A cline within an ecotype of the yellow monkeyflower, *Mimulus guttatus*

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ABSTRACT

Premise: A key goal of evolutionary biologists is to understand how and why genetic variation is partitioned within species. In the yellow monkeyflower, *Mimulus guttatus* (syn. *Erythranthe guttata*), coastal perennial populations collectively constitute a single genetically and morphologically differentiated ecotype compared to inland populations of *M. guttatus*. While the distinctiveness of the coastal ecotype has now been well documented, there is also variation in environmental factors across the range of the coastal ecotype that could drive differentiation among its component populations in a more continuous way.

Methods: Based on previous observations of a potential cline within this ecotype, we quantified plant height across coastal perennial accessions from 66 total populations in a greenhouse common garden experiment. To evaluate possible environmental factors driving the relationship between trait variation and latitude, we regressed height against multiple climatic factors, including temperature, precipitation, and coastal wind speeds.

Results: The most significant relationship discovered in our experiment was a negative correlation between plant height and latitude. Plant height correlated negatively with annual precipitation and mean wind speed. Height was positively correlated with mean annual temperature. We hypothesize that one or more of these climatic factors drove the evolution of clinal variation within the coastal ecotype.

Conclusion: Overall, our study illustrates the complexity of how the distribution of environmental variation can simultaneously drive the evolution of distinct ecotypes as well as continuous clines within those ecotypes. These results are discussed in the context of the classic criticisms of ecotypes being intermediates in the process of speciation.

KEY WORDS: Local adaptation, salt spray, *Erythranthe guttata*, wind, speciation

INTRODUCTION

Understanding the formation of new species has long fascinated biologists (Darwin 1859; Wallace 1865). However, the path by which one species becomes two is complex and has been the subject of controversy (Coyne & Orr 2004). Of particular importance have been debates about the nature of the continuum along which new species are formed (Dres & Mallet 2002; Hendry et al. 2009; Nosil 2012; Gould & Lowry 2016; Stankowski & Ravinet 2021). In plants, much of this controversy has long been dominated by debates over the role of ecotypes in the formation of new species (Lowry 2012).

A century ago, Turesson (1922a,b) recognized that adaptive phenotypic variation in plants was often highly divergent in correlated sets of traits when comparing groups of populations derived from different habitats. Turesson referred to these groups of phenotypically similar, locally-adapted populations as ecotypes (Turesson 1922a,b; Gregor et al. 1936). Several researchers following Turesson found the concept of ecotypes useful for describing the distribution of adaptive genetic variation within species (Gregor et al. 1936; Clausen et al. 1948; Clausen 1951; Böcher 1967; Heywood 2011). Further, these researchers viewed partially reproductively isolated ecotypes as an intermediate stage in the process of speciation and a way to directly link natural selection to the formation of new species (reviewed in Lowry 2012). However, other researchers doubted the validity of Turesson's ecotype concept and argued that clines (Huxley 1938) best represented how adaptive genetic variation is partitioned across the geographic distribution of a species' range (Langlet 1963; 1971; Stebbins 1980).

While some recent authors have concluded that the cline versus ecotype controversy has been resolved because adaptive genetic variation can be partitioned different ways depending on the distribution of selective factors across the range of a species (Linhart and Grant 1996; Briggs and Walters 2016), multiple questions still remain. Foremost among these questions is whether ecotypes do indeed represent intermediate stages in the formation of new species. Both Quinn (1978) and Levin (1993) argued that there was no way that ecotypes could be intermediate stages in the process of speciation because, in their view, formation of geographically widespread ecotypes would require uniform habitats to also be widespread across large geographic regions. This led Levin to conclude that most speciation in plants occurs in localized, isolated populations (Levin 1993). However, if it can be shown that ecotypes can evolve despite a lack of uniformity of habitat conditions, it would support the hypothesis that widespread ecotypes are indeed a stage in the process of the formation of new species.

In this study, we examined whether there is a clinal distribution of phenotypic variation within a widespread ecotype of the yellow monkeyflower, *Mimulus guttatus* (syn. *Erythrante guttata*; Barker et al. 2012). *M. guttatus* exhibits high levels of genetic and phenotypic variation across its extensive range across western North America, which makes it a particularly useful system for studying local adaptation (Vickery 1978; Lowry et al. 2008). Coastal perennial populations of *M. guttatus*, occurring from southern California to southern Washington State, collectively comprise a distinct ecotype based on population genetic data (Lowry et al. 2008; Twyford and Friedman 2015). This ecotype has been designated as a variety (*M. guttatus var. grandis* Greene) or species (*M. grandis* syn. *E. grandis*; Barker et al. 2012, Nesom 2012, 2014) by taxonomists. Despite

these taxonomic designations due to their morphological distinctiveness, the coastal perennial ecotype is highly interfertile with the inland annual ecotype of *M. guttatus* (Lowry and Willis 2010). Further, there were only four fixed genetic differences between the coastal perennial and inland annual populations out of 29,693,578 SNPs surveyed in a population genetic study of this system (Gould et al. 2017). Based on these results, we believe that it is best to think of the coastal perennial populations as an intraspecific ecotype within the *Mimulus guttatus* species complex, which is the result of non-fixed differences in allele frequencies at many loci across the genome (Lowry and Willis 2010; Twyford and Friedman 2015; Gould et al. 2017).

Several previous studies of local adaptation in *M. guttatus* have found striking genetically-based phenotypic differences between the coastal perennial ecotype and nearby inland populations in the western United States (Vickery 1952; Hall et al. 2006, 2010; Lowry et al. 2008; Oneal et al. 2014). The level of morphological and genetic differentiation of these ecotypes can be seen clearly in previously published principal component plots (Fig. 3 of Lowry 2012 and Fig. S2 of Twyford & Friedman 2015). Many of the inland populations of *M. guttatus* have an annual life-history, especially those that occur in inland habitats that completely dry out during summer months (Vickery 1952; Clausen and Heisey 1958; Wu et al. 2008; Lowry et al. 2008). It should be noted that many of the inland populations, especially far inland, occur as perennials (Coughlin et al. 2021). In contrast to the inland annuals, the coastal perennial ecotype occurs in coastal habitats that are regularly damp and foggy during summer months (Vickery 1952; Clausen and Heisey 1958; Hitchcock and Cronquist 1973; Hall and Willis 2006; Lowry et al. 2008). In greenhouse common garden experiments, accessions derived from coastal habitats had thicker stems, many more prostrate stoloniferous branches, later flowering times, and produced far more

111 herbivore defensive compounds than nearby inland annual populations (Hall et al. 2006, 2010;
112 Lowry et al. 2008; Lowry and Willis 2010; Oneal et al. 2014; Lowry et al. 2019). Much of the
113 trait differences between the annual and perennial ecotypes contribute to local adaptation (Lowry
114 et al. 2008, 2019; Lowry & Willis 2010; Popovic & Lowry 2019) and are caused by a few large
115 effect loci, including a large chromosomal inversion polymorphism on chromosome 8 (Lowry
116 and Willis 2010; Oneal et al. 2014; Twyford & Friedman 2015; Lowry et al. 2019).

117

118 While there is both phenotypic and genetic divergence between ecotypes of *M. guttatus*, it is
119 unclear to what extent genetic variation is present within individual ecotypes – either due to
120 adaptation to widespread environmental gradients or idiosyncratic with regard to geographic
121 space. However, a previous pilot study suggested the possibility that there may be a cline in plant
122 height within the coastal ecotype (Lowry 2010). Since that initial study, dozens of additional
123 coastal *M. guttatus* populations have been collected, which provides an opportunity for a more
124 comprehensive survey of variation over the entire geographic range of the coastal perennial
125 ecotype.

126

127 In this study, we tested the hypothesis that clinal phenotypic variation can occur within ecotypes
128 of a species. Specifically, we investigated several populations of coastal perennial *M. guttatus*
129 from across the ecotype's range to determine whether there was indeed a latitudinal cline in plant
130 traits. To establish potential sources of selection, we examined whether there is a relationship
131 between the phenotypes of the accessions and the climates from where they were collected in
132 nature. Through these efforts, we attempted to understand whether clinal trait variation occurred

133 within a distinct ecotype, which would support the hypothesis that the evolution of
134 geographically widespread ecotypes does not require a widespread distribution of uniform
135 habitats, as argued by Quinn (1978) and Levin (1993).

136

137 **METHODS**

138 ***Germplasm for experiments –***

139 The seeds used in these experiments originated from natural population collections made
140 between 2004 and 2016. Seeds were either collected directly from plants in the field or collected
141 from plants that had been brought from the field back to the greenhouses at Duke University
142 (Durham, North Carolina, USA) or Michigan State University (East Lansing, Michigan, USA).
143 All seeds used for the experiment were grown for at least one generation in the greenhouse prior
144 to the study. For each population, we collected location description information, habitat
145 description, and GPS coordinates. Photos of the plants in the field for each population have been
146 posted on iNaturalist and vouchers for a subset of the populations were deposited at the
147 California Academy of Sciences. Permits were obtained for collections on public lands, where
148 permission was required. All maternal families used in this study are currently stored at
149 Michigan State University in airtight containers with silica gel as a desiccant.

150 ***Greenhouse Experiment –***

151 To determine whether there was clinal variation in *M. guttatus* traits, we conducted an
152 experiment in a controlled greenhouse environment. We conducted the greenhouse experiment in
153 the Plant Ecology Field Lab greenhouse at Kellogg Biological Station (KBS, Hickory Corners,
154 MI, USA). Seeds from coastal perennial *M. guttatus* (N = 402 individuals, 397 seed families, 66

155 populations) were planted with SUREMIX (Michigan Grower Products, Inc., Galesburg,
156 Michigan, USA) in 3.5 sq. in. pots without prior cold stratification. We included a variable
157 number of seed families per population (Range = 1-15, Mean = 6.09 families) and all families
158 but five were represented by a single individual (BON-2, BON-4, CPT-1, CPT-5, and CRU-1
159 each had two individuals measured). More information on the populations used in this
160 experiment and the coordinates of the original population locations can be found in Table S1 in
161 the supplement. All germinating seedlings except the centermost plant were removed from the
162 pot and remaining individuals were watered every other day throughout the experiment.
163 Germination and flowering dates were recorded to determine flowering time; plant flowering
164 height (cm), basal leaf width (cm), corolla length (mm), and corolla width (mm) were measured
165 at the time of first flowering.

166 ***Climate Data –***

167 To investigate potential environmental drivers of adaptive phenotypic variation, we extracted
168 climatic data for each population's collection location of origin from the WorldClim Bioclimatic
169 variables database (Fick and Hijmans 2017). This data was imported and compiled using R
170 (v.3.6.0, R Core Team 2019) using the *stats*, *nlme, raster*, and *sp* packages.

171

172 In addition to the Bioclim data, we extracted oceanic wind speed data from along the US Pacific
173 Coast using the National Renewable Energy Laboratory's Wind Prospector website, which
174 featured interactive online maps of wind speeds surveyed by NREL (King et al. 2014; Draxl et
175 al. 2015a,b). We used Pacific Monthly Offshore Wind Speed map layers to collect wind speeds
176 for each month from the site nearest to shore (8 km offshore at the closest; Draxl et al. 2015b) at

the latitudes of each of the coastal perennial populations. The wind speeds found through the NREL Wind Prospector were the averages for each 1.2 km X 1.2 km offshore site and were modeled at 100 m (Draxl et al. 2017). Due to unavailability of data outside of the contiguous US, populations found in British Columbia and Alaska (denoted by population codes ALA and LBG) were not included in climate data analyses. Monthly wind speed averages accessible via NREL were based on observations and statistical analyses over the course of seven years (King et al. 2014; Draxl et al. 2015a,b), which we used to calculate quarterly and annual wind speeds for each population collection location. We then matched these climate variables with their corresponding coordinates and population codes for further analysis.

Statistical Analysis –

To test whether there was a relationship between plant traits and the latitude of origin (location from which each population accession was collected), we conducted linear regression modeling in R using the base *stats* package and visualized our results using the *ggplot2* and *viridis* packages.

Although we predicted that latitude would correlate with plant height across space, we wanted to evaluate which climatic variables correlated with latitude might ultimately be driving observed clinal patterns. Climate variables are often highly correlated with each other, so we conducted a principal components analysis (PCA) to reduce the dimensionality of the data prior to further analyses. Climate data, described above, was extracted for each population and we performed the PCA in R using the *factoextra* and *stats* packages. We utilized all principal components (PCs) with an eigenvalue greater than 1, following the Kaiser criterion (Kaiser 1960). We then

conducted regression analyses of plant height against each of these PCs, again using linear models in the base R *stats* package. All data organization, analysis, and visualization were conducted in R Studio.

RESULTS

Trait correlations with latitude –

We found a significant negative relationship between latitude of population origin and plant height within the coastal perennial ecotype in our greenhouse experiment (*slope* = -1.12, R^2 = 0.37, $F_{1,64}$ = 36.83, $P < 0.001$; Figs. 1, 2). Based on our linear modeling, we found that every degree latitude further north translated to a roughly 1 cm decrease in plant height under greenhouse conditions. In addition to the strong relationship with height, we found significant, but much weaker relationships between population latitude and flowering time (*slope* = 0.48, R^2 = 0.07, $F_{1,64}$ = 4.54, P = 0.037), basal leaf width (*slope* = -0.19, R^2 = 0.09, $F_{1,64}$ = 6.59, P = 0.013), corolla width (*slope* = -0.32, R^2 = 0.13, $F_{1,64}$ = 9.84, P = 0.003). The relationship between latitude and corolla length was not significant (*slope* = -0.19, R^2 = 0.05, $F_{1,64}$ = 3.61, P = 0.062). Because the correlation between plant height and latitude was so much stronger than for the other traits, we focused the rest of our analyses on the possible causes of this correlation.

The relationship of climatic factors and plant height –

In our greenhouse experiment, we found that climate PC1 through PC3 all had eigenvalues greater than 1. In total, those three PCs accounted for 89.74% of cumulative variance in climate factors. PC1 explained the most variation at 55.93%, followed by PC2 with 22.94%, and PC3 with 10.88%. PC1 was significantly negatively correlated with plant height in a linear regression model (*slope* = -1.25, *Multiple* R^2 = 0.39, $F_{1,62}$ = 39.53, $P < 0.001$). Due to the nonlinear

relationship between wind speeds and latitude, we also assessed nonlinear regression models and found that the relationship between PC1 and plant height was best explained by a quadratic regression model as determined via AIC (*Multiple* $R^2 = 0.42$, $F_{2,61} = 21.78$, $P < 0.001$; *AIC linear* = 410.27, *AIC quadratic* = 409.34). The quadratic regression yields the same qualitative relationships between plant height and the climate factors as the linear model; we have included a visualization of the quadratic model in the supplement (Fig. S1). None of the other PCs were significantly correlated with plant height ($P > 0.05$).

We evaluated which climatic factors loaded onto PC1. Heavily loaded climate variables included Annual Precipitation, Average Annual Temperature, Precipitation Seasonality, Precipitation of the Warmest Quarter, Precipitation of the Wettest Quarter, Precipitation of the Coldest Quarter, Precipitation of the Wettest Month, and Precipitation of the Driest Quarter; Average Wind Speeds in Quarter 1 (January, February, March) and Quarter 4 (October, November, December) were not as heavily loaded, but were still represented on PC1 (Table S2). A higher value of PC1 indicates lower Average Annual Temperature and Precipitation Seasonality, but higher Annual Precipitation, Precipitation of the Warmest Quarter, Precipitation of the Wettest Quarter, Precipitation of the Coldest Quarter, Precipitation of the Wettest Month, Precipitation of the Driest Quarter, and Average Wind Speeds in Quarter 1 and Quarter 4 (Table S2).

Latitude strongly predicts climate for coastal populations –

While latitude can be a weak predictor of climate, especially for continental populations, we suspected that it might be highly correlated with climate in our study, as all populations were distributed latitudinally along a coastline. To assess how well latitude predicts climate for these

243 populations, we regressed the extracted principal components from our greenhouse experiment
244 against latitude. Our analysis confirmed that many of the climate variables involved were highly
245 correlated with latitude. PC1 was the only principal component significantly associated with
246 latitude and the two variables had a positive relationship (*slope* = 0.85, *Multiple* R^2 = 0.91, $F_{1,62}$
247 = 652.1, $P < 0.001$). To determine how well variation in individual climatic factors was predicted
248 by latitude, we regressed all the climate factors that were highly loaded on PC1 individually
249 against latitude (Table 1).

250

251 ***Patterns of wind speed along the west coast of North America –***

252 Plants are well known to respond to wind through changes in growth form (Grace 1977;
253 Gardiner et al. 2016; Telewski 2021). This, combined with the observation of wind speed
254 loading onto the first PC of climate, led us to evaluate the patterns of wind speed variation more
255 carefully across the populations that we studied. We found that, in general, annual wind speeds
256 increase with latitude (*slope* = 0.07, *Multiple* R^2 = 0.06, $F_{1,76}$ = 4.465, P = 0.038). Average wind
257 speeds in Quarter 1 (*slope* = 0.19, *Multiple* R^2 = 0.46, $F_{1,76}$ = 65.9, $P < 0.001$) and Quarter 4
258 (*slope* = 0.27, *Multiple* R^2 = 0.50, $F_{1,76}$ = 75.32, $P < 0.001$) of the year follow this pattern closely,
259 although Quarter 2 wind speeds (*slope* = -0.20, *Multiple* R^2 = 0.20, $F_{1,76}$ = 19.1, $P < 0.001$)
260 showed a negative relationship with latitude. Quarter 3 wind speeds were not correlated with
261 latitude (*slope* = 0.05, *Multiple* R^2 = 0.01, $F_{1,76}$ = 0.57, $P > 0.05$). A key realization from this
262 analysis is that patterns of wind speed variation shift dramatically across seasons and have a
263 striking non-linear relationship with latitude. This nonlinear relationship can be observed in
264 visualizations of annual and quarterly average wind speeds: after initially increasing with
265 latitude, average wind speeds generally decrease with latitude for populations north of southern

Oregon (42º N) (Fig. 3). Across seasons, wind speeds slow down during the summer much more in the southern end of the range of the coastal populations, while remaining high in northern California and southern Oregon throughout the summer months.

DISCUSSION

In this study, we found a significant negative relationship between latitude and genetically-based differences in plant height within the coastal ecotype of *M. guttatus*. This clinal pattern was likely driven by adaptation to climate variables that are correlated with latitude along the coast of the Pacific Ocean. However, field experimentation will be necessary to test whether height influences fitness in an environmentally-dependent way. Populations of *M. guttatus* collected from the southern end of the range were repeatedly found to be taller in common garden experiments than more northern populations. These southern populations generally experience higher temperatures, less precipitation, and lower velocity winds than more northern populations, especially during summer months. The results of this study further reinforce the idea that genetically-based phenotypic variation can be simultaneously partitioned in disjunct (among ecotypes) and continuous (clinal) ways. Overall, our results have implications for the evolution of adaptive genetic variation in this system, as well as general implications for the longstanding criticism of the hypothesis that ecotypes are intermediates in the evolution of plant species.

The finding of clines within ecotypes helps shape our understanding of the evolution of new plant species –

Understanding the patterns by which genetic variation is partitioned among populations within a species across a geographic landscape has been a matter of considerable debate, especially in the classic plant evolutionary biological literature (reviewed in Lowry 2012). It is reasonable to hypothesize that the partitioning of that genetic variation is a function of how environmental variation is partitioned across the same landscape. However, several previous researchers have argued against the existence of ecotypes and that only clinal variation exists within species (Langlet 1971; Quinn 1978; Stebbins 1980; Levin 1993).

A series of prior studies has clearly established that the coastal populations of *Mimulus guttatus* from central California to southern Washington state collectively comprise a genetically distinct, geographically widespread ecotype (Lowry et al. 2008; Lowry and Willis 2010; Oneal et al. 2014; Twyford and Friedman 2015). This coastal perennial ecotype clusters as a distinct group from the rest of the *Mimulus guttatus* species complex based on both a set of morphological traits and allele frequencies of loci across the genome (Lowry 2012; Oneal et al. 2014; Twyford and Friedman 2015) but has not accumulated appreciable divergence through fixed genetic differences (Gould et al. 2017).

While genomic data has allowed us to address the classic criticism about the existence of geographically widespread ecotypes made by Langlet (1971) and others, it is the discovery of clines within ecotypes that allows us to address doubts about how those ecotypes could come into existence in the first place. Both Quinn (1978) and Levin (1993) expressed doubt about the evolution of geographically widespread ecotypes evolving within species because they

309 hypothesized that uniform habitats would also necessarily be widespread to facilitate the
310 concerted evolution of new widespread ecotypes. Levin (1993) took this argument a step further
311 and argued that most speciation in plants must be local and thus, follow a model of peripatric
312 speciation. Levin's argument was built off those of Raven (1980), who concluded that there was
313 no logical way that geographically widespread ecotypes or subspecies could eventually evolve
314 into distinct species.

315

316 The level of environmental change across the range of the coastal ecotype of *M. guttatus*
317 demonstrates that habitats with uniform environments are not necessary for the evolution of
318 geographically widespread ecotypes. Further, the finding of a cline within the coastal ecotype
319 suggests that that nonuniformity of climate across the range of an ecotype can drive adaptative
320 phenotypic variation within an ecotype, while other shared climate factors simultaneously
321 maintain the divergence of that ecotype from the rest of a species complex. Indeed, this should
322 not be a surprising conclusion, as clines within coastal ecotypes/species have been observed in
323 the classic literature for plant systems such as *Plantago maritima* (Gregor 1930, 1938) and
324 *Achillea borealis* (Clausen et al. 1948).

325

326 Overall, the conclusion that widespread ecotypes exist as intermediates in the evolution of new
327 species fits the emerging modern paradigm of speciation based on recent results from genomic
328 studies. In those studies, there is increasing evidence that introgression (i.e., gene flow) occurs at
329 various stages during the process of species splitting into distinct phylogenetic lineages
330 (Reviewed in Mallet et al. 2015; Taylor and Larson 2019; Edelman and Mallet 2021). This

model of speciation with gene flow is not the same as classic models of sympatric speciation, which require consistent gene flow throughout the entire process of speciation (Coyne and Orr 2004). Instead, recent genomic studies suggest that it takes considerable time, with successive bouts of sympatry and allopatry for distinct, fully reproductively isolated phylogenetic lineages to become established (Mallet et al. 2015; Taylor and Lason 2019; Edelman and Malet 2021). Further, a recent review by Levin (2012) supports the long period over which the speciation process is completed in plants. During the long period over which new lineages evolve, ecotypes/subspecies will evolve clines and locally adapted populations within their boundaries. Many of these ecotypes/subspecies will go extinct or merge fully back into broader species complexes. Others will eventually become fully reproductively isolated lineages, but only after multiple bouts of gene flow with other members of the species complex. In the case of the coastal perennial ecotype of *M. guttatus*, the population genetic evidence suggests that it was derived at a single point in time from the rest of the species complex and then spread along the Pacific coast, adapting to local environments as it spread (Lowry et al. 2008; Twyford & Friedman 2015). During and following the spread of the coastal ecotype, there has likely been appreciable gene flow with other parts of the species complex, which is further supported by there only being four fixed nucleotide differences between these groups out of millions of SNPs (Gould et al. 2017).

The environmental factors responsible for the cline in plant height –

While we have demonstrated that a cline in plant height does occur within the coastal ecotype of *Mimulus guttatus*, the question remains as to what environmental factors have driven this cline. Though our analysis identified multiple factors correlated with both latitude and plant height,

these factors must be carefully considered, as they are autocorrelated with each other and not all of them may have an impact on the selective advantage of plant height.

Other researchers studying the evolution of ecotypes in the same region have found similar patterns in plant height variation along the Pacific coast of North America. These studies were far less comprehensive, but are useful, as they suggest that the patterns observed within the coastal ecotype of *M. guttatus* are not unique. For example, Clausen et al. (1948) found similar genetic variation with the coastal ecotype of *Achillea borealis* when comparing populations in Northern California, where populations north of the San Francisco Bay were shorter in stature than those from farther south in California in common garden experiments.

The causes of the latitudinal cline of plant height cannot be determined by this study alone and likely are complex. A global study of plant heights collected from an array of ecosystems found that there is a strong negative relationship between latitude and plant height (Moles et al. 2009). However, none of the environmental variables evaluated in that study were very strongly correlated with plant height. For our intraspecific study, temperature and precipitation were the most highly correlated with latitude. Both of these variables could directly or indirectly be driving the evolution of plant height in this system. For example, the longer growing season in the southern end of the range of the coastal perennial ecotype could lead to greater competition, which would favor greater height. A positive relationship of temperature and plant height has been observed within species for altitudinal gradients (Totland & Birks 1996; Macek & Leps

2008; Pellissier et al. 2010), but other factors could also drive the overall negative association of elevation and height.

Another possible contributor of the cline in plant height could be the spatial-temporal distribution of winds speeds along the Pacific coast. While less closely correlated with latitude, due to its non-linear distribution, wind and salt spray have long been thought to drive the evolution of the stature of coastal plant species. For example, Boyce (1954) recognized that this compact growth form is advantageous for coastal plants because it helps them to avoid the impacts of salt spray by being more sheltered from the wind. Onshore wind from the ocean can be intense along the Pacific Coast, depending on the location and time of year (Grace 1977; Gilliam 2002). The intensity of wind speed increases with the distance above the ground (Grace 1977; Jaffe and Forbes 1993). While wind can be damaging by itself, it is particularly damaging to plants along ocean coastlines because it contains aerosolized oceanic salt spray (Boyce 1954; Barbour 1978; Cheplick and Demetri 1999; Griffiths et al. 2006; Kouali et al. 2017; Du and Hesp 2020). Across the range of the coastal perennial ecotype, wind speeds and wave energy that drive salt spray are greater in the spring (April, May) than the summer (July, August) (Gilliam 2002; Garcia-Reyes and Largier 2012). However, those winds speeds dissipate most during the summer in the region south of the San Francisco, especially in more protected areas, like the southern Monterey Bay (Fig. 2b), where plants in the field reach up to two meters in height (Lowry *personal observation*). In Northern California and Southern Oregon, high coastal windspeeds remain elevated throughout the summer months, which coincides with when this species flowers. Since reproductive structures are often the most sensitive to salt spray (Griffiths et al. 2006), maintaining a compact growth form when flowering under higher windspeed/salt spray

conditions is likely to be advantageous. While this hypothesis works well to explain the results for populations from central California to southern Oregon, it does not hold up for central and Northern Oregon, where windspeeds are generally reduced during summer months. We are currently conducting experiments to establish whether reduced height is an adaptation to wind and salt spray in common garden experiments in the field at a windy coastal location.

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AUTHOR CONTRIBUTIONS

DBL conceived of and oversaw the experiments and analyses. TZ conducted the experiments and analyses. Both authors wrote the manuscript.

DATA AVAILABILITY STATEMENT

All data will be made available at publication, either as supplementary information or on Dryad.

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563

564 **TABLES**

Table 1. Regressions of climate factors that were highly-loaded on PC1 against latitude

Climate Factors		Estimate	F	Multiple R^2	P
	Annual Precipitation	0.048	242.2	0.7962	< 2.2e-16
	Coldest Quarter Precipitation	0.1082	143.3	0.698	< 2.2e-16
	Driest Quarter Precipitation	0.5965	372.9	0.8574	< 2.2e-16
	Mean Annual Temperature	-2.538	315.9	0.8359	< 2.2e-16
	Mean Wind Speed, 1st Quarter	2.3456	47.42	0.4334	3.38E-09
	Mean Wind Speed, 4th Quarter	1.8108	67.49	0.5212	1.68E-11
	Precipitation Seasonality	-0.2974	553.1	0.8992	< 2.2e-16
	Warmest Quarter Precipitation	0.6029	430.6	0.8741	< 2.2e-16
	Wettest Month Precipitation	0.3004	143.6	0.6984	< 2.2e-16
	Wettest Quarter Precipitation	0.1023	169.2	0.7318	< 2.2e-16

565 Note: F-statistic degrees of freedom were 1,62

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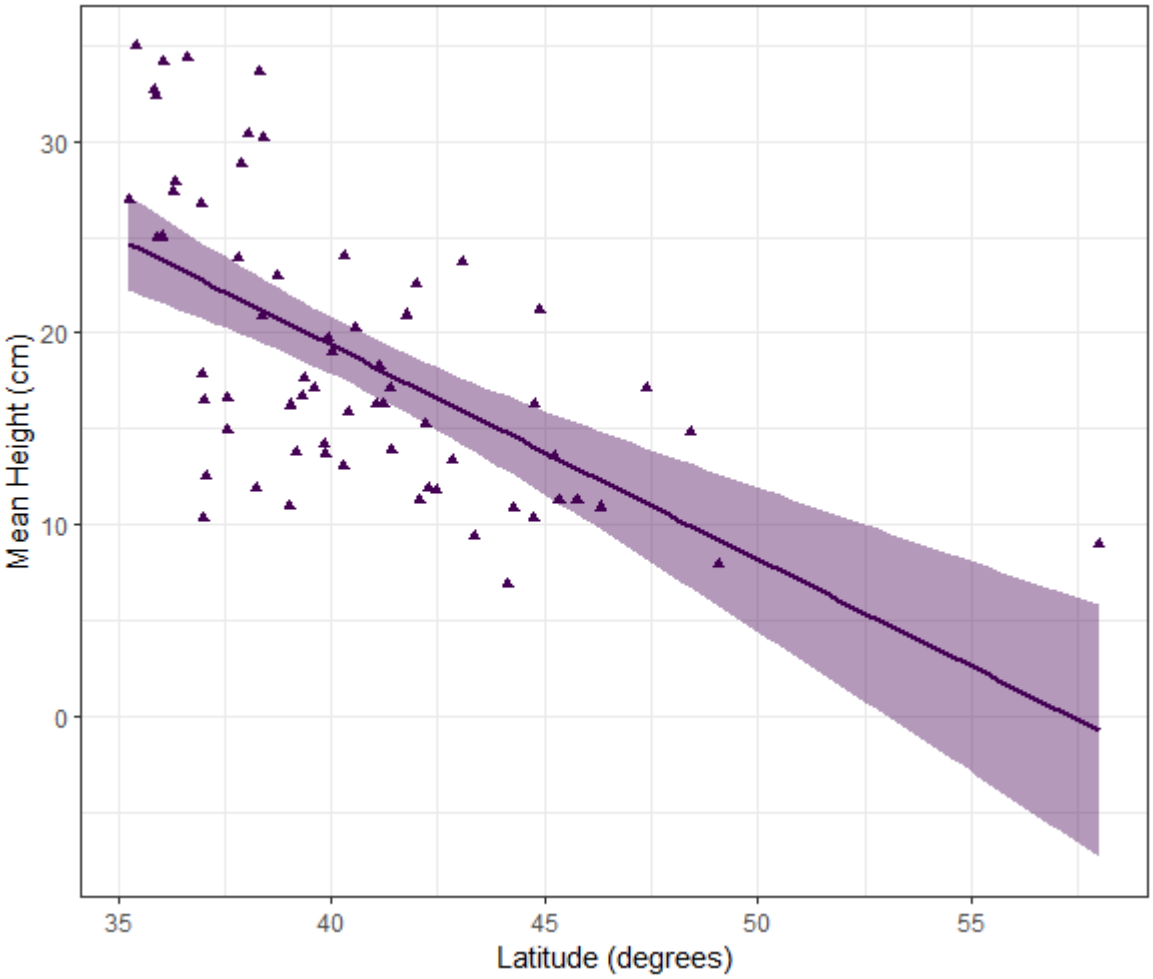
575 **FIGURES**



576

577 **Figure 1.** Mean *M. guttatus* population height decreases significantly with latitude ($P < 0.01$).

578 Relationship of latitude of origin (in degrees) for *M. guttatus* populations and mean individual

579 heights (cm) of these populations was determined by linear regression.

580

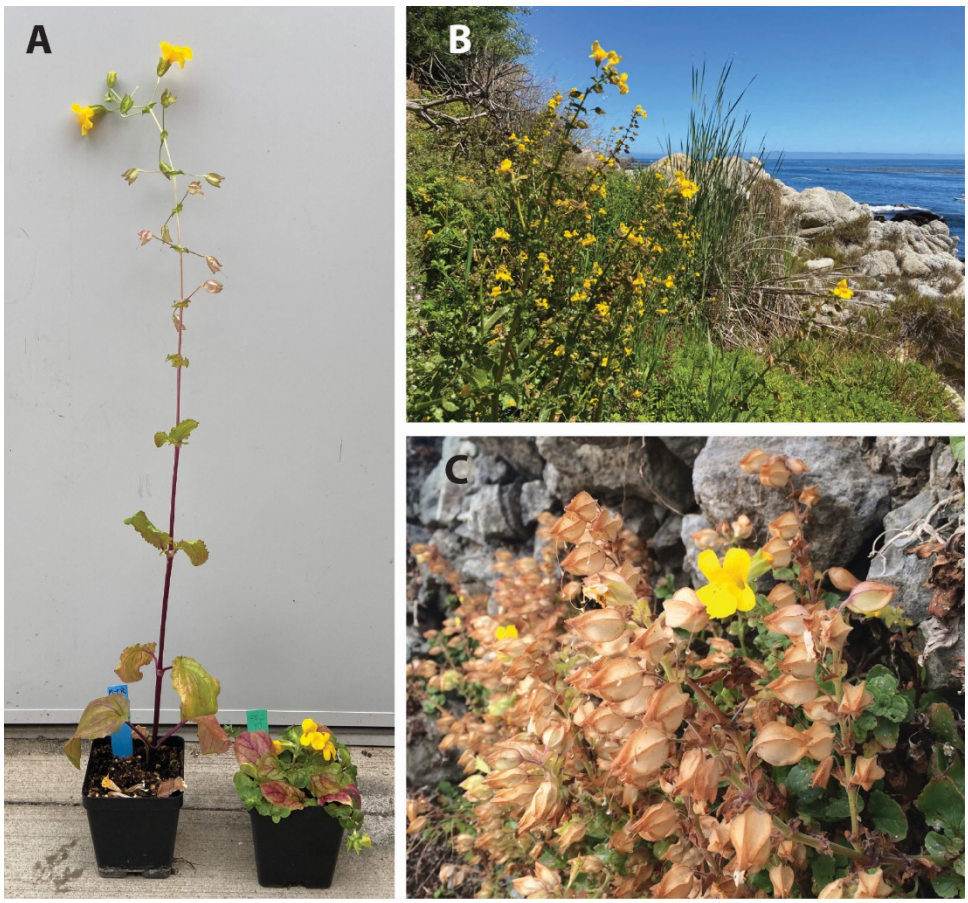


Figure 2. Variation in plant height within the coastal perennial ecotype in *Mimulus guttatus.* A) Divergence in height between plants from the Central California coast (left) and Oregon (right) when grown in a greenhouse common garden experiment. B) Tall plants growing along the Central California coast reach up to 2 meters in height. C) Plants growing along the coast in Oregon are more compact in growth form.

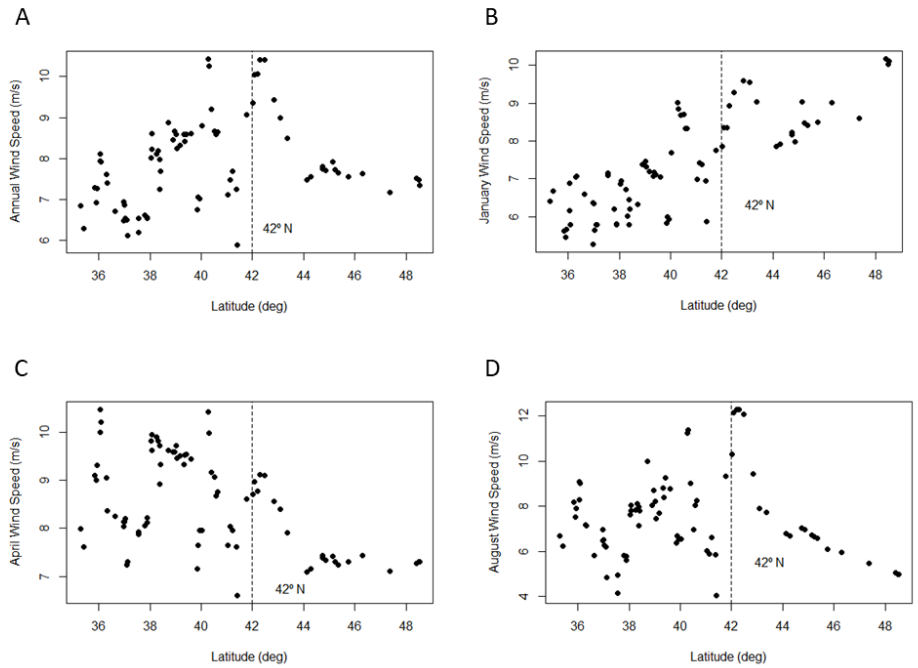


Figure 3. Mean wind speeds (m/s) along the United States Pacific Coast reported annually (A), during January (B), April (C), and August (D). The dashed line indicates 42° N, the latitude of the Oregon coast at which wind speeds start declining going further north.

601 **SUPPLEMENTARY MATERIAL**

602 ***Pilot experiment methods –***

603 We initially conducted a pilot experiment with fewer plants to begin our investigation of
604 potential clines in *M. guttatus* phenotypes. We planted seeds collected from coastal perennial *M.*
605 *guttatus* according to maternal family (*N* = 135 individuals, 135 seed families, 56 populations) in
606 3.5 sq. in. pots with SUREMIX (Michigan Grower Products, Inc., Galesburg, Michigan, USA).
607 We included a variable number of seed families per population for the pilot experiment as well
608 (Range = 1-4, Mean = 2.41 families); each seed family was represented by one individual. We
609 randomized these pots into flats (17 pots per flat) and stratified the seeds in a dark cold room (set
610 to 4º C) for a week to encourage more uniform germination before being transferred to the
611 greenhouses at Michigan State University. All germinating seedlings were removed from each
612 pot, except for the centermost plant. Plants were bottom-watered approximately every other day
613 throughout the remainder of the experiment. Plants were grown in the greenhouses with 16 h of
614 supplemental lighting and room temperature set to 22º C. We recorded approximate germination
615 dates and flowering dates, and measured plant flowering height (cm) and stem width (mm) for
616 each plant at the time of first flower.

617

618 We also gathered climate data for each population via the WorldClim database (Fick and
619 Hijmans 2017) and approximated offshore wind velocity at each population's latitude using the
620 NREL Wind Prospector (King et al. 2014; Draxl et al. 2015a,b). Details of these procedures can
621 be found in the main Methods section of this study.

622

To assess the relationship of plant traits with population latitude and environmental variables, we conducted linear regression and PCA analyses as described in the main Methods section of this study. All analyses were performed with R statistical software (v.3.6.0, R Core Team 2019).

Pilot experiment results –

In our pilot experiment, we found that there was a significant negative relationship between latitude of origin and mean height in coastal perennial *M. guttatus* populations (*slope* = -0.95, *Multiple* R^2 = 0.24, $F_{1,54}$ = 16.71, $P < 0.001$; Fig. S2). We also found significant relationships between latitude of origin and flowering time (*slope* = 0.46, *Multiple* R^2 = 0.09, $F_{1,54}$ = 5.39, P = 0.024) and stem width (*slope* = -0.14, *Multiple* R^2 = 0.24, $F_{1,53}$ = 16.34, $P < 0.001$). The relationship between latitude and plant height was the strongest and so was the focus of our subsequent analyses.

To gain further insight into the possible causes of the negative relationship between latitude and plant height, we conducted principal component analyses of climate factors. For our pilot experiment, we focused our analyses on climate PC1 through PC4, as all of them had eigenvalues greater than 1. These four PCs accounted for 94.55% of cumulative variance in the climate factors; PC1 explained 59.03% of the variation, followed by PC2 at 19.57%, PC3 at 11.13%, and PC4 at 4.82%. We found that PC1 had a significant negative relationship with plant height (*slope* = -0.72, *Multiple* R^2 = 0.22, $F_{1,54}$ = 14.97, $P < 0.001$). We assessed nonlinear models for PC1 due to the nonlinear relationship of wind speed and latitude but confirmed that the linear model fit the data best (*AIC linear* = 346.75, *AIC quadratic* = 348.40, *AIC cubic* = 348.14). The other three PCs were not significantly correlated with plant height under linear

models ($P > 0.05$), but PC4 had a significant, positive, nonlinear relationship with plant height that was fit best by a quartic model (*Multiple* $R^2 = 0.20$, $F_{4,51} = 3.12$, $P = 0.023$; *AIC quartic* = 354.21, *AIC quintic* = 355.21). We focused our initial analyses on PC1, as it explained the most variation in climate variables.

Given that PC1 had a significant negative correlation with height, we investigated which climate variables were most highly loaded on this PC axis. The most heavily loaded variables on PC1 were Annual Precipitation, Average Annual Temperature, Precipitation of the Wettest Quarter, and Precipitation of the Coldest Quarter. Annual Temperature was negatively correlated with PC1, while all others were positively correlated. While not as heavily loaded, Average Wind Speeds in Quarter 1 (January, February, March) and Quarter 4 (October, November, December) were both positively correlated with PC1. Overall, a higher value of PC1 indicates more precipitation, greater wind speeds, and lower temperatures. More specific information regarding loadings of individual climate variables on PC1 can be found in Table S3.

Using the principal components from our pilot experiment, we found that PC1 had a very strong positive relationship with latitude (*slope* = 0.76, *Multiple* $R^2 = 0.93$, $F_{1,54} = 689$, $P < 0.001$). The other PCs were not significantly correlated with latitude ($P > 0.05$). We also regressed climate factors that were highly-loaded on PC1 against latitude to determine how well variation in each individual climate factor was predicted by latitude (Table S4).

Supplemental tables and figures –

Table S1. *M. guttatus* population codes, coordinates, and experiments in which they were used

PopCode	Latitude	Longitude	Experiment
ALA	58.00433	-135.7425	Main
BCB	36.06285	-121.5922	Main
BHE	38.30703	-123.0584	Both
BLN	42.83772	-124.5601	Both
BND	43.07971	-124.43	Both
BON	37.00016	-122.182	Both
BRK	42.06429	-124.3011	Both
BYR	43.34902	-124.3458	Both
CAB	39.3592	-123.8168	Both
CKI	45.24213	-123.9687	Main
CMD	40.40923	-124.392	Both
CML	41.04636	-124.1226	Both
COV	40.02157	-124.0688	Both
CPT	36.9746	-121.9451	Both
CRD	36.30686	-121.8503	Both
CRU	35.84372	-121.4039	Both
CVG	38.3723	-123.0552	Both
CVR	38.3684	-123.0606	Pilot
DAV	37.02498	-122.2175	Both
DIL	38.25167	-122.9678	Main
DIS	46.3053	-124.072	Both
FPT	37.80918	-122.4751	Both
FRA	39.0083	-123.694	Main
GBM	41.37863	-124.0696	Both
GRE	37.07795	-122.2677	Both
GRZ	40.56885	-124.2118	Both
GWC	37.56394	-122.5138	Both
HEC	44.13536	-124.1235	Both
HOC	47.3854	-123.1473	Main
HUM	39.92726	-123.7608	Main
LAG	41.23157	-124.1064	Both
LBG	49.07185	-125.7676	Main
LJA	39.86053	-123.9024	Both
LOK	45.33825	-123.9771	Both
MTO	40.28343	-124.3596	Both
MTR	37.5517	-122.5137	Both
MUR	37.87685	-122.5578	Pilot
MXX	40.31068	-124.2671	Both
NAV	39.1869	-123.7572	Main
OPB	42.46402	-124.4229	Both
ORO	35.27335	-120.8891	Both
OSW	45.76108	-123.9666	Both
OTT	44.75224	-124.0641	Main
OZF	38.95578	-123.6603	Pilot
PCR	41.39856	-124.0688	Main
PGR	36.62799	-121.9208	Both
PPT	41.13709	-124.1519	Both
PTL	42.28225	-124.405	Both
PTR	38.04861	-122.8694	Both
PTU	44.27916	-124.1121	Both
PUN	44.74484	-124.06	Both
SCC	48.41262	-124.0121	Main
SDB	35.92127	-121.4668	Both
SIM	44.87659	-124.0383	Both
SRN	38.72593	-123.4805	Both
STB	37.89132	-122.6347	Both
SUR	36.33194	-121.8887	Both
SWB	39.03598	-123.6905	Main
TSU	41.75983	-124.2221	Both
USB	39.83218	-123.8493	Both
VIC	36.04834	-121.5885	Both
WCK	42.00256	-124.2129	Both
WCR	35.89838	-121.4613	Both
WEV	35.42313	-120.7887	Both
WOD	39.31239	-123.8029	Both
WPT	42.21213	-124.3767	Both
WPV	39.59383	-123.7854	Both
WSZ	36.96233	-122.0764	Both
WTB	38.40525	-123.0961	Both

Table S2. Principal Component Loadings and Coordinates (main experiment)

Climate Variables	PC1 loading	PC2 loading	PC3 loading	PC4 loading	PC5 loading	PC6 loading	PC1 coordinate	PC2 coordinate	PC3 coordinate	PC4 coordinate	PC5 coordinate	PC6 coordinate
Q1Wind	4.384	3.231	2.450	0.798	1.877	22.373	0.767	0.422	-0.253	-0.086	-0.109	0.333
Q2Wind	0.377	3.641	24.884	1.098	1.983	2.719	-0.225	0.448	-0.806	-0.101	-0.112	-0.116
Q3Wind	0.864	5.442	18.105	0.010	12.041	0.433	0.340	0.547	-0.687	0.010	0.277	0.046
Q4Wind	5.145	3.190	1.449	0.018	0.005	12.299	0.831	0.419	-0.195	-0.013	0.005	0.247
WindAnnual	1.596	6.095	16.446	0.269	0.522	2.907	0.463	0.579	-0.655	-0.050	0.058	0.120
AnnTemp	6.718	0.178	0.004	5.306	4.888	0.561	-0.950	0.099	-0.010	0.222	0.176	0.053
DiurnalRange	3.750	3.966	4.855	9.517	5.135	3.523	-0.709	-0.467	-0.356	-0.298	0.181	-0.132
Isothermality	4.421	3.269	0.468	9.902	8.410	8.684	-0.770	0.424	-0.111	-0.303	0.231	-0.208
TempSeasonality	0.954	14.064	2.417	0.824	1.063	3.116	0.358	-0.880	-0.251	0.088	-0.082	0.124
MaxTemp_WarmMonth	3.038	8.346	4.420	0.298	0.511	0.164	-0.639	-0.678	-0.340	0.053	0.057	0.029
MinTemp_ColdMonth	1.637	9.751	1.577	20.106	0.357	0.310	-0.469	0.733	0.203	0.432	0.048	0.039
TempAnnRange	0.731	13.520	5.007	2.228	0.102	0.007	-0.313	-0.863	-0.362	-0.144	0.025	0.006
TempWetQuarter	4.866	5.247	0.123	2.212	3.877	0.153	-0.808	0.537	0.057	0.143	0.157	-0.028
TempDryQuarter	3.107	6.851	2.838	9.788	1.745	4.823	-0.646	-0.614	-0.272	0.302	0.105	0.155
TempWarmQuarter	3.698	5.727	1.939	10.363	3.207	3.699	-0.705	-0.561	-0.225	0.310	0.143	0.136
TempColdQuarter	5.138	4.426	0.299	1.718	5.688	0.081	-0.830	0.494	0.088	0.126	0.190	-0.020
AnnPrecip	6.749	0.125	0.721	3.517	0.341	6.057	0.952	-0.083	-0.137	0.181	0.047	-0.173
PrecipWetMonth	6.122	0.119	1.761	7.843	0.064	8.729	0.906	-0.081	-0.214	0.270	-0.020	-0.208
PrecipDryMonth	5.143	1.347	3.158	1.271	15.456	1.690	0.831	-0.272	0.287	-0.109	0.313	0.092
PrecipSeasonality	6.483	0.156	0.821	0.850	5.404	0.390	-0.933	0.093	-0.146	0.089	-0.185	-0.044
PrecipWetQuarter	6.377	0.129	1.524	5.520	0.017	8.189	0.925	-0.084	-0.199	0.227	-0.010	-0.202
PrecipDryQuarter	6.108	0.575	1.644	0.052	15.698	0.027	0.905	-0.178	0.207	-0.022	0.316	0.012
PrecipWarmQuarter	6.408	0.492	1.111	0.059	11.442	0.008	0.927	-0.164	0.170	-0.023	0.270	-0.006
PrecipColdQuarter	6.186	0.116	1.979	6.429	0.165	9.057	0.911	-0.080	-0.227	0.245	-0.032	-0.212

Table S3. Principal Component Loadings and Coordinates (pilot experiment)

Climate Variables	PC1 loading	PC2 loading	PC3 loading	PC4 loading	PC1 coordinate	PC2 coordinate	PC3 coordinate	PC4 coordinate
Q1Wind	4.745	3.893	1.893	0.007	0.820	-0.428	0.225	0.009
Q2Wind	0.012	2.341	29.701	0.048	-0.042	-0.332	0.891	0.024
Q3Wind	1.774	3.333	17.748	0.010	0.501	-0.396	0.689	-0.011
Q4Wind	5.280	3.177	1.023	0.021	0.865	-0.386	0.165	-0.015
WindAnnual	2.591	4.445	15.244	0.000	0.606	-0.457	0.638	0.000
AnnTemp	6.343	0.220	0.011	4.128	-0.948	-0.102	-0.017	-0.218
DiurnalRange	3.497	4.888	5.302	9.528	-0.704	0.479	0.376	0.332
Isothermality	3.898	2.213	1.848	18.127	-0.743	-0.322	0.222	0.458
TempSeasonality	0.463	17.639	1.789	3.794	0.256	0.910	0.219	-0.209
MaxTemp_WarmMonth	3.926	7.093	3.338	0.355	-0.746	0.577	0.299	-0.064
MinTemp_ColdMonth	1.178	11.607	2.268	17.630	-0.409	-0.738	-0.246	-0.452
TempAnnRange	1.453	13.620	4.744	1.651	-0.454	0.800	0.356	0.138
TempWetQuarter	4.751	5.731	0.096	1.339	-0.820	-0.519	-0.051	-0.124
TempDryQuarter	3.991	4.896	1.199	11.223	-0.752	0.480	0.179	-0.360
TempWarmQuarter	4.441	3.987	0.643	10.368	-0.793	0.433	0.131	-0.346
TempColdQuarter	5.005	4.811	0.266	0.633	-0.842	-0.475	-0.084	-0.086
AnnPrecip	6.536	0.439	0.375	2.297	0.962	0.144	0.100	-0.163
PrecipWetMonth	5.966	0.425	1.100	5.906	0.919	0.141	0.171	-0.261
PrecipDryMonth	4.448	2.012	4.369	1.957	0.794	0.307	-0.342	0.150
PrecipSeasonality	5.964	0.319	1.005	1.231	-0.919	-0.122	0.164	-0.119
PrecipWetQuarter	6.228	0.423	0.946	4.013	0.939	0.141	0.159	-0.215
PrecipDryQuarter	5.561	1.154	2.309	0.467	0.888	0.233	-0.248	0.073
PrecipWarmQuarter	5.863	0.904	1.535	0.471	0.911	0.206	-0.203	0.074
PrecipColdQuarter	6.085	0.431	1.248	4.795	0.928	0.142	0.183	-0.235

Table S4. Regressions of climate factors that were highly-loaded on PC1 against latitude (pilot experiment)

Climate Factors	Estimate	F	Multiple R^2	P
Annual Precipitation	0.0459	401.9	0.8816	< 2.2e-16
Coldest Quarter Precipitation	0.1038	200.9	0.7882	< 2.2e-16
Driest Quarter Precipitation	0.5854	436.9	0.89	< 2.2e-16
Mean Annual Temperature	-2.3367	237.2	0.8146	< 2.2e-16
Mean Temperature, Coldest Quarter	-1.8891	163.2	0.7514	< 2.2e-16
Mean Wind Speed, 1st Quarter	2.1737	50.06	0.4811	3.11E-09
Mean Wind Speed, 4th Quarter	1.6486	74.6	0.5801	9.37E-12
Precipitation Seasonality	-0.2794	607.2	0.9183	< 2.2e-16
Warmest Quarter Precipitation	0.5861	470.4	0.897	< 2.2e-16
Wettest Month Precipitation	0.2877	191.4	0.7799	< 2.2e-16
Wettest Quarter Precipitation	0.0978	239.6	0.8161	< 2.2e-16

Note: F-statistic degrees of freedom were 1,54

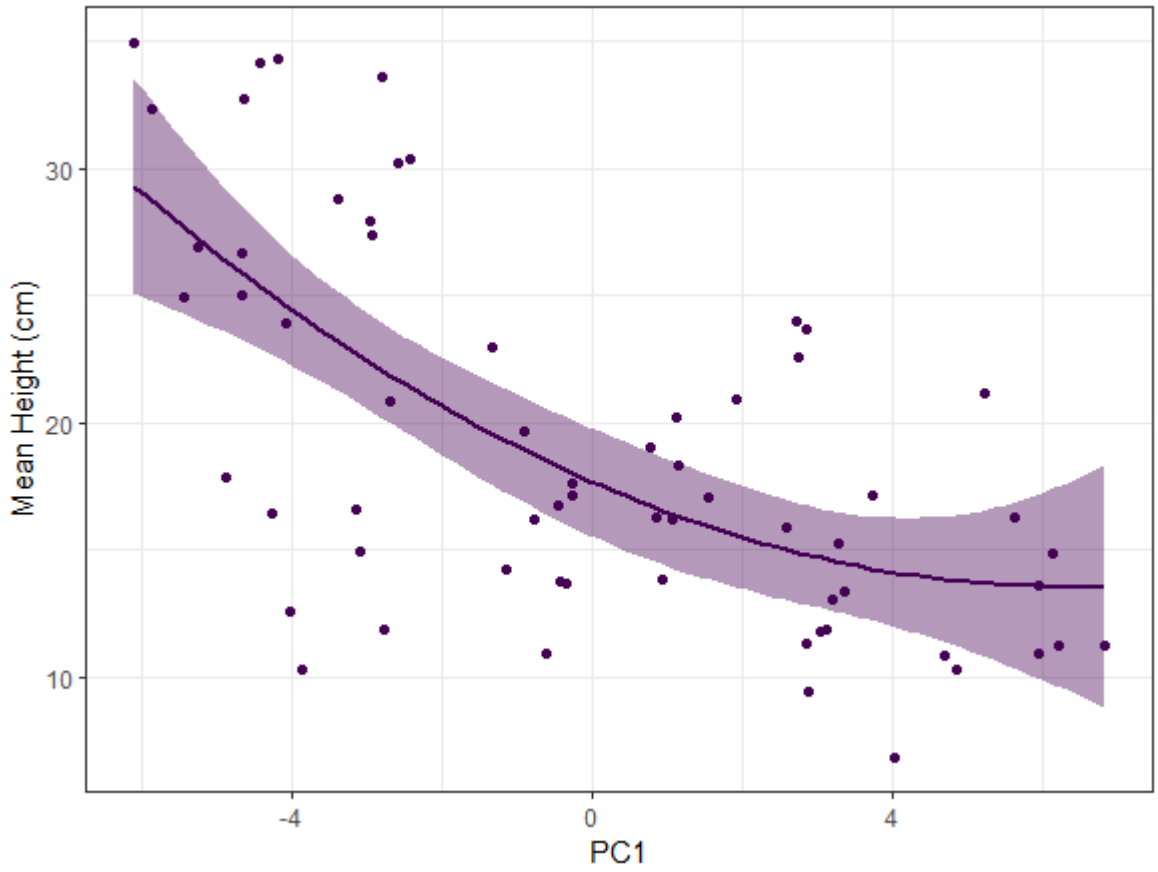


Figure S1. Mean *M. guttatus* population height decreased significantly and non-linearly with increasing values of climate PC1 in the main experiment ($P < 0.001$). The figure displays the quadratic relationship between climate PC1 and plant height, which was the best fitting model as determined by AIC.

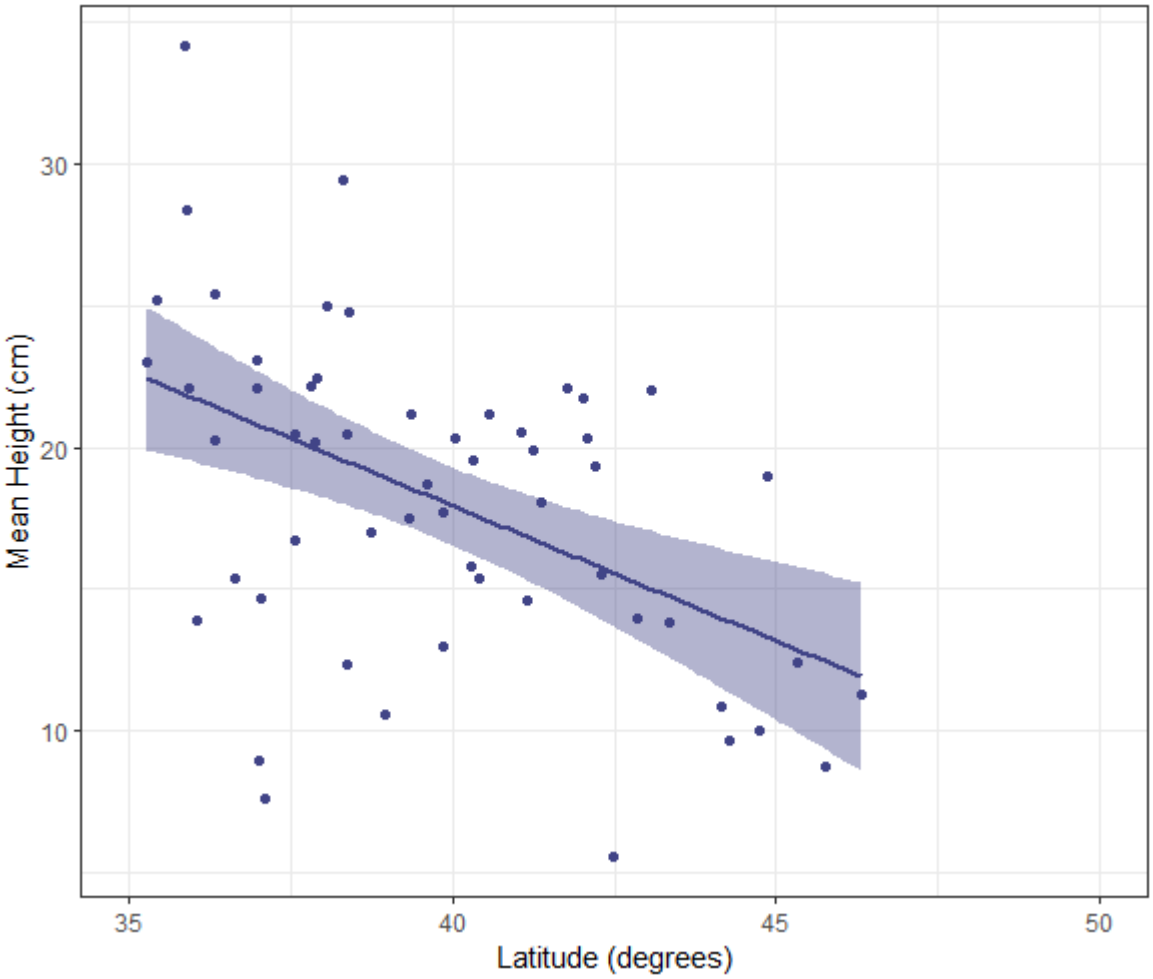


Figure S2. Mean *M. guttatus* population height decreased significantly with latitude of origin in the pilot experiment ($P < 0.01$). The relationship between latitude (in degrees) and mean height in *M. guttatus* populations was determined by linear regression.