along stream habitats as *M. cardinalis* is riparian. We identified climatic factors that most strongly predicted latitude of occurrences (Climate-Latitude variables) and traits (Climate-Trait variables), as shown for hypothetical data in plots at the bottom of the figure.



**Figure 3:** Trait variation, from fast to slow growth, is closely associated with latitude. Each point is a population's latitude of origin (x-axis) and position along the slow to fast growth axis (y-axis), defined as Principal Component 1 of four traits (see Material and Methods). The line and 95% confidence intervals were estimated using linear regression.



**Figure 4:** Climatic variation integrated over climatic neighborhood is closely correlated with latitude of *M. cardinalis* and trait variation. A. Using Random Forest regression, we identified 16 climatic variables significantly (high importance) associated with latitude of *M. cardinalis* occurrences. B. Only one of the most important Climate-Latitude variables (in bold) was among the most important Climate-TraitPC1 variables. Note that the Importance values in A and B are not comparable because the dependent variables (Latitude and Trait PC1, respectively) are on different scales. Climatic variables (left of A; right of B) are defined by four qualities: Climatic factor – Temperature (Temp), Precipitation (Prec), Heating degree-days (Heating), Snow (precipitation as snow); Temporal scale – Annual, Coldest quarter (Cold Quar), Warmest Quarter (Warm Quar), Wettest quarter (Wet Quar), Driest Quarter (Dry Quar), or Seasonality; Type – 30-year average (Avg.) or coefficient of variation (Var.); Spatial scale – local or 62-km radius climatic neighborhood.



**Figure 5:** Variation in precipitation is correlated with TraitPC1 and latitude. A. Greater values of TraitPC1 are associated with greater interannual variation in precipitation of the wettest quarter. This was the most important Climate-Latitude variable, but not among the most important Climate-TraitPC1 variables. B. However, a closely related parameter, interannual variation in precipitation seasonality, was among the most important Climate-TraitPC1 variables. C. Across focal populations, variation in precipitation of the wettest quarter and seasonality are closely correlated. D. Southern populations of *M. cardinalis* experience much greater interannual variation in precipitation. In all panels, we report climatic neighborhood values (see Material and Methods). Regression lines, 95% confidence intervals, and coefficients of determination ( $R^2$ ) were calculated using linear regression.

569 **Supporting Information**

570 **Supporting Tables**

**Table S1:** Initial size of seedlings did not vary among Populations, Families, or Treatments. We used a censored Gaussian model of initial size at the outset of the experiment (longest leaf length of the first true leaves). The model was censored because we could not accurately measure leaves less than 0.25 mm with digital callipers (217 of 702, 30.9%, were too small). We fit models using a Bayesian MCMC method implemented using the MCMCglmm function with default priors in the R package **MCMCglmm** version 2.17 (Hadfield, 2010). We estimated the posterior distribution from 1000 samples of an MCMC chain run for  $10^5$  steps after a  $10^4$  step burn-in. We used step-wise backward elimination procedure to find the best-supported model according to Deviance Information Criterion (DIC).

Model	Random	DIC
Population + Water + Temperature + Population:Water + Population:Temperature + Water:Temperature + Population:Water:Temperature	Family	1638
Population + Water + Temperature + Population:Water + Population:Temperature + Water:Temperature	Family	1605.2
Population + Water + Temperature + Population:Water + Population:Temperature	Family	1603.4
Population + Water + Temperature + Population:Water + Water:Temperature	Family	1577.5
Population + Water + Temperature + Population:Temperature + Water:Temperature	Family	1579.9
Population + Water + Temperature + Population:Water	Family	1577.3
Population + Water + Temperature + Water:Temperature	Family	1550.5
Population + Water + Temperature	Family	1549.3
Population + Water	Family	1541.7
Population + Temperature	Family	1546.8
Water + Temperature	Family	1551.1
Population	Family	1541.9
Water	Family	1543.9
-	Family	1541.7
-	-	1538.3



**Table S2:** Climatic variables used

Abbreviation	Climate variable
DD_0	degree-days below 0°C(chilling degree-days)
DD5	degree-days above 5°C(growing degree-days)
DD_18	degree-days below 18°C(heating degree-days)
DD18	degree-days above 18°C(cooling degree-days)
NFFD	number of frost-free days
PAS	precipitation as snow (mm) between August in previous year and July in current
Eref	Hargreaves reference evaporation (mm)
CMD	Hargreaves climatic moisture deficit (mm)
RH	mean annual relative humidity
bio1	annual mean temperature
bio2	mean diurnal range (mean of monthly (max temp - min temp))
bio3	isothermality (bio2/bio7) (* 100)
bio4	temperature seasonality (standard deviation *100)
bio5	max temperature of warmest month
bio6	min temperature of coldest month
bio7	temperature annual range (bio5-bio6)
bio8	mean temperature of wettest quarter
bio9	mean temperature of driest quarter
bio10	mean temperature of warmest quarter
bio11	mean temperature of coldest quarter
bio12	annual precipitation
bio15	precipitation seasonality (coefficient of variation)
bio16	precipitation of wettest quarter
bio17	precipitation of driest quarter
bio18	precipitation of warmest quarter
bio19	precipitation of coldest quarter

**Table S3:** Analysis of variance (ANOVA) table on leaf expansion rate (LER) using **lmerTest** (Kuznetsova et al., 2016). Family and Block were included as random effects. Abbreviations: SS = sum of squares; MS = mean sum of squares (SS / df1); df1 = numerator degrees of freedom; df2 = denominator degrees of freedom.

	SS	MS	df1	df2	<i>F-value</i>	<i>P-value</i>
Day to Germination	12.12	12.12	1	637	35.21	$4.9 \times 10^{-9}$
Population	22.22	1.48	15	118	4.3	$2.5 \times 10^{-6}$
Temperature	80.42	80.42	1	5	233.61	$2.6 \times 10^{-5}$
Water	4.1	4.1	1	5	11.92	0.019
Temperature $\times$ Water	0.03	0.03	1	4	0.07	0.801
Population $\times$ Temperature	2.76	0.18	15	547	0.53	0.925
Population $\times$ Water	9.66	0.64	15	562	1.87	0.024
Population $\times$ Temperature $\times$ Water	4.11	0.27	15	530	0.78	0.700

**Table S4:** Analysis of variance (ANOVA) table on stem elongation rate (SER) using **lmerTest** (Kuznetsova et al., 2016). Family and Block were included as random effects. Abbreviations: SS = sum of squares; MS = mean sum of squares (SS / df1); df1 = numerator degrees of freedom; df2 = denominator degrees of freedom.

	SS	MS	df1	df2	<i>F-value</i>	<i>P-value</i>
Day to Germination	3.6	3.6	1	662	21.1	$5.1 \times 10^{-6}$
Population	12	0.8	15	113	4.7	$5.8 \times 10^{-7}$
Temperature	12.4	12.4	1	6	72.8	$1.5 \times 10^{-4}$
Water	0.6	0.6	1	5	3.7	0.113
Temperature $\times$ Water	0.9	0.9	1	4	5.2	0.093
Population $\times$ Temperature	3.6	0.2	15	549	1.4	0.126
Population $\times$ Water	2.8	0.2	15	536	1.1	0.330
Population $\times$ Temperature $\times$ Water	1.5	0.1	15	518	0.6	0.874

**Table S5:** Analysis of variance (ANOVA) table on photosynthetic rate using **lmerTest** (Kuznetsova et al., 2016). Family and Block were included as random effects. Abbreviations: SS = sum of squares; MS = mean sum of squares (SS / df1); df1 = numerator degrees of freedom; df2 = denominator degrees of freedom.

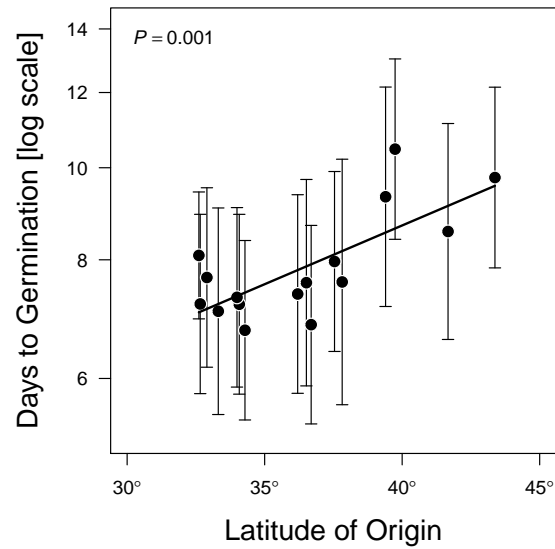
	SS	MS	df1	df2	<i>F-value</i>	<i>P-value</i>
Population	347.7	23.2	15	78	3.02	$7.5 \times 10^{-4}$
Temperature	134.1	134.1	1	6	17.46	$6.4 \times 10^{-3}$
Water	51	51	1	4	6.64	0.066
Temperature $\times$ Water	0.7	0.7	1	3	0.09	0.781
Population $\times$ Temperature	218.6	14.6	15	263	1.9	0.024
Population $\times$ Water	87.7	5.8	15	233	0.76	0.724
Population $\times$ Temperature $\times$ Water	91.4	6.1	15	208	0.79	0.686

**Table S6:** Analysis of deviance table on the probability of mortality by the end of the experiment using Type-II Wald  $\chi^2$  tests in the R package **car** (Fox and Weisberg, 2011). Family and Block were included as random effects. Abbreviations: df = degrees of freedom

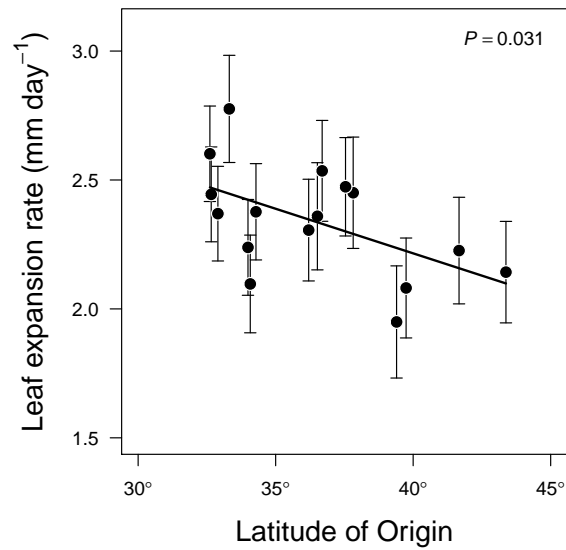
	$\chi^2$	df	<i>P-value</i>
Population	32	31	0.419
Temperature	31.8	6	$1.8 \times 10^{-5}$
Water	69.2	12	$4.6 \times 10^{-10}$
Temperature $\times$ Water	20.7	1	$5.3 \times 10^{-6}$
Population $\times$ Temperature	5.6	15	0.985
Population $\times$ Water	8.6	15	0.897
Population $\times$ Temperature $\times$ Water	0.2	15	1.000

**Table S7:** Important climatic variables predicting latitude of *M. cardinalis* populations ('Climate-Latitude') and the first principal component of traits measured in a common garden ('Climate-TraitPC1'). Local climatic variables were measured from the exact location of collection; neighborhood climatic variables were averaged from a 62-km neighborhood around population (see Material and Methods). Importance and significance were determined using the variable selection using random forests (VSURF) algorithm (see Material and Methods). Climatic variables are described in Table S2.  $\mu$  signifies the mean of the climate variables from 1981–2010;  $\sigma$  indicates coefficient of variation among years.

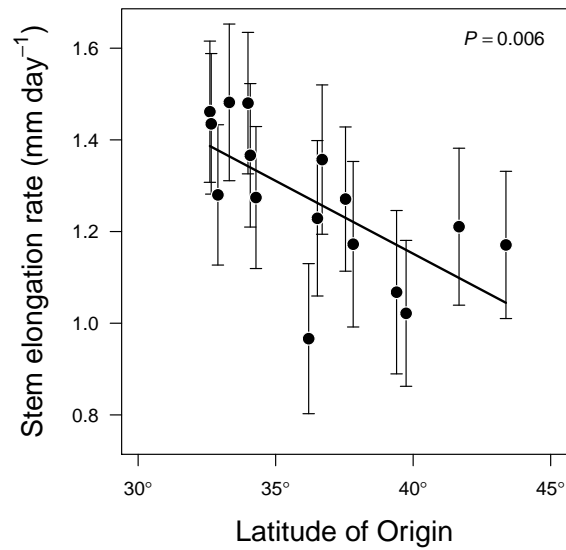
Climate-Latitude variables	Climate-TraitPC1 variables
Precipitation of wettest quarter ( $\sigma$ , neighborhood)	Mean diurnal range ( $\sigma$ , neighborhood)
Annual precipitation ( $\mu$ , neighborhood)	Mean temperature of coldest quarter ( $\sigma$ , neighborhood)
Precipitation of wettest quarter ( $\mu$ , neighborhood)	Precipitation seasonality ( $\sigma$ , neighborhood)
Mean temperature of coldest quarter ( $\sigma$ , neighborhood)	
Annual precipitation ( $\sigma$ , neighborhood)	
Precipitation of driest quarter ( $\mu$ , neighborhood)	
Precipitation of coldest quarter ( $\sigma$ , neighborhood)	
Hargreaves climatic moisture deficit ( $\mu$ , neighborhood)	
Precipitation of warmest quarter ( $\mu$ , neighborhood)	
Precipitation seasonality ( $\mu$ , neighborhood)	
Precipitation of warmest quarter ( $\sigma$ , neighborhood)	
Heating degree-days ( $\sigma$ , neighborhood)	
Precipitation of driest quarter ( $\sigma$ , neighborhood)	
Number of frost-free days ( $\sigma$ , neighborhood)	
Mean temperature of wettest quarter ( $\sigma$ , neighborhood)	
Precipitation as snow ( $\sigma$ , neighborhood)	



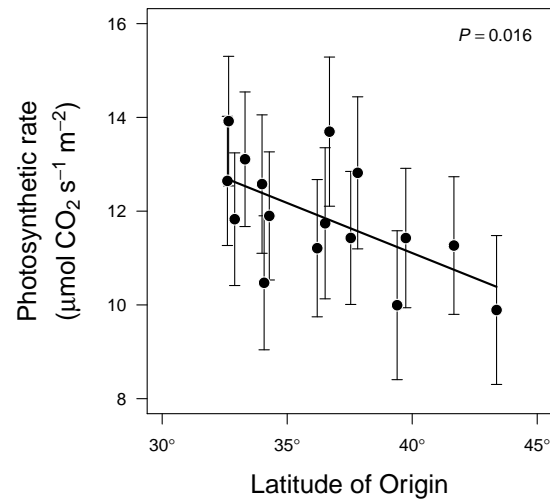
**Figure S1:** Southern populations germinate faster. Each point is a population of *M. cardinalis* showing its latitude of origin (x-axis) and model-predicted days to germination in days under growth chamber conditions (see Material and Methods). Bars around each point are 95% confidence intervals. Predicted time to germination and confidence intervals are based on survival regression (see Materials and Materials). The line is the linear regression of  $\log(\text{model-predicted days to germination}) \sim \text{latitude}$ . The  $P$ -value of the regression is given in the upper left corner.



**Figure S2:** Southern populations grow faster. Each point is a population of *M. cardinalis* showing its latitude of origin (x-axis) and model-predicted leaf expansion rate during the rosette phase. Bars around each point are 95% confidence intervals. Predicted leaf expansion rate based least-square mean estimates and confidence intervals were calculated from linear mixed-effects models (see Materials and Materials). The line is the linear regression of model-predicated leaf expansion rate  $\sim$  latitude. The  $P$ -value of the regression is given in the upper right corner.

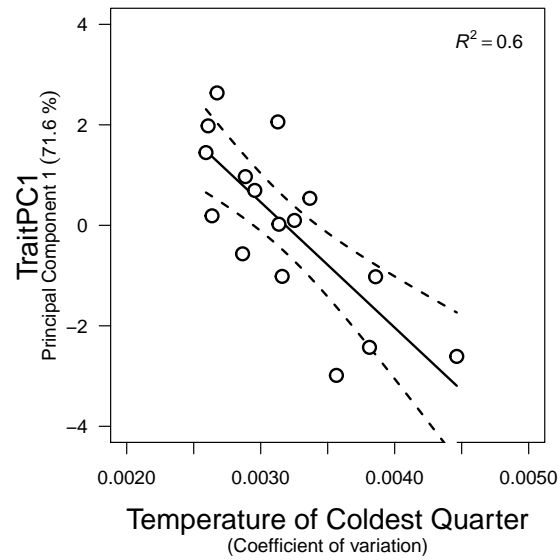


**Figure S3:** Southern populations grow faster. Each point is a population of *M. cardinalis* showing its latitude of origin (x-axis) and model-predicted stem elongation rate. Bars around each point are 95% confidence intervals. Predicted stem elongation rate based least-square mean estimates and confidence intervals were calculated from linear mixed-effects models (see Materials and Materials). The line is the linear regression of model-predicated stem elongation rate  $\sim$  latitude. The  $P$ -value of the regression is given in the upper right corner.



**Figure S4:** Southern populations photosynthesize faster. Each point is a population of *M. cardinalis* showing its latitude of origin (x-axis) and model-predicted instantaneous photosynthetic rate. Bars around each point are 95% confidence intervals. Predicted photosynthetic rates based least-square mean estimates and confidence intervals were calculated from linear mixed-effects models (see Materials and Materials). The line is the linear regression of model-predicted photosynthetic rate  $\sim$  latitude. The  $P$ -value of the regression is given in the upper right corner.





**Figure S5:** Trait variation, from fast to slow growth, is closely associated with neighborhood variation in temperature of the coldest quarter ( $\text{bio11}_\sigma$ ). Each point is a population coefficient of variation in  $\text{bio11}$  averaged over a 62-km climatic neighborhood (x-axis) and position along the slow to fast growth axis (y-axis), defined as Principal Component 1 of four traits (see Material and Methods). The line and 95% confidence intervals were estimated using linear regression.

## 572 Supporting Material and Methods

### 573 Temperature treatments

574 We simulated typical growing season (June 1 - August 15) air temperatures at the two most  
575 thermally divergent focal sites in our study, Whitewater Canyon (WWC, Hot) and Little  
576 Jameson (LIJ, Cool). We downloaded daily interpolated mean, minimum, and maximum  
577 air temperature from 13 years (2000-2012) at both sites from ClimateWNA (Wang et al.,  
578 2012). This range was chosen because seeds used in the experiment were collected around  
579 2012, thus their presence in that location at that time suggests that populations were able  
580 to persist there for at least some years before collection. Monthly temperatures from Cli-  
581 mateWNA are highly correlated with the air temperature recorded from data loggers in  
582 the field at these sites (A. Angert, unpub. data). Hence, the ClimateWNA temperature  
583 profiles are similar to actual thermal regimes experienced by *M. cardinalis* in nature. We  
584 simulated realistic temperature regimes by calculating the mean temperature trend from  
585 June to August using LOESS (Cleveland et al., 1992). The residuals were highly autocor-  
586 related at both sites (warmer than average days are typically followed by more warm days)  
587 and there was strong correlation ( $r = 0.65$ ) between sites (warm days in WWC were also  
588 warm in LIJ). The ‘VARselect’ function in the **vars** package for R (Pfaff, 2008) indicated  
589 that a lag two Vector Autoregression (VAR(2)) model best captured the within-site auto-  
590 correlation as well as between-site correlation in residuals. We fit and simulated from the  
591 VAR(2) model using the package **dse** (Gilbert, 2014) in R. Simulated data closely resem-  
592 bled the autocorrelation and between-site correlation of the actual data. From simulated  
593 mean temperature, we next selected minimum and maximum daily temperatures. Mean,  
594 min, and max temperature were highly correlated at both sites. We chose min and max  
595 temperatures using site-specific fitted linear models between mean, max, and min tem-  
596 perature, with additional variation given by normally distributed random deviates with  
597 variance equal to the residual variance of the linear models. For each day, the nighttime

598 (22:00 - 6:00) chamber temperature was set to the simulated minimum temperature. Dur-  
599 ing the middle of the day, temperature was set to the simulated maximum temperature,  
600 with a variable period of transition between min and max so that the average temperature  
601 was equal the simulated mean temperature.

## 602 **Watering treatments**

603 For watering treatments, we simulated two extreme types of streams where *M. cardinalis*  
604 grows. In the well-watered treatment, we simulated a large stream that never goes dry  
605 during the summer growing season. In the drought treatment, we simulated a small stream  
606 that has ample flow at the beginning of the season due to rain and snow melt, but gradually  
607 dries down through the summer. In both treatments, plants were bottom-watered using  
608 water chilled to 7.5°C. Plants in the well-watered treatment were fully saturated every two  
609 hours during the day. Watering in the drought treatment gradually declined from every  
610 two hours to every day between May 20 (36 days after sowing) and 10 June (57 days after  
611 sowing). Simultaneously, the amount of bottom-watering per flood decreased, such that  
612 only the bottom of the cone-tainers were wetted by the end of the experiment.