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Ocean exposure and latitude drive multiple clines within the coastal perennial 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 constitute a single genetically and morphologically differentiated ecotype compared to inland *M. guttatus* populations. While the coastal ecotype's distinctiveness has now been well documented, there is also environmental variation across the ecotype's range that could drive more continuous differentiation among its component populations.

Methods: Based on previous observations of a potential cline within this ecotype, we quantified plant height, among other traits, across coastal perennial accessions from 74 populations in a greenhouse common garden experiment. To evaluate potential drivers of the relationship between trait variation and latitude, we regressed height against multiple climatic factors, including temperature, precipitation, and coastal wind speeds. We also accounted for exposure to the open ocean in all analyses.

Results: Multiple traits were correlated with latitude of origin, but none more than plant height. Height was negatively correlated with latitude, and plants directly exposed to the open ocean were shorter than those protected from coastal winds. Further analyses revealed that height was correlated with climatic factors (precipitation, temperature, and wind speeds) that were autocorrelated with latitude. We hypothesize that one or more of these climatic factors drove the evolution of latitudinal clinal variation within the coastal ecotype.

Conclusions: Overall, our study illustrates the complexity of how the distribution of environmental variation can simultaneously drive the evolution of both distinct ecotypes *and* continuous clines within those ecotypes.

KEYWORDS

abiotic stress, Erythranthe guttata, local adaptation, salt spray, wind

Over a century ago, Turesson (1922a, 1922b) 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, 1922b; 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

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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 many contemporary biologists recognize the utility of concepts like ecotypes and/or clines to describe the distribution of adaptive phenotypic variation within species, there is still a lack of clarity about the full extent to which phenotypic variation is distributed across the complexity of the natural landscape (Lowry et al., 2014; Briggs and Walters, 2016). In particular, questions remain about the extent to which geographically widespread ecotypes themselves may harbor clines in phenotypic variation. This question was of major importance to the biosystematists like Clausen, Keck, Hiesey (Clausen et al., 1948), and their contemporaries (Gregor, 1930, 1938), but has since received limited attention.

In this study, we examined the distribution of phenotypic variation within a widespread ecotype of the yellow monkeyflower, *Mimulus guttatus* (Phrymaceae, syn. *Erythranthe guttata*; Barker et al., 2012). *Mimulus guttatus* exhibits high levels of genetic and phenotypic variation across its extensive range spanning western North America, which makes it a particularly useful system for studying how local adaptation shapes the distribution of adaptive genetic variation across space (Vickery, 1978; Lowry et al., 2008). Coastal perennial populations of *M. guttatus*, occurring from southern California to southern Washington, 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 single-nucleotide polymorphisms (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 *M. 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). Inland *M. guttatus* populations of the coastal mountain ranges of western North America primarily 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; Lowry et al., 2008; Wu et al., 2008). Although inland populations in the coast ranges are annuals, perennial populations occur with some frequency in areas that maintain soil water through the summer months (van Kleunen, 2007; Oneal et al., 2014; Twyford and Friedman, 2015). The occurrence of inland perennial populations increases farther inland, especially at higher elevations in the Sierra Nevada and Cascade Mountain ranges (Coughlan et al., 2021). The level of morphological differentiation between coastal perennial and inland annual ecotypes is immense (Lowry et al., 2008). Further, coastal perennial populations are separated genetically from inland populations, both annual and perennial, in previously published principal component plots (Lowry, 2012: Figure 3; Twyford and Friedman, 2015: fig. S2). 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 herbivore defensive compounds than nearby inland annual populations (Hall et al., 2006, 2010; Lowry et al., 2008, 2019; Lowry and Willis, 2010; Oneal et al., 2014).

Many of the trait differences between the inland annual and coastal perennial ecotypes contribute to local adaptation (Lowry et al., 2008, 2019; Lowry and Willis, 2010; Popovic and Lowry, 2020) and are caused by a few large effect loci, including a large chromosomal inversion polymorphism on chromosome 8 (Lowry and Willis, 2010; Oneal et al., 2014; Twyford and Friedman, 2015; Lowry et al., 2019). Inland perennial populations of *M. guttatus* consistently have the same orientation of the chromosomal inversion as coastal perennial populations (Lowry and Willis, 2010). Indeed, one of the few regions of the genome that is highly differentiated between inland annual and inland perennial populations is the chromosome 8 inversion (Oneal et al., 2014; Twyford and Friedman, 2015). Here, we focus on phenotypic variation only among coastal perennial populations, which we assume all share the same chromosome 8 orientation, based on previous studies (Lowry et al., 2008; Oneal et al., 2014; Twyford and Friedman, 2015; Gould et al., 2017; Kollar et al., 2024 [preprint]).

While there is both phenotypic and genetic divergence between ecotypes of *M. guttatus*, it is unclear to what extent genetic variation is present within individual ecotypes—either due to adaptation to widespread environmental gradients or idiosyncratic with regard to geographic space. However, a previous pilot study suggested the possibility that there may be a latitudinal cline in plant height within the coastal ecotype (Lowry, 2010). Since that initial study, dozens of additional coastal *M. guttatus* populations have been collected (Figure 1), which provides an opportunity for

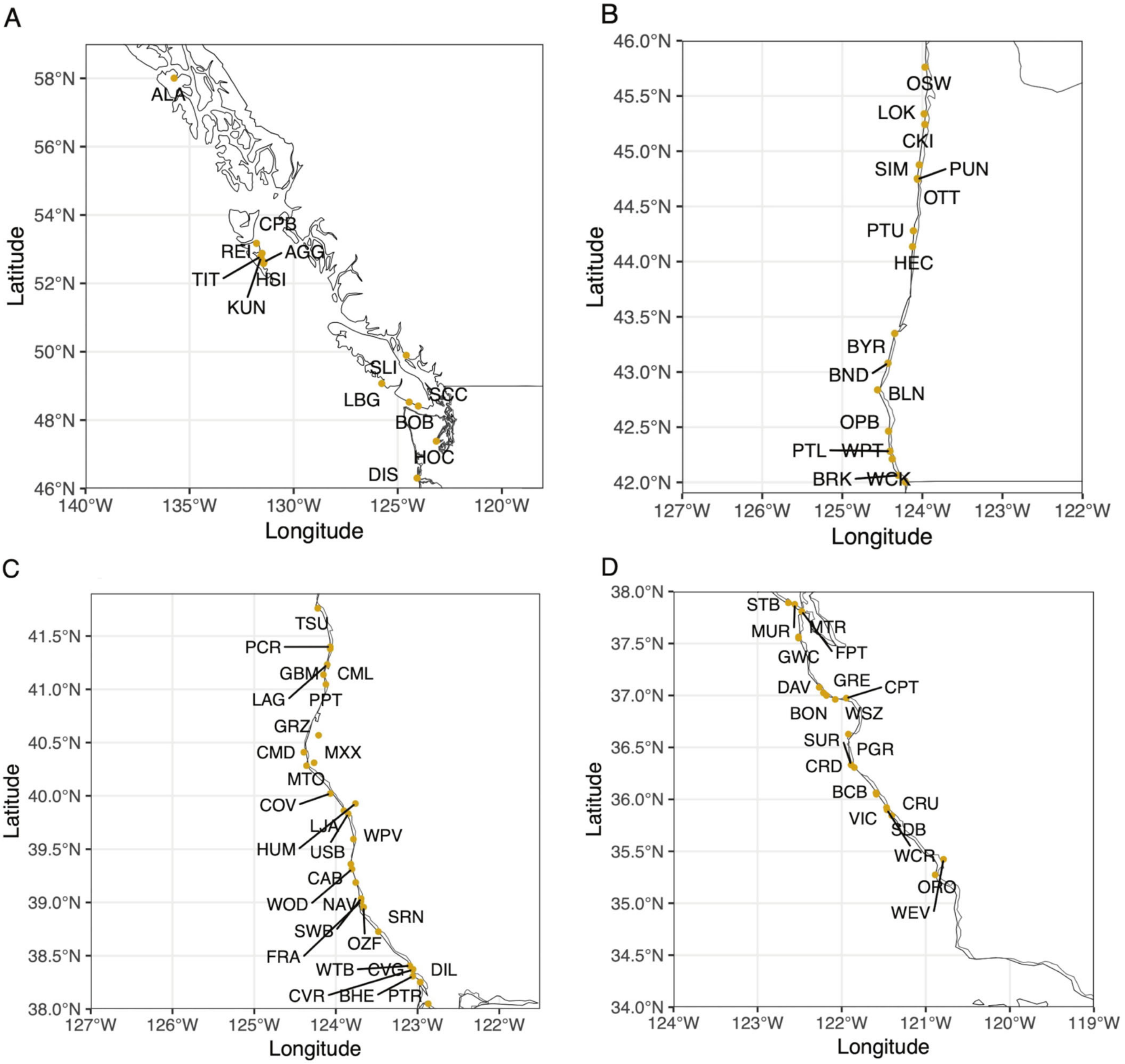

FIGURE 1 Population locations for coastal perennial *Mimulus guttatus* in (A) Washington, British Columbia, and Alaska; (B) Oregon; (C) northern California; and (D) southern California. These maps include populations grown in both the main and pilot studies.

a more comprehensive survey of variation over the entire geographic range of the coastal perennial ecotype.

We tested the hypothesis that there is a latitudinal cline in phenotypic variation within the coastal ecotype. We simultaneously evaluated the role of exposure of the coastal perennial populations to the open ocean on trait variation. To establish potential sources of selection, we examined whether there is a relationship between the phenotypes of the accessions and the climates of the locations from which they were collected in nature. Through these efforts, we attempted to understand whether clinal trait variation occurred within the distinctive coastal perennial ecotype.

MATERIALS AND METHODS

Germplasm for experiments

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

Greenhouse experiment

To determine whether there was clinal variation in *M. guttatus* traits, we conducted an experiment in a controlled greenhouse environment. We conducted the greenhouse experiment in the Plant Ecology Field Lab greenhouse at Kellogg Biological Station (Hickory Corners, Michigan, USA). Seeds from coastal perennial *M. guttatus* ($N = 423$ individuals, 418 seed families, 74 populations) were planted with SUREMIX (Michigan Grower Products, Galesburg, Michigan, USA) in 3.5 sq. in. pots without prior cold stratification. We included a variable number of seed families per population (range: 1–15, mean = 5.72 families), and all families but five were represented by a single individual (BON-2, BON-4, CPT-1, CPT-5, and CRU-1 each had two individuals measured). More information on the populations used in this experiment, plus the coordinates and maps of the original population locations, can be found in Figure 1 and Appendix S1: Table S1. All germinating seedlings except the centermost plant were removed from the pot, and remaining individuals were watered every other day throughout the experiment. Germination and flowering dates were recorded to determine flowering time; plant height at first flower (cm), width of the second set of true leaves (cm), corolla length (mm), and corolla width (mm) were measured at the time of first flowering.

Climate data

To investigate potential environmental drivers of adaptive phenotypic variation, we extracted climatic data for each population's collection location of origin from the WorldClim bioclimatic variables database (Fick and Hijmans, 2017). These data were imported and compiled in R version 4.2.1 (R Core Team, 2022) using the packages “stats” version 4.2.1, “nlme” version 3.1-157, “raster” version 3.6-26, and “sp” version 2.1-3.

In addition to the Bioclim data, we extracted oceanic wind speed data from along the U.S. Pacific coast using the National Renewable Energy Laboratory's (NREL) Wind Prospector website, which featured interactive online maps of wind speeds surveyed by NREL (King et al., 2014; Draxl et al., 2015a, b). We used Pacific Monthly Offshore Wind Speed map layers to collect wind speeds 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 × 1.2 km offshore site and were modeled at 100 m (Draxl et al., 2017). Due to unavailability of wind data outside of the contiguous USA, 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

Prior to data analyses, we calculated mean trait values for each population to avoid pseudoreplication. Our analyses included a binary factor to identify whether each population was exposed to (1) or protected (0) from the open ocean. Exposed populations were located on the cliffs, headlands, and seeps just adjacent to the open Pacific Ocean. In contrast, protected populations were not directly exposed to the open ocean by being located in inland roadside ditches and lagoons or on the coastlines of Washington, British Columbia, and Alaska in locations where they were protected from the open ocean by other landmasses. To test whether there was a relationship between plant traits and the latitude of origin (location from which each population accession was collected), we modeled trait values against latitude with the binary predictor for ocean exposure via linear regression in R using the “stats” package, evaluated predictor significance via analysis of variance (ANOVA) using the “car” package version 3.1-2, and visualized our results in “ggplot2” version 3.5.0. We modeled both additive and interactive relationships between the predictors for each trait, but in each case found that the additive model was a better descriptor of the data per Akaike information criterion (AIC) and ANOVA model comparison (both in the “stats” package).

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 component analysis (PCA) to reduce the dimensionality of the data prior to further analyses. We extracted climate data, described above, for each population and performed the PCA in R using the package “factoextra” version 1.0.7 and the “stats” package. We evaluated all principal components (PCs) with an eigenvalue > 1, following the Kaiser criterion (Kaiser, 1960). We then conducted regression analyses of population mean plant height against each of these PCs individually and with all as factors in the same model, again including the exposure factor and using linear models in the "stats" package. We also evaluated models with interactions and polynomial fits and selected the best descriptors for the data via AIC and ANOVA model comparison. For all regression analyses, we evaluated residuals with the package "DHARMa" version 0.4.6 and confirmed via visual inspection that residuals were normally distributed for all models. All data organization, analyses, and visualizations were conducted in R Studio (2023.12.0 + 369 "Ocean Storm" Release) (Posit Team, 2023).

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 ($R^2 = 0.43$, $F_{2,71} = 26.83$, $P < 0.001$; Figures 2 and 3). Based on our linear modeling, we found that every degree latitude farther north translated to a ~ 1 cm decrease in plant height under greenhouse conditions. We also found a significant difference in the height-latitude relationship depending on whether populations were highly exposed to the wind and salt spray from the open Pacific Ocean. In general, protected populations were taller than those exposed to the open ocean (Table 1; Figure 2). In addition to the strong relationship with height, we found significant but much weaker relationships between population latitude and width of the second set of true leaves ($R^2 = 0.12$, $F_{2,71} = 4.83$, $P = 0.011$), corolla width ($R^2 = 0.12$, $F_{2,63} = 4.38$, $P = 0.017$), and corolla length ($R^2 = 0.17$, $F_{2,63} = 6.51$, $P = 0.003$). Leaf width generally decreased with latitude, as did corolla width and length; none of these models included a significant term for ocean exposure (Table 1). The relationship of latitude to flowering time ($R^2 = 0.03$, $F_{2,71} = 0.97$, $P = 0.383$) was not significant. 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.

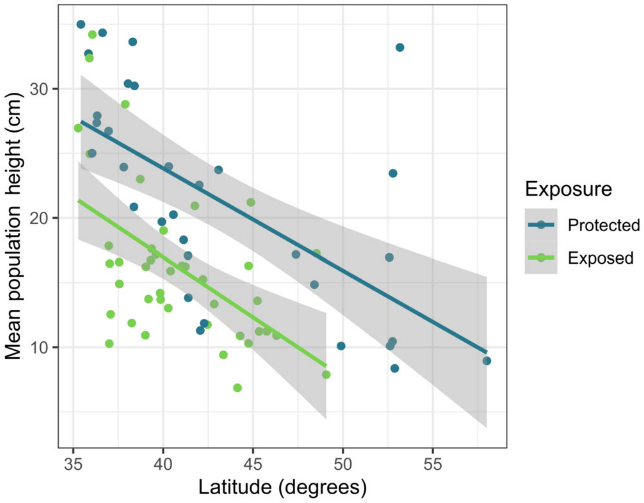

FIGURE 2 Mean *Mimulus guttatus* population height decreases significantly with latitude ($P < 0.001$), and exposed populations have shorter average heights than protected populations ($P < 0.001$); both population groups express the same relationship between height and latitude of origin. Relationship of latitude of origin (in degrees) for *M. guttatus* populations and mean individual heights (cm) of these populations was determined by linear regression.

The relationship of climatic factors and plant height

In our greenhouse experiment, we found that climate PC1 through PC3 all had eigenvalues > 1. In total, those three PCs accounted for 89.99% of cumulative variance in climate factors. PC1 explained the most variation at 56.39%, followed by PC2 with 22.81%, and PC3 with 10.79%. PC1 was significantly negatively correlated with plant height in a linear regression model (PC1 estimate = −1.09, exposure estimate = −5.69, multiple $R^2 = 0.52$, $F_{2,62} = 33.05$, $P < 0.001$). 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 $R^2 = 0.57$, $F_{3,61} = 27.23$, $P < 0.001$; AIC, linear = 402.52, AIC, quadratic = 396.46). The quadratic regression yielded the same qualitative relationships between mean population height and the climate factors as the linear model; height decreased with increasing values of PC1 (Figure 4). We also evaluated regression models of the remaining PCs against plant height. While each showed a significant relationship with height, PCs 2 and 3 included only significant terms for ocean exposure (Table 2). However, our model with the PC1 square term and ocean exposure as predictors was a better descriptor of the data based on AIC than models with PC2 and PC3 (Table 2). We also constructed a linear model with PCs 1–3 and exposure as predictors to compare its fit with our quadratic PC1 model, but yet again found PC1 to best describe the data via AIC (AIC, PC1 = 396.46, AIC, all PCs = 396.82). Following our tests for model fit, we focused on PC1's loadings for the remainder of our interpretations.

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 (Appendix S1: Table S2). Higher values of PC1 indicated lower

TABLE 1 ANOVA and linear regression summaries for *Mimulus guttatus* traits in the main greenhouse.

	Parameter summaries						**Full model summaries**			
Trait	**Predictor**	**Estimate**	**Sum sq.**	**df**	***F***	***P***	**Multiple R^2**	**df**	***F***	***P***
Flowering time (days)	Latitude	−0.0633	7.6	1, 71	0.120	0.7301	0.0267	2,71	0.974	0.3825
	Exposure	2.3836	98.4	1, 71	1.551	0.2171				
2nd leaf width (cm)	Latitude	−0.1633	50.56	1, 71	9.661	0.0027**	0.1198	2,71	4.83	0.0108*
	Exposure	−0.3736	2.42	1, 71	0.462	0.4990				
Corolla width (mm)	Latitude	−0.2036	71.82	1, 63	5.636	0.0207*	0.1221	2,63	4.383	0.0165*
	Exposure	1.0034	15.13	1, 63	1.187	0.2800				
Corolla length (mm)	Latitude	−0.2106	76.84	1, 63	7.008	0.0102*	0.1713	2,63	6.511	0.0027**
	Exposure	1.4334	30.87	1, 63	2.816	0.0983				
Height (cm)	Latitude	−0.8297	1305.94	1, 71	39.017	2.738e−08***	0.4304	2,71	26.83	2.099e-09***
	Exposure	−7.0716	865.79	1, 71	25.867	2.863e-06***				

Asterisks denote significance at P < 0.05 (*), P < 0.01 (**), and P < 0.001 (***).

TABLE 2 ANOVA, linear regression, and AIC summaries for models evaluating the relationship of selected principal components to *Mimulus guttatus* height in the main greenhouse.

	Parameter summaries						**Full model summaries**				
Principal component	**Predictor**	**Estimate**	**Sum sq.**	**df**	***F***	***P***	**Multiple R^2**	**df**	***F***	***P***	**AIC**
PC1	(Dim. 1)^2	13.923	1193.90	2, 61	25.044	1.147e-08***	0.5725	3, 61	27.23	2.679e-11***	**396.462**
	Exposure	−6.048	533.47	1, 61	22.381	1.368e-05***					
PC2	Dim. 2	0.0520	0.94	1, 62	0.022	0.883	0.2217	2, 62	8.831	4.219e-04***	433.403
	Exposure	−7.0733	752.57	1, 62	17.628	8.736e-05***					
PC3	Dim. 3	0.0663	0.71	1, 62	0.017	0.898	0.2217	2, 62	8.828	4.23e-4***	433.408
	Exposure	−7.0856	743.63	1, 62	17.417	9.524e-05***					

Asterisks denote significance at P < 0.5 (*), P < 0.01 (**), and P < 0.001 (***). Lowest AIC is in bold.

average annual temperature and precipitation seasonality, but greater 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 (Appendix S1: Table S2). Therefore, any population at a location corresponding to a high value of PC1 experienced lower temperatures, higher amounts and greater consistency of precipitation, and faster wind speeds than populations at sites with a low PC1 value.

Latitude strongly predicts climate for coastal populations

To assess how well latitude predicts climate at the sites of our study populations, we regressed the extracted principal components from our greenhouse experiment against latitude. Our analysis confirmed that many of the climate variables involved were highly correlated with latitude. PC1 was the only principal component significantly associated with latitude, and the two variables had a positive relationship (slope = 0.89, multiple $R^2 = 0.92$, $F_{1,63} = 704.9$, $P < 0.001$). To determine how well variation in individual climatic factors was predicted by latitude, we regressed all the climate factors that were highly loaded on PC1 individually against latitude (Table 3).

Patterns of wind speed along the west coast of North America

Plants are well known to respond to wind through changes in growth form (Grace, 1977; Gardiner et al., 2016; Telewski, 2021). This, combined with the observation of wind speed loading onto the first PC of climate, led us to evaluate the patterns of wind speed variation more carefully across the populations that we studied (Figure 5). We found that, in general, annual wind speeds increase with latitude (slope = 0.07, multiple $R^2 = 0.06$, $F_{1,76} = 4.465$, $P = 0.038$). However, the relationship between wind speeds and latitude varied dramatically based on time of year. Average wind speeds in quarter 1

TABLE 3 Regressions of climate factors that were highly loaded on PC1 against latitude.

Climate factors	Estimate	F	Multiple R^2	P
Annual precipitation	0.0495	231	0.7857	<2.2e-16
Coldest quarter precipitation	0.1116	136.4	0.684	<2.2e-16
Driest quarter precipitation	0.6088	405.3	0.8655	<2.2e-16
Mean annual temperature	−2.5849	350.2	0.8475	<2.2e-16
Mean wind speed, 1st quarter	2.4760	53.32	0.4584	5.94E-10
Mean wind speed, 4th quarter	1.8632	64	0.5039	3.57E-11
Precipitation seasonality	−0.3049	549.7	0.8972	<2.2e-16
Warmest quarter precipitation	0.6092	483.8	0.8848	<2.2e-16
Wettest month precipitation	0.3099	137.1	0.6852	<2.2e-16
Wettest quarter precipitation	0.1054	159.6	0.7169	<2.2e-16

(slope = 0.19, multiple $R^2 = 0.46$, $F_{1,76} = 65.9$, $P < 0.001$) and quarter 4 (slope = 0.27, multiple $R^2 = 0.50$, $F_{1,76} = 75.32$, $P < 0.001$) of the year were strongly positively correlated with latitude. In contrast, quarter 2 wind speeds (slope = −0.20, multiple $R^2 = 0.20$, $F_{1,76} = 19.1$, $P < 0.001$) showed a negative relationship with latitude and quarter 3 wind speeds were not correlated with latitude (slope = 0.05, multiple $R^2 = 0.01$, $F_{1,76} = 0.57$, $P > 0.05$).

Finally, we constructed a linear model to evaluate how mean population height varied with annual mean wind speeds for each population from which accessions were collected. We also included the ocean exposure term in this model. While annual mean wind speed had only a marginally significant effect on plant height (estimate = −1.29, $P = 0.073$), open ocean exposure made a clear difference (estimate = −6.98, $P < 0.001$) and the model was a significant fit for the data (multiple $R^2 = 0.26$, $F_{2,62} = 10.95$, $P < 0.001$). In general, *M. guttatus* populations exposed to the open ocean were shorter than their protected counterparts (Appendix S1: Figure S1).

DISCUSSION

We found a significant role of latitude and exposure to the open ocean in predicting genetically based trait variation among populations of the coastal perennial ecotype of *M. guttatus*. The trait most affected by these two factors was plant height, which was smaller for populations collected from locations exposed to the open ocean and was negatively correlated with latitude. Multiple environmental factors may have contributed to the evolution of these patterns of trait variation. These southern populations generally experience higher temperatures, less precipitation, and lower-velocity winds than more northern populations, but this can vary based on the time of year. Further, populations exposed to the open ocean often experience greater wind speeds and more toxic salt spray, which is a byproduct of the wave energy of the ocean (Boyce, 1954; Du and Hesp, 2020). The results of this study further reinforce the idea that genetically based phenotypic variation can be simultaneously partitioned in disjunct (among coastal perennial and inland annual ecotypes) and continuous, clinal ways (within ecotypes). We found similar overall results in a pilot experiment (see Appendix S1), indicating that our findings are replicable.

Phenotypic variation is partitioned at multiple levels within 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 *M. guttatus* from central California to southern Washington 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 *M. 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). Our results demonstrate that while the coastal perennial ecotype is strikingly divergent from the inland annual ecotype, it also harbors genetically based phenotypic variation that is partitioned along two major environmental gradients. Latitudinal clines within ecotypes/species have been observed in the classic literature for plant systems such as *Plantago maritima* (Gregor, 1930, 1938) and *Achillea borealis* (Clausen et al., 1948). Similarly, differences in traits including plant height have been found recently for coastal plants that occur on dunes versus headlands in *Senecio lautus* (Roda et al., 2013; James et al., 2021). However, in contrast to *M. guttatus*, the evolution of short headland versus tall dune ecotypes in *S. lautus* has occurred independently through repeated parallel evolution (Roda et al., 2013; James et al., 2021) instead of occurring all within a single coastal ecotype. 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 and 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 geographic distribution of trait variation

While we have demonstrated that clines in plant height occur within the coastal ecotype of *M. guttatus*, the question remains as to what environmental factors have driven these clines. 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. Regardless of which environmental variables dictate the shape of the height cline, we have shown that exposure to the open ocean is predictive of shorter plant height in *M. guttatus* populations (Figure 2; Appendix S1: Figure S1). These results are consistent with classic observations by Boyce (1954) and recent studies in *Setaria viridis* (Itoh, 2021; Itoh et al., 2024).

FIGURE 3 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 in a protected area of Monterey Bay along the central California coast reach ≤2 m in height. (C) Plants exposed to the open ocean in Oregon are much more compact in growth form.

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 be driving, directly or indirectly, 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 between temperature and plant height has been observed within species for altitudinal gradients (Totland and Birks, 1996; Macek and Leps, 2008; Pellissier et al., 2010), but other factors could also drive the overall negative association of elevation and height. Whatever combination of factors drove the relationship of plant height and latitude, those same factors did not drive the evolution of a clinal relationship in other often locally adaptive traits like flowering time.

Perhaps the most compelling factor driving patterns of plant height is the combination of wind and salt spray. While wind is less closely correlated with latitude than other environmental factors, whether a population is exposed to the open ocean or is protected from salt spray by being located slightly inland can have a large effect on the impacts of wind and salt spray on plants (Barbour, 1978; Du and Hesp, 2020). 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 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). In winter months (quarters 1 and 4; e.g., Figure 5B), there is a strong correlation between latitude and wind speeds, which could play a role in the evolution of shorter plant heights in more northern populations of this perennial plant ecotype. However, wind speed patterns are complex and their relationship with latitude shifts greatly over the year. 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 in the summer (July–August) (Gilliam, 2002; García-Reyes and Largier, 2012). However, those wind speeds dissipate most during summer in the region south of San Francisco, especially in more protected areas like the southern Monterey Bay (Figure 3B), where plants in the field reach up ≤2 m in height (D. B. Lowry, personal observation). In northern California and southern Oregon, high coastal wind speeds remain elevated throughout the summer months, which coincides with the time 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 wind speed or salt spray conditions is likely to be advantageous. Field experimentation will be necessary to deconstruct how the time of year interacts with potential selection imposed by wind speeds and salt spray.

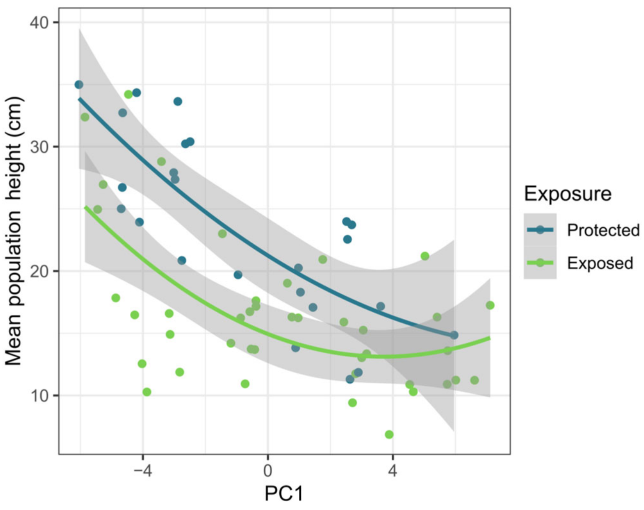

FIGURE 4 Mean *Mimulus guttatus* population height decreased significantly and nonlinearly with increasing values of climate PC1 in the main experiment ($P < 0.001$); exposed populations were also significantly shorter than protected populations but maintained the same relationship to PC1 ($P < 0.001$). The figure displays the quadratic relationship between climate PC1 and plant height, which was the best-fitting model as determined by AIC.

CONCLUSIONS

Overall, our study demonstrates that there is dramatic and likely important phenotypic variation distributed within distinct ecotypes contained within species. Therefore, as in other species, like switchgrass (*Panicum virgatum*; Lowry

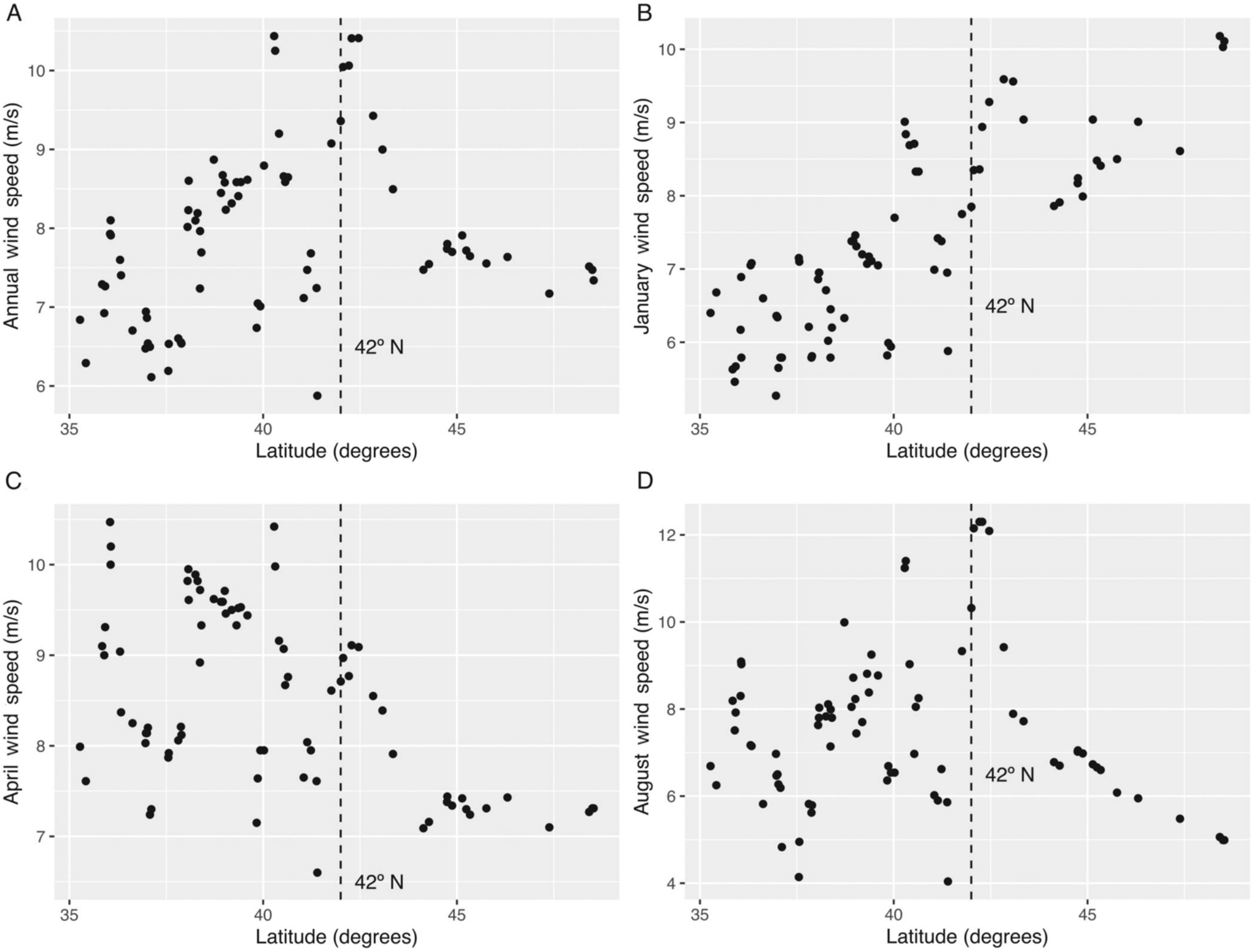

FIGURE 5 Mean wind speeds (m/s) along the U.S. Pacific coast reported annually (A), during January (B), April (C), and August (D). The dashed line indicates 42° N, the latitude of the Oregon coast at which wind speeds start declining going farther north.

et al., 2014), it is critical to account for both ecotypic and clinal variation when conceptualizing how functional genetic variation is distributed within a species. The exact causes of the clines within the coastal perennial ecotype of *M. guttatus* are still unclear, especially for trait correlations with latitude. However, the fact that exposure to the open ocean is predictive of plant height suggests that wind and salt spray may play a crucial role in driving genetically based variation in plant height in this system.

AUTHOR CONTRIBUTIONS

D.B.L. conceived of and oversaw the experiments and analyses. T.Z. conducted the experiments and analyses. Both authors wrote the manuscript.

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DATA AVAILABILITY STATEMENT

All data are available as supplementary information or on Dryad at https://doi.org/10.5061/dryad.4tmpg4fkh (Zambiasi and Lowry, 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Pilot experiment and supplementary tables/figures.

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