

```

# The path to the ms directory may need to be changed for different users
# Manuscript files
pathMS <- "~/Google Drive/CardLocalAdaptation/ms" # Chris' MacBook
# pathMS <- "~/Documents/CardAdapt/ms" # Chris' Linux laptop

# Data files for paper
pathDat <- paste(pathMS, "/Data", sep = "")

# Figures for paper
pathFig <- paste(pathMS, "/Figures", sep = "")

# Tables for paper
pathTab <- paste(pathMS, "/Tables", sep = "")

# Small function to make statistical significance asterisks
sigStar <- function(pvalue)
{
  if (pvalue >= 0.05) return("")
  if (pvalue < 0.05 & pvalue >= 0.01) return("*")
  if (pvalue < 0.01 & pvalue >= 0.001) return("**")
  if (pvalue < 0.001) return("***")
}

# Color palette
# Primary color:

primShade0 <- rgb(1, 0, 0.286)
primShade1 <- rgb(1, 0.643, 0.745)
primShade2 <- rgb(1, 0.439, 0.6)
primShade3 <- rgb(0.765, 0, 0.22)
primShade4 <- rgb(0.588, 0, 0.169)

# Secondary color (1):

sec1shade0 <- rgb(0.008, 0.957, 1)
sec1shade1 <- rgb(0.643, 0.984, 1)
sec1shade2 <- rgb(0.443, 0.976, 1)
sec1shade3 <- rgb(0, 0.49, 0.514)
sec1shade4 <- rgb(0, 0.376, 0.396)

# Secondary color (2):

sec2shade0 <- rgb(0.988, 1, 0)
sec2shade1 <- rgb(0.996, 1, 0.643)
sec2shade2 <- rgb(0.992, 1, 0.439)
sec2shade3 <- rgb(0.835, 0.847, 0)
sec2shade4 <- rgb(0.643, 0.651, 0)

```

(working title) Local Physiological Adaptation in *Mimulus cardinalis*

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April 29, 2015

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Abstract

Introduction

Local adaptation is one of the most ubiquitous observations in nature: organisms perform well in their natal environment, but poorly outside it. Local adaptation within species most often involves genetic differences in ecologically relevant traits caused by selection to different environments, leading to predictable trait-environment correlations that persist in a common garden. Although local adaptation is well-supported by data from reciprocal transplant experiments, trait-environment associations, and ecological genetics/genomics, predicting which traits respond to selection exceeds our understanding. In particular, the role of physiological traits is poorly understood, even though physiology explains adaptation to the abiotic environment, which we need to understand as a baseline for anticipating how organisms will respond to climate change. A related problem is identifying the exact selective agents, such as abiotic factors like soil water availability or biotic interactions [etc.]... Here, we simultaneously investigate which traits and causal selective agents explain range-wide patterns of local physiological adaptation to climate in *Mimulus*, a model genus for local adaptation studies.

One surprising pattern is that populations adapt to different environments by adjusting their life history to stay within the same fundamental physiological niche rather than have the niche itself evolve. For example, mosquitoes adjust diapause length with latitude rather than evolve altered ... **[CDM: *what other examples could we include here?*]** This suggests that in many situations it is evolutionarily easier to change life history rather than fundamental physiological tolerance.

We also know little about the selective agents, such as climatic factors or biotic interactions, that are most important in driving adaptive divergence. In certain situations, such as pollinator shifts and edaphic adaptation, it is straightforward to identify putative se-

lective agents and test predicted mechanisms. However, it is more challenging for cases in which traits vary with less specific factors like mean annual precipitation or latitude. For example, body size clines (larger size at higher latitude) have evolved repeatedly on three continents in *Drosophila subobscura*, strongly indicating selection. But which one or more of the many climatic factors that covary with latitude actually controls selection on body size? Selection might be even more complex for species that live over multiple seasons, meaning that environmental variation rather than the average environment may be a selective agent.

Linking physiological traits to potentially complex patterns of local adaptation requires integrating multiple lines of evidence from comparative, experimental, genomic studies under both lab and field conditions. Many classic and contemporary studies of local adaptation have been conducted using species from genus *Mimulus* because of its natural history, easy propagation, and genetic/genomic resources. ... Conspicuous absence of phys mechanism

1. ‘intrinsic’ physiological variation 2. variation in plasticity 3. what ecological factors explain divergence (i.e. putative selective agents underlying local adaptation)

Conclude that observations are best explained by stabilizing selection on the fundamental niche, but spatially varying divergent selection on coordinated life history and physiological traits.

Methods

Population Selection

We used 16 populations from throughout the range of *M. cardinalis* (Table 1). Seeds were collected in the field [CDM: Amy, can you explain seed collection methods?].

Table 1: 16 Focal populations

Name	Region	Demo?	Pop gen?	Latitude	Longtiude	Elevation (mas)
HAU	South Margin	yes	yes	32.657	−116.532	799
CTC	South Margin	yes	no	32.609	−116.7	267
CUR	South Margin	yes	yes	32.9	−116.585	1180
GRP	South Margin	no	no	33.314	−116.871	1577
WWC	Transverse	yes	yes	33.994	−116.665	705
MIL	Transverse	yes	no	34.077	−116.873	2050
WFM	Transverse	yes	no	34.284	−117.378	1120
NMT	South Sierras	yes	yes?	36.201	−118.651	1314
PRD	South Sierras	yes	yes	36.518	−118.759	926
RWD	South Sierras	yes	yes	36.691	−118.91	1727
WNA	Central Sierras	yes	yes	37.541	−119.649	1224
RBW	Central Sierras	yes	no	37.819	−120.007	876
MYU	North Sierras	yes	yes	39.397	−121.082	455
LIJ	North Sierras	yes	yes	39.743	−120.704	1603
DPC	North Coast	yes	yes	41.668	−123.11	707
RCC	North Margin	yes	yes	43.374	−122.957	326

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

Germination was recorded daily from one to two weeks after sowing, and every few days thereafter. On 5 May (21 days after sowing), seedlings were transferred to one of two MODEL Growth Chambers (Convion, 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 S#).

Treatments

We imposed four treatments, a fully-factorial cross of two temperature treatments and two watering treatments. Because growth chambers cannot be subdivided, one chamber was assigned to the Hot treatment and another to the Cool treatment. Within each chamber, there were two Well-watered blocks and two Drought blocks. A detailed description of treatments is given below [CDM: *should some be moved to supplement?*] and summarized in Fig 1. The irradiance in both chambers was approximately $400 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. The growth chambers did not control humidity, but because of watering and high plant transpiration rates, the relative humidity was quite high in both temperature treatments (data not shown).

Temperature treatments

We simulated typical growing season (June 1 - August 15) air temperatures at the two most thermally divergent focal sites in our study, Whitewater Canyon (Hot) and Little Jameson (Cool). We downloaded daily interpolated mean, minimum, and maximum air temperature from 13 years (2000-2012) at both sites from ClimateWNA (Wang et al., 2012). Daily temperatures from ClimateWNA are highly correlated with the air temperature

recorded from data loggers in the field at these sites (A. Angert, unpub. data). Hence, the ClimateWNA temperature profiles are similar to actual thermal regimes experienced by *M. cardinalis* in nature. We simulated realistic temperature regimes by calculating the mean temperature trend from June to August using LOESS (?). The residuals were highly autocorrelated at both sites (warmer than average days are typically followed by more warm days) and there was strong correlation ($r = 0.65$) between sites (warm days in WWC were also warm in LLJ). The ‘VARselect’ function in the **vars** package for R (?) indicated that a lag two Vector Autoregression (VAR(2)) model best captured the within-site autocorrelation as well as between-site correlation in residuals. We fit and simulated from the VAR(2) model using the package **dse** (Gilbert and D., 2014) in R. Simulated data closely resembled the autocorrelation and between-site correlation of the actual data. From simulated mean temperature, we next selected minimum and maximum daily temperatures. Mean, min, and max temperature were highly correlated at both sites. We chose min and max temperatures using site-specific fitted linear models between mean, max, and min temperature, with additional variation given by normally-distributed random deviates with variance equal to the residual variance of the linear models. For each day, the nighttime (22:00 - 6:00) chamber temperature was set to the simulated minimum temperature. During the middle of the day, temperature was set to the simulated maximum temperature, with a variable period of transition between min and max so that the average temperature was equal the simulated mean temperature.

Watering treatments

For watering treatments, we simulated two extreme types of streams where *M. cardinalis* grows. In the well-watered treatment, we simulated a large stream that never goes dry during the summer growing season. In the drought treatment, we simulated a small stream that has ample flow at the beginning of the season, but gradually dries down as the winter snow pack melts. In both treatments, plants were bottom-watered using water chilled to

7.5 [check] by MAKE AND MODEL OF CHILLER. Plants in the well-watered treatment were fully saturated every two hours during the day. Watering in the drought treatment gradually declined from every two hours to every day between May 20 (36 days after sowing) and 10 June (57 days after sowing). Simultaneously, the amount of bottom-watering per flood decreased, such that only the bottom of the cone-tainers were wetted by the end of the experiment.

[CDM: *Are there any data from California about soil water content through the season?*]

```
library(scales) # for color transparency
exptlDes <- read.csv(paste(pathDat, "/ExperimentalDesign.csv", sep = ""))

pdf(paste(pathFig, "/ExperimentalDesignBase.pdf", sep = ""), 7, 5)
par(mai = rep(1, 4))
plot(0, 0, type = "n", xlim = c(0, max(exptlDes$DayN)), ylim = c(-1, 41),
      axes = F, frame.plot = T, cex.lab = 1.5, xlab = "Key treatments and measur",
      ylab = expression(paste("Temperature (", phantom()*degree, "C)")))

# Temperature axis
axis(2, at = seq(0, 40, 10), lwd = 0, lwd.ticks = 1, las = 1,
      labels = c(expression(0*degree), expression(10*degree), expression(20*degree),
                  expression(30*degree), expression(40*degree)))

# Axis of time on top
mtext("Days after sowing", side = 3, line = 3.5, cex = 1.5)
mtext("(Date for climate simulation)", side = 3, line = 2.25, cex = 1.5)
axis(3, at = seq(0, 90, 30), lwd = 0, lwd.ticks = 1)
axis(3, at = c(17, 47, 78), lwd = 0, lwd.ticks = 1, line = -0.5,
      labels = c("(June)", "(July)", "(August)"))
```



```

# Indicate sowing, beginning of temperature and drought treatments
abline(v = 0, col = "grey", lwd = 2, lty = 2)
mtext("Sow", side = 1, at = 0, line = 0)
abline(v = 17, col = "grey", lwd = 2, lty = 2)
mtext("Temp\nTreatment", side = 1, at = 17, line = 1)
abline(v = 37, col = "grey", lwd = 2, lty = 2)
mtext("Drought\nTreatment", side = 1, at = 37, line = 1)

# Polygon of Hot treatment, min and max
with(exptlDes[18:94, ], polygon(c(17:93, 93:17), c(Tmin_hot, rev(Tmax_hot)),
  border = NA, col = alpha(primShade0, 0.25)))

# Polygon of Cool treatment, min and max
with(exptlDes[18:94, ], polygon(c(17:93, 93:17), c(Tmin_cool, rev(Tmax_cool)),
  border = NA, col = alpha(sec1shade0, 0.25)))

# Lines with average temperature
with(exptlDes[18:94, ], points(17:93, Tavg_hot, lwd = 2, type = "l",
  col = primShade3))
with(exptlDes[18:94, ], points(17:93, Tavg_cool, lwd = 2, type = "l",
  col = sec1shade3))

# Rug indicating measurment timing
rect(6, -2, 17, 0, border = "white", col = "grey")
with(exptlDes, text(11.5, -1, labels = "Germ", col = "white", font = 3,
  cex = 0.9))

```

```

with(exptlDes, rect(min(which(LERmeas)), -2, max(which(LERmeas)), 0,
  border = "white", col = "grey"))
with(exptlDes, text(min((which(LERmeas)) + max(which(LERmeas)))) / 2, -1,
  labels = "LER", col = "white", font = 3, cex = 0.9))

with(exptlDes, rect(min(which(SERmeas)), 0, max(which(SERmeas)), 2,
  border = "white", col = "grey"))
with(exptlDes, text(min((which(SERmeas)) + max(which(SERmeas)))) / 2, 1,
  labels = "SER", col = "white", font = 3, cex = 0.9))

with(exptlDes, rect(min(which(Harvest)), -2, max(which(Harvest)), 0,
  border = "white", col = "grey"))
with(exptlDes, text(min((which(Harvest)) + max(which(Harvest)))) / 2, -1,
  labels = "Harvest", col = "white", font = 3, cex = 0.9))

with(exptlDes, text(min((which(PhotoMeas)) + max(which(PhotoMeas)))) / 2, 5,
  labels = "Photo", col = "grey", font = 3, cex = 0.9))
with(exptlDes, rect(min(which(PhotoMeas)), 2, max(which(PhotoMeas)), 4,
  border = "white", col = "grey"))
#with(exptlDes, text(min((which(PhotoMeas)) + max(which(PhotoMeas)))) / 2, 3,
# labels = "Photo", col = "white", font = 3, cex = 0.9))

dev.off()

## pdf
## 2

# Legend

```

```

pdf(paste(pathFig, "/ExperimentalDesignLegend.pdf", sep = ""), 1.5, 2)
par(mai = rep(0, 4))
plot(0, 0, type = "n", xlim = c(0, 1.5), ylim = c(0, 1), axes = F)

polygon(c(0, 0.5, 1, 1, 0.5, 0), c(0.8, 1, 1, 0.55, 0.55, 0.35), border = NA,
        col = alpha(primShade0, 0.25))
polygon(c(0, 0.5, 1, 1, 0.5, 0), c(0.6, 0.45, 0.45, 0, 0, 0.15), border = NA,
        col = alpha(sec1shade0, 0.25))
points(c(0, 0.5, 1), c(0.575, 0.775, 0.775), lwd = 2, type = "l", col = primShade3)
points(c(0, 0.5, 1), c(0.375, 0.225, 0.225), lwd = 2, type = "l", col = sec1shade3)
text(0.5, 0.9, labels = "Hot", col = primShade3)
text(0.5, 0.1, labels = "Cool", col = sec1shade3)
text(1.25, 1, labels = "Max", col = alpha(primShade0, 0.25))
text(1.25, 0.55, labels = "Min", col = alpha(primShade0, 0.25))
text(1.25, 0.775, labels = "Mean", col = primShade3)

text(1.25, 0, labels = "Min", col = alpha(sec1shade0, 0.25))
text(1.25, 0.45, labels = "Max", col = alpha(sec1shade0, 0.25))
text(1.25, 0.225, labels = "Mean", col = sec1shade3)

dev.off()

## pdf
## 2

```

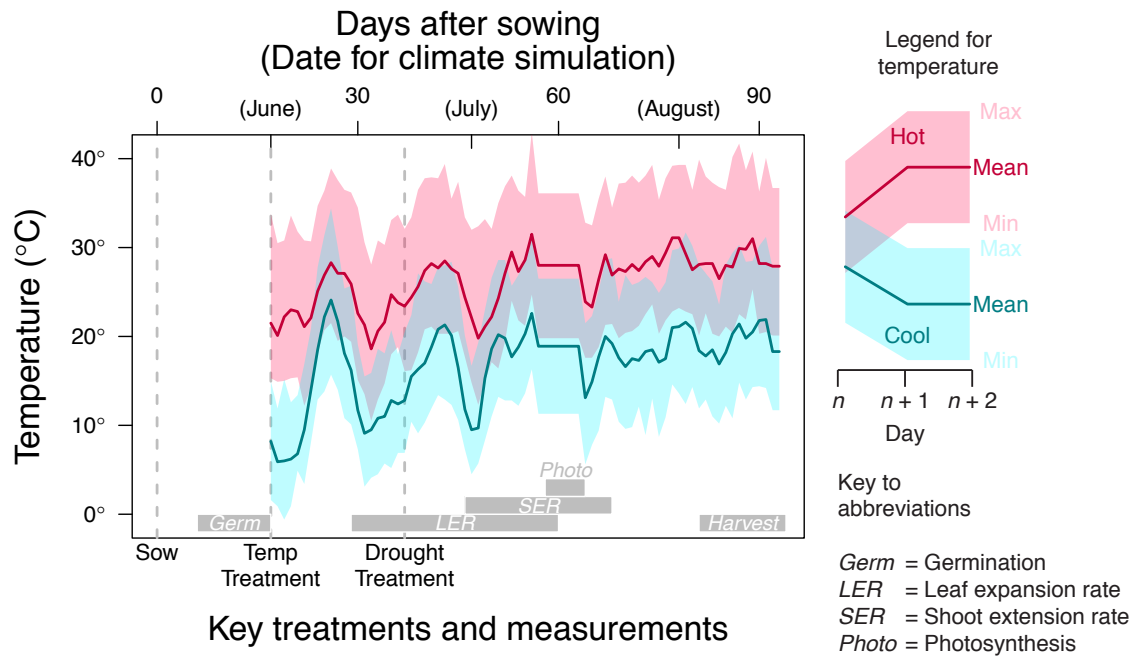


Figure 1: CAPTION

Growth and photosynthesis

Day of germination We tested for population variation in germination rate, measured as Days to Germination, using a lognormal survival model fit using the `survreg` function in the R package **survival** version 2.38 (Therneau, 2015). The model was fit with Population as a fixed effect and Family as random effect using a Γ frailty function. The significance of the Population effect was determined using analysis of deviance.

```
library(survival)

pathMS <- "~/Google Drive/CardLocalAdaptation/ms"

pathDat <- paste(pathMS, "/Data", sep = "")

master <- read.csv(paste(pathDat, "/MasterDatasheet_out.csv", sep = ""),
  row.names = 1)
```

Table 2: Traits

Trait	Units
Day of germination	day
Leaf expansion rate	mm day ⁻¹
Shoot elongation rate	mm or cm day ⁻¹
Harvest dry mass	g
Photosynthetic rate	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
Mortality	Probability?

```

# Create Surv object

germ <- with(subset(master, !is.na(master$MinGermDay)), Surv(MinGermDay, MaxGermDay,
  type = "interval2"))

# lognormal produced best fit to data

fitGerm <- survreg(germ ~ PopID + frailty(Family, sparse = F),
  data = subset(master, !is.na(master$MinGermDay)), dist = "lognormal")
suppressWarnings(anova(fitGerm)) # significant family and population effects

##                                Df Deviance Resid. Df    -2*LL
## NULL                          NA         NA   700.0000 2796.417
## PopID                        15.0000  151.9495   685.0000 2644.468
## frailty(Family, sparse = F) 106.9738  254.7233   578.0262 2389.745
##                                Pr(>Chi)
## NULL                          NA
## PopID                        9.887489e-25
## frailty(Family, sparse = F) 4.530519e-14

```

Growth rate: leaf expansion and shoot elongation We censused longest leaf length > 1 mm on 10 days (twice per week) between 12 May and 12 June (28 to 59 days after sowing). We ceased measuring leaf length once it appeared to asymptote and growth shifted to shoot elongation. We also censused plant height on 7 days (twice per week) between 29 May and 20 June (45 to 67 days after sowing). Both leaf expansion and shoot elongation were modeled as a second-order polynomials of time with individual coefficients (separate for leaf and shoot growth) using empirical Bayes' estimates from linear mixed-effects models fit using the R package **lme4** version 1.1-7 (Bates et al., 2014).

```
library(lme4)

## Loading required package: Matrix
## Loading required package: Rcpp

library(lmerTest)

##
## Attaching package: 'lmerTest'
##
## The following object is masked from 'package:lme4':
##
##   lmer
##
## The following object is masked from 'package:stats':
##
##   step

# New (blank) datasheet on which to add data
newdata <- read.csv(paste(pathDat, "/MasterDatasheet_in.csv", sep = ""))

# Read in LER data
```

```

LERdata <- read.csv(paste(pathDat, "/LERdata.csv", sep = ""))

#
# Empirical Bayes Approach: get coefficients for every plant
#

# suppressWarnings(mmLER <- lmer(log(LLL) ~ poly(DayN, 2, raw = T) +
# (poly(DayN, 2, raw = T)|indiv), data = LERdata))
# save(mmLER, file = paste(pathDat, "/mmLER.RData", sep = ""))
load(file = paste(pathDat, "/mmLER.RData", sep = ""))

# Extract coefficients and add to datasheet
Y <- t(unlist(fixef(mmLER)) + t(ranef(mmLER)$indiv))[match(newdata$indiv,
  rownames(ranef(mmLER)$indiv)), ]
colnames(Y) <- c("b0_LER", "b1_LER", "b2_LER")
newdata <- cbind(newdata, Y)

# Model-based LER

# Timeframe for Cool treatment: Day 0 - 31
# Timeframe for Hot treatment: Day 0 - 24
newdata$LERstart <- exp(newdata$b0_LER)
newdata$LERend <- exp(newdata$b0_LER +
  newdata$b1_LER * ifelse(newdata$TempTrt == "Cool",
  newdata$b2_LER * ifelse(newdata$TempTrt == "Cool",
newdata$LER_AbsGrowth <- (newdata$LERend - newdata$LERstart) /
  ifelse(newdata$TempTrt == "Cool", 31, 24)

```

```

# Mixed model ANOVA with family as random factor fit using step down procedure
mm <- lmer(LER_AbsGrowth ~ AvgGermDay + PopID * TempTrt * WaterTrt + (1|Family),
           data = master)

fitLER <- step(mm, reduce.random = F) # includes pop, temp, h2o, population x water
# fitLER <- step(mm, ddf = "Kenward-Roger", reduce.random = F) # same result using

# Read in SER data
SERdata <- read.csv(paste(pathDat, "/SERdata.csv", sep = ""))

#
# Empirical Bayes Approach: get coefficients for every plant
#

# suppressWarnings(mmSER <- lmer(log(height + 1) ~ poly(DayN, 2, raw = T) +
# (poly(DayN, 2, raw = T)|indiv), data = SERdata))
# save(mmSER, file = paste(pathDat, "/mmSER.RData", sep = ""))
load(file = paste(pathDat, "/mmSER.RData", sep = ""))

# Extract coefficients and add to datasheet
Y <- t(unlist(fixef(mmSER)) + t(ranef(mmSER)$indiv))[match(newdata$indiv,
                  rownames(ranef(mmSER)$indiv)), ]
colnames(Y) <- c("b0_SER", "b1_SER", "b2_SER")
newdata <- cbind(newdata, Y)

# Model-based SER

# Timeframe for Cool treatment: Day 0 - 31
# Timeframe for Hot treatment: Day 0 - 24

```



```

newdata$SERstart <- exp(newdata$b0_SER)
newdata$SERend <- exp(newdata$b0_SER +
                      newdata$b1_SER * ifelse(newdata$TempTrt == "Cool",
                      newdata$b2_SER * ifelse(newdata$TempTrt == "Cool",
newdata$SER_AbsGrowth <- (newdata$SERend - newdata$SERstart) /
                      ifelse(newdata$TempTrt == "Cool", 31, 24)

mm <- lmer(SER_AbsGrowth ~ AvgGermDay + PopID * TempTrt * WaterTrt +
          (1|Family), data = master)

fitSER <- step(mm, reduce.random = F) #includes pop, temp, h2o, temp x water

```

Photosynthesis During the week of 10 to 16 June (57 to 63 days after sowing), we measured daytime photosynthetic rate and stomatal conductance on a subset of 329 plants evenly spread between treatments and families within populations. The youngest, fully-expanded leaf acclimated for 3 minutes to reach steady state in a 6 cm² chamber of a LI-COR 6400XT Portable Photosynthesis System (LI-COR Biosciences, Lincoln, Nebraska). All measurements were made at ambient light (400 $\mu\text{mol m}^{-2} \text{s}^{-1}$), temperature, and moderate relative humidity. During this period, we suspended normal day-to-day temperature fluctuations and set daytime temperatures to its average for that period (Cool: 26.5°; Hot: 36.1° [CDM: *get exact temps used*]) so that all plants within a temperature treatment were measured under the same conditions.

```

# Photosynthetic rate
mm <- lmer(Photo ~ PopID * TempTrt * WaterTrt + (1|Family),
          data = subset(master, !is.na(master$Photo)))
fitPhoto <- step(mm, reduce.random = F)

```

```

# Intrinsic photosynthetic rate
# = residuals of Conductance - Photosynthesis regression
# Removed one outlier (may change if data are rearranged!)

mm <- lmer(resPhoto ~ PopID * TempTrt * WaterTrt + (1|Family),
  data = subset(master, !is.na(master$Photo))[-52, ])
fitIntrnPhoto <- step(mm, reduce.random = F)

```

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

```

library(car)

fitMort <- glm(DiedFromStress ~ PopID * TempTrt * WaterTrt,
  data = subset(master, !is.na(master$DiedFromStress)), family = "quasibinomial")

Anova(fitMort, type = 2)

## Analysis of Deviance Table (Type II tests)
##
## Response: DiedFromStress
##
##              LR Chisq Df Pr(>Chisq)
## PopID          38.169 15  0.0008519 ***
## TempTrt        247.763  1 < 2.2e-16 ***
## WaterTrt        79.508  1 < 2.2e-16 ***
## PopID:TempTrt    26.188 15  0.0360962 *
## PopID:WaterTrt   11.547 15  0.7129658

```

```
## TempTrt:WaterTrt          48.543   1  3.231e-12 ***
## PopID:TempTrt:WaterTrt    8.685  15  0.8934160
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Biomass (show correlation between growth rate and biomass?)

Intrinsic variation and plasticity

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

Principal components of germination, growth, and photosynthesis

[maybe this goes in Results?] We summarized population-level coefficients, after factoring out Treatment and other effects, using principal components.

```

popMeans <- data.frame(
  germ = c(coef(fitGerm)[1], coef(fitGerm)[1] + coef(fitGerm)[2:16]),
  LER = fitLER$lsmeans.table[1:16, "Estimate"],
  SER = fitSER$lsmeans.table[1:16, "Estimate"],
  photo = fitIntrnPhoto$lsmeans.table[1:16, "Estimate"])

traitPC <- prcomp(popMeans, center = T, scale. = T)
focClim$PC1 <- traitPC$x[, "PC1"]
focClimSA$PC1 <- traitPC$x[, "PC1"]

# Percent variance explained by first principal component
pve <- paste(round(100 * traitPC$sdev[1] ^ 2 / sum(traitPC$sdev ^ 2), 1), "%")

# Plot latitude versus PC1
pdf(paste(pathFig, "/Figure_PC1vLat.pdf", sep = ""), 5.5, 5)
par(mai = c(1, 1.5, 0.25, 0.25))
plot(focClim$Lat, focClim$PC1, xlim = c(32, 44), ylim = c(-4, 4), axes = F,
     frame.plot = T, type = "n", xlab = "", ylab = "")
title(xlab = "Latitude", cex.lab = 1.5)
title(ylab = substitute(plain(Principal~Component~1)~group("(", x, ")"),
     list(x = pve)))
axis(1, at = seq(32, 44, 4), labels = c(expression(32*degree),
     expression(36*degree), expression(40*degree), expression(44*degree)),
     lwd = 0, lwd.ticks = 1)
axis(2, at = seq(-4, 4, 2), las = 1, lwd = 0, lwd.ticks = 1)
fitLat <- lm(PC1 ~ Latitude, data = focClim)

```

```

# Label y-axis from slow to fast growth
mtext(c("Slow\nGrowth", "Fast\nGrowth"), 2, at = c(-4, 4), cex = 1.25,
      font = 3, las = 1, line = 4, adj = 0.5)

# polygon of confidence intervals
x <- seq(min(focClim$Latitude), max(focClim$Latitude), l = 1e3)
u <- predict(fitLat, new = data.frame(Latitude = x))
s <- sqrt(sapply(x, function(X) t(c(1, X)) %*% vcov(fitLat) %*% c(1, X)))
confInt <- data.frame(lowerCI = qnorm(0.025, u, s), upperCI = qnorm(0.975, u, s))
polygon(c(x, rev(x)), c(confInt$lowerCI, rev(confInt$upperCI)),
       col = "grey80", border = NA)
lines(x, confInt$lowerCI, lwd = 2, lty = 2)
lines(x, confInt$upperCI, lwd = 2, lty = 2)
lines(range(focClim$Lat), rev(range(predict(fitLat))), lwd = 2)
points(focClim$Lat, focClim$PC1, pch = 21, col = "black", bg = primShade0, cex = 1.5,
      lwd = 2)
dev.off()

## pdf
## 2

# plot axes could be range maps with areas highlighted (x-axis) and short to tall plants
#plot(focClim$Lat, traitPC1x[, "PC1"])
#cor.test(focClim$Lat, traitPC1x[, "PC1"])
#cor.test(popMeans[, 4], traitPC1x[, "PC1"])
#plot(focClim$bio16_cva, traitPC1x[, "PC1"])

```

```
# R data.frame of 178 M. cardinalis occurrences, thinned to correct for sampling bias, and
load(paste(pathDat, "/thinnedOccClimate.RData", sep = ""))
```

Selective agents and environmental correlates

As with previous studies in *Mimulus* and many other species [CITES, (?), mosquitoes, what else?], latitude *per se* predicted trait variation more than many obvious environmental variables (e.g. temperature or precipitation) that covary with latitude. We used Random Forest regression (Liaw and Wiener, 2002) to identify putative climatic factors underlying trait-latitude associations in *M. cardinalis*. We did this by looking for overlap between climatic variables that best predict latitude of *M. cardinalis* occurrence records and climatic variables that best predict trait variation across our 16 focal populations. For brevity, we refer to these as Climate-Latitude and Climate-Trait variables. We selected Climate-Latitude and Climate-Trait variables independently using Random Forest (VSURF algorithm in the R **VSURF** version 0.8.2 (Genuer et al., 2014)). From VSURF models, we kept only variables selected for prediction, the most stringent criteria.

```
library(VSURF)

## Loading required package: randomForest
## randomForest 4.6-10
## Type rfNews() to see new features/changes/bug fixes.
## Loading required package: rpart
## Loading required package: doParallel
## Loading required package: foreach
## Loading required package: iterators
## Loading required package: parallel

# Load VSURF results of climatic variables associated with latitude at cardinalis occurrence
```

```

# Note that occurrences have been randomly thinned to account for variation in sampling ef
load(paste(pathDat, "/clim2lat_fit_t1.RData", sep = "")) # fit_t1
vsurfLat <- fit_t1

# Load climVars
load(paste(pathDat, "/ExploratoryCimateVariables.RData", sep = ""))

# Figure of variable importance for latitude
pdf(paste(pathFig, "/Figure_clim2lat_imp.pdf", sep = ""), 4, 5)
par(mar = c(5, 6, 1, 1))
plot(0, 0, type = "n", xlim = c(0, ceiling(vsurfLat$ord.imp$x)[1]),
      ylim = c(1, length(vsurfLat$vselect.pred)), axes = F, frame.plot = T,
      xlab = "", ylab = "")

title(xlab = "Climate-Latitude\nImportance", line = 4, cex.lab = 1.5)
for (i in 1:length(vsurfLat$vselect.pred))
{
  lines(x = c(-1, length(vsurfLat$vselect.pred)), y = c(i, i), col = "grey")
}
points(vsurfLat$ord.imp$x[1:length(vsurfLat$vselect.pred)],
        length(vsurfLat$vselect.pred):1, pch = 21, col = "black", bg = "white", cex = 1.5)
  # mtext(climVars[vsurfLat$vselect.pred], side = 2,
# at = length(vsurfLat$vselect.pred):1, las = 1, line = 1)
  axis(1, lwd = 0, lwd.ticks = 1)
  dev.off()

## pdf
## 2

```

```

# Variable selection using random forests (VSURF)
linmod.PC1 <- as.formula(paste("PC1 ~ ", paste(climVars, collapse = " + "), sep = ""))
set.seed(69647)
vsurfPC1 <- VSURF.parallel(linmod.PC1, data = focClim[, c("PC1", climVars)],
  ncores = 3)

## Warning in VSURF.parallel.formula(linmod.PC1, data = focClim[, c("PC1", :
VSURF with a formula-type call outputs selected variables
## which are indices of the input matrix based on the formula:
## you may reorder these to get indices of the original data

vsurfPC1_SA <- VSURF.parallel(linmod.PC1, data = focClimSA[, c("PC1", climVars)],
  ncores = 3)

## Warning in VSURF.parallel.formula(linmod.PC1, data = focClimSA[, c("PC1",
: VSURF with a formula-type call outputs selected variables
## which are indices of the input matrix based on the formula:
## you may reorder these to get indices of the original data

# Plot most important climatic predictor versus PC1
pdf(paste(pathFig, "/Figure_bio16cva_v_Lat.pdf", sep = ""), 5, 5)
par(mar = c(5, 5, 1, 1))
plot(focClim$bio16_cva, focClim$PC1, xlim = c(0.25, 0.55), ylim = c(-4, 4), axes = F,
  frame.plot = T, type = "n", xlab = "", ylab = "")
title(xlab = "Precipitation of Wettest Quarter", cex.lab = 1.5)
title(xlab = "(Coefficient of variation)", cex.lab = 1, line = 4)
title(ylab = substitute(plain(Principal~Component~1)~group("(", x, ")")),
  list(x = pve))
axis(1, lwd = 0, lwd.ticks = 1)
axis(2, at = seq(-4, 4, 2), las = 1, lwd = 0, lwd.ticks = 1)

```



```

fitBio16cva <- lm(PC1 ~ bio16_cva, data = focClim)

# polygon of confidence intervals
x <- seq(min(focClim$bio16_cva), max(focClim$bio16_cva), l = 1e3)
u <- predict(fitBio16cva, new = data.frame(bio16_cva = x))
s <- sqrt(sapply(x, function(X) t(c(1, X)) %*% vcov(fitBio16cva) %*% c(1, X)))
confInt <- data.frame(lowerCI = qnorm(0.025, u, s), upperCI = qnorm(0.975, u, s))
polygon(c(x, rev(x)), c(confInt$lowerCI, rev(confInt$upperCI)),
  col = "grey80", border = NA)
lines(x, confInt$lowerCI, lwd = 2, lty = 2)
lines(x, confInt$upperCI, lwd = 2, lty = 2)
lines(range(focClim$bio16_cva), range(predict(fitBio16cva)), lwd = 2)
points(focClim$bio16_cva, focClim$PC1, pch = 21, col = "black", bg = primShade0,
  cex = 1.5, lwd = 2)
dev.off()

## pdf
## 2

# Overlap between latitude predictors and phenotype predictors
#### NEED TO COMPARE RESULTS FROM POINT DATA AND SPATIALLY AVERAGED CLIMATE!

imp2 <- climVars[vsurfPC1$vselect.pred]
# imp2 <- climVars[vsurfPC1_SAF$vselect.pred]
nImpPC1 <- length(imp2)

vsurfLat$vselect.pred

## [1] 45 43 42 20 47 41 46 39 19 13

imp1 <- climVars[vsurfLat$vselect.pred]

```

```

nImpLat <- length(imp1)

# Figure of variable importance for traits
pdf(paste(pathFig, "/Figure_clim2pc1_imp.pdf", sep = ""), 4, 5)
par(mar = c(5, 1, 1, 6))

plot(0, 0, type = "n", xlim = c(0, 0.3), ylim = c(1, nImpLat),
     axes = F, frame.plot = T, xlab = "", ylab = "")
title(xlab = "Climate-Trait PC1\\nImportance", line = 4, cex.lab = 1.5)
for (i in (nImpLat:(nImpLat - nImpPC1 + 1)))
{
  lines(x = c(-100, 100), y = c(i, i), col = "grey")
}
points(vsurfPC1$ord.imp$x[1:nImpPC1], nImpLat:(nImpLat - nImpPC1 + 1),
       pch = 21, col = "black", bg = "white", cex = 1.5)
# mtext(climVars[vsurfPC1$varselect.pred], side = 4, las = 1, line = 1,
# at = nImpLat:(nImpLat - nImpPC1 + 1),
# font = ifelse(vsurfPC1$varselect.pred %in% vsurfLat$varselect.pred, 2, 1))
axis(1, lwd = 0, lwd.ticks = 1, at = seq(0, 0.3, 0.1))
dev.off()

## pdf
## 2

```

For these analyses, we compiled a representative set of 178 recent (since 2000) known *M. cardinalis* occurrences. These occurrences were thinned by 50% to correct for uneven sampling from a comprehensive set of herbarium records and an exhaustive field survey in 2010-11 (Angert, 2015). For each occurrence, we used a 90m digital elevation model from

HydroSHEDS (Lehner et al., 2006) to extract elevation. Monthly interpolated climate layers were calculated using ClimateWNA (Wang et al., 2012), which accurately down-scales 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** (Hijmans et al., 2014). In total, we included 24 climate variables, 9 from ClimateWNA and 15 bioclimatic variables (Table 5). 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.

Results

A coordinated latitudinal cline in germination, growth, and photosynthesis

Genetic differences between populations affecting original function and performance point to traits that may be involved in local adaptation. Using a common garden design, we identified strong genetic differences in germination, growth, and photosynthetic rate among populations of *M. cardinalis* (Table 3). A single principal component captured 74.2

Remarkably little evidence for variation in plasticity

Genotype \times environment interactions are also a common signature of local adaptation [CITE]. For example, populations from more stressful environments may tradeoff reduced growth rate under benign conditions for the ability to tolerate stress by maintaining pos-

Table 3: Population, treatment effects

	Germination survreg	Leaf expansion lmer	Shoot elongation lmer	Photosynthesis lmer	Intrin. Photo lmer
Population	***	***	***	***	***
Temperature	NA	***	***	***	
Water	NA	***	***	***	
Pop \times Temp	NA			*	
Pop \times Water	NA	*			
Temp \times Water	NA		***		
Pop \times Temp \times Water	NA				

itive growth rates and/or surviving through adverse conditions. We found remarkably little evidence for this pattern in *M. cardinalis*. There was only one statistically significant Population \times Treatment interaction (Table 3), but this idiosyncratic result was weak and would not have survived correction for multiple testing. Otherwise, populations responded remarkably similarly to treatments: faster growth in the hot treatment, slower growth in the dry treatment, and high mortality in the hot, dry treatment (Table 3). Note that interactions were calculated after factoring out intrinsic trait differences, necessarily reducing statistical power to detect significant interactions relative to main effects. However, the fact that the Population and Treatment effects were highly significant ($P \ll 0.001$ in most cases) suggests that statistical power alone cannot explain why we failed to detect Population \times Treatment interactions.

Climatic variability best explains phenotypic divergence

Latitudinal clines are common, but it is often difficult to ascribe this variation to a particular selective agent. For *M. cardinalis*, interannual variation in precipitation over the past 30 years is very closely related to the latitude of recently-recorded occurrences of this species (Fig. 3A). The two most important Climate-Latitude variables were also strong predictors of position along the fast-slow growth axis (Fig. 3B). This overlap between

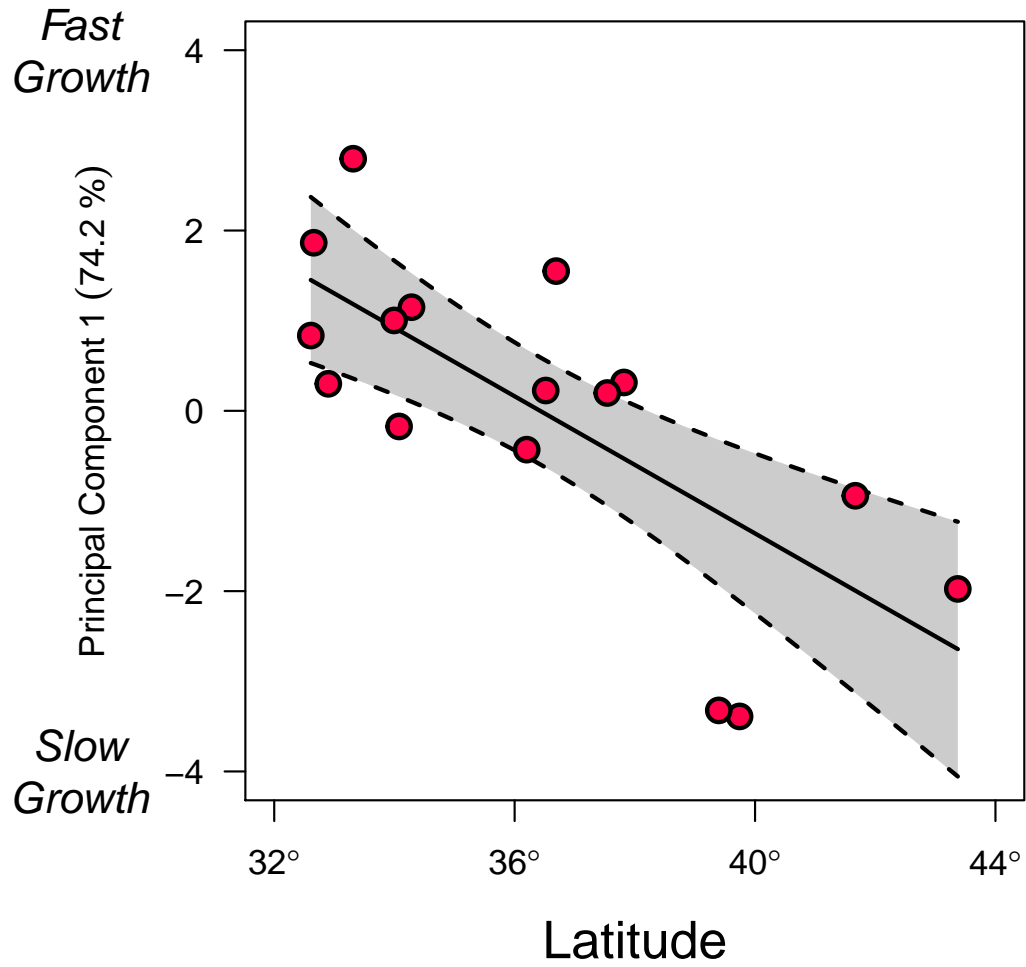


Figure 2: Trait variation, from fast to slow growth, is closely associated with latitude. Each point is a population, its latitude of origin a position along the slow to fast growth axis, defined as Principal Component 1 of five traits (see Methods). The line and 95% confidence intervals (grey polygon)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

Climate-Latitude and Climate-Trait variables suggests that interannual variation in precipitation is an important selective agent in *M. cardinalis*. Specifically, we hypothesize that more frequent droughts (greater precipitation cv) in Southern populations selects for an ‘annual-ized’ life history, as we detail in the Discussion. We must qualify these results

because our analysis obviously cannot rule out that alternative variables not included in the analysis may be more important.

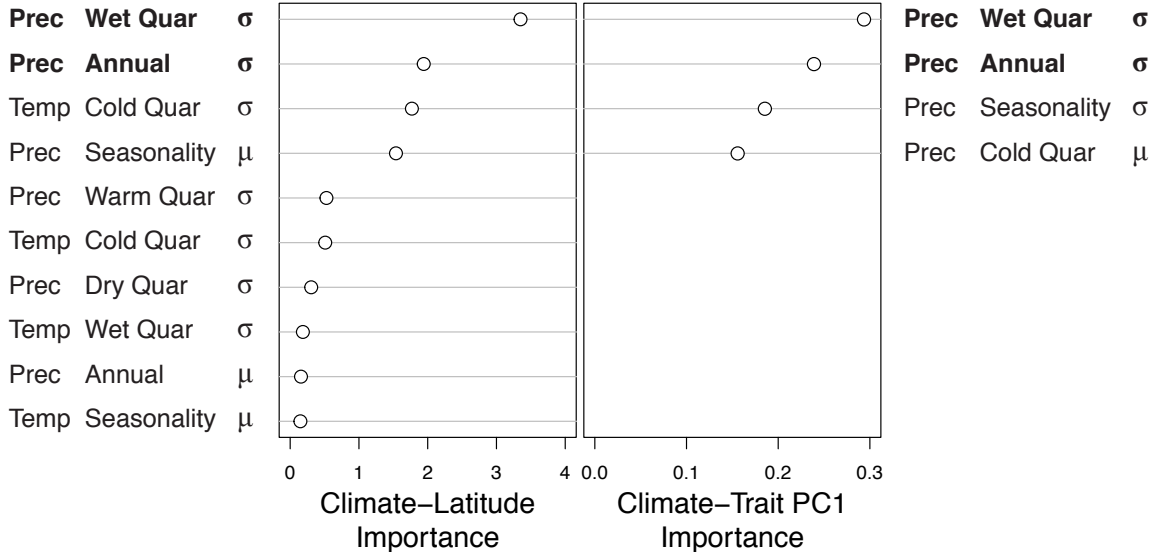


Figure 3: Interannual variation in precipitation is closely correlated with latitude and trait variation. A. Using Random Forest regression, we identified 10 climatic variables significantly (high importance) associated with latitude of *M. cardinalis* occurrences. B. The two most important Climate-Latitude variables were also the two most important Climate-Trait variables. Note that the Importance values in A and B are not comparable because the dependent variables (Latitude and Trait PC1, respectively) are on different scales. Climatic variables (left of A; right of B) are defined by three qualities: Climatic factor – Temperature (Temp) or Precipitation (Prec); Temporal scale – Annual, Coldest quarter (Cold Quar), Warmest Quarter (Warm Quar), Wettest quarter (Wet Quar), Dry Quarter (Dry Quar), or Seasonality; Summary statistic – average (μ) or coefficient of variation (σ)

Discussion

The observation that populations are locally adapted to their environment is old and well-documented in many species. New frontiers in local adaptation research seek a general explanation for why local adaptation often succeeds but sometimes fails, why certain traits

respond to selection whereas others are constrained, and what environmental drivers are most important [CITES such as Kawecki]. Understanding why and how populations have adapted to different environments in the past is especially significant now as a scientific basis for predicting how species' ranges will shift under climate change [CITES, (Catullo et al., 2015)]. In this study, we found evidence for one of two common signature of local adaptation, pointing to both phenotypic lability and constraint. In particular, our data suggest that photosynthesis and intrinsic growth rate vary among populations, whereas the fundamental abiotic niche is more conserved, and that climatic variation may be a more important selective agent than the average. Next, we tie these results into the broader threads of evolutionary theory in order to figure out what factors explain the trait variation we see and that we do not.

As long as environments vary and there exists genetic variation in fitness, why shouldn't local adapted genotypes evolve? Evolutionary theory indicates that the shape of fitness tradeoffs, demography, and gene flow can constrain adaptation (??). Specifically, adaptive variation cannot be maintained by spatially varying selection if tradeoffs are too strong, demography is strong asymmetric, and/or maladaptive gene flow is too high. In *M. cardinalis* we found substantial genetic variation among populations along a phenotypic axis from fast to slow growth that varied over a large spatial scale (Fig. 2). If this variation is adaptive, it suggests that the fitness tradeoff between doing well in low versus high latitude environments is not too strong nor swamped by demographic asymmetry or maladaptive gene flow. That is, alleles favoured at one latitude are not strongly selected against when they flow to another population, allowing locally adaptive genetic variation to be maintained by spatial heterogeneous selection. We also know from previous work that population size does not vary strongly with latitude [CDM: Amy - is this correct? JPs pop gen data seemed to suggest little difference in effective pop size across the range. Is this also true of census pop size?]. 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.

Another possibility we could have seen is that southern populations, which appear to experience more frequent drought years (see next section), could have evolved the ability to tolerate drought better than northern populations, thereby expanding the fundamental niche of the species as a whole. We found no evidence for this; all populations responded to drought and temperature treatments similarly (Table 3). We hypothesize that evolution of the fundamental niche may be constrained by a combination of strong fitness tradeoffs, demographic asymmetry, and gene flow. Riparian habitats where *M. cardinalis* live are highly heterogeneous at small spatial scales. Plants in the stream never have to tolerate drought whereas plants only a few meters away may experience extreme drought since there is little direct precipitation during the growing season in Mediterranean climates of Western North America. But alleles that confer greater drought tolerance may be quite costly in well-watered soils, and vice versa, leading to strong fitness tradeoffs. Such tradeoffs promote specialization to one soil type or another, thereby inhibiting the evolution of broad environmental tolerance within a population. Demography and gene flow may reinforce niche conservatism. A new mutant with increased drought tolerance that can survive at the resource-poor margin of a population will be demographically overwhelmed by the larger census populations that can be maintained in higher resource environments. Finally, gene flow, which is generally high among *M. cardinalis* populations within the same ecoregion (?), 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 via altered fundamental niche.

4. Selective agent - unpredictability. Explain Reiterate result other supporting evidence:
 - ENM models are better for habitat suitability for S pops when using data from 5 years;
 better for Northern populations using 30 yr (M. Bayly & A. Angert, unpub. data) - greater
 variance in recruit size in S pops -

There is a lot of interest in understanding how organisms will respond to changes in climatic variation, not just changes in the average climate. Our data indeed suggest that variation

may be more important than the mean.

But what we really need to understand is why certain types of traits are repeatedly targets of natural selection, whereas other traits are constrained.

5. Future work and broad conclusions. Supports general conclusions that traits related to timing and growth are evolutionarily labile whereas those related to the fundamental niche are constrained (cites like Emery et al. (2012); Emery and Ackerly (2014)). Next, we are testing whether phenotypic variation and constraint can be predicted by the shape of tradeoffs, as predicted by evolutionary theory,

How organisms adapt depends on the grain of the environment, ..., and gene flow between populations

Possibilities: 1. no trait variation: no environmental variation, high gene flow, generalist (convex) 2. variation in mean: 3. variation in plasticity: convex?,

our data are most consistent with: - concave tradeoffs, fine-grained (coarse+concave would predict alt morphs), high gene flow

Acknowledgements

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References

- Angert, A. L., 2015. *Cardinalis* enm. TBD 1:1–1.
- Bates, D., M. Maechler, B. Bolker, and S. Walker, 2014. lme4: Linear mixed-effects models using Eigen and S4. URL <http://CRAN.R-project.org/package=lme4>. R package version 1.1-7.

- Catullo, R. A., S. Ferrier, and A. A. Hoffmann, 2015. Extending spatial modelling of climate-change responses beyond the realised niche: estimating, and accommodating, physiological limits and adaptive evolution. *Global Ecology and Biogeography* .
- Emery, N. C. and D. D. Ackerly, 2014. Ecological release exposes genetically based niche variation. *Ecology Letters* 17:1149–1157.
- Emery, N. C., E. J. Forrestel, G. Jui, M. S. Park, B. G. Baldwin, and D. D. Ackerly, 2012. Niche evolution across spatial scales: climate and habitat specialization in California *lasthenia* (Asteraceae). *Ecology* 93:S151–S166.
- Genuer, R., J.-M. Poggi, and C. Tuleau-Malot, 2014. VSURF: Variable Selection Using Random Forests. URL <http://CRAN.R-project.org/package=VSURF>. R package version 0.8.2.
- Gilbert and P. D., 2014. Brief User’s Guide: Dynamic Systems Estimation. URL <http://cran.r-project.org/web/packages/dse/vignettes/dse-guide.pdf>.
- Hadfield, J. D., 2010. Mcmc methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software* 33:1–22. URL <http://www.jstatsoft.org/v33/i02/>.
- Hijmans, R. J., S. Phillips, J. Leathwick, and J. Elith, 2014. dismo: Species distribution modeling. URL <http://CRAN.R-project.org/package=dismo>. R package version 1.0-5.
- Kuznetsova, A., P. Bruun Brockhoff, and R. Haubo Bojesen Christensen, 2014. lmerTest: Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package). URL <http://CRAN.R-project.org/package=lmerTest>. R package version 2.0-11.
- Lehner, B., K. Verdin, and A. Jarvis, 2006. HydroSHEDS technical documentation. World Wildlife Fund, Washington, D.C. URL www.worldwildlife.org/hydrosheds.
- Liaw, A. and M. Wiener, 2002. Classification and regression by randomforest. *R News*

2:18–22. URL <http://CRAN.R-project.org/doc/Rnews/>.

Therneau, T. M., 2015. survival: A Package for Survival Analysis in S. URL <http://CRAN.R-project.org/package=survival>. R package version 2.38.

United States Geological Survey, 2015. National Hydrogeography Dataset. United States Geological Survey, Washington, D.C. URL <http://nhd.usgs.gov/index.html>.

Wang, T., A. Hamann, D. L. Spittlehouse, and T. Q. Murdock, 2012. ClimateWNA – high-resolution spatial climate data for western North America. Journal of Applied Meteorology and Climatology 51:16–29.

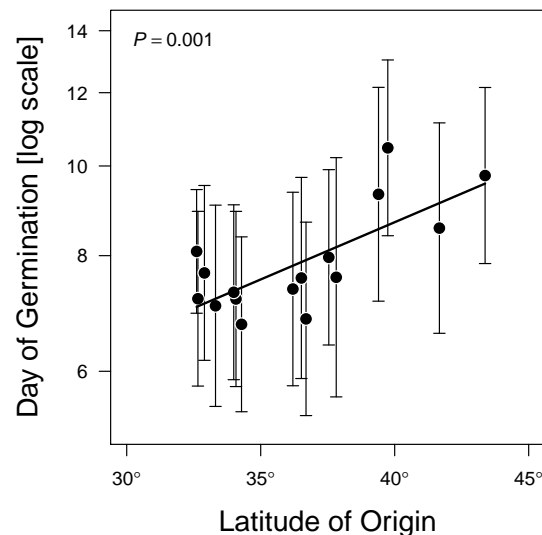


Figure 4: CAPTION

Spatially-averaged climate

Selection at given site might not reflect simply the local environment, but rather the average environment in a surrounding region, since alleles will experience selection across populations linked by migration. We therefore calculated spatially averaged climate means and

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

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

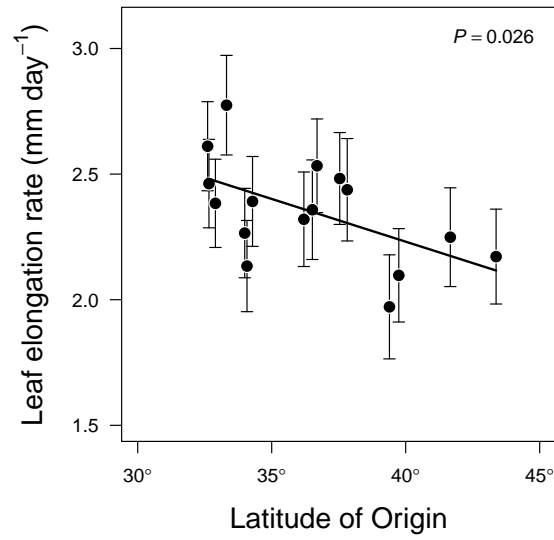


Figure 5: CAPTION

coefficients of variation. We sampled climate at 1000 random points (at 90-m resolution) within a 10 km buffer around focal populations. Since *M. cardinalis* is found exclusively in riparian areas, we only selected points along streams using the National Hydrogeoraphy Dataset (United States Geological Survey, 2015). Climatic means and CVs were weighted by their climatic suitability as determined using a multimodel ensemble average of ecological niche models (Angert, 2015).

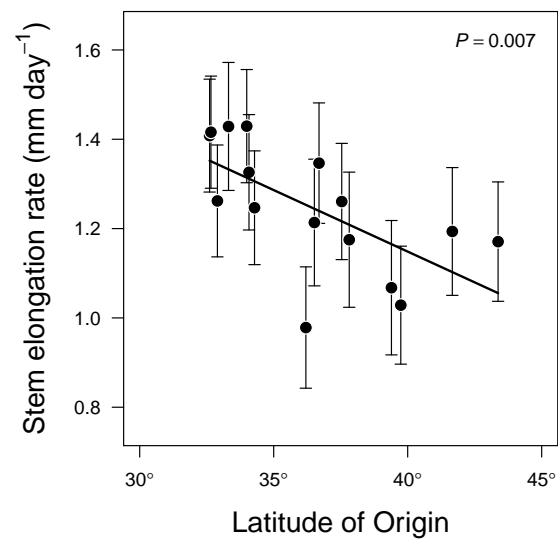


Figure 6: CAPTION

Table 5: Climatic variables

Climate variable	Abbreviation
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 climativ moisture deficit (mm)
MAR	mean annual solar radiation (NOTE: removing because too many missing values)
RH	mean annual relative humidity
bio1	Annual Mean Temperature
bio2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
bio3	Isothermality (bio2/bio7) (* 100)
bio4	Temperature Seasonality (standard deviation *100)
bio5	Max Temperature of Warmest Month
bio6	Min Temperature of Coldest Month
bio7	Temperature Annual Range (bio5-bio6)
bio8	Mean Temperature of Wettest Quarter
bio9	Mean Temperature of Driest Quarter
bio10	Mean Temperature of Warmest Quarter
bio11	Mean Temperature of Coldest Quarter
bio12	Annual Precipitation
bio15	Precipitation Seasonality (Coefficient of Variation)
bio16	Precipitation of Wettest Quarter
bio17	Precipitation of Driest Quarter
bio18	Precipitation of Warmest Quarter
bio19	Precipitation of Coldest Quarter

Table 6: CAPTION

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

Table 7: CAPTION

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