

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

Christopher D. Muir<sup>1,\*</sup> and Amy L. Angert<sup>1,2</sup>

<sup>1</sup> Biodiversity Research Centre and Department of Botany, University of British Columbia, Vancouver, BC, Canada

<sup>2</sup> Department of Zoology, University of British Columbia, Vancouver, BC, Canada

\*corresponding author: Chris Muir, [chrisdmuir@gmail.com](mailto:chrisdmuir@gmail.com)

Running Head: Latitudinal cline and climate in *Erythranthe*

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## Abstract

Local adaptation is commonly observed in nature: organisms perform well in their natal environment, but poorly outside it. Correlations 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 is challenging. Here, we investigated a latitudinal cline in growth and photosynthesis across 16 populations of the perennial herb *Erythranthe 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 Mediterranean climates become more variable due to climate change.

## Introduction

1 Local adaptation has been documented within numerous species; populations generally  
2 have higher fitness in their native environment, but perform poorly outside it (Schluter,  
3 2000; Leimu and Fischer, 2008; Hereford, 2009). However, the prevalence of local adapta-  
4 tion remains difficult to assess because researchers rarely test for local adaptation unless  
5 there are obvious phenotypic or environmental differences (but see Hereford and Winn  
6 2008). When local adaptation occurs, it frequently leads to clines in both phenotypes and

7 allele frequencies when selection varies over environmental gradients (Huxley, 1938; Endler,  
8 1977; Barton, 1999). Phenotypic differences between populations along a cline often have  
9 a genetic basis and can be studied in a common garden (Turesson, 1922; Clausen et al.,  
10 1940; Hiesey et al., 1942). Despite a long history of studying local adaptation and clines,  
11 it remains challenging to identify exactly which traits are under selection and which differ  
12 for nonadaptive reasons. In particular, the role that physiological differences play in local  
13 adaptation is poorly understood, despite the fact that physiology is frequently assumed to  
14 explain adaptation to the abiotic environment. A related problem is identifying which of  
15 the myriad and often covarying aspects of the environment cause spatially varying selective  
16 pressures.

17 When populations are locally adapted, reaction norms for fitness will cross, such that local  
18 genotypes have higher fitness than foreign genotypes and rank orders change across envi-  
19 ronments (Kawecki and Ebert, 2004). The traits that underlie local adaptation, however,  
20 need not mirror this pattern. Populations can have fixed genetic differences conferring  
21 trait values that are adaptive at home but neutral or maladaptive away. Alternatively,  
22 the ability to plastically respond to a particular environment or the magnitude of response  
23 to an environment could be adaptive. We distinguish between these patterns of adaptive  
24 trait differences by referring to ‘genetic variation’ in trait means and ‘genetic variation in  
25 plasticity’, respectively. Genetic variation in plasticity is synonymous with genotype-by-  
26 environment interactions, or simply ( $G \times E$ ). Genetic variation in trait means and plasticity  
27 are both involved in adaptation. For example, genetic variation in photoperiod responses  
28 (Blackman et al., 2011) and developmental rate (Stinchcombe et al., 2004) allow organisms  
29 to properly time their life history with the local environment. Conversely, sun and shade  
30 plants do not have intrinsically higher or lower rates of carbon assimilation, but rather,  
31 genetic variation in plasticity cause sun plants to assimilate more under high light and  
32 shade plants under low light (Givnish, 1988). In plants especially, we know little about the  
33 prevalence and adaptive significance of variation in fundamental physiological traits like

34 photosynthesis and their impact on plant performance (Flood et al., 2011).

35 A basic approach to identify candidate traits underlying local adaptation is to find asso-  
36 ciations between traits and environments. Either genetic variation in trait means and/or  
37 plasticity should vary clinally along environmental gradients. Indeed, clines in ecologically  
38 important traits are widespread in nature (Endler, 1977) and often adaptive, but in most  
39 cases the selective agent is unknown. For example, in *Drosophila* numerous latitudinal  
40 clines exist for traits like thermal tolerance (Hoffmann et al., 2002), body size (Coyne and  
41 Beecham (1987) and references therein), and life history (Schmidt et al., 2005). Some  
42 *Drosophila* clines have evolved multiple times (Oakeshott et al. (1982); Huey et al. (2000),  
43 see also Bradshaw and Holzapfel (2001)) or shifted in response to climate change (Umina  
44 et al., 2005), evincing climatic adaptation. Similarly, plant species exhibit latitudinal clines  
45 in traits like flowering time (Stinchcombe et al., 2004), cyanogenesis (Kooyers and Olsen,  
46 2012), leaf morphology (Hopkins et al., 2008; Stock et al., 2014), and drought resistance  
47 (Kooyers et al., 2015) that likely relate to climatic variation.

48 Despite the fact that latitudinal clines have been studied for a long time, latitude *per se*  
49 cannot be a selective agent. Latitude may be strongly correlated with one or two key  
50 climatic variables, such as temperature, precipitation, or growing degree-days. Latitude  
51 may also correlate with the strength of biotic interactions (Schemske et al., 2009) or other  
52 nonclimatic aspects of the environment, though as we explain below, we do not yet have  
53 compelling data that these are important in our study system. Hence, we focus on whether  
54 latitude could be an effective proxy for an underlying climatic driver, in which case we  
55 would expect a yet stronger relationship between traits and the key climatic variable(s)  
56 driving selection. Alternatively, latitude may be more strongly related to traits than any  
57 single climatic variable for at least two reasons. First, latitude may be correlated with  
58 several climatic agents of selection that are individually weak, but add up to a strong  
59 latitudinal cline. Alternatively, gene flow among neighbouring populations could smooth  
60 out local climatic effects, since alleles will experience selection across populations linked

61 by migration (Slatkin, 1978; Paul et al., 2011; Hadfield, 2016). We refer to this as the  
62 ‘climatic neighborhood’. For example, in mountainous regions average temperature at  
63 a given latitude varies widely, but in aggregate, a lower latitude set of populations will  
64 experience warmer climate than a higher latitude one. Thus, any particular low latitude  
65 population would be warm-adapted, even if it was located in a cooler (e.g. high elevation)  
66 site. Because many climatic factors vary latitudinally, and which climatic factors vary  
67 latitudinally changes over the earth’s surface (e.g. coastal vs. continental), dissecting the  
68 evolution of latitudinal clines across many species will help identify generalities, such as  
69 whether thermal tolerance maxima or seasonal timing is more important (Bradshaw and  
70 Holzapfel, 2008), and whether local or regional climate shapes selective pressures.

71 In this study, we investigated two major questions: 1) whether genetic variation in physi-  
72 ological trait means or plasticity corresponds with latitude; and 2) what climatic factor(s)  
73 could plausibly be responsible for latitudinal clines. Within question 2, we tested three  
74 hypotheses outlined in the previous paragraph: latitudinal clines are explained by a single  
75 dominant climatic factor, multiple climatic factors, or the climatic neighborhood expe-  
76 rienced by nearby population connected through gene flow. These hypotheses are not  
77 mutually exclusive since, for example, single or multiple factors in a climatic neighborhood  
78 may lead to latitudinal clines. We focused on climate because climate often determines  
79 where species are found and also can exert strong selection on populations within species.  
80 We acknowledge that other abiotic and biotic factors could contribute to selection and  
81 the overall pattern of local adaptation. Furthermore, there is a compelling need to know  
82 how populations are (or are not) locally adapted to climate so as to predict how they will  
83 respond to climate change (Aitken and Whitlock, 2013).

84 We examined these questions in *Erythranthe cardinalis* (formerly *Mimulus cardinalis* [Ne-  
85 som 2014]) because linking physiological traits to potentially complex patterns of local  
86 adaptation requires integrating multiple lines of evidence from comparative, experimental,  
87 and genomic studies under both lab and field conditions. Many classic and contemporary

studies of local adaptation use *Mimulus sensu lato* species because of their natural history, easy propagation, 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 deficiency of links between local adaptation and physiological mechanisms (Angert, 2006; Angert et al., 2008; Wu et al., 2010; Wright et al., 2013). We measured genetic variation in trait means and plasticity in response to temperature and drought among 16 populations distributed over 10.7° of latitude. We found a latitudinal cline of trait means in photosynthesis and growth, but little evidence for variation in plasticity. Interannual variation in precipitation and temperature are associated with this axis of variation, suggesting that climatic variance rather than mean may be an important driver of local adaptation in *E. cardinalis*. The climatic neighborhoods around populations explained trait variation better than local climate, indicating that latitudinal clines may be common because latitude integrates effects of selection on populations connected through gene flow. We place these findings in the context of life history theory and consider future directions in the Discussion.

## Material and Methods

Data and annotated source code to reproduce these analyses and manuscript are available on GitHub (<https://github.com/cdmuir/card-cline>).

### Population Selection

*E. cardinalis* is a perennial forb native to the Western US (California and Oregon). It is predominantly outcrossing, self-compatible, and pollinated primarily by hummingbirds. We used 16 populations from throughout the range of *E. cardinalis* (Table 1). These populations were intentionally chosen to span much of the climatic range of the species

111 based on all known occurrences (see below). Seeds were collected in the field from mature,  
 112 undehisced fruit left open for 2-4 weeks to dry, then stored at room temperature. To  
 113 control for maternal effects, we grew a large number of field-derived seeds in the greenhouse  
 114 and generated seed families for this experiment by haphazardly crossing individuals from  
 115 the same population. We selected seed families to maximize the number of field-derived  
 116 individuals represented. Thus, we used seeds from 154 greenhouse-derived seed families,  
 117 4–12 (mean = 9.6, median = 12) families per population.

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

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

## 118 Plant propagation

119 On 14 April, 2014, 3-5 seeds per family were sown directly on sand (Quikrete Play Sand,  
 120 Georgia, USA) watered to field capacity in RLC4 Ray Leach cone-tainers placed in RL98  
 121 98-well trays (Stuewe & Sons, Inc., Oregon, USA). We used pure sand because *E. cardinalis*

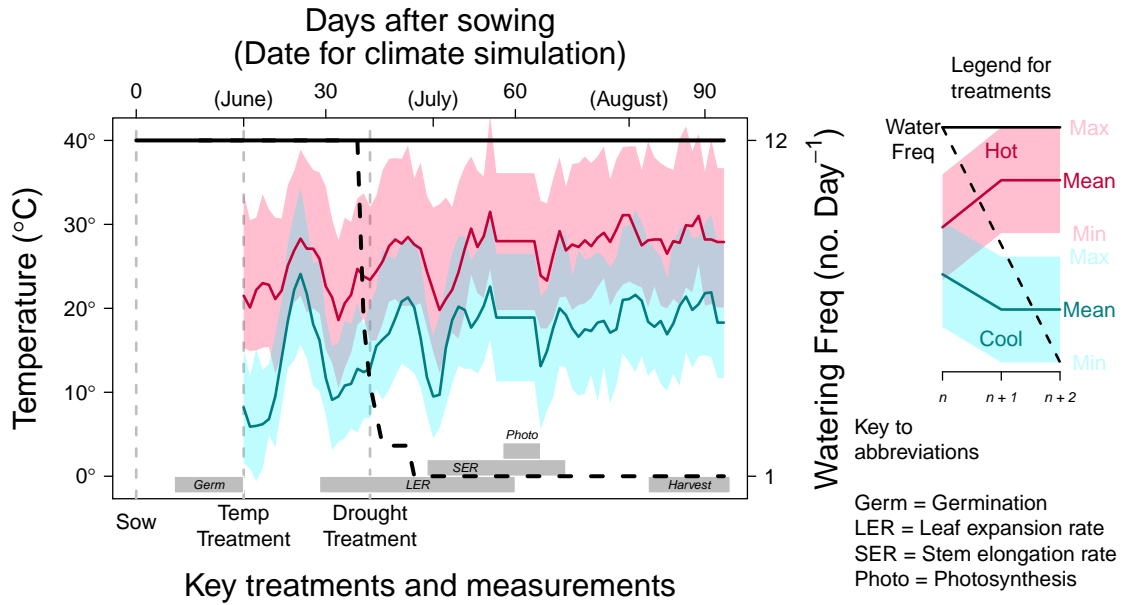
typically grows in sandy, riparian soils (A. Angert, pers. obs.). Two jumbo-sized cotton balls at the bottom of cone-tainers prevented sand from washing out. Cone-tainers sat in medium-sized flow trays (FLOWTMD, Stuewe & Sons, Inc., Oregon, USA) to continuously bottom-water plants during germination in greenhouses at the University British Columbia campus in Vancouver, Canada (49°15' N, 123°15' W). Mistlers 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. We recorded germination daily between one to two weeks after sowing, and every 2-3 days thereafter. On 5 May (21 days after sowing), we transferred seedlings to one of two growth chambers (Model E-15 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°C, night: 16°C) 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).

## **Temperature and drought 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 we 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 provided in the Supplemental Materials and Methods 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 photosynthetically active radiation in both chambers was



approximately  $400 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  and set to a 16:8 light:dark cycle to simulate summer growing conditions. 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). Lower humidity would have made the drought more severe, but low soil moisture is stressful in and of itself. The total number of plants in each treatment was:  $n_{\text{cool,dry}} = 169$ ;  $n_{\text{cool,ww}} = 174$ ;  $n_{\text{hot,dry}} = 176$ ;  $n_{\text{hot,ww}} = 183$ . Each population had 8–12 individuals per treatment level (mean = 11, median = 11).



**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 temperature (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.

154 **Trait measurements**

155 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	cm day <sup>-1</sup>
Photosynthetic rate	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
Mortality	probability of death

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

164 **Growth rate: leaf expansion and stem elongation** We measured growth rate dur-  
 165 ing two phases: leaf expansion and stem elongation. Growth measurements were taken  
 166 during the early vegetative stage. We censused leaf length twice per week shortly after  
 167 the emergence of true leaves from 12 May – 12 June (28–59 days after sowing), resulting  
 168 in 10 measurements. We ceased measuring leaf length once it appeared to asymptote and  
 169 growth shifted to stem elongation. We also censused plant height on 7 occasions (twice  
 170 per week) between 29 May and 20 June (45 to 67 days after sowing) until plants began  
 171 to initiate floral buds. Thus all growth measurements occurred during the vegetative, pre-

172 reproductive phase. Both leaf expansion and stem elongation were modelled separately  
173 as second-order polynomials. We used empirical Bayes' estimates of growth for each indi-  
174 vidual plant from linear mixed-effects models fit with the R package **lme4** version 1.1-12  
175 (Bates et al., 2015).

176 **Photosynthesis** During the week of 10 to 16 June (57 to 63 days after sowing), we  
177 measured daytime photosynthetic rate on a subset of 329 plants evenly spread between  
178 treatments and families within populations. The youngest, fully-expanded leaf acclimated  
179 for 3 minutes to reach steady state in a 6-cm<sup>2</sup> chamber of a LI-COR 6400XT Portable Pho-  
180 tosynthesis System (LI-COR Biosciences, Lincoln, Nebraska). We made all measurements  
181 at ambient light (400  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  of photosynthetically active radiation), atmospheric  
182 CO<sub>2</sub> (400 ppm), temperature, and moderate relative humidity. All measurements were  
183 taken between 9:00 AM and 5:00 PM (3 hours after lights turned on and 5 hours before  
184 lights turned off). During this period, we suspended normal day-to-day temperature fluc-  
185 tuations and set daytime temperatures to the average for that period (Cool: 26.5°; Hot:  
186 36.1°) so that all plants within a temperature level could be measured under the same con-  
187 ditions. We measured photosynthesis after dry down had progressed to assess differences  
188 in photosynthetic responses to drought.

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

## Genetic variation in trait means and plasticity

For all traits (Table 2) except germination (see above), we tested for Population, Treatment (Temperature, Water, and Temperature  $\times$  Water), and Population  $\times$  Treatment interactions (Population  $\times$  Temperature, Population  $\times$  Water, and Population  $\times$  Temperature  $\times$  Water). We interpreted significant Population effects to indicate genetic variation in trait means and Population  $\times$  Treatment interactions to indicate genetic variation in plasticity. As mentioned above, we used survival and GLMM models for germination rate and mortality, respectively. For all other traits, we used mixed model ANOVAs with Family and Block included as random factors. We fit models using restricted maximum likelihood 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). We used Satterthwaite’s approximation to calculate denominator degrees of freedom for  $F$ -tests. We also included days to germination as a covariate in growth analyses. To ensure that Population and Treatment effects were specific to a particular growth phase, we included germination day as a covariate in leaf expansion and stem elongation analyses.

Failure to detect a significant effect could be the result of Type-2 error, so we complemented step-wise ANOVA (see above) by comparing effect sizes calculated in the full model. The full model contains all main effects, two-way interactions (Population  $\times$  Temperature, Population  $\times$  Water), a three-way interaction (Population  $\times$  Temperature  $\times$  Water), and random effects. For linear mixed-effects models (leaf expansion, stem elongation, and photosynthesis) we used mean-squared error as a measure of effect size; for GLMM (mortality) we used  $\chi^2$  as a measure of effect size. We did not include germination rate because no Population  $\times$  Treatment effects were estimated. The difference in effect size of Population versus Population  $\times$  Treatment is:

$$\Delta \text{Effect Size}_{\text{Pop}-(\text{Pop} \times \text{Trt})} = \text{Effect Size}_{\text{Pop}} - \text{Effect Size}_{\text{Pop} \times \text{Trt}}$$

220 We calculated  $\Delta \text{Effect Size}_{\text{Pop}-(\text{Pop} \times \text{Trt})}$  for all two- and three-way Population  $\times$  Treat-  
 221 ment interactions for each trait. To determine whether  $\Delta \text{Effect Size}_{\text{Pop}-(\text{Pop} \times \text{Trt})}$  was sig-  
 222 nificantly different than 0, we calculated 95% confidence intervals using 1000 parametric  
 223 bootstrap samples simulated from fitted models. If the 95% confidence interval for a given  
 224  $\Delta \text{Effect Size}_{\text{Pop}-(\text{Pop} \times \text{Trt})}$ , was greater than zero then we concluded that the Population  
 225 effect size was significantly larger than the Population  $\times$  Treatment effect size, and vice  
 226 versa if the confidence interval was less than zero. If the confidence spanned zero, then  
 227 the effect sizes are not significantly different.

## 228 **Principal components of germination, growth, and photosynthesis**

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

## 234 **Identifying putative selective agents**

235 Latitudinal clines are common, but it is often difficult to ascribe this variation to a par-  
 236 ticular selective agent. To reiterate, we tested three non-mutually exclusive hypotheses  
 237 about how such latitudinal clines emerge: 1) one or two climatic variables explain latitudi-  
 238 nal trait variation; 2) latitude is a proxy for multiple climatic factors that together shape  
 239 trait variation; and 3) latitude integrates selection in a broader climatic neighborhood. We  
 240 found that a population's position along TraitPC1 correlated strongly with the latitude of

241 origin (see Results) and next used Random Forest regression (Liaw and Wiener, 2002) to  
 242 identify putative climatic factors underlying trait-latitude associations in *E. cardinalis*. We  
 243 reasoned that if we identified a single climatic factor that explained more trait variation  
 244 than latitude, then this would suggest that factor is a key selective agent underlying the  
 245 latitudinal cline (Hypothesis 1). On the other hand, if multiple climatic factors together  
 246 are necessary to explain trait variation, then this would suggest that many climatic factors  
 247 together have imposed selection for the latitudinal cline (Hypothesis 2). We hereafter refer  
 248 to factors identified in this analysis as ‘Climate-TraitPC1’ variables.

249 To test Hypothesis 3 about climatic neighborhoods driving selection, we directly competed  
 250 local with neighborhood climate. The logic is that if the climatic analysis can identify  
 251 candidate climatic factors important for local adaptation, then stronger correlations with  
 252 neighbourhood climate would suggest a role for gene flow. We used the immediate collection  
 253 location for local climate. For climate neighborhoods, we sampled climate at 1000 random  
 254 points (at 90-m resolution) within a 62-km radius buffer around the collection and took the  
 255 average. We chose this buffer radius based on population genetic structure, as inferred from  
 256  $\approx 25,000$  restriction-site associated SNPs among 49 populations from across the range (Paul  
 257 et al., In review). Spatial autocorrelation in allele frequencies persists for 62 km. However  
 258 radii of 10 km<sup>2</sup> and 100 km<sup>2</sup> resulted in similar outcomes (data not shown). Since *E.*  
 259 *cardinalis* is found exclusively in riparian areas, we only selected points along streams using  
 260 the National Hydrography Dataset (United States Geological Survey, 2015). Climatic  
 261 means and variances (see below) were weighted by their climatic suitability as determined  
 262 using a multimodel ensemble average of ecological niche models (Angert et al., 2016).  
 263 In addition to competing local and neighborhood climate, we compared the univariate  
 264 correlation between local and neighborhood climate with TraitPC1 and Latitude using  
 265 paired *t*-tests. We adjusted degrees of freedom to account for the fact that many climatic  
 266 factors are highly correlated and not independent. Specifically, we calculated the effective  
 267 number of independent climatic factors ( $M_{\text{eff}}$ ) using the formula  $M_{\text{eff}} = 1 + (M - 1)(1 -$

268  $\text{Var}(\lambda)/M$ ) (Chevrud, 2001), where  $M$  is the original number of climatic factors and  $\lambda$  are  
269 the eigenvalues of the correlation matrix of all climatic factors.

270 To help eliminate potentially spurious correlations between TraitPC1 and climate, we tested  
271 for overlap between climatic variables that best predict latitude of all *E. cardinalis* occur-  
272 rence records (see detail below), not just the 16 focal populations. We refer to these climatic  
273 factors as ‘Climate-Latitude’ variables. The logic is that climatic factors associated with  
274 both TraitPC1 and latitude for all populations are more likely to be important selective  
275 agents than climatic factors that happen to correlate with TraitPC1 but do not covary with  
276 latitude throughout the *E. cardinalis* range. If a climatic factor is driving the latitudinal  
277 cline in TraitPC1, then we expect that climatic factor will correlate strongly with lati-  
278 tude of occurrence localities. Therefore, we did not consider Climate-TraitPC1 variables  
279 to be candidate selective agents unless the same or very similar variable was found in the  
280 Climate-Latitude analysis. However, we do not interpret potential selective agents iden-  
281 tified in Climate-Latitude analyses alone, because the goal was to explain the latitudinal  
282 clines in traits, not all aspects of climate that vary with latitude.

283 We selected Climate-Latitude and Climate-TraitPC1 variables independently using Vari-  
284 able Selection Using Random Forest (VSURF) algorithm in the R package **VSURF** version  
285 1.0.3 (Genuer et al., 2016). Random Forest regression is useful for cases like ours when  
286 the number of potential predictors is similar to or greater than the number of observations  
287 (‘high  $p$ , low  $n$ ’ problem). VSURF is a multistep algorithm that progressively retains or  
288 eliminates variables based on their importance over regression trees in the forest. Variable  
289 importance is defined as the average amount a climate variable reduces mean-squared er-  
290 ror in the predicted response (TraitPC1 or Latitude), compared to a randomly permuted  
291 dataset, across all trees in the random forest (see Genuer et al. [2015] for further detail).  
292 Hence, VSURF automatically eliminates unimportant and redundant variables based on  
293 the data without having to arbitrarily choose among colinear climate variables before the  
294 analysis. We kept only variables selected for prediction, the most stringent criterion. We

visually depict how we selected climatic variables in Fig 2.

For Climate-Latitude analyses, we compiled a representative set of 356 recent (since 2000) known *E. cardinalis* occurrences from a comprehensive set of herbarium records and an exhaustive field survey in 2010-11 (Angert et al., 2016). These occurrences were thinned by 50% to correct for uneven sampling. For both Climate-TraitPC1 analyses (16 focal populations) and Climate-Latitude (many populations), we used a 90-m digital elevation model from HydroSHEDS (Lehner et al., 2006) to extract elevation. Monthly interpolated climate layers were calculated using ClimateWNA version 5.30 (Wang et al., 2012), which accurately downscales climate data specifically for the rugged topography of western North America. For each occurrence, we calculated bioclimatic variables using the biovars function in the R package **dismo** version 1.1-1 (Hijmans et al., 2016). We included 24 climatic factors, 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, and annual temperature range. For each variable, we calculated both a 30-year normal by averaging 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 (CV). In total, the VSURF algorithm selected among 96 climate variables: 24 climatic factors  $\times$  2 types (30-year average and CV)  $\times$  2 spatial scales (local and neighborhood).



## 316 Results

### 317 A coordinated latitudinal cline in germination, growth, and photosynthe- 318 sis

319 There are strong genetically-based trait differences in time to germination, growth, and  
320 photosynthetic rate among populations of *E. cardinalis*, as evidenced by large and signif-  
321 icant population effects for these traits (Table 3). A single principal component captured  
322 71.6 % of the trait variation among populations, defining an axis of variation from fast to  
323 slow growth. A population’s position along this axis strongly covaried with its latitude of  
324 origin; southern populations grew faster than northern populations (Fig 3). There were  
325 similar latitudinal clines for individual traits underlying PC1 (Figures S1 to 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				

## 326 Little evidence for variation in plasticity

327 In contrast to the genetic variation in trait means described above, we found little evidence  
328 of G×E in *E. cardinalis*. There were only two statistically significant Population × Treat-  
329 ment interactions (Table 3, Fig. S5), but these were not strong compared to Population  
330 and Temperature effects. Otherwise, populations responded similarly to treatments: faster  
331 growth in the hot treatment, slower growth in the dry treatment, and high mortality in  
332 the hot, dry treatment (Table 3). Complete ANOVA tables are available in the Supporting  
333 Information (Tables S3 to S6).

334 The effect size of Population was significantly larger than that for Population × Treat-  
335 ment interactions (Fig. S6) in most cases. For leaf expansion, Population had a signifi-  
336 cantly larger effect size than Population × Treatment interactions in 2 of 3 comparisons  
337 (Fig. S6A). For stem elongation (Fig. S6B) and mortality (Fig. S6D), Population effect  
338 sizes were significantly larger than all Population × Treatment interactions. For Photosyn-  
339 thesis, Population and Population × Treatment effect sizes were not significantly different  
340 (Fig. S6C), presumably because we had a smaller sample size.

## 341 Neighborhood climatic variability best explains latitudinal cline

342 Interannual variation in climate averaged over each populations’s climatic neighborhood  
343 correlated most strongly with trait variation and latitude of *E. cardinalis* occurrences  
344 (Fig. 4, Table S7). All 16 Climate-Latitude and 3 Climate-TraitPC1 variables were neigh-  
345 borhood rather than local variables (Fig. 4). In fact, neighborhood climate almost always  
346 correlated better with TraitPC1 and Latitude than local climate (Fig. 5). On average,  
347 neighborhood Climate-TraitPC1 correlation coefficients were 0.16 higher than correlations  
348 with local-scale climate variables (paired *t*-test,  $t = 7.87$ , d.f. = 33.6,  $P = 3.94 \times 10^{-9}$ ).  
349 Likewise, neighborhood Climate-Latitude correlation coefficients were 0.13 higher than

350 those for local-scale climate (paired  $t$ -test,  $t = 6.71$ , d.f. = 36.8,  $P = 7.22 \times 10^{-8}$ ). Among  
 351 Climate-Latitude and Climate-TraitPC1 variables, neighborhood climatic variability over  
 352 30 years (1981–2010) in either winter precipitation (bio16 $_{\sigma}$ ) and/or temperature (bio11 $_{\sigma}$ )  
 353 are the strongest candidates to explain the latitudinal cline in *E. cardinalis* (see Table S2  
 354 for a key to climate variable abbreviations). Note that the coefficient of variation of a  
 355 climatic factor is subscripted with  $\sigma$  whereas the mean is subscripted with  $\mu$ . More specif-  
 356 ically, greater winter precipitation variability and lower winter temperature variability are  
 357 associated with Southern latitudes and higher TraitPC1 values (Fig. 6A,B). Neighborhood  
 358 interannual variation in winter precipitation (bio16 $_{\sigma}$ ) was the most important Climate-  
 359 Latitude variable (Fig. 4A). However, neighborhood bio16 $_{\sigma}$  did not overlap with Climate-  
 360 TraitPC1 variables (Fig. 4B). We nevertheless consider it a plausible candidate for two  
 361 reasons. First, neighborhood bio16 $_{\sigma}$  correlated strongly with TraitPC1 (Fig. 6A). Second,  
 362 one of the most important Climate-TraitPC1 variables (neighborhood bio15 $_{\sigma}$ ; Fig. 6B,C)  
 363 is very similar to bio16 $_{\sigma}$ . In Mediterranean climates like California, most precipitation  
 364 occurs in the wettest quarter (winter), so years with low winter precipitation also have  
 365 low precipitation seasonality. Hence, highly variable year-to-year winter precipitation at  
 366 lower latitude (Fig. 6D) is closely associated with large swings in precipitation seasonality  
 367 (Fig. 6C).

368 Interannual variation in temperature of the coldest quarter (neighborhood bio11 $_{\sigma}$ ) is an-  
 369 other plausible candidate because it was the only variable in both Climate-Latitude and  
 370 Climate-TraitPC1 analyses (Fig. 4). Neighborhood bio11 $_{\sigma}$  explained more variation in  
 371 TraitPC1 than latitude (latitude  $r^2 = 0.55$  vs. bio11 $_{\sigma}$   $r^2 = 0.6$ ; Fig. S7), whereas neigh-  
 372 borhood bio16 $_{\sigma}$  did slightly worse (bio16 $_{\sigma}$   $r^2 = 0.49$ ). Models using bio15 $_{\sigma}$  or bio11 $_{\sigma}$  to  
 373 predict TraitPC1 also had significantly lower Akaike Information Criteria (AIC) than the  
 374 latitude model (AIC of different models – bio15 $_{\sigma}$ : 48.5; bio11 $_{\sigma}$ : 52.4; latitude: 54.5). The  
 375 best two-factor model including both neighborhood bio15 $_{\sigma}$  and bio11 $_{\sigma}$  did not significantly  
 376 improve explanatory power ( $r^2 = 0.71$ , AIC= 49.2). In summary, either variation in precip-

377 itation or temperature seasonality may be important selective agents, but there is no strong  
378 evidence that they are both important. The most important Climate-TraitPC1 variable,  
379 neighborhood variation in mean diurnal range ( $\text{bio2}_\sigma$ ; Fig. 4B) did not have any obvious  
380 similarity to Climate-Latitude variables. Given the large number of potential associations,  
381 we therefore think this may be a spuriously strong relationship.

## 382 Discussion

383 We found evidence for one of two common signatures of local adaptation in the perennial  
384 herb *Erythranthe cardinalis*. Latitudinal clines in germination rate, photosynthesis, and  
385 growth suggest adaptive differentiation in important physiological traits of the species.  
386 However, we caution that these are candidate adaptive traits and that we cannot yet rule  
387 out nonadaptive demographic processes such as a recent range expansion toward higher lat-  
388 itude (Paul et al., In review; Sheth and Angert, 2017). In contrast, we found little evidence  
389 for variation in plasticity to temperature or drought. Due to low replication within families,  
390 we did not have power to assess within-population genotype-by-environment interactions,  
391 which may be present. As we discuss below, low variation in plasticity among popula-  
392 tions may indicate that some dimensions of the fundamental abiotic niche are relatively  
393 conserved. Note that statistical power to detect significant plasticity is lower than that  
394 for differences in trait means, but the effect size of variation in plasticity was significantly  
395 less than that for trait means in most cases (Fig. S6). Finally, our results suggest that  
396 neighborhood-scale climate and interannual variation are more important selective agents  
397 than local averages. In the paragraphs that follow, we tie these results into the broader  
398 threads of evolutionary theory that might help explain why variation in physiological trait  
399 means changes clinally, whereas plastic responses to temperature and drought are relatively  
400 static. One caveat to bear in mind is that we are limited by the size of the climate grid  
401 ( $\approx 90 \text{ m}^2$ ) and therefore unable to detect very fine-scale local adaptation.

402 Evolutionary theory indicates that the shape of fitness tradeoffs, demography, and gene flow  
403 can constrain adaptation (Levins, 1968; Ronce and Kirkpatrick, 2001; Lenormand, 2002)  
404 and hence the type of variation maintained within species. Specifically, adaptive variation  
405 can be maintained by spatially varying selection if tradeoffs are not too strong, demography  
406 is symmetric, and/or maladaptive gene flow is low. Strong tradeoffs can prevent local  
407 adaptation in spatially variable environments because selection favors habitat specialists  
408 that track a specific habitat regardless of its frequency in the environment (Levins, 1968).  
409 For example, a riparian specialist may experience similar selection in rivers of high rainfall  
410 regions and deserts, even though the habitat is much rarer in the latter. In *E. cardinalis* we  
411 found substantial genetically based variation among populations along a phenotypic axis  
412 from fast to slow growth that varied over a large spatial scale (Fig. 3). If this variation  
413 is adaptive, it suggests one of several possibilities to investigate in the future: the fitness  
414 tradeoff between low versus high latitude environments is not too strong nor swamped  
415 by demographic asymmetry or maladaptive gene flow. That is, alleles favoured at one  
416 latitude are not strongly selected against when they flow to another population, allowing  
417 locally adaptive genetic variation to be maintained by spatially heterogeneous selection.  
418 We also know from previous work that population size does not vary strongly with latitude  
419 (Angert, unpub. data). Gene flow appears to be high, but attenuates at broad spatial  
420 scales, especially between southern ( $< 35^{\circ}\text{N}$ ) and northern portions of the range (Paul  
421 et al., In review).

422 Nevertheless, local gene flow from similar environments may shape how selection varies  
423 with latitude. Theory predicts that populations will not be perfectly adapted to their  
424 immediate habitat when there is gene flow from surrounding populations with different  
425 optima (Lenormand, 2002). With spatial heterogeneity and gene flow, traits will not covary  
426 perfectly with the local optimum (Slatkin, 1978; Paul et al., 2011; Hadfield, 2016), but  
427 should instead better match the average environment experienced by nearby populations  
428 connected through gene flow, which we refer to as the climatic neighborhood. Gene flow

429 and spatial heterogeneity may therefore be important in maintaining genetic variation  
430 (Yeaman and Jarvis, 2006). As this hypothesis predicts, climatic neighborhoods (62-km  
431 buffer around populations) correlated with traits and latitude of occurrences better than  
432 local climate (Fig. 4). We interpret this as suggestive evidence that gene flow between  
433 neighboring *E. cardinalis* populations shapes selection – populations are locally adapted to  
434 prevailing climate in their neighborhood, but perhaps not perfectly adapted to their local  
435 climate. This may not greatly constrain local adaptation because local and neighborhood  
436 climate values were generally similar in *E. cardinalis* populations (Fig. 5), at least at the  
437 resolution of ClimateWNA (90 m<sup>2</sup>). Therefore, we would predict in reciprocal transplants  
438 that populations whose local climate is farther from their neighborhood average would be  
439 less well adapted than those close to their neighborhood average.

440 It is reasonable to predict that southern populations, which appear to experience more  
441 frequent drought years (see below), might have physiological adaptations to respond to  
442 drought stress to survive and grow in drier soil. We found little evidence for this type of  
443 drought tolerance; all populations responded to drought and temperature similarly (Ta-  
444 ble 3). Plants grew faster in the Hot treatment, but there was little effect of drought on  
445 growth. Rather, the effects of drought took longer to materialize but resulted in high mor-  
446 tality, especially in the Hot treatment. However, there was no differential mortality among  
447 populations in this treatment. Although our results indicate that this axis of the species  
448 niche may be constrained, plants have multiple ways to resist drought through both toler-  
449 ance and escape (Ludlow, 1989; Kooyers, 2015). Next, we consider why drought tolerance  
450 may be less important in local adaptation than a form of escape for this species.

451 We hypothesize that tolerance to dry soil may be constrained by a combination of strong  
452 fitness tradeoffs, demographic asymmetry, and gene flow. Soil moisture in riparian habitats  
453 where *E. cardinalis* lives is highly heterogeneous at very small spatial scales (several me-  
454 ters). Plants in the stream never have to tolerate drought whereas plants only a few meters  
455 away may experience extreme drought since there is little direct precipitation during the

growing season in Mediterranean climates of western North America. We hypothesize that alleles conferring greater drought tolerance may be quite costly in well-watered soils, and *vice versa*, leading to strong fitness tradeoffs. Such tradeoffs would promote specialization to one soil moisture or another, thereby inhibiting the evolution of broad environmental tolerance within a population. Demography and gene flow may reinforce niche conservatism. A new mutant with increased drought tolerance that could survive at the resource-poor margin of a population would likely be demographically overwhelmed by the larger census populations that can be maintained in higher-resource environments. Infrequent wet years may also produce most seeds, so selection is weighted towards alleles that have high fitness in the wet environment, even if dry years are more frequent (Templeton and Levin, 1979; Brown and Venable, 1986). However, demographic asymmetry should equally hinder the evolution of both drought tolerance and escape, so it should not explain why one mechanism evolves but not the other. Finally, gene flow, which is generally high among *E. cardinalis* populations within the same ecoregion (Paul et al., In review), will thwart local adaptation and reinforce specialization. Thus, the spatial grain of the environment, demographic asymmetry, and gene flow may conspire to constrain local adaptation along this environmental axis. Consistent with this hypothesis, recent record-setting droughts have caused the decline or even local extinction of some natural populations of *E. cardinalis* (Sheth and Angert, 2017).

In sum, these results indicate that genetic differences in physiology and growth are better candidates than plastic responses to temperature and drought as mediators of local adaptation to climate in *E. cardinalis*. Next, we would like to understand why variation in these particular traits may be adaptive. We argue that temporally more variable environments, as experienced by southern populations, select for a more ‘annualized’ life-history strategy, a form of drought escape. Demographic observations in natural populations of *E. cardinalis* reveal that southern populations tend to flower earlier at a smaller size, while northern populations invest more in vegetative growth (Sheth and Angert, 2017). In this

experiment, the fastest growing plants began producing flowers in  $\sim 60$  days (data not shown), suggesting that rapid vegetative development may likewise affect flowering time. The association between position along the ‘fast-slow’ continuum and associated traits in *E. cardinalis* is similar to interspecific relationships between growth, functional traits, and life history (Adler et al., 2014; Salguero-Gómez et al., 2016). However, we cannot exclude unexplored factors (e.g. edaphic conditions, competitors, pollinators, etc.) which may also contribute to the latitudinal cline.

Greater investment in aboveground growth, as opposed to belowground storage for future seasons, may be favoured in climates with more frequent drought years, but maladaptive in climates with more consistent precipitation. In a stable environment where winter survivorship is assured in most years, failure to store resources may reduce lifetime fitness. But for perennial herbs in Mediterranean climates, a dry winter (rainy season) can kill the rhizomes (underground stems that store nutrients for future growth) before emergence or aboveground stems before flowering. If drought years occur frequently enough, selection may favour the fast-growing strategy because there is no advantage to storage if drought kills plants before flowering. Considering life-history strategy as a continuum from no storage (annual) to lots of storage (perennial), we hypothesize that the optimal allocation to aboveground growth is more ‘annualized’ in southern climates that have greater interannual variation in precipitation. This is a form of drought escape in that plants are investing more reproduction in the present to avoid possible drought in subsequent years, but is distinct from classic drought escape syndromes in which plants speed up development early in the season before the onset of drought.

The hypothesis that greater precipitation variability selects for an annualized life history is tentative, but consistent with theory and data from other species. Life history theory shows that less variable environments are one factor that favours the evolution of perenniality (Stearns, 1976; Iwasa and Cohen, 1989; Friedman and Rubin, 2015). Populations of the perennial *Plantago asiatica* show a similar latitudinal cline in growth and allocation to



510 storage (Sawada et al., 1994) but attribute the cline to variation in growing season length.  
 511 There are also life history clines in the closely related species *E. guttata*, but the underlying  
 512 traits and climatic drivers are quite different. Annual *E. guttata* flower sooner and produce  
 513 fewer stolons in response to climates with shorter seasons and more intense summer drought  
 514 (Lowry and Willis, 2010; Friedman et al., 2015; Kooyers et al., 2015). In contrast, there are  
 515 no truly annual (monocarpic and semelparous) populations of *E. cardinalis*. Rather, our  
 516 hypothesis states that climatic variability selects on quantitative variation in allocation to  
 517 growth versus storage.

518 In summary, we found evidence for a coordinated latitudinal cline in germination rate,  
 519 photosynthesis, and growth, suggesting local adaptation. We therefore predict to find dif-  
 520 ferent optima for these traits in different climates. We did not find evidence that the  
 521 relative performance of populations shifts with temperature or watering regime, suggesting  
 522 relatively little variation in plasticity. Exploratory analysis implicate that more variable  
 523 precipitation regimes at lower latitude could drive much of the latitudinal cline, though  
 524 other climatic factors could also contribute. Interestingly, the climatic neighborhood may  
 525 shape selective pressures more than local climate. In the future, we will use field exper-  
 526 iments to test whether greater variation in precipitation selects for faster growth and if  
 527 selection on temperature/drought responses does not vary among populations. By doing  
 528 so, we aim to understand why certain physiological and developmental mechanisms, but  
 529 not others, contribute to local adaptation.

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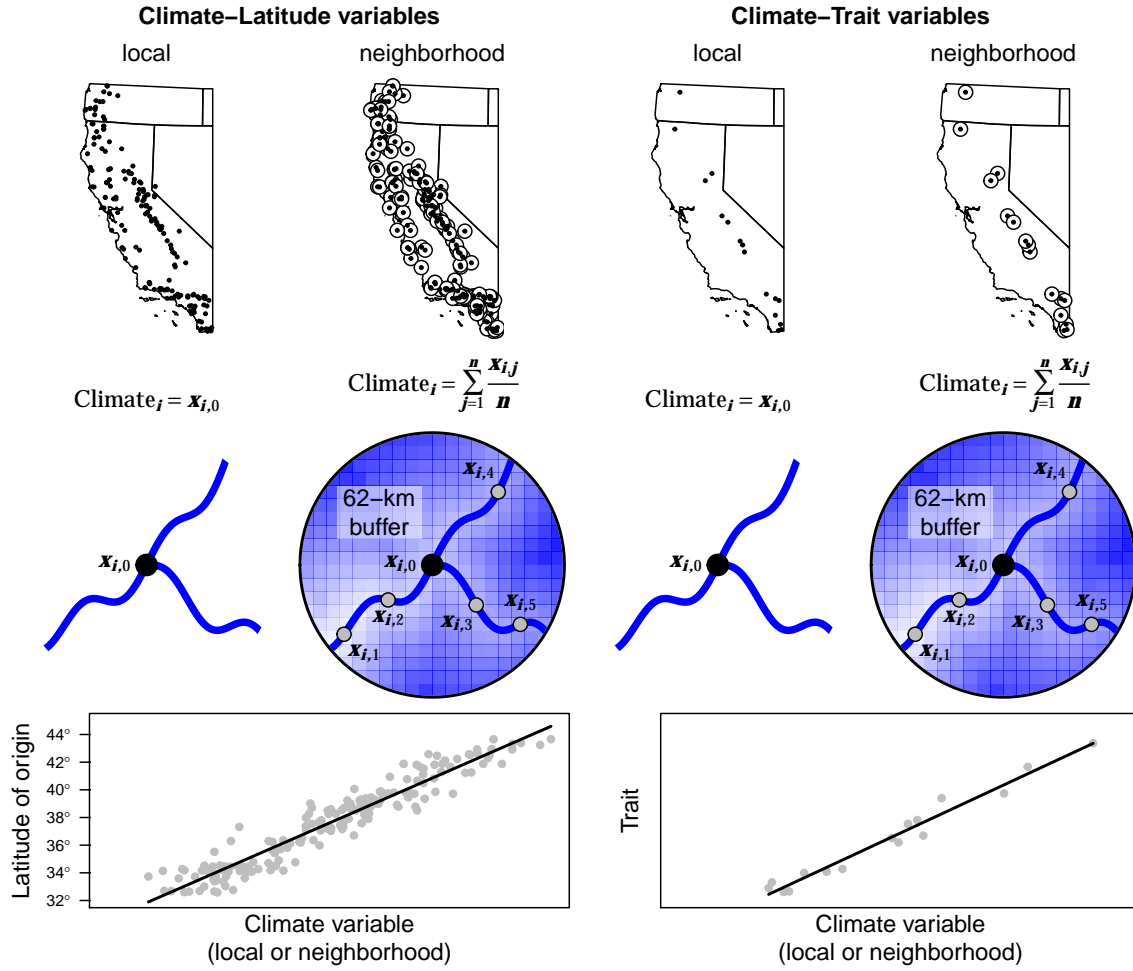
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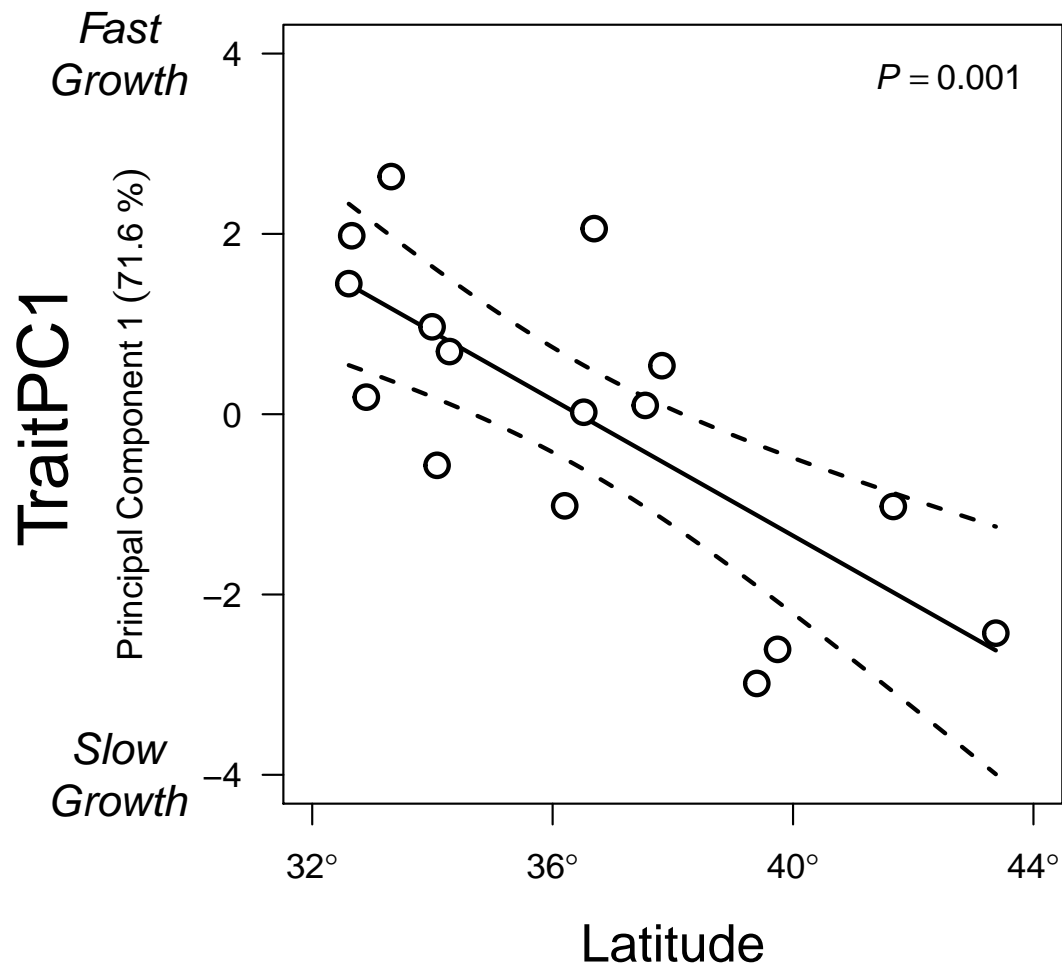
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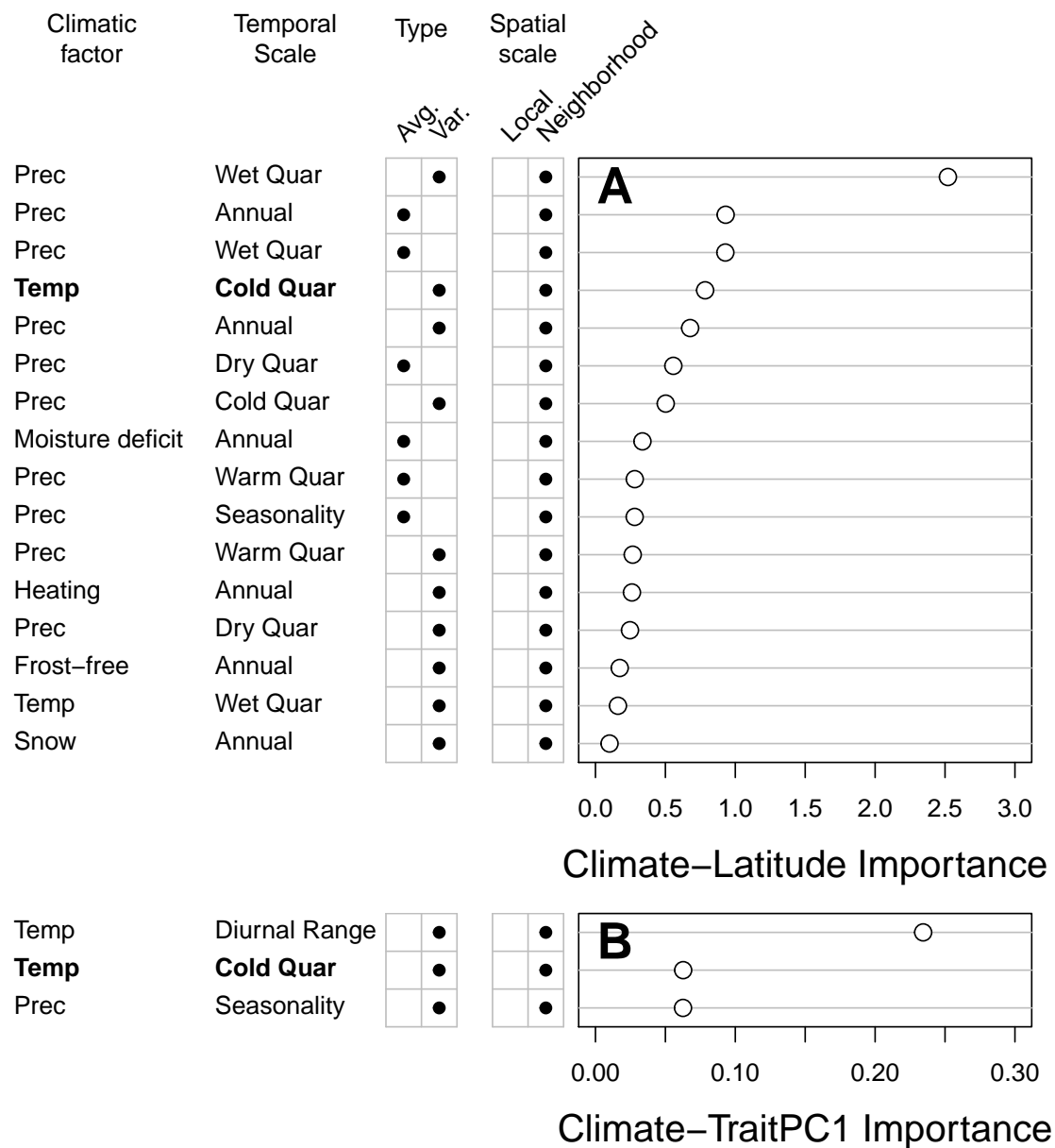
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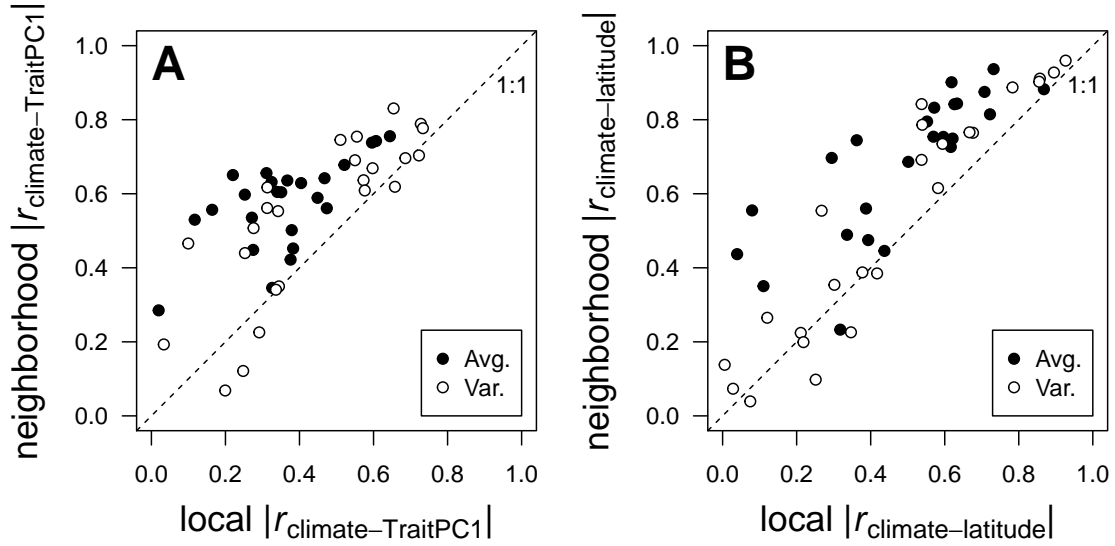
**Figure 2:** Overview of method for identifying putative climatic selective agents underlying latitudinal cline. We looked for climate variables that explained both the latitude of 356 *E. cardinalis* occurrences ('Climate-Latitude variables') and traits ('Climate-Trait variables'). For Climate-Latitude 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 *E. 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.



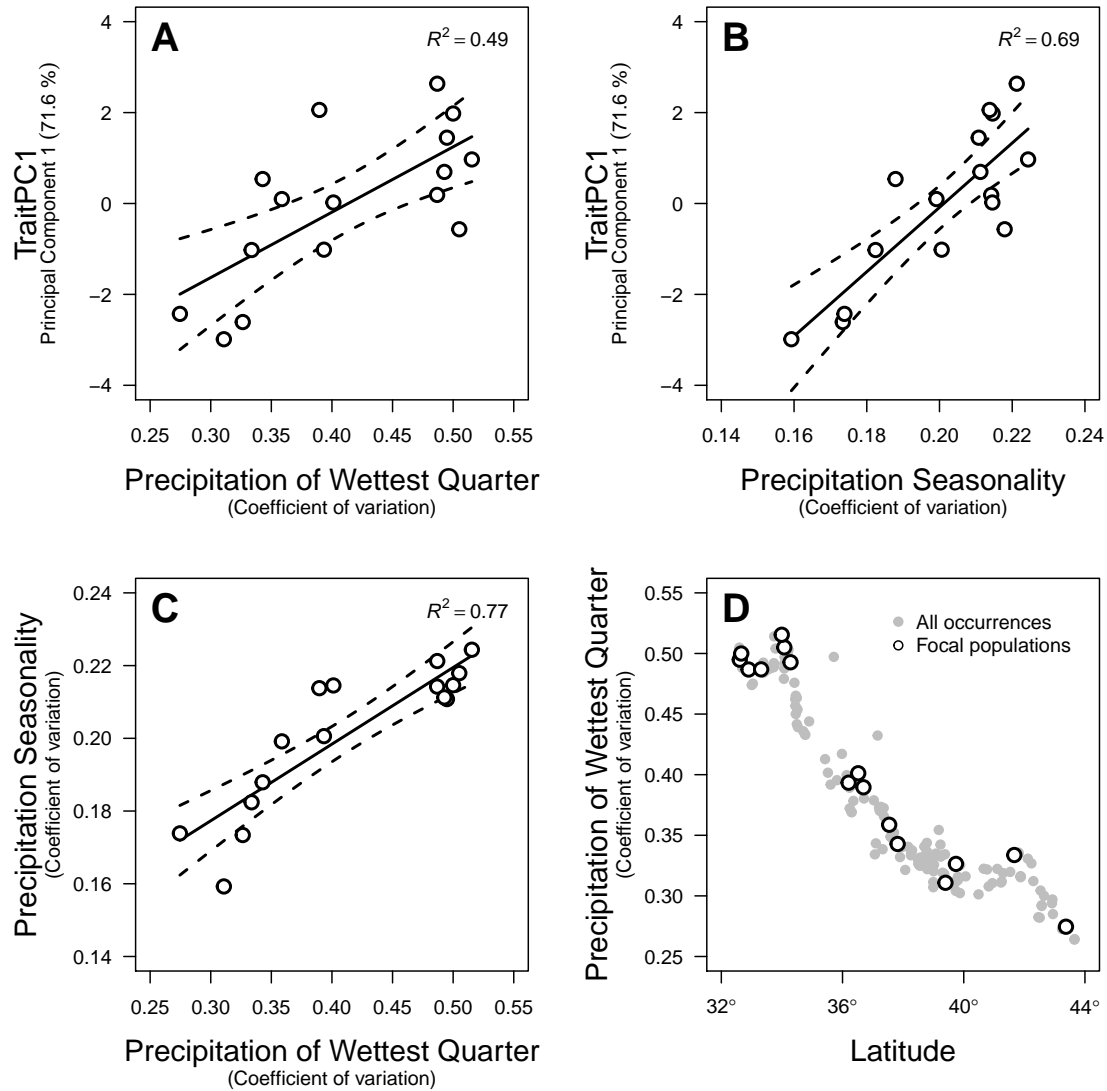
**Figure 3:** Trait variation, from fast to slow growth, is closely associated with latitude. Each point is a population's latitude of origin (x-axis) and position along the slow to fast growth axis (y-axis), defined as Principal Component 1 of four traits (see Material and Methods). The line and 95%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



**Figure 4:** Climatic variation integrated over climatic neighborhood is closely correlated with latitude of *E. cardinalis* and trait variation. A. Using Random Forest regression, we identified 16 climatic variables significantly (high importance) associated with latitude of *E. cardinalis* occurrences. B. Only one of the most important Climate-Latitude variables (in bold) was among the most important Climate-TraitPC1 variables. Variable importance is defined as the average amount a climate variable reduces mean-squared error in the predicted response (TraitPC1 or Latitude), compared to a randomly permuted dataset, across all trees in the random forest (see Genuer et al. [2015] for further detail). 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:** Neighborhood climate predicts TraitPC1 ('Climate-trait', panel A) and Latitude of occurrences ('Climate-latitude', panel B) better than local climate. Each point is the absolute value of the Pearson correlation coefficient ( $|r|$ ) between TraitPC1 (A) or latitude (B) for 24 climatic factors, for which we used both the 30-year mean (closed circles) and coefficient of variation (open circles). Most points lie above the 1:1 line, indicating stronger correlations with neighborhood compared to local climate. Neighborhood climate was integrated over a 62-km radius around focal populations (see text for further detail).



**Figure 6:** 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 *E. 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.

710 **Supporting Information**

711 **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.

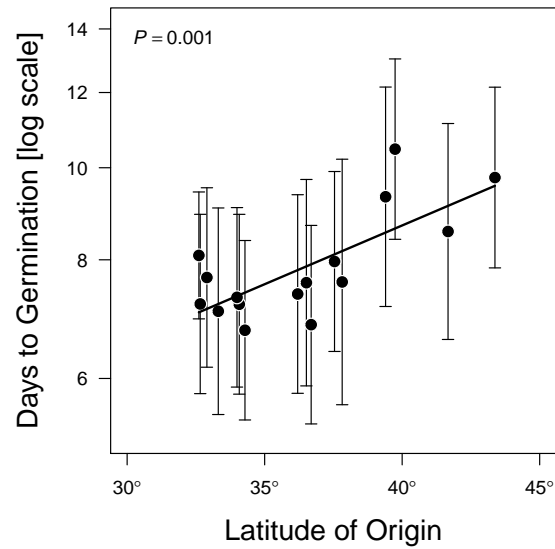
	SS	MS	df1	df2	<i>F-value</i>	<i>P-value</i>
Population	347.7	23.2	15	78	3.02	$7.5 \times 10^{-4}$
Temperature	134.1	134.1	1	6	17.46	$6.4 \times 10^{-3}$
Water	51	51	1	4	6.64	0.066
Temperature $\times$ Water	0.7	0.7	1	3	0.09	0.781
Population $\times$ Temperature	218.6	14.6	15	263	1.9	0.024
Population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

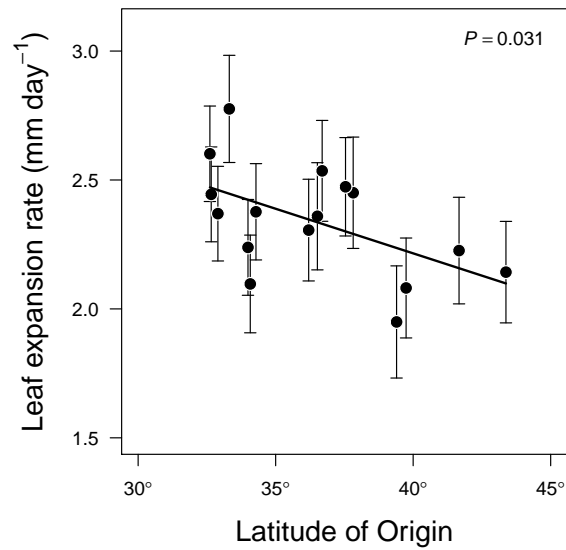
	$\chi^2$	df	<i>P-value</i>
Population	32	31	0.419
Temperature	31.8	6	$1.8 \times 10^{-5}$
Water	69.2	12	$4.6 \times 10^{-10}$
Temperature $\times$ Water	20.7	1	$5.3 \times 10^{-6}$
Population $\times$ Temperature	5.6	15	0.985
Population $\times$ Water	8.6	15	0.897
Population $\times$ Temperature $\times$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

**Table S7:** Important climatic variables predicting latitude of *E. 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.

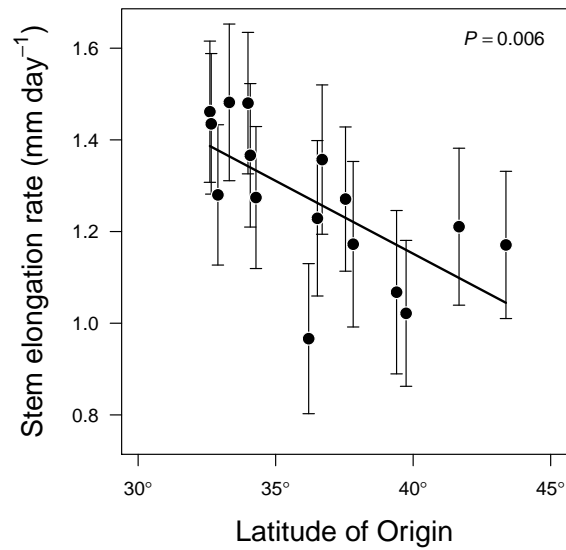
Climate-Latitude variables	Climate-TraitPC1 variables
Precipitation of wettest quarter ( $\sigma$ , neighborhood)	Mean diurnal range ( $\sigma$ , neighborhood)
Annual precipitation ( $\mu$ , neighborhood)	Mean temperature of coldest quarter ( $\sigma$ , neighborhood)
Precipitation of wettest quarter ( $\mu$ , 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)	



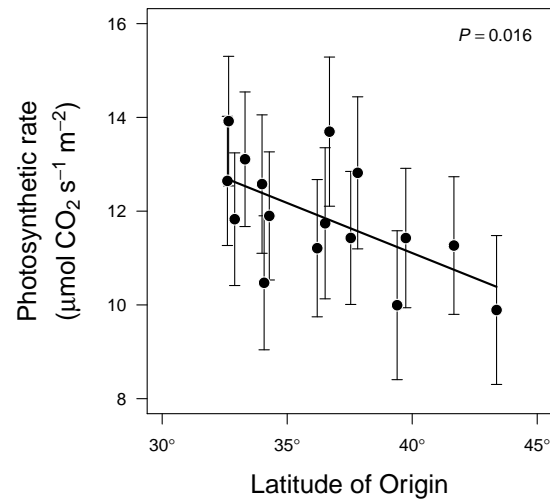
**Figure S1:** Southern populations germinate faster. Each point is a population of *E. cardinalis* showing its latitude of origin (x-axis) and model-predicted days to germination in days under growth chamber conditions (see Material and Methods). Bars around each point are 95% confidence intervals. 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 *E. 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-predicted leaf expansion rate  $\sim$  latitude. The  $P$ -value of the regression is given in the upper right corner.

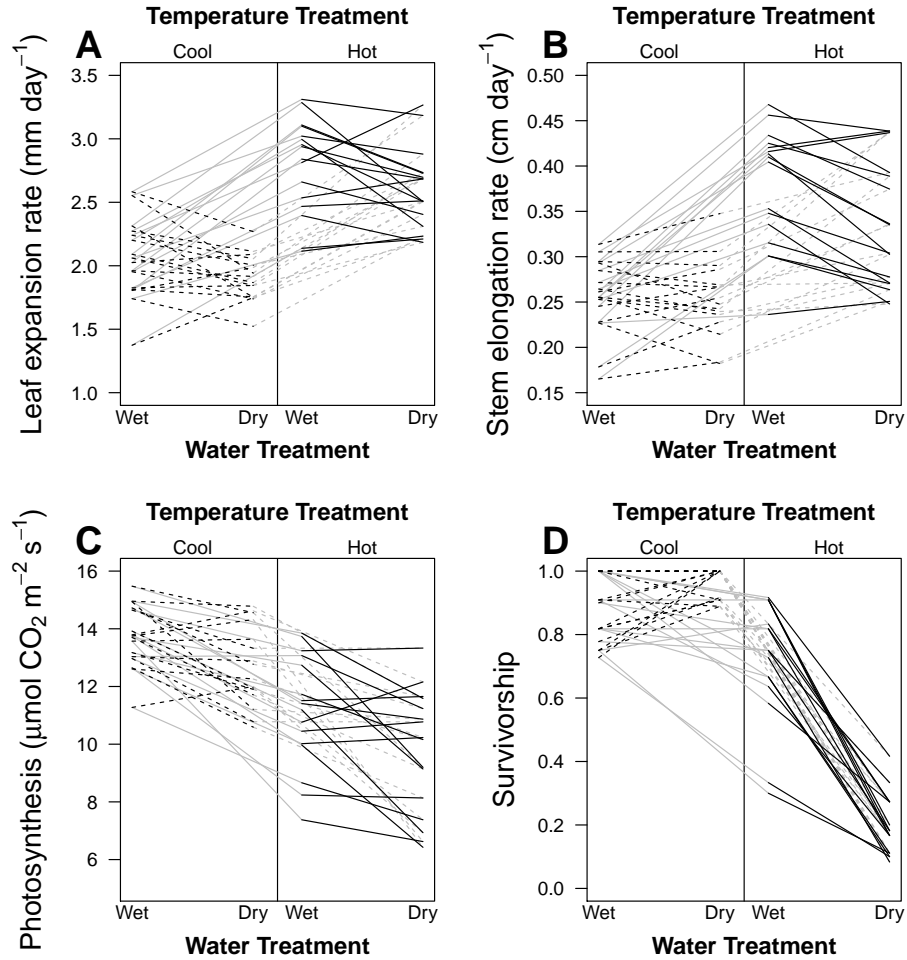


**Figure S3:** Southern populations grow faster. Each point is a population of *E. cardinalis* showing its latitude of origin (x-axis) and model-predicted stem elongation rate. Bars around each point are 95% confidence intervals. Predicted stem elongation rate based least-square mean estimates and confidence 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.

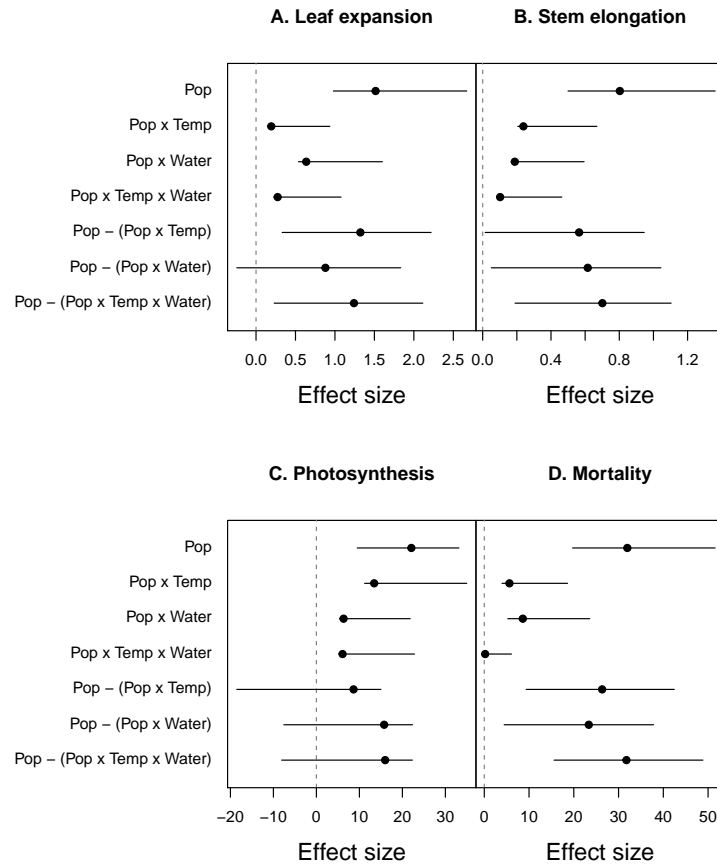


**Figure S4:** Southern populations photosynthesize faster. Each point is a population of *E. cardinalis* showing its latitude of origin (x-axis) and model-predicted instantaneous photosynthetic rate. Bars around each point are 95% confidence intervals. Predicted photosynthetic rates based least-square 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.

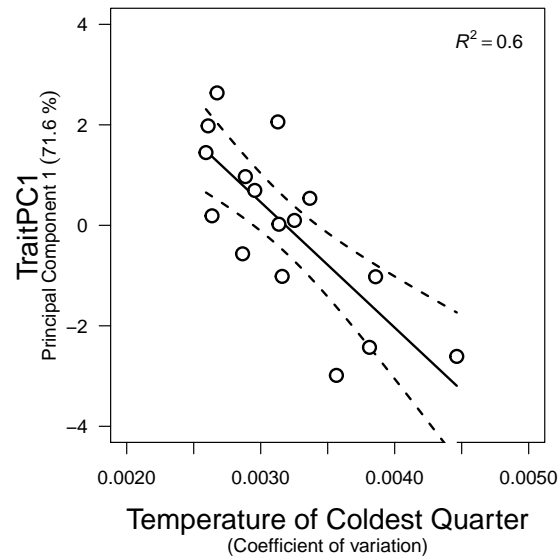




**Figure S5:** Reaction norms signify little Population  $\times$  Treatment interactions. For all panels, black lines represent population-level reaction norms from Wet to Dry in the Cool temperature treatment (dashed black lines) and Hot temperature treatment (solid black lines); gray lines represent reaction norms from Cool to Hot in the Wet treatment (solid gray lines) and Dry treatment (dashed gray lines). The responses shown are (A) leaf expansion rate, (B) stem elongation rate, (C) photosynthesis, and (D) survivorship (= 1 - mortality).



**Figure S6:** Population effect sizes are usually larger than Population  $\times$  Treatment effect sizes. In each panel, we plot estimated effect size (points) and 95% confidence intervals (lines) inferred using parametric bootstrap (see Materials and Methods). At the top, we plot the effect sizes of Population ('Pop'), two-way interactions between Population and Temperature ('Pop  $\times$  Temp') or Water ('Pop  $\times$  Water'), and the three-way interaction between Population, Temperature, and Water ('Pop  $\times$  Temp  $\times$  Water'). Below that we plot the difference in the Population minus the Population  $\times$  Treatment effect size (e.g. 'Pop - (Pop  $\times$  Temp)'). When confidence intervals do not overlap zero (dashed line), this means that Population has a significantly greater effect size than the interaction. Effect sizes were measured using unstandardized mean square error for linear mixed-effects models (leaf expansion, stem elongation, and photosynthesis) and  $\chi^2$  for GLMM (mortality). Hence, the effect size values are not comparable between different traits.



**Figure S7:** 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.

## 713 Supporting Material and Methods

### 714 Temperature treatments

715 We simulated typical growing season (June 1 - August 15) air temperatures at the two most  
716 thermally divergent focal sites in our study, Whitewater Canyon (WWC, Hot) and Little  
717 Jameson (LIJ, Cool). We downloaded daily interpolated mean, minimum, and maximum  
718 air temperature from 13 years (2000-2012) at both sites from ClimateWNA (Wang et al.,  
719 2012). This range was chosen because seeds used in the experiment were collected around  
720 2012, thus their presence in that location at that time suggests that populations were able  
721 to persist there for at least some years before collection. Monthly temperatures from Cli-  
722 mateWNA are highly correlated with the air temperature recorded from data loggers in  
723 the field at these sites (A. Angert, unpub. data). Hence, the ClimateWNA temperature  
724 profiles are similar to actual thermal regimes experienced by *E. cardinalis* in nature. We  
725 simulated realistic temperature regimes by calculating the mean temperature trend from  
726 June to August using LOESS (Cleveland et al., 1992). The residuals were highly autocor-  
727 related at both sites (warmer than average days are typically followed by more warm days)  
728 and there was strong correlation ( $r = 0.65$ ) between sites (warm days in WWC were also  
729 warm in LIJ). The ‘VARselect’ function in the **vars** package for R (Pfaff, 2008) indicated  
730 that a lag two Vector Autoregression (VAR(2)) model best captured the within-site auto-  
731 correlation as well as between-site correlation in residuals. We fit and simulated from the  
732 VAR(2) model using the package **dse** (Gilbert, 2014) in R. Simulated data closely resem-  
733 bled the autocorrelation and between-site correlation of the actual data. From simulated  
734 mean temperature, we next selected minimum and maximum daily temperatures. Mean,  
735 min, and max temperature were highly correlated at both sites. We chose min and max  
736 temperatures using site-specific fitted linear models between mean, max, and min tem-  
737 perature, with additional variation given by normally distributed random deviates with  
738 variance equal to the residual variance of the linear models. For each day, the nighttime

739 (22:00 - 6:00) chamber temperature was set to the simulated minimum temperature. Dur-  
740 ing the middle of the day, temperature was set to the simulated maximum temperature,  
741 with a variable period of transition between min and max so that the average temperature  
742 was equal the simulated mean temperature.

### 743 **Watering treatments**

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