

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

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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 favour 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

Local adaptation has been documented within numerous species; populations generally have higher fitness in their native environment, but perform poorly outside it (Schluter, 2000; Leimu & Fischer, 2008; Hereford, 2009). However, the prevalence of local adaptation remains difficult to assess because researchers rarely test for local adaptation unless there are obvious phenotypic or environmental differences (but see Hereford & Winn, 2008). When local adaptation occurs, it frequently leads to clines in both phenotypes and allele frequencies when selection varies over environmental gradients (Huxley, 1938; Endler, 1977; Barton, 1999). Phenotypic differences between populations along a cline 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 challenging 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. A related problem is identifying which of the myriad and often covarying aspects of the environment cause spatially varying selective pressures.

When populations are locally adapted, reaction norms for fitness will cross, such that local genotypes have higher fitness than foreign genotypes and rank orders change across environments (Kawecki & Ebert, 2004). The traits that underlie local adaptation, however, need not mirror this pattern. Populations can have fixed genetic differences conferring trait values that are adaptive at home but neutral or maladaptive away. Alternatively, the ability to plastically respond to a particular environment or the magnitude of response to an environment could be adaptive. We distinguish between these patterns of adaptive trait differences by referring to ‘genetic variation in trait means’ and ‘genetic variation in plasticity’, respectively. Genetic variation in plasticity is synonymous with genotype-by-environment interactions, or simply ($G \times E$). Genetic variation in trait means and genetic variation plasticity are both involved in adaptation. For example, genetic variation in photoperiod responses (Blackman *et al.*, 2011) and developmental rate (Stinchcombe *et al.*,

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2004) allows 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, genetic variation in plasticity causes sun plants to assimilate more under high light and shade plants under low light (Givnish, 1988). In plants especially, we know little about the prevalence and adaptive significance of variation in fundamental physiological traits like photosynthesis and their impact on plant performance (Flood *et al.*, 2011).

A basic approach to identify candidate traits underlying local adaptation is to find associations between traits and environments. Either genetic variation in trait means and/or plasticity 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 & 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 & 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 & Olsen, 2012), leaf morphology (Hopkins *et al.*, 2008; Stock *et al.*, 2014) and drought resistance (Kooyers *et al.*, 2015) that likely relate to climatic variation.

Despite the fact that latitudinal clines have been studied for a long time, latitude *per se* cannot be a selective agent. Latitude may be strongly correlated with one or two key climatic variables, such as temperature, precipitation or growing degree-days. Latitude may also correlate with the strength of biotic interactions (Schemske *et al.*, 2009) or other nonclimatic aspects of the environment, though as we explain below, we do not yet have compelling data that these are important in our study system. Hence, we focus on whether latitude could be an effective proxy for an underlying climatic driver, in which case we would expect a yet stronger relationship between traits and the key climatic variable(s) driving selection. Alternatively, latitude may be more strongly related to traits than any single climatic variable for at least two reasons. First, latitude may be correlated with several climatic agents of selection that are individually weak, but add up to a strong latitudinal cline. Alternatively, gene flow among neighbouring populations could smooth out local climatic effects, because alleles will experience selection across populations linked by migration (Slatkin, 1978; Paul *et al.*, 2011; Hadfield, 2016). We refer to this as the 'climatic neighbourhood'. For example, in mountainous regions average temperature at a given latitude

varies widely, but in aggregate, a lower latitude set of populations will experience warmer climate than a higher latitude one. Thus, any particular low latitude population would be warm-adapted, even if it was located in a cooler (e.g. high elevation) site. 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 identify generalities, such as whether thermal tolerance maxima or seasonal timing is more important (Bradshaw & Holzapfel, 2008), and whether local or regional climate shapes selective pressures.

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

We examined these questions in *Erythranthe cardinalis* (formerly *Mimulus cardinalis* [Nesom, 2014]) because linking physiological traits to potentially complex patterns of local adaptation requires integrating multiple lines of evidence from comparative, experimental and genomic studies under both laboratory 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 & Schemske, 2003; Wu *et al.*, 2008; Lowry & 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 is 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 neighbourhoods 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.

Materials 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 United States (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 based on all known occurrences (see below). Although the elevation range of *E. cardinalis* compresses slightly from south to north, latitude is not strongly confounded by elevation among our focal populations. The correlation between Latitude and Elevation among the 16 focal populations was weak ($r = -0.25$, $P = 0.34$). Seeds were collected in the field from mature, indehiscent fruits left open for 2–4 weeks to dry, then stored at room temperature. To control for maternal effects, we grew a large number of field-derived seeds in the greenhouse and generated seed families for this experiment by haphazardly crossing individuals from the same population. We selected

seed families to maximize the number of field-derived individuals represented. Thus, we used seeds from 154 greenhouse-derived seed families, 4–12 (mean = 9.6, median = 12) families per population.

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 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.) 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 centre-most plant. 702 of 768 (91.4%) cone-tainers had plants that could be used in the experiment. We allowed one week at constant, nonstressful 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).

Table 1 Latitude, longitude and elevation (mas = metres above sea level) of 16 focal populations used in this study.

Name	Latitude	Longitude	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

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 Appendix S1 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

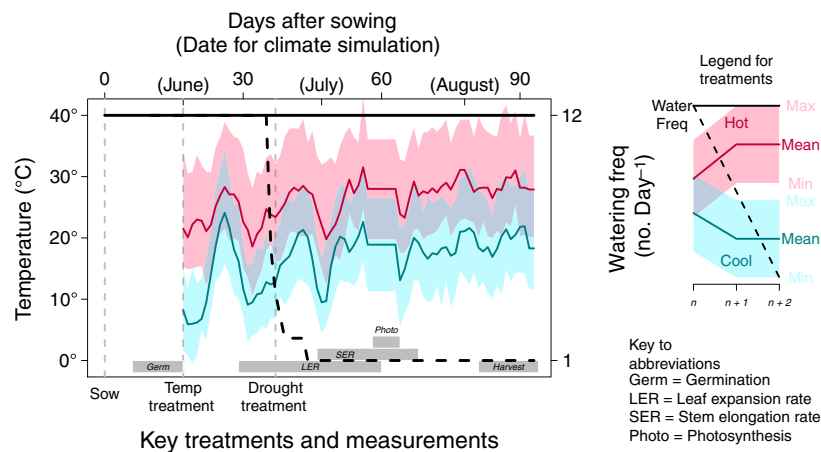


Fig. 1 Overview of experimental treatments and timing of key trait measurements. All plants germinated within 21 days of sowing. At that time, we began temperature treatments (left axis), simulating a typical June–August weather pattern at hot (red) and cool (blue) sites. The bold lines track the average daily temperatures. Within each day, there was a maximum daytime temperature (top of translucent polygons) and minimum night-time temperature (bottom of translucent polygons). The drought treatment commenced later by ramping down the frequency of bottom-watering episodes (dashed black line; right axis), whereas 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 Materials and Methods.

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 as follows: $n_{\text{cool,dry}} = 169$; $n_{\text{cool,ww}} = 174$; $n_{\text{hot,dry}} = 176$; $n_{\text{hot,ww}} = 183$. Each population had 8–12 individuals per treatment level (mean = 11, median = 11).

Trait measurements

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

Days to 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). We treated Population as a fixed effect and family as random effect using a Γ frailty function. Statistical significance of the Population effect was determined using

analysis of deviance. Note that, unlike other traits discussed below, we did not include block, treatment or population \times Treatment interactions because during germination plants had not been placed into blocks and treatments had not yet been applied.

Growth rate: leaf expansion and stem elongation

We measured growth rate during two phases: leaf expansion and stem elongation. Growth measurements were taken during the early vegetative stage. We censused leaf length twice per week shortly after the emergence of true leaves from 12 May to 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 stem elongation. We also censused plant height on seven occasions (twice per week) between 29 May and 20 June (45–67 days after sowing) until plants began to initiate floral buds. Thus, all growth measurements occurred during the vegetative, prereproductive phase. Both leaf expansion and stem elongation were modelled separately as second-order polynomials. We used empirical Bayes' estimates of growth for each individual plant from linear mixed-effects models fit with the R package **lme4** version 1.1-12 (Bates *et al.*, 2015).

Photosynthesis

During the week of 10–16 June (57–63 days after sowing), we measured daytime photosynthetic rate on a subset of 329 plants evenly spread between treatments and families within populations. The youngest, fully expanded leaf acclimated for 3 min to reach steady state in a 6-cm² chamber of a LI-COR 6400XT Portable

Table 2 Key traits measured in this study.

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

Photosynthesis System (LI-COR Biosciences, Lincoln, Nebraska, USA). We made all measurements at ambient light ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation), atmospheric CO_2 (400 ppm), temperature and moderate relative humidity. All measurements were taken between 9:00 AM and 5:00 PM (3 h after lights turned on and 5 h before lights turned off). During this period, we suspended normal day-to-day temperature fluctuations and set daytime temperatures to the average for that period (Cool: 26.5° ; Hot: 36.1°) so that all plants within a temperature level could be measured under the same conditions. We measured photosynthesis after dry down had progressed to assess differences in photosynthetic responses to drought.

Mortality

We assayed mortality during twice-weekly growth measurements. We analysed the probability of surviving until the end of the experiment as a function of population, treatment and their interactions using a generalized linear mixed model (GLMM) assuming binomially distributed errors. We included Family and Block as random effects. We assessed significance of fixed effects using type-II analysis of deviance with Wald χ^2 tests in the R package **car** (Fox & 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 χ^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 vs. Population \times Treatment is:

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

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

Principal components of germination, growth, and photosynthesis

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

Identifying putative selective agents

Latitudinal clines are common, but it is often difficult to ascribe this variation to a particular selective agent. To reiterate, we tested three nonmutually exclusive hypotheses about how such latitudinal clines emerge: (1) one or two climatic variables explain latitudinal trait variation; (2) latitude is a proxy for multiple climatic factors that together shape trait variation; and (3) latitude integrates selection in a broader climatic neighbourhood. We found that a population's position along TraitPC1 correlated strongly with the latitude of origin (see Results) and next used Random Forest regression (Liaw & Wiener, 2002) to identify putative climatic factors underlying trait-latitude associations in *E. cardinalis*. We reasoned that if we identified a single climatic factor that explained more trait variation than latitude, then this would suggest that factor is a key selective

agent underlying the latitudinal cline (Hypothesis 1). On the other hand, if multiple climatic factors together are necessary to explain trait variation, then this would suggest that many climatic factors together have imposed selection for the latitudinal cline (Hypothesis 2). We hereafter refer to factors identified in this analysis as ‘Climate-TraitPC1’ variables.

To test Hypothesis 3 about climatic neighbourhoods driving selection, we directly compared local with neighbourhood climate. The logic is that if the climatic analysis can identify candidate climatic factors important for local adaptation, then stronger correlations with neighbourhood climate would suggest a role for gene flow. We used the immediate collection location for local climate. For climate neighbourhoods, we sampled climate at 1000 random points (at 90-m resolution) within a 62-km radius buffer around the collection and took the average. We chose this buffer radius based on population genetic structure, as inferred from $\approx 25\,000$ restriction-site associated SNPs among 49 populations from across the range (J.R. Paul, T.L. Parchman, B. Econopoulou, C.A. Buerkle & A.L. Angert, unpublished). Spatial autocorrelation in allele frequencies persists for 62 km. However, radii of 10 km² and 100 km² resulted in similar outcomes (data not shown). As *E. cardinalis* is found exclusively in riparian areas, we only selected points along streams using the National Hydrography Dataset (United States Geological Survey, 2015). Climatic means and variances (see below) were weighted by their climatic suitability as determined using a multimodel ensemble average of ecological niche models (Angert *et al.*, 2016). In addition to competing local and neighbourhood climate, we compared the univariate correlation between local and neighbourhood climate with TraitPC1 and Latitude using paired *t*-tests. We adjusted degrees of freedom to account for the fact that many climatic factors are highly correlated and not independent. Specifically, we calculated the effective number of independent climatic factors (M_{eff}) using the formula $M_{\text{eff}} = 1 + (M - 1)(1 - \text{Var}(\lambda)/M)$ (Chevrud, 2001), where M is the original number of climatic factors and λ are the eigenvalues of the correlation matrix of all climatic factors.

To help eliminate potentially spurious correlations between TraitPC1 and climate, we tested for overlap between climatic variables that best predict latitude of all *E. cardinalis* occurrence records (see detail below), not just the 16 focal populations. We refer to these climatic factors as ‘Climate-Latitude’ variables. The logic is that climatic factors associated with both TraitPC1 and latitude for all populations are more likely to be important selective agents than climatic factors that happen to correlate with TraitPC1 but do not covary with latitude throughout the *E. cardinalis* range. If a climatic factor is driving the latitudinal cline in TraitPC1, then we expect that climatic factor will correlate strongly with latitude of occurrence localities. Therefore, we did not consider Climate-TraitPC1 variables to be candidate

selective agents unless the same or very similar variable was found in the Climate-Latitude analysis. However, we do not interpret potential selective agents identified in Climate-Latitude analyses alone, because the goal was to explain the latitudinal clines in traits, not all aspects of climate that vary with latitude.

We selected Climate-Latitude and Climate-TraitPC1 variables independently using Variable Selection Using Random Forest (VSURF) algorithm in the R package **VSURF** version 1.0.3 (Genuer *et al.*, 2016). Random Forest regression is useful for cases like ours when the number of potential predictors is similar to or greater than the number of observations (‘high *p*, low *n*’ problem). VSURF is a multistep algorithm that progressively retains or eliminates variables based on their importance over regression trees in the forest. 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 data set, across all trees in the random forest (see Genuer *et al.*, 2015 for further detail). Hence, VSURF automatically eliminates unimportant and redundant variables based on the data without having to arbitrarily choose among colinear climate variables before the 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

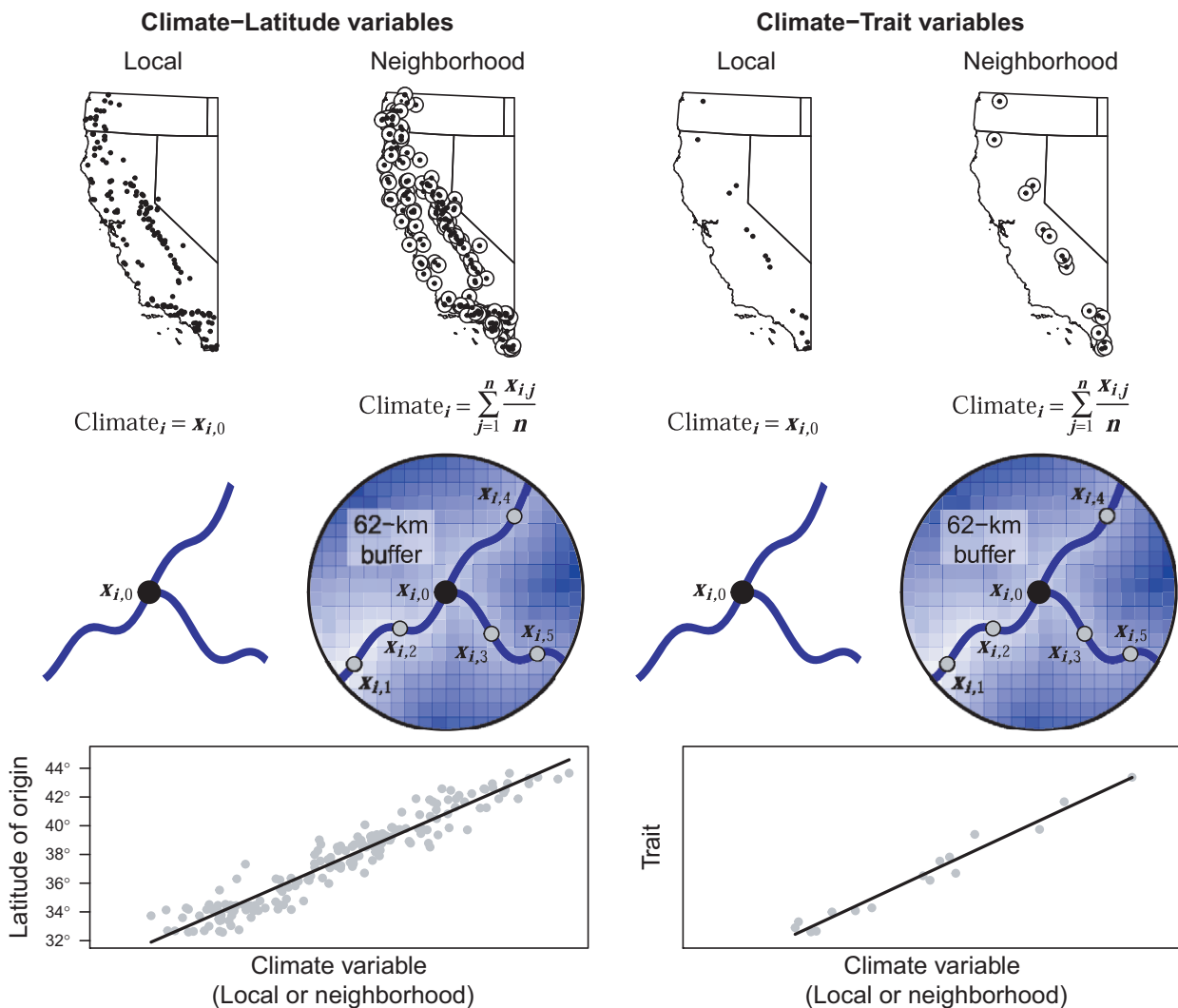


Fig. 2 Overview of method for identifying putative climatic selective agents underlying latitudinal cline. We looked for climate variables that explained both the latitude of 356 *E. cardinalis* occurrences ('Climate-Latitude variables') and traits ('Climate-Trait variables'). For Climate-Latitude variables, 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 neighbourhood climate. Local climate refers to climate only from where a population was collected ($x_{i,0}$). Neighbourhood climate was calculated as the average over 1000 points in a 62-km radius climatic neighbourhood ($x_{i,1}, x_{i,2}, \dots$), 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.

variables: 24 climatic factors \times 2 types (30-year average and CV) \times 2 spatial scales (local and neighbourhood).

Results

A coordinated latitudinal cline in germination, growth and photosynthesis

There are strong genetically based trait differences in time to germination, growth and photosynthetic rate

among populations of *E. cardinalis*, as evidenced by large and significant population effects for these traits (Table 3). A single principal component captured 71.6% of the trait variation among populations, defining an axis of variation from fast to slow growth. A population's position along this axis strongly covaried with its latitude of origin; southern populations grew faster than northern populations (Fig. 3). There were similar latitudinal clines for individual traits underlying PC1 (Figs S1–S4).

Table 3 Summary of Population, Treatment and Population \times Treatment effects. We used different statistical modelling 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				

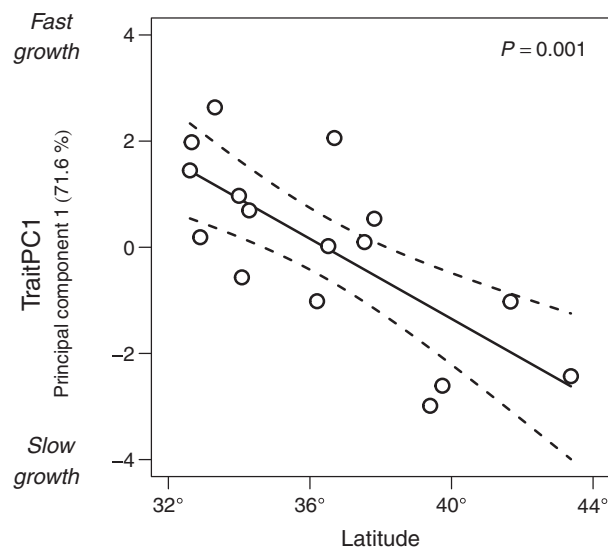


Fig. 3 Trait variation, from fast to slow growth, is closely associated with latitude. Each point is a population's latitude of origin (x-axis) and position along the slow to fast growth axis (y-axis), defined as Principal Component 1 of four traits (see Materials and Methods). The line and 95% confidence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

Little evidence for variation in plasticity

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

The effect size of Population was significantly larger than that for Population \times Treatment interactions (Fig. S6) in most cases. For leaf expansion, Population had a significantly larger effect size than Population \times Treatment interactions in two of three comparisons (Fig. S6a). For stem elongation (Fig. S6b) and mortality (Fig. S6d), population effect sizes were significantly larger than all Population \times Treatment interactions. For photosynthesis, Population and Population \times Treatment effect sizes were not significantly different (Fig. S6c), presumably because we had a smaller sample size.

Neighbourhood climatic variability best explains latitudinal cline

Interannual variation in climate averaged over each population's climatic neighbourhood correlated most strongly with trait variation and latitude of *E. cardinalis* occurrences (Fig. 4, Table S7). All 16 Climate-Latitude and 3 Climate-TraitPC1 variables were neighbourhood rather than local variables (Fig. 4). In fact, neighbourhood climate almost always correlated better with TraitPC1 and Latitude than local climate (Fig. 5). On average, neighbourhood Climate-TraitPC1 correlation coefficients were 0.16 higher than correlations with local-scale climate variables (paired t -test, $t = 7.87$, d.f. = 33.6, $P = 3.94 \times 10^{-9}$). Likewise, neighbourhood Climate-Latitude correlation coefficients were 0.13 higher than those for local-scale climate (paired t -test, $t = 6.71$, d.f. = 36.8, $P = 7.22 \times 10^{-8}$). Among Climate-Latitude and Climate-TraitPC1 variables, neighbourhood climatic variability over 30 years (1981–2010) in either winter precipitation (bio16 σ) and/or temperature (bio11 σ) are the strongest candidates to explain the latitudinal cline in *E. cardinalis* (see Table S2 for a key to climate variable abbreviations). Note that the coefficient of variation of a climatic factor is subscripted with σ , whereas the mean is subscripted with μ . More specifically, greater winter precipitation variability and lower

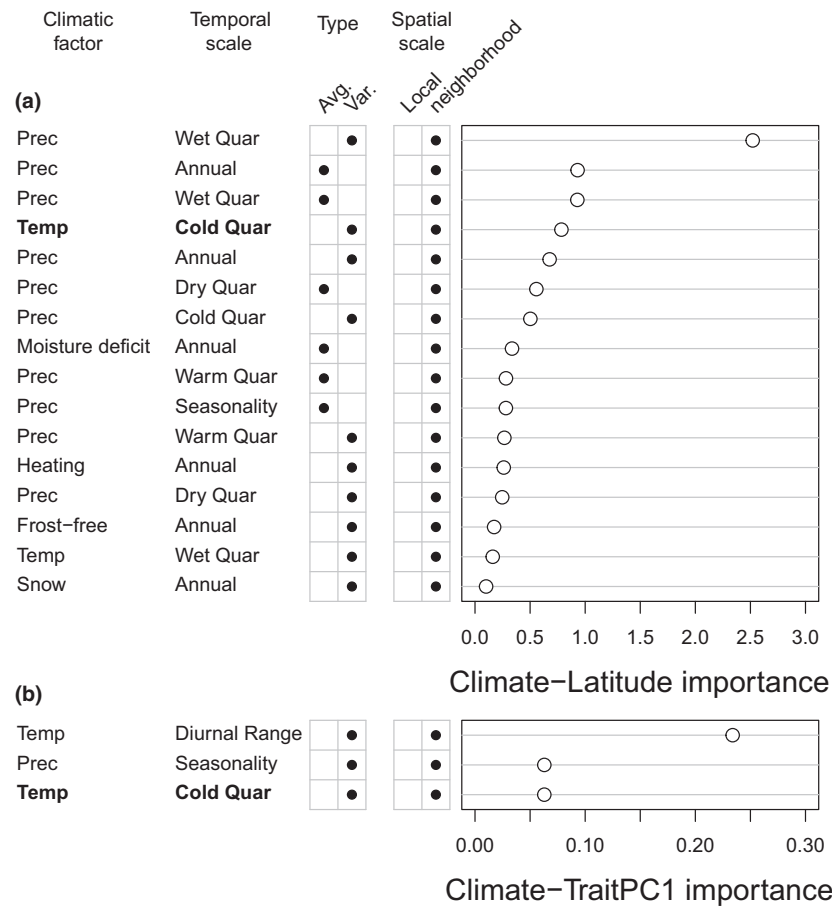


Fig. 4 Climatic variation integrated over climatic neighbourhood is closely correlated with latitude of *E. cardinalis* and trait variation. (a) Using Random Forest regression, we identified 16 climatic variables significantly (high importance) associated with latitude of *E. cardinalis* occurrences. (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 neighbourhood.

winter temperature variability are associated with southern latitudes and higher TraitPC1 values (Fig. 6a, b). Neighbourhood interannual variation in winter precipitation (bio16_σ) was the most important Climate-Latitude variable (Fig. 4a). However, neighbourhood bio16_σ did not overlap with Climate-TraitPC1 variables (Fig. 4b). We nevertheless consider it a plausible candidate for two reasons. First, neighbourhood bio16_σ correlated strongly with TraitPC1 (Fig. 6a). Second, one of the most important Climate-TraitPC1 variables (neighbourhood bio15_σ ; Fig. 6b,c) is very similar to bio16_σ . In Mediterranean climates like California, most precipitation occurs in the wettest quarter (winter), so years with low winter precipitation also have low

precipitation seasonality. Hence, highly variable year-to-year winter precipitation at lower latitude (Fig. 6d) is closely associated with large swings in precipitation seasonality (Fig. 6c).

Interannual variation in temperature of the coldest quarter (neighbourhood bio11_σ) is another plausible candidate because it was the only variable in both Climate-Latitude and Climate-TraitPC1 analyses (Fig. 4). Neighbourhood bio11_σ explained more variation in TraitPC1 than latitude (latitude $r^2 = 0.55$ vs. bio11_σ $r^2 = 0.6$; Fig. S7), whereas neighbourhood bio16_σ did slightly worse (bio16_σ $r^2 = 0.49$). Models using bio15_σ or bio11_σ to predict TraitPC1 also had significantly lower Akaike information criteria (AIC) than the

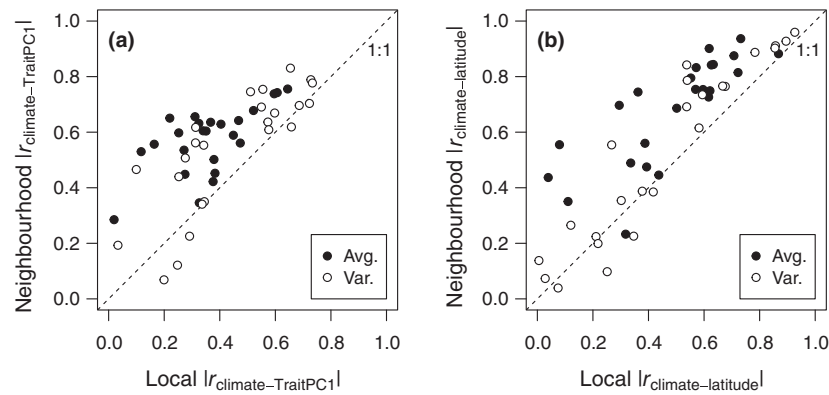


Fig. 5 Neighbourhood climate predicts TraitPC1 ('Climate-trait', panel a) and Latitude of occurrences ('Climate-latitude', panel b) better than local climate. Each point is the absolute value of the Pearson correlation coefficient ($|r|$) between TraitPC1 (a) or latitude (b) for 24 climatic factors, 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 neighbourhood compared to local climate. Neighbourhood climate was integrated over a 62-km radius around focal populations (see text for further detail).

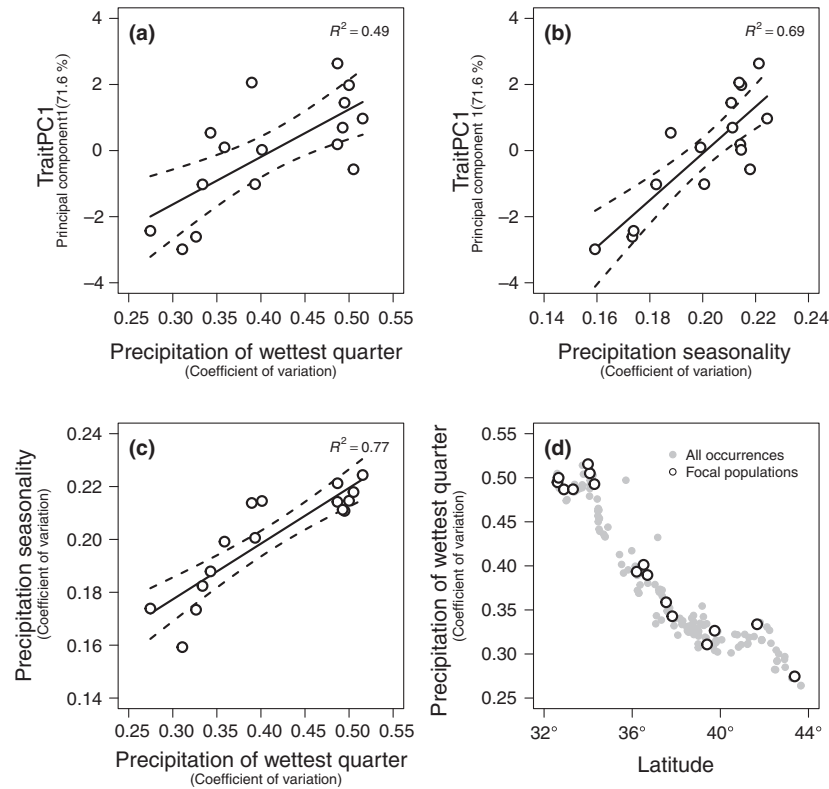


Fig. 6 Variation in precipitation is correlated with TraitPC1 and latitude. (a) Greater values of TraitPC1 are associated with greater interannual variation in precipitation of the wettest quarter. This was the most important Climate-Latitude variable, but not among the most important Climate-TraitPC1 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 neighbourhood values (see Materials and Methods). Regression lines, 95% confidence intervals and coefficients of determination (R^2) were calculated using linear regression.

latitude model (AIC of different models – bio15_σ: 48.5; bio11_σ: 52.4; latitude: 54.5). The best two-factor model including both neighbourhood bio15_σ and bio11_σ did not significantly improve explanatory power ($r^2 = 0.71$, AIC = 49.2). In summary, either variation in precipitation or temperature seasonality may be important selective agents, but there is no strong evidence that they are both important. The most important Climate-TraitPC1 variable, neighbourhood variation in mean diurnal range (bio2_σ; Fig. 4b) did not have any obvious similarity to Climate-Latitude variables. Given the large number of potential associations, we therefore think this may be a spuriously strong relationship.

Discussion

We found evidence for one of two common signatures of local adaptation in the perennial herb *Erythranthe cardinalis*. Latitudinal clines in germination rate, photosynthesis and growth suggest adaptive differentiation in important physiological traits of the species. However, we caution that these are candidate adaptive traits and that we cannot yet rule out nonadaptive demographic processes such as a recent range expansion towards higher latitude (J.R. Paul, T.L. Parchman, B. Econopoulou, C.A. Buerkle & A.L. Angert, unpublished; Sheth & Angert, 2017). In contrast, we found little evidence for variation in plasticity to temperature or drought. Due to low replication within families, we did not have power to assess within-population genotype-by-environment interactions, which may be present. As we discuss below, low variation in plasticity among populations may indicate that some dimensions of the fundamental abiotic niche are relatively conserved. Note that statistical power to detect significant plasticity is lower than that for differences in trait means, but the effect size of variation in plasticity was significantly less than that for trait means in most cases (Fig. S6). Finally, our results suggest that neighbourhood-scale climate and interannual variation are more important selective agents than local averages. In the paragraphs that follow, we tie these results into the broader threads of evolutionary theory that might help explain why variation in physiological trait means changes clinally, whereas plastic responses to temperature and drought are relatively static. One caveat to bear in mind is that we are limited by the size of the climate grid ($\approx 90 \text{ m}^2$) and therefore unable to detect very fine-scale local adaptation.

Evolutionary theory indicates that the shape of fitness trade-offs, demography and gene flow can constrain adaptation (Levins, 1968; Ronce & Kirkpatrick, 2001; Lenormand, 2002), and hence, the type of variation maintained within species. Specifically, adaptive variation can be maintained by spatially varying selection if trade-offs are not too strong, demography is symmetric, and/or maladaptive gene flow is low. Strong

trade-offs can prevent local adaptation in spatially variable environments because selection favours habitat specialists that track a specific habitat regardless of its frequency in the environment (Levins, 1968). For example, a riparian specialist may experience similar selection in rivers of high rainfall regions and deserts, even though the habitat is much rarer in the latter. In *E. cardinalis*, we found substantial genetically based variation among populations along a phenotypic axis from fast to slow growth that varied over a large spatial scale (Fig. 3). If this variation is adaptive, it suggests one of several possibilities to investigate in the future: the fitness trade-off between low vs. high latitude environments is not too strong nor swamped by demographic asymmetry or maladaptive gene flow. That is, alleles favoured at one latitude are not strongly selected against when they flow to another population, allowing locally adaptive genetic variation to be maintained by spatially heterogeneous selection. We also know from previous work that population size does not vary strongly with latitude (Angert, unpub. data). Gene flow appears to be high, but attenuates at broad spatial scales, especially between southern ($< 35^\circ\text{N}$) and northern portions of the range (J.R. Paul, T.L. Parchman, B. Econopoulou, C.A. Buerkle & A.L. Angert, unpublished).

Nevertheless, local gene flow from similar environments may shape how selection varies with latitude. Theory predicts that populations will not be perfectly adapted to their immediate habitat when there is gene flow from surrounding populations with different optima (Lenormand, 2002). With spatial heterogeneity and gene flow, traits will not covary perfectly with the local optimum (Slatkin, 1978; Paul *et al.*, 2011; Hadfield, 2016), but should instead better match the average environment experienced by nearby populations connected through gene flow, which we refer to as the climatic neighbourhood. Gene flow and spatial heterogeneity may therefore be important in maintaining genetic variation (Yeaman & Jarvis, 2006). As this hypothesis predicts, climatic neighbourhoods (62-km buffer around populations) correlated with traits and latitude of occurrences better than local climate (Fig. 4). We interpret this as suggestive evidence that gene flow between neighbouring *E. cardinalis* populations shapes selection – populations are locally adapted to prevailing climate in their neighbourhood, but perhaps not perfectly adapted to their local climate. This may not greatly constrain local adaptation because local and neighbourhood climate values were generally similar in *E. cardinalis* populations (Fig. 5), at least at the resolution of ClimateWNA (90 m^2). Therefore, we would predict in reciprocal transplants that populations whose local climate is farther from their neighbourhood average would be less well adapted than those close to their neighbourhood average.

An alternative, nonbiological, explanation for why neighbourhood climate correlates better than local

climate is that averaging climate over a wider area integrates out error in ClimateWNA values. ClimateWNA interpolates between weather stations using a fine-scale digital elevation model to accurately downscale climate data (Wang *et al.*, 2012), but there is obviously some error. If the error were randomly distributed about the true climatic values, then averaging climate over a larger sample of points could give a more precise estimate, which might explain why traits and latitude correlate better with climatic neighbourhood. For this technical artefact to be important, the measurement error in local climate would have to be large relative to true variation in climate among locations within the climatic neighbourhoods. We do not know the relative magnitude of these factors, but future work should compare ClimateWNA-predicted local climate with actual plant-level measurements to estimate the magnitude of this error.

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

We hypothesize that tolerance to dry soil may be constrained by a combination of strong fitness trade-offs, demographic asymmetry and gene flow. Soil moisture in riparian habitats where *E. cardinalis* lives is highly heterogeneous at very small spatial scales (several metres). Plants in the stream never have to tolerate drought, whereas plants only a few metres away may experience extreme drought as 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 trade-offs. Such trade-offs 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 & Levin, 1979; Brown & 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 (J.R. Paul, T.L. Parchman, B. Econopoulou, C.A. Buerkle & A.L. Angert, unpublished), 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 & 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, whereas northern populations invest more in vegetative growth (Sheth & Angert, 2017). In this experiment, the fastest growing plants began producing flowers in ~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 & Cohen, 1989; Friedman & Rubin, 2015). Populations of the perennial *Plantago asiatica* show a similar latitudinal cline in growth and allocation to storage (Sawada *et al.*, 1994) but attribute the cline to variation in growing season length. There are also life history clines in the closely related species *E. guttata*, but the underlying traits and climatic drivers are quite different. Annual *E. guttata* flower sooner and produce fewer stolons in response to climates with shorter seasons and more intense summer drought (Lowry & Willis, 2010; Friedman *et al.*, 2015; Kooyers *et al.*, 2015). In contrast, there are no truly annual (monocarpic and semelparous) populations of *E. cardinalis*. Rather, our hypothesis states that climatic variability selects on quantitative variation in allocation to growth vs. storage.

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

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Data are currently available on GitHub and Dryad (Muir and Angert, 2017).

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1 Initial size of seedlings did not vary among Populations, Families, or Treatments.

Table S2 Climatic variables used.

Table S3 Analysis of variance (ANOVA) table on leaf expansion rate (LER) using **lmerTest** (Kuznetsova et al., 2016).

Table S4 Analysis of variance (ANOVA) table on stem elongation rate (SER) using **lmerTest** (Kuznetsova et al., 2016).

Table S5 Analysis of variance (ANOVA) table on photosynthetic rate using **lmerTest** (Kuznetsova et al., 2016).

Table S6 Analysis of deviance table on the probability of mortality by the end of the experiment using Type-II Wald χ^2 tests in the R package **car** (Fox and Weisberg, 2011).

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').

Figure S1 Southern populations germinate faster.

Figure S2 Southern populations grow faster.

Figure S3 Southern populations grow faster.

Figure S4 Southern populations photosynthesize faster.

Figure S5 Reaction norms signify little Population \times Treatment interactions.

Figure S6 Population effect sizes are usually larger than Population \times Treatment effect sizes.

Figure S7 Trait variation, from fast to slow growth, is closely associated with neighborhood variation in temperature of the coldest quarter (bio11_σ).

Appendix S1 Supporting material and methods.

Data deposited at Dryad: <https://doi.org/10.5061/dryad.67n73>

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