Grow with the flow: faster growth is associated with more variable precipitation in a perennial herb

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[**CDM:** *Chris' comments*]
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Abstract

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

Introduction

Local adaptation within species is ubiquitous; populations generally have higher fitness in their native environment, but perform poorly outside it (Schluter, 2000; Hereford, 2009). Local adaptation also frequently leads to clines in both phenotypes and allele frequencies when selection varies over environmental gradients (Huxley, 1938; Endler, 1977). Phenotypic differences between populations along the cline most often have a genetic basis and can be studied in a common garden (Turesson, 1922; Clausen et al., 1940; Hiesey et al., 1942). Despite a long history of studying local adaptation and clines, it remains to challegenging to identify exactly which traits are under selection and which differ for nonadaptive reasons. In particular, the role that physiological differences play in local adaptation is poorly understood, despite the fact that physiology is frequently assumed to explain adaptation to the abiotic environment. We need to understand physiological adaptations within species as a baseline for anticipating how organisms will respond to climate change. A related problem is identifying which features of the environment, abiotic factors like soil water availability or biotic interactions, cause spatially varying selective pressures. Here, we examine physiological trait variation and possible selective agents in the perennail herb *Mimulus cardinalis* Douglas ex Benth. (Phrymaceae), a model system for local adaptation studies.

There are two basic approaches one can use to identify candidate traits underlying local adaptation in a common garden. First, if genetically-based trait differences between populations vary clinally with environmental differences, this may point to traits important for local adaptation. Second, genotype by environment interactions could indicate that variation in plasticity mediates local adaptation. We distinguish between these signatures of local adaptation by referring to 'intrinsic' and 'plastic' trait variation, respectively. There are classic cases adaptation involving both intrinsic and plastic trait variation. For example, intrinsic differences in critical photoperiod [CITE] and developmental rate (Stinchcombe

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et al., 2004) allow organisms to properly time their life history with the local environment. Conversely, sun and shade plants do not have intrinsically higher or lower rates of carbon assimilation, but rather, genotype by environment interactions cause sun plants to assimilate more under high light and shade plants under low light (Givnish, 1988).

Either instrinsic and/or plastic variation should vary clinally along environmental gradients. Indeed, clines in ecologically important traits are widespread in nature (Endler, 1977) and often adaptive, but in most cases the selective agent is unknown. For example, in *Drosophila* numerous latitudinal clines exist for traits like thermal tolerance (Hoffmann et al., 2002), body size (Coyne and Beecham (1987) and references therein), and life history (Schmidt et al., 2005). Some *Drosophila* clines have evolved multiple times (Oakeshott et al. (1982); Huey et al. (2000), see also Bradshaw and Holzapfel (2001)) or shifted in response to climate change (Umina et al., 2005), evincing climatic adaptation. Similarly, plant species exhibit latitudinal clines in traits like flowering time (Stinchcombe et al., 2004), cyanogenesis (Kooyers and Olsen, 2012), and leaf morphology (Hopkins et al., 2008) that likely related to climatic variation. Despite the fact that latitudinal clines in particular have been studied for a long time, the exact climatic factors (e.g. minimum or maximum temperature, growing season length, strength of biotic interactions) is rarely known. Because many climatic factors vary latitudinally, and which climatic factors vary latitudinally changes over the earth's surface (e.g. coastal vs. continental), dissecting the evolution of latitudinal clines across many species will help biologists identify generalities, such as whether thermal tolerance maxima or seasonal timing is more important (Bradshaw and Holzapfel, 2008). In plants especially, we know little about the prevelance and adaptive significance of clinal variation in fundamental physiological traits like photosynthesis and the impact on plant performance.

In this study, we address these gaps by asking whether intrinsic or plastic physiological trait variation corresponds with latitude and what climatic factor(s) could plausibly be repsonsible for latitudinal clines in a focal species, *Mimulus cardinalis*. We chose this species because

linking physiological traits to potentially complex patterns of local adaptation requires integrating multiple lines of evidence from comparative, experimental, genomic studies under both lab and field conditions. Many classic and contemporary studies of local adaptation use species from genus *Mimulus* because of its natural history, easy propogation, and genetic/genomic resources (Clausen et al., 1940; Hiesey et al., 1971; Bradshaw and Schemske, 2003; Wu et al., 2008; Lowry and Willis, 2010; Wright et al., 2013). Yet, there is a conspicuous deficieny of links between local adaptation and physiological mechanisms (Angert, 2006; Angert et al., 2008; Wu et al., 2010). We measured genetic and genotype by environment variation in response to temperature and drought among 16 populations distributed over 10.7°of latitude. We found a latitudinal cline of intrinsic differences in photosynthesis and growth, but no evidence for variation in plasticity. Interannual variation in precipitaiton and/or precipitation seasonality is associated this axis of variation, suggesting that climatic variance rather than mean may be an important driver of local adaptation in *M. cardinalis*. We place these findings in the context of life history theory and consider future directions in the Discussion.

Methods

Population Selection

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

Plant propagation

On 14 April, 2014, 3-5 seeds per family were sown directly on sand (Quikrete Play Sand, Georgia, USA) watered to field capacity in RLC4 Ray Leach cone-tainers placed in RL98

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

Name	Region	Latitude	Longtiude	Elevation (mas)
HAU	South Margin	32.657	-116.532	799
CTC	South Margin	32.609	-116.7	267
CUR	South Margin	32.9	-116.585	1180
GRP	South Margin	33.314	-116.871	1577
WWC	Transverse	33.994	-116.665	705
MIL	Transverse	34.077	-116.873	2050
WFM	Transverse	34.284	-117.378	1120
NMT	South Sierras	36.201	-118.651	1314
PRD	South Sierras	36.518	-118.759	926
RWD	South Sierras	36.691	-118.91	1727
WNA	Central Sierras	37.541	-119.649	1224
RBW	Central Sierras	37.819	-120.007	876
MYU	North Sierras	39.397	-121.082	455
LIJ	North Sierras	39.743	-120.704	1603
DPC	North Coast	41.668	-123.11	707
RCC	North Margin	43.374	-122.957	326

98-well trays (Stuewe & Sons, Inc., Oregon, USA). We used pure sand both to facilitate root-washing and because *M. cardinalis* typically grows in sandy, riparian soils (A. Angert, pers. obs.). Two jumbo-sized cotton balls at the bottom of cone-tainers prevented sand from washing out. Cone-tainers were continuously bottom-watered during germination by placing them in medium-sized flow trays (FLOWTMD, Stuewe & Sons, Inc., Oregon, USA) filled part way with water, placed on benches in greenhouses at the University British Columbia campus in Vancouver, Canada (49°15' N, 123°15' W). Misters thoroughly wetted the top of the sand every two hours during the day. Most seeds germinated between 1 and 2 weeks, but we allowed 3 weeks before transferring seedlings to growth chambers. Germination was recorded daily from one to two weeks after sowing, and every few days thereafter. On 5 May (21 days after sowing), seedlings were transferred to one of two MODEL Growth Chambers (Conviron, Manitoba, Canada). We thinned seedlings to one

plant per cone-tainer, leaving the center-most plant. 702 of 768 (91.4%) had plants that could be used in the experiment. We allowed one week at constant, non stressful conditions (day: 20℃, night: 16℃) for plants to acclimate to growth chambers before starting treatments. The initial size of seedlings, measured as the length of the first true leaves, did not differ between populations, families, or treatments Table S1.

Treatments

We imposed four treatments, a fully-factorial cross of two temperature levels and two watering levels. The temperature levels closely simulated an average growing season at the thermal extremes of the species range, which designate as Hot and Cool treatments. Watering levels contrasted a perennial and seasonal stream, which we refer to as Well-watered and Drought treatments. A detailed description of treatments is described in the Supplemental Information and summarized in Fig 1. Because growth chambers cannot be subdivided, one chamber was assigned to the Hot treatment level and another to the Cool treatment level. Within each chamber, there were two Well-watered blocks and two Drought blocks. The irradiance in both chambers was approximately 400 μmol quanta m^{-2} s^{-1}. The growth chambers did not control humidity, but because of watering and high plant transpiration rates, the relative humidity was quite high in both temperature levels (data not shown).

Growth and photosynthesis

Day of germination We tested for population variation in germination rate, measured as Days to Germination, using a lognormal survival model fit using the survreg function in the R package **survival** version 2.38 (Therneau, 2015). The model was fit with Population as a fixed effect and Family as random effect using a Γ frailty function. The signifcance of the Population effect was determined using analysis of deviance.
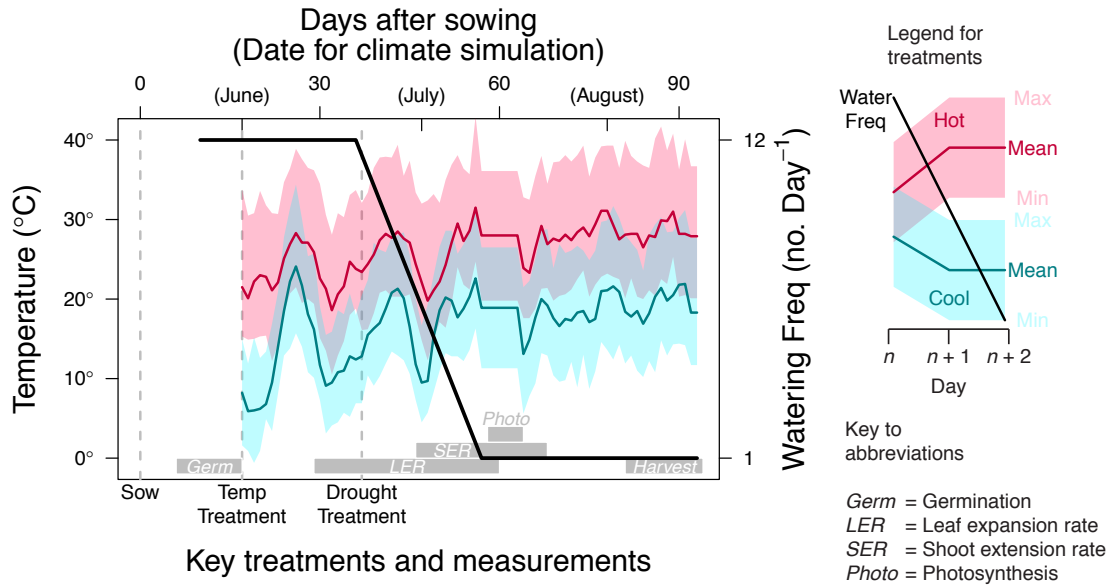
Figure 1: Overview of experimental treatments and timing of key trait measurements. All plants germinated within 21 days of sowing. At that time, we began temperature treatments (left axis), simulating a typical June-August weather pattern at Hot (red) and Cold (blue) sites. The bold lines track the 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 (black line; right axis). Grey boxes on the bottom of the plot outline the period of key measurements described in the Methods.

Growth rate: leaf expansion and shoot elongation   We censused leaf length twice per week from 12 May – 12 June (28–59 days after sowing), resulting in 10 measurements. We ceased measuring leaf length once it appeared to asymptote and growth shifted to shoot elongation. We also censused plant height on 7 days (twice per week) between 29 May and 20 June (45 to 67 days after sowing). Both leaf expansion and shoot elongation were modeled as a second-order polynomials of time with individual coefficients (separate for leaf and shoot growth) using empirical Bayes' estimates from linear mixed-effects models fit using the R package **lme4** version 1.1-7 (Bates et al., 2014).

Table 2: Key traits measured in this study.

Trait	Units
Day of germination	day
Leaf expansion rate	mm day^{-1}
Shoot elongation rate	mm or cm day^{-1}
Harvest dry mass	g
Photosynthetic rate	μmol CO$_2$ m^{-2} s^{-1}
Mortality	[**CDM:** *Probability?*]

Photosynthesis   During the week of 10 to 16 June (57 to 63 days after sowing), we measured daytime photosynthetic rate and stomatal conductance on a subset of 329 plants evenly spread between treatments and families within populations. The youngest, fully-expanded leaf [**CDM:** *this is what I did...not sure exactly which node, as I didn't record that when I did measurements*] acclimated for 3 minutes to reach steady state in a 6 cm^2 chamber of a LI-COR 6400XT Portable Photosynthesis System (LI-COR Biosciences, Lincoln, Nebraska). All measurements were made at ambient light (400 μmol m^{-2} s^{-1}), temperature, and moderate relative humidity. During this period, we suspended normal day-to-day temperature fluctuations and set daytime temperatures to its average for that period (Cool: 26.5°; Hot: 36.1° so that all plants within a temperature level were measured under the same conditions.

Mortality   We assayed mortality during twice-weekly growth measurements. We could not get GLMM with Family effects to converge, so we used GLM with a quasibinomial error structure and assessed signifiance using Type 2 Analysis of Deviance with the R package **car**.

Biomass at harvest   [**CDM:** *show correlation between growth rate and biomass?*]

Intrinsic variation and plasticity

For all traits (Table 2) we tested for Population, Treatment, and Population × Treatment interactions. We interpreted significant Population effects to indicate intrinsic variation and Population by Treatment effects to indicate variation in plasticity. As mentioned above, we survival and GLM models for germination rate and mortality, respectively. For all other traits, we used mixed model ANOVAs with Family included as a random factor. Models were fit by restricted maximum likelihood using lmer from the R package **lme4** (Bates et al., 2014). Significant fixed effect terms were selected using a step-wise backward elimination procedure implemented with the step function in the R package **lmerTest** version 2.0-11 (Kuznetsova et al., 2014). Denominator degrees of freedom for F-tests were estimated using Satterthwaite's approximation. Significant Population effect indicate intrinsic trait differences; significant Population × Treatment effects indicate population differences in plasticity. For growth rate, we also accounted for differences in germination rate by including day of germination as a factor.

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 (PCA). The first principal component of these traits (Trait PC1) loaded positively with germination, growth, and photostynthetic rate, therefore we define this as a phenotypic axis dilineating fast and slow growing populations.

Selective agents and environmental correlates

We found that a population's position along PC1 correlated strongly with the latitude or origin (see Results). Since latitude *per se* cannot be a selective agent, we considered three possible explanations. First, latitude may be strongly correlated, in this species, with one or two climatic variables, such as temperature, precipitation, or growing degree-days. Second, latitude may be correlated with several climatic agents of selection that are individually weak, but add up to a strong latiduinal cline. Third, gene flow among neighboring populations could smooth out local climatic effects, since alleles will experience selection across populations linked by migration. For example, average temperature of *M. cardinalis* populations at a given latitude varies widely, but in aggregate, a Southern metapopulation experiences warmer climate than a Northern one. Thus, any particular Southern population would be warm-adapted, even if it was located in cooler (e.g. high elevation) site.

To evaluate support for these hypotheses, we used Random Forest regression (Liaw and Wiener, 2002) to identify putative climatic factors underlying trait-latitude associations in *M. cardinalis*. We looked for overlap between climatic variables that best predict latitude of *M. cadinalis* occurence records and a separate analysis of climatic variables that best predict trait variation across our 16 focal populations. For brevity, we refer to these as Climate-Latitude and Climate-Trait variables. We selected Climate-Latitude and Climate-Trait variables independently using Random Forest (VSURF algorithm in the R **VSURF** version 0.8.2 (Genuer et al., 2014)). From VSURF models, we kept only variables selected for prediction, the most stringent criterion.

The first hypothesis predicts that there should be one or two Climate-Latitude and Climate-Trait associations that are strongly correlated, whereas the second hypothesis predicts that several climatic variables should be weakly correlated. To test the third hypothesis about gene flow smoothing out local climatic variation, we repeated the same procedure to identify

Climate-Trait variables as above, except that we used spatially averaged climate variables. We sampled climate at 1000 random points (at 90-m resolution) within a 100-km buffer around focal populations. We chose this buffer size because neutral genetic differentiation increases slowly with geographic distance, indicating significant gene flow between nearby populations (Paul et al., 2015). Since *M. cardinalis* is found exclusively in riparian areas, we only selected points along streams using the National Hydrogeoraphy Dataset (United States Geological Survey, 2015). Climatic means and CVs were weighted by their climatic suitability as determined using a multimodel ensemble average of ecological niche models (Angert, 2015). For clarity, we distinguish between analyses where climate is inferred from a single point ('point estimated Climate-Trait') versus averaged across a 100-km buffer ('spatially averaged Climate-Trait').

For these analyses, we compiled a representative set of 178 recent (since 2000) known *M. cardinalis* occurences. These occurences 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, 2015). For each occurence, we used a 90m digital elevation model from HydroSHEDS (Lehner et al., 2006) to extract elevation. Monthly interpolated climate layers were calculated using ClimateWNA (Wang et al., 2012), which accurately downscales climate data specifically for the rugged topography of western North America. For each occurence, we calculated bioclimatic variables using the biovars function in the R package **dismo** (Hijmans et al., 2014). In total, we included 24 climate variables, 9 from ClimateWNA and 15 bioclimatic variables (Table S2). The bioclimatic variables included all permutations of two climatic factors, temperature and precipitation, and six temporal scales (annual average, coldest quarter, warmest quarter, wettest quarter, driest quarter, or seasonality) as well as mean diurnal range, isothermality, annual temperature range. For each variable, we calculated both a 30-year normal by averging annual values between 1981 and 2010 and 30-year coefficient of variation, a standardized metric of interannual climatic variation. Temperatures were converted to Kelvin to be on a ratio scale appropriate for

calculating the coefficient of variation.

Results

A coordinated latitudinal cline in germination, growth, and photosynthesis

Using a common garden, we identified strong genetically-based trait differences in time to germination, growth, and photosynthetic rate among populations of *M. cardinalis*, as evidenced by large and highly significant population effects (Table 3). A single principal component captured 74.2 % of the trait variation among populations, defining an axis of variation from fast to slow growth (Fig 2). As we explain below, intrinsic differences between populations in terms of plant function (photosynthesis) and performance (growth) contrasted greatly with little variation in plasticity.

Table 3: Summary of Population, Treatment, and Population × Treatment effects. We used different statistical modeling for the diverse traits assayed – glm: generalized linear model using R (R Core Team, 2015); lmer: linear mixed model using the R package **lme4** (Bates et al., 2014); survreg: survival regression using the R package **survival** (Therneau, 2015). Note that temperature and water treatments were imposed after germination, hence are not application to this trait. Key to statistical significance: *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

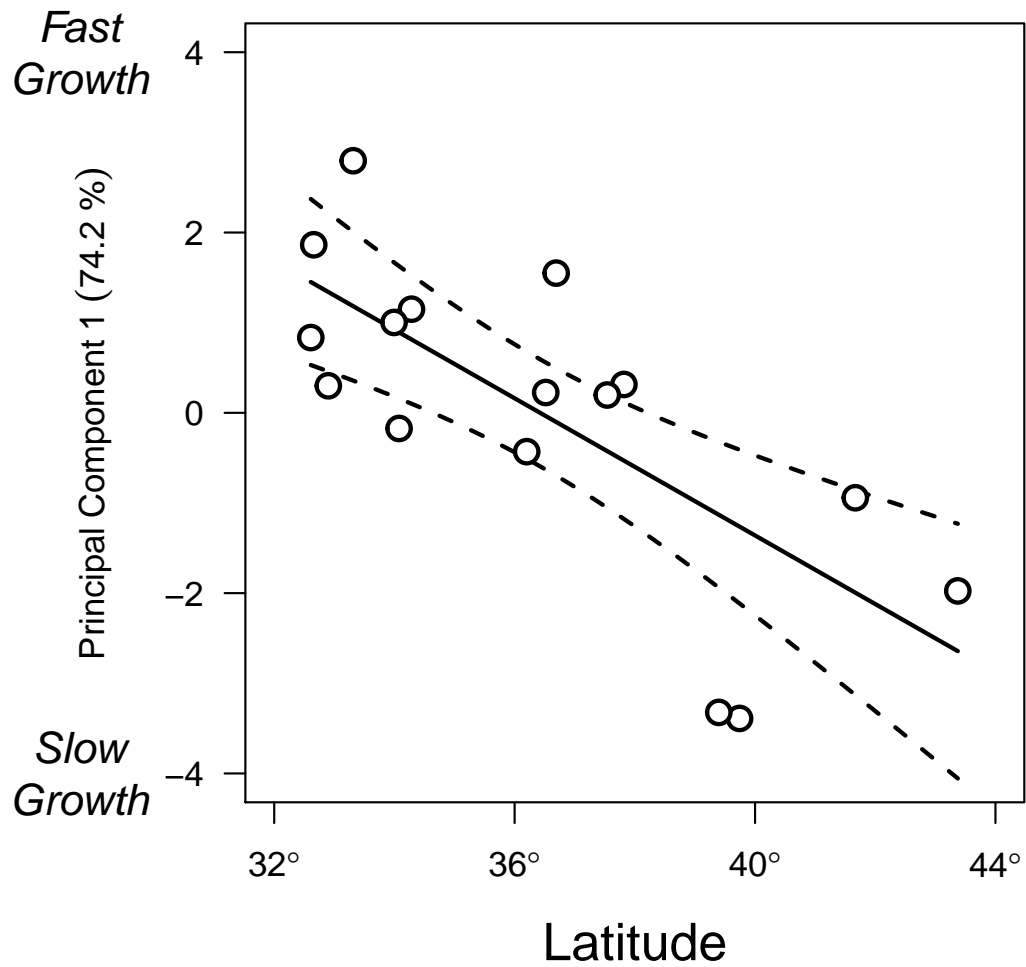| Trait Statistical model	Germination survreg	Leaf expansion lmer	Shoot elongation lmer	Photosynthesis lmer	Intrin. Photo lmer	Mortality glm
Population	***	***	***	***	***	***
Temperature	NA	***	***	***		***
Water	NA	***	***	***		***
Pop × Temp	NA			*		*
Pop × Water	NA	*				
Temp × Water	NA		***			***
Pop × Temp × Water	NA					

Figure 2: Trait variation, from fast to slow growth, is closely associated with latitude. Each point is a population, its latitude of origin a position along the slow to fast growth axis, defined as Principal Component 1 of five traits (see Methods). The line and 95% confidence intervals were estimated using standard linear regression.

Remarkably little evidence for variation in plasticity

Genotype $\times$ environment interactions are also a common signature of local adaptation. For example, populations from more stressful environments may tradeoff reduced growth rate

under benign conditions for the ability to tolerate stress by maintaining positive growth rates and/or surviving through adverse conditions. We found remarkably little evidence for this pattern in *M. cardinalis*. There was only one statistically significant Population $\times$ Treatment interaction (Table 3), but this idiosyncratic result was weak and would not have survived correction for multiple testing. Otherwise, populations responded remarkably 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 Treatment effects were 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.

Climatic variability best explains phenotypic divergence

Latitudinal clines are common, but it is often difficult to ascribe this variation to a particular selective agent. For *M. cardinalis*, interannual variation in precipitation over the past 30 years is very closely related to the latitude of recently recorded occurences of this species (Fig. 3A). Between year variation in precipitation was also strongly correlated with position along the fast-slow growth axis, but climate based on point estimates gave somewhat different results than climate based on spatially averaged values. The two most important point estimated Climate-Latitude variables were also strong predictors of position along the fast-slow growth axis (Fig. 3B). For spatially averaged climate, interannual variation in precipitation seasonality was the only climatic variable selected, (Fig. S5) [**CDM:** *Do we need a figure for this?*]. Overall, there was good but imperfect agreement between the weight assigned to climatic variables using point estimated and spatially averaged climate (Fig. ??) [**CDM:** *This figure will show strong, positive correlation between weights given*

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to climatic variables from point estimated and spatially averaged analyses].

Overlap between Climate-Latitude and point estimated Climate-Trait variables suggests that interannual variation in precipitation is an important selective agent in *M. cardinalis*. Specifically, we hypothesize that more frequent droughts (greater precipitation cv) in Southern populations selects for an 'annual-ized' life history, as we detail in the Discussion. Even though the spatially averaged Climate-Trait variable was not among the Climate-Latitude variables, it points to a similar conclusion. Specifically, this analysis showed that greater interannual variation in precipitation seasonality, which is also driven by precipitation variability (i.e. more frequent drought years) in Southern California, is associated with faster growth in southern populations. We must qualify these results because our analysis obviously cannot rule out that alternative variables not included in the analysis may be more important.

Discussion

In this study, we found evidence for one of two common signatures of local adaptation. A latitudinal cline in traits such as germination rate, photosynthesis, and growth, suggests 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 may indicate that the fundamental abiotic niche is relatively conserved [**ALA:** *the results that underlie this statement need more emphasis in the methods*]. Finally, we found that climatic variation between years may be a more important selective agent than the 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 photosynthesis and growth varies clinally, but plastic responses to temperature and drought are relatively conserved.
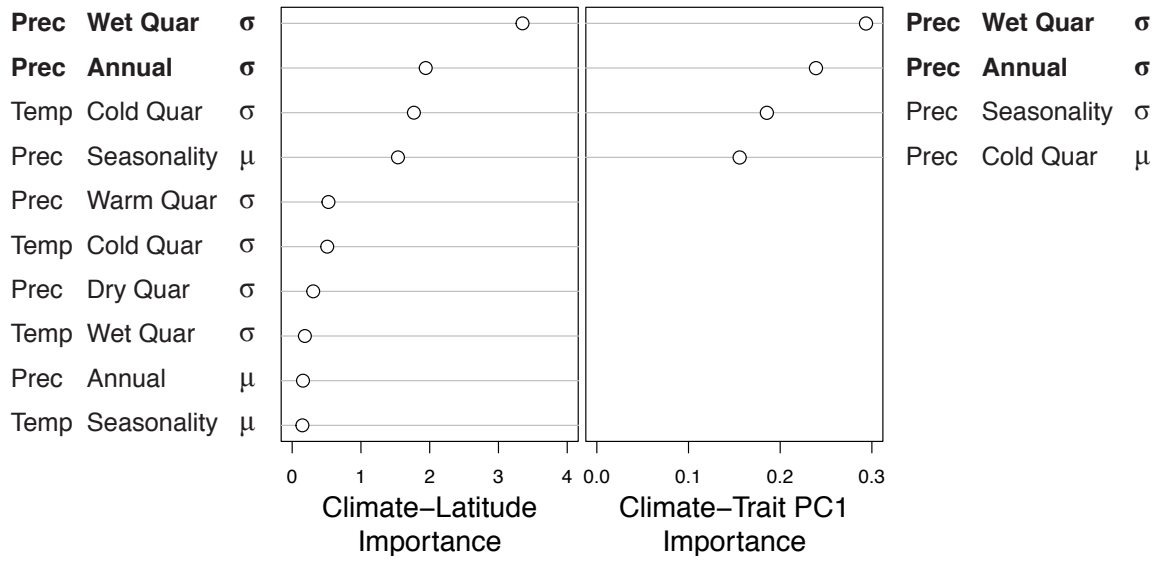
Figure 3: Interannual variation in precipitation is closely correlated with latitude and trait variation. A. Using Random Forest regression, we identified 10 climatic variables significantly (high importance) associated with latitude of *M. cardinalis* occurences. B. The two most important Climate-Latitude variables were also the two most important Climate-Trait 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 three qualities: Climatic factor – Temperature (Temp) or Precipitation (Prec); Temporal scale – Annual, Coldest quarter (Cold Quar), Warmest Quarter (Warm Quar), Wettest quarter (Wet Quar), Driest Quarter (Dry Quar), or Seasonality; Summary statistic – average (μ) or coefficient of variation (σ)

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 genetic variation among populations along a phenotypic axis from fast to slow growth that varied over a large spatial scale (Fig. 2). If this variation is adaptive, it suggests that the fitness tradeoff between doing well in low versus high latitude environments is not too strong nor

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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 spatial heterogenous 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 ($< 35°$N) and Northern portions of the range (Paul et al., 2015).

Another possibility we could have seen is that southern populations, which appear to experience more frequent drought years (see next section), could have evolved the ability to tolerate drought better than northern populations, thereby expanding the fundamental niche of the species as a whole. We found no evidence for this; all populations responded to drought and temperature similarly (Table 3). We hypothesize that evolution of the fundamental niche may be constrained by a combination of strong fitness tradeoffs, demographic asymmetry, and gene flow. Riparian habitats where *M. cardinalis* live are highly heterogeneous at small spatial scales. Plants in the stream never have to tolerate drought whereas plants only a few meters away may experience extreme drought since there is little direct precipitation during the growing season in Meditteranean climates of western North America. But alleles that confer greater drought tolerance may be quite costly in well-watered soils, and vice versa, leading to strong fitness tradeoffs. Such tradeoffs promote specialization to one soil type or another, thereby inhibiting the evolution of broad environmental tolerance within a population. Demography and gene flow may reinforce niche conservatism [**ALA:** *also bring in idea of temporal source sink: most individuals made in wet years, even if infrequent, so selection weighted towards wet environment even if dry states are frequent*]. A new mutant with increased drought tolerance that can survive at the resource-poor margin of a population will be demographically overwhelmed by the larger census populations that can be maintained in higher resource environments. Finally, gene flow, which is generally high among *M. cardinalis* populations within the same ecoregion (Paul et al., 2015), will thwart local adaptation and reinforce specialization. Thus, the

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spatial grain of the environment, demographic asymmetry, and gene flow may conspire to constrain local adaptation via altered fundamental niche.

Based on the available data, interannual variation in annual or winter precipitation or precipitaiton seasonality (these are closely correlated in Mediterranean climates) may be the selective agent driving variation in growth and photosynthesis. Variation in precipitation was best predicted latitude of recent *M. cardinalis* occurences and trait variation along the fast-slow growth continuum (Fig. 3). A life history tradeoff between allocation to growth in the current year at the expense of future years could explain this pattern. In southern populations with more frequent droughts capable of killing rhizomes, a more annualized strategy could be favored. Conversely, in more predictable northern environments, lifetime fitness may be optimized when a significant fraction of assimilate is allocated below ground for future years. Although this hypothesis remains to be directly tested, a few indepdent lines of evidence are consistent with it. Preliminary surveys suggest that northern populations not only grow slower, but also produce greater numbers of rhizomes (C.D. Muir, unpub. data), suggesting an allocation tradeoff. Ecological niche models also show that occurence of southern populations is best predicted by recent climate (< 5 years), whereas northern occurences are best predicted by climate over the previous 30 years (M. Bayly & A. Angert, unpub. data). Finally, demographic surveys of natural populations show greater variation in the size recruits in southern populations, suggesting higher maximum growth rates under natural conditions (M. Bayly & A. Angert, unpub. data). There is a lot of interest in understanding how organisms will respond to changes in climatic variation, not just changes in the average climate. Our data indeed suggest that variation may be more important than the mean.

[**CDM:** *I am still working a paragraph or two linking these results to empirical results in other systems and climatic adaptaiton in Mimulus sect Simiolus.*]

Acknowledgements

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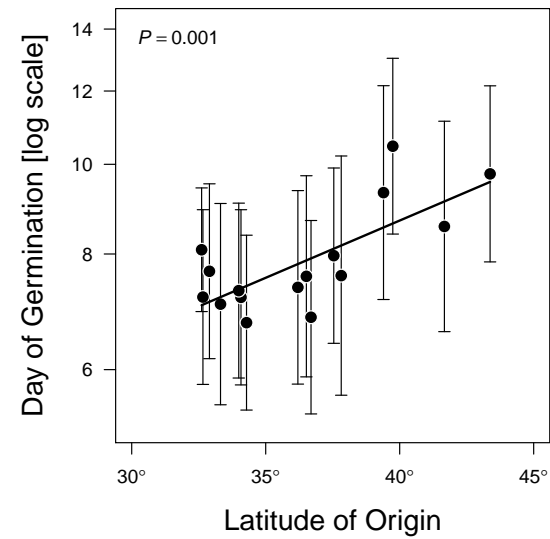
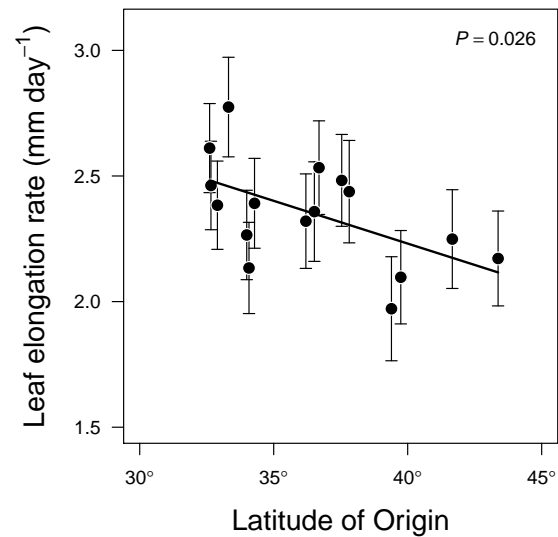# Supporting Information

Figure S1: CAPTION

Figure S2: CAPTION

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 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
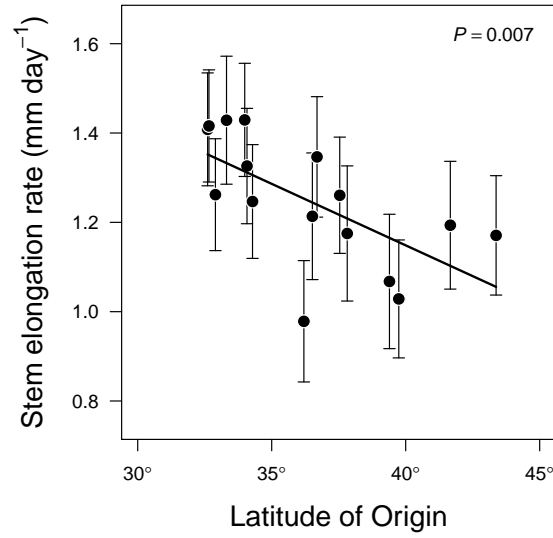
Figure S3: CAPTION

Temperature treatments

We simulated typical growing season (June 1 - August 15) air temperatures at the two most thermally divergent focal sites in our study, Whitewater Canyon (Hot) and Little Jameson (Cool). We downloaded daily interpolated mean, minimum, and maximum air temperature from 13 years (2000-2012) at both sites from ClimateWNA (Wang et al., 2012). This range was chosen because seeds used in the experiment were collected around 2012, thus their presence in that location at that time suggests that populations were able to persist there for at least some years before collection. Monthly temperatures from ClimateWNA are highly correlated with the air temperature recorded from data loggers in the field at these sites (A. Angert, unpub. data). Hence, the ClimateWNA temperature profiles are similar to actual thermal regimes experienced by *M. cardinalis* in nature. We simulated realistic temperature regimes by calculating the mean temperature trend from June to August

using LOESS (Cleveland et al., 1992). The residuals were highly autocorrelated at both sites (warmer than average days are typically followed by more warm days) and there was strong correlation ($r = 0.65$) between sites (warm days in WWC were also warm in LIJ). The 'VARselect' function in the **vars** package for R (Pfaff, 2008) indicated that a lag two Vector Autoregression (VAR(2)) model best captured the within-site autocorrelation as well as between-site correlation in residuals. We fit and simulated from the VAR(2) model using the package **dse** (Gilbert, 2014) in R. Simulated data closely resembled the autocorrelation and between-site correlation of the actual data. From simulated mean temperature, we next selected minimum and maximum daily temperatures. Mean, min, and max temperature were highly correlated at both sites. We chose min and max temperatures using site-specific fitted linear models between mean, max, and min temperature, with additional variation given by normally-distributed random deviates with variance equal to the residual variance of the linear models. For each day, the nighttime (22:00 - 6:00) chamber temperature was set to the simulated minimum temperature. During the middle of the day, temperature was set to the simulated maximum temperature, with a variable period of transition between min and max so that the average temperature was equal the simulated mean temperature.

Watering treatments

For watering treatments, we simulated two extreme types of streams where *M. cardinalis* grows. In the well-watered treatment, we simulated a large stream that never goes dry during the summer growing season. In the drought treatment, we simulated a small stream that has ample flow at the beginning of the season, but gradually dries down through the summer. In both treatments, plants were bottom-watered using water chilled to 7.5 [check] by MAKE AND MODEL OF CHILLER. Plants in the well-watered treatment were fully saturated every two hours during the day. Watering in the drought treatment gradually declined from every two hours to every day between May 20 (36 days after sowing) and

10 June (57 days after sowing). Simultaneously, the amount of bottom-watering per flood decreased, such that only the bottom of the cone-tainers were wetted by the end of the experiment.

Table S2: Climatic variables

Climate variable	Abbreviation
DD_0	degree-days below 0℃(chilling degree-days)
DD5	degree-days above 5℃(growing degree-days)
DD_18	degree-days below 18℃(heating degree-days)
DD18	degree-days above 18℃(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 climativ moisture deficit (mm)
MAR	mean annual solar radiation (NOTE: removing because too many missing values)
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: CAPTION

	SS	MS	df1	df2	F	P
Day of Germination	18.2	18.2	1	633.1	35.2	4.78×10^{-9}
Population	22.3	1.5	15	121.5	4.3	2.57×10^{-6}
Temperature	114.4	114.4	1	567.7	352.3	1.64×10^{-61}
Water	4.2	4.2	1	567.7	12.6	4.12×10^{-4}
Population × Temperature	3.1	0.2	15	553.3	0.6	0.86
Population × Water	8.9	0.6	15	567.8	1.8	0.03
Temperature × Water	0	0	1	550.5	0	0.99
Population × Temperature × Water	4	0.3	15	535.4	0.8	0.69

Table S4: CAPTION

	SS	MS	df1	df2	F	P
Day of Germination	6.2	6.2	1	656.2	20.9	5.9×10^{-6}
Population	10	0.7	15	109.9	4.3	3.69×10^{-6}
Temperature	70.3	70.3	1	568	445.3	2.05×10^{-73}
Water	2.9	2.9	1	567.7	17.8	2.92×10^{-5}
Population × Temperature	2.7	0.2	15	552.7	1.2	0.25
Population × Water	2.6	0.2	15	539.8	1.1	0.33
Temperature × Water	2.3	2.3	1	565.2	14.7	1.37×10^{-4}
Population × Temperature × Water	1.5	0.1	15	521.4	0.6	0.86

Table S5: Important climatic variables predicting latitude of *M. cardinalis* populations ('Climate-Latitude') and the first principal component of traits measured in a common garden ('Climate-Trait'). Importance and significance were determined using the variable selection using random forests (VSURF) algorithm (see 'Methods'). Climatic variables are described in Table S2. μ signifies the mean of the climate variables from 1981–2010; σ indicates coefficient of variation among years

Climate-Latitude variables	Climate-Trait variables Point estimated	Spatially averaged
Precipitation of wettest quarter (σ)	Precipitation of wettest quarter (σ)	Precipitation seasonality (σ)
Annual precipitation (σ)	Annual precipitation (σ)	
Mean temperature of coldest quarter (σ)	Precipitation seasonality (σ)	
Precipitation seasonality (μ)	Precipitation of wettest quarter (μ)	
Precipitation of warmest quarter (σ)		
Mean temperature of warmest quarter (σ)		
Precipitation of driest quarter (σ)		
Mean temperature of wettest quarter (σ)		
Annual precipitation (μ)		
Temperature seasonality (μ)		