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DIFFERENTIATION IN PHENOTYPIC PLASTICITY TO CLIMATE AMONG *ERIOGONUM FASCICULATUM* SUBSP. *FOLIOLOSUM* (POLYGONACEAE) POPULATIONS IN CALIFORNIA

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ABSTRACT

California, a hotspot of plant biodiversity, is projected to experience a significant increase in average temperature by the end of the century, from +1.5°C under low emissions to +4°C under medium-high emissions models. This warming could severely affect unique, endemic ecosystems like the chaparral. Phenotypic plasticity, the capacity of organisms to express different phenotypes in response to environmental cues, may be an important means by which plants adjust to climate change. Here, we examine differentiation in two traits related to climate adaptation, leaf length and trichome density, previously observed to differ qualitatively between coastal and inland populations of *Eriogonum fasciculatum* subsp. *foliolosum* (Nutt.) S.Stokes. We quantitatively verify these leaf trait differences, assess whether they are plastic to temperature, and also assess whether coastal and inland populations express this plasticity differently. We performed field collections, established a novel procedure for vegetative propagation of woody cuttings, and grew cuttings and germinated seeds in temperature treatments simulating current conditions and those projected by future climate models. Our results revealed that leaf traits of both populations respond plastically to temperature, and we also found differentiation for the plasticity expressed by the two populations. Population genetic analyses detected limited genetic differentiation and high gene flow between these two populations, indicating that the observed differentiation in plasticity may be locally adaptive. Together, these findings suggest that this system may exemplify genetic accommodation, in which the evolution of plasticity mediates adaptation, and imply that variation in plasticity is relevant to conservation strategies for this plant and other California native chaparral shrubs in the face of climate change.

Key Words: California Buckwheat, chaparral, *Eriogonum fasciculatum*, genetic accommodation, local adaptation, phenotypic plasticity, trichomes, vegetative propagation.

The California Floristic Province is one of the few global biodiversity "hotspots". Of the more than 5500 distinct species, subspecies, or varieties of native flora found here, 40% (2387) are endemic; their native ranges are restricted to only this area (Loarie et al. 2008). Many of these endemic taxa are found in chaparral, a habitat characterized by hot, dry summers and rainy winters typical of Mediterranean climates. Chaparral landscapes are dominated by low-lying woody evergreen shrubs adapted to grow rapidly during the rainy season and then withstand hot summer temperatures accompanied by periodic drought and fire regimes (Cooper 1922). Due to their hardiness and ability to cope with a changing environment throughout their development, chaparral plants present strong model systems to study how plants respond to environmental change.

The California climate is projected to change considerably over the next century. Simulations that apply Intergovernmental Panel on Climate Change Fourth Assessment predictions (IPCC 2007) to generate models with different sensitivity to forcings (such as CO_2 levels) under different emissions scenarios predict that by end-of-century California will experience an increase in mean temperature somewhere from 1.5°C in the most optimistic, low emissions scenario to 4.5°C assuming medium-high emissions (Cayan et al. 2008). These changes to the climate are projected to have drastic effects on California flora. For instance, Rehfeldt et al. (2006) predicted that only 22% of the geographic envelope surveyed will harbor the same communities as at present.

Plant populations may be able to persist in the new and changing conditions by dispersal to new suitable habitats or by adaptation. However, these processes may require many years, especially for species with short dispersal distances or long

generation times (Dullinger et al. 2004; Chevin et al. 2010). In the short term, phenotypic plasticity, the ability of one genotype to exhibit different phenotypes when exposed to different environmental conditions, may be the most important line of defense for a population against local extirpation in the face of climate change (Nicotra et al. 2010; Gratani 2014). If plastic responses are sufficient to promote population persistence, subsequent generations may then be able to adapt genetically to the new environment through natural selection, a possible scenario known as the Baldwin effect (Crispo 2007). Many ecophysiological traits demonstrate phenotypic plasticity to climatic factors, including temperature (Ackerly et al. 2000). Because plants in Mediterranean environments are adapted to experience ample seasonal environmental heterogeneity in temperature and precipitation, phenotypic plasticity likely plays a key role in regulating the morphologies, physiologies, and phenologies of chaparral plants. Indeed, plants in the matorral, a Mediterranean ecosystem in Spain similar to the California chaparral, exhibit phenotypic plasticity for multiple traits as a seasonal response to the hot, dry summers when plants experience high solar radiation, high temperatures, and water stress (Zunzunegui et al. 2007).

In the California chaparral, the widespread evergreen shrub *Eriogonum fasciculatum* Benth., commonly called California Buckwheat, exhibits substantial phenotypic variation and is present in disparate environments (Montalvo 2004; Montalvo and Beyers 2010). Four common genetically distinct subspecies have been defined as ecotypes locally adapted to particular habitats, including coastal *E. fasciculatum* subsp. *fasciculatum* and interior *E. fasciculatum* subsp. *polifolium* (Benth.) S.Stokes (Cole 1967). One of the most widespread varieties, *Eriogonum fasciculatum* subsp. *foliolosum* (Nutt.) S.Stokes occurs in both coastal and inland habitats. It is tetraploid ($2n = 80$) relative to the other subspecies ($2n = 40$), likely as a consequence of an allopolyploidy event involving hybridization of subsp. *polifolium* and subsp. *fasciculatum* (Stebbins and Major 1965; Sanchez and Kron 2008).

Although much phenotypic variation is partitioned discretely among different subspecies of *E. fasciculatum*, with each genetically distinct ecotype occupying specific habitats, continuous phenotypic differentiation is also observed among proximate populations of the same subspecies, possibly due to phenotypic plasticity. For instance, coastal and inland populations of *E. fasciculatum* subsp. *foliolosum* were qualitatively observed to have distinct morphological traits in the Santa Monica Mountains of Southern California. Coastal populations of *E. fasciculatum* have fewer hairs known as trichomes on the leaf surface, and the trichome density increases in a cline going inland (Cole 1967). Leaf pubescence is often an adaptation that protects the leaf from solar radiation and high temperatures, as well as deters herbivores (Ehleringer et al. 1976), and this character is often plastic (Holeski 2007). Cole (1967) also observed that leaf length, a trait related to thermoregulation through impacts on leaf shape and area (Nicotra et al. 2011), differed between inland and coastal populations.

Eriogonum fasciculatum subsp. *foliolosum* is the most geographically widespread subspecies and occurs in multiple environments. Therefore, Cole (1967) hypothesized that the variation in leaf pubescence and length observed among coastal and inland populations of this subspecies results from plasticity. Trichomes and leaf length are quantifiable and can indicate physiological functions such as transpiration and photosynthetic rates (Bickford 2016). Plasticity for trichome density and leaf length, if present, may be in response to different temperature cues between coastal and inland sites. Given projected climatic changes, understanding how this subspecies responds to temperature change could help inform future conservation strategies.

Here, we aim (1) to confirm whether the pattern observed by Cole (1967) persists and (2) to determine whether *Eriogonum fasciculatum* subsp. *foliolosum* exhibits phenotypic plasticity for trichome density and leaf length in response to variation in temperature as hypothesized. To do so, we took a multipronged approach that involved field observations, vegetative propagation, direct growth from seed, and population genetics. Cuttings and seeds were collected from inland and coastal populations in Topanga Canyon State Park (Los Angeles Co.), a site where all populations are potentially interbreeding because there is no habitat fragmentation between the coastal and inland sites. To examine plasticity of vegetative characters in response to temperature, clonal cuttings and seedlings were grown in a common garden experiment in growth chambers under different temperature treatments that simulated future climate scenarios. In addition, we surveyed genetic diversity and characterized population structure to determine whether any genetically based phenotypic differentiation in trait means or plasticity persists despite gene flow, which would indicate this differentiation may reflect local adaptation.

Methods

Field Collections

At Topanga Canyon State Park (Los Angeles Co.), 15 coastal individuals were systematically sampled along a transect at Los Liones Trail, and 15 inland individuals were sampled along Santa Ynez Canyon Trail (Fig. 1A). Collections were made during the late morning and afternoon of 28 October 2018. The high and low temperatures that day, 84°F (29°C) and 69°F (21°C), respectively, were relatively warm for that month, which had average high and low temperatures of 75°F (24°C) and 62°F (17°C), respectively, at those locations (NOAA NCEI, 2018,

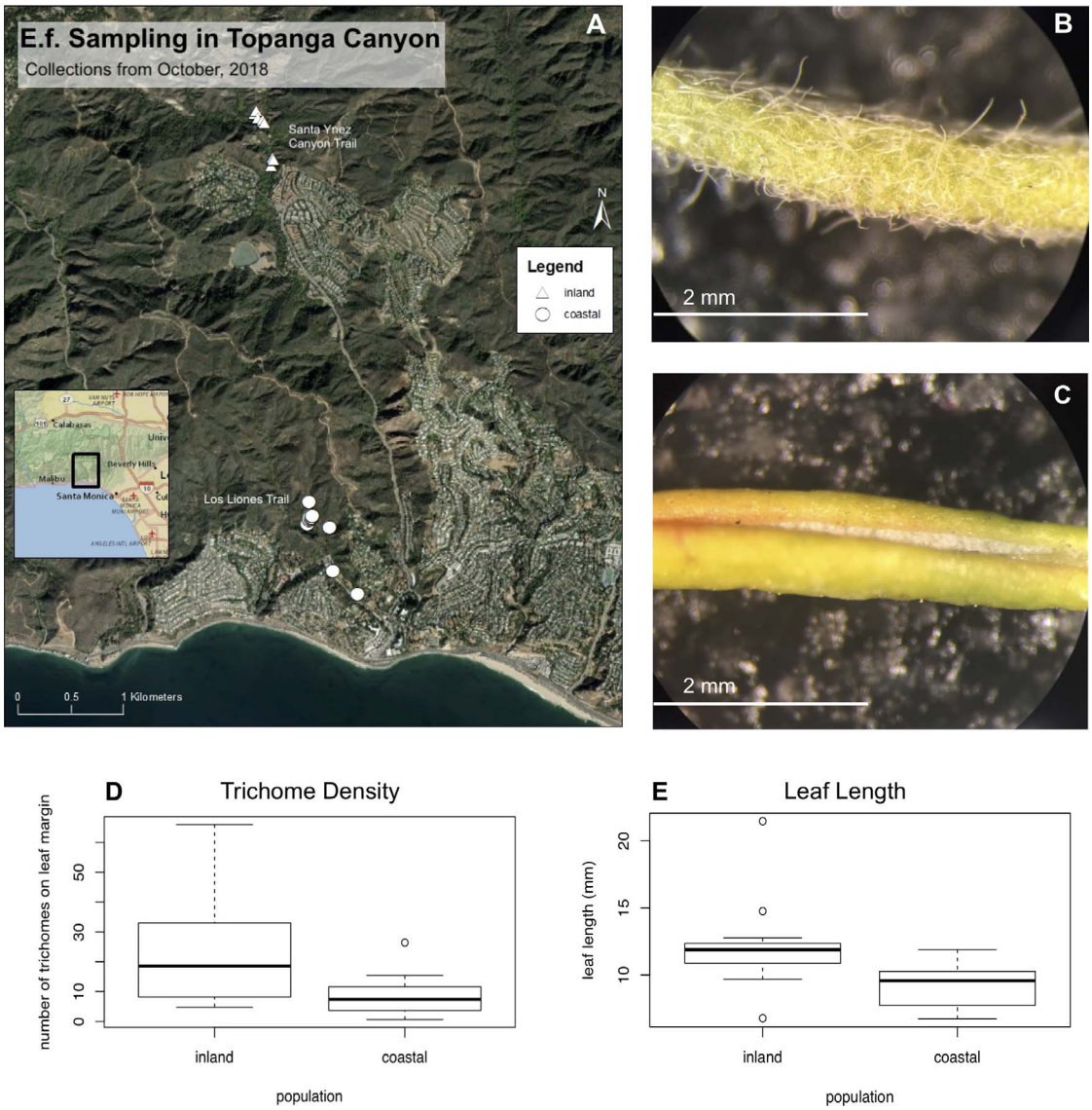

FIG. 1. (A) Sampling locations of *E. fasciculatum* plants. Inland plants (triangles) sampled along Santa Ynez Canyon Trail (~34.0804094°N, –118.5662666°W) and coastal plants (circles) sampled along Los Liones Trail (~34.0461893°N, –118.5597197°W) in Topanga Canyon State Park (Los Angeles Co.). Differences in trichomes between plants collected from (B) inland and (C) coastal populations. Distributions of (D) trichome density and (E) leaf length for the coastal and inland populations of *E. fasciculatum* in the field. Boxplots show medians (line), upper and lower quantiles (box), minimums and maximums (whiskers), and outliers.

Malibu Hills station). Collections were made from plants at least 10 m apart unless too few plants were present at a site to do so. For each of the 15 plants at each location, six stem cuttings ~15 cm in length and with approximately seven nodes of healthy leaves were obtained using sharp scissors. Five cuttings were prepared for propagation by storing them in a moist paper towel in a cooler, and the remaining cutting was dried and preserved. We collected three to six dried calyxes (depending on size) per plant and stored the unprocessed seeds in coin envelopes.

Vegetative Propagation

No protocol for vegetatively propagating wild *Eriogonum* cuttings has been previously published, but the plants have been successfully propagated in restoration and horticultural settings (D. Kopp, U.S. Forest Service, personal communication). The following procedure was accomplished by synthesizing some of these techniques, especially drawing on those applied at Moosa Creek Nursery (S. Kraus, Moosa Creek Nursery, personal communication).

TABLE 1. PROPAGATION AND GERMINATION RATES FOR INLAND AND COASTAL POPULATIONS OF *E. FASCICULATUM* IN THE TEMPERATURE TREATMENTS AND CHI-SQUARE CONTINGENCY TEST RESULTS. n = the number of seedlings that germinated total across all conditions from that source population. Significant P-values ($\alpha = 0.05$) are indicated in bold.

Population	Propagation success rate	Germination rate: greenhouse	Germination rate: warm	Germination rate: cool	n	χ^2 (df)	P-value
Inland	61.33%	26.67%	26.67%	40.0%	14	0.829 (2)	<0.95
Coastal	68.0%	33.33%	6.67%	66.67%	16	11.832 (2)	**<0.005**

Within 24 hr of collecting a cutting, leaves were removed from the lower ~4 cm of its stem, and a fresh diagonal cut was made near its lower end using sharp scissors. The remaining lower ~3 cm of the stem was then dipped in a 1:4 by volume bleach : water solution to prevent fungal growth and then immersed for 3–5 s in a 1:5 dilution of commercially available rooting hormone solution (Dip'n'Grow, Clackamas, OR). Cuttings were then inserted into wetted 100% perlite and placed on a misting bench. After 3 wk of growth on the mist bench, most stems had rooted sufficiently to transplant. Perlite was then removed from the roots, and the cuttings were transplanted into individual 5 × 10 × 10 cm pots with a well-draining potting mix (Sunshine #4, Sungro, Agawam, MA). The cuttings were then grown under the specific temperature regimens described below.

This procedure yielded a fairly high rate of successful establishment: 61.33% and 68.0% of cuttings rooted for inland and coastal stems, respectively (Table 1). On average, the cuttings accumulated 10.71 cm of new stem growth during the first month and 11.18 cm during the second month across all populations and treatments.

Seed Processing and Germination

For each envelope of calyxes collected from the field, seeds were separated from chaff using a sieve. We then germinated ~20 seeds per parent plant by gently pressing them into the surface of moist, well-draining potting soil in ~5 × 10 × 10 cm pots so they were exposed to light, which is necessary for germination (Montalvo and Beyers 2010). Pots were gently top watered as needed to maintain a moist soil surface throughout germination, which took place either in the growth chamber or greenhouse conditions.

Growth Chamber Experiment

Ten cuttings and 15 seeds from plants from each population (one inland, one coastal) were grown together in two E15 growth chambers (Conviron, Winnipeg, Manitoba, Canada) and in a greenhouse (60 cuttings and seeds from 90 plants in total across the three growth conditions). The chambers were both set to 12.5 hr of light per day, mimicking spring conditions best for germination, but each was set to different temperature regimens to simulate future climate change. The cooler growth chamber was set to 22°C d : 12°C nights, and the warmer chamber was set to 25°C d : 12°C nights. After 1 mo of growth, the daytime temperatures in both chambers were increased by 1.5°C (i.e., 23.5°C for the cool chamber, and 26.5°C for the warm chamber). Thus, we observed growth of genetically identical cuttings and of seeds from the same 30 plants in four experimental temperature conditions: 22, 23.5, 25, and 26.5°C. Treatments simulated projected climate change above a baseline of the current springtime average temperature of 22°C, by +0, +1.5, +3, and +4.5°C, respectively. Leaf samples were taken from the propagules for phenotyping initially before the treatments started (12/05/2018), after 1 mo of growth at the first experimental temperature (01/14/2019), and after one more month of growth at the second (02/17/2019). To ensure leaves were initiated in the second month, samples were only taken from the top 10 cm of new stem growth. Due to differential germination rates, leaf samples were only obtained from the seedlings after 2 mo of growth in each chamber and the greenhouse. Precise temperature measurements of greenhouse temperatures were not available. However, outside temperatures remained largely consistent on average for the duration of the experiment, averaging 52.0°F (11.1°C) and 51.7°F (10.9°C) during the first and second months, respectively (NOAA NCEI, 2018, Berkeley Station).

Phenotyping

Based on preliminary sampling, we determined that the average trichome density obtained from five leaves per stem serves as a representative measurement of this trait, which may vary greatly even among leaves on an individual stem. Trichome density was measured against a black background under a dissecting scope at 50× power along a 4 mm section of leaf (the diameter of the field of view; Fig. 1B). We counted the white trichomes protruding from both sides of the leaf margin. Because *E. fasciculatum* leaves are curled, we measured leaf length from base to tip with a ruler as a proxy for leaf size.

Population Genetics

To assess population structure, we first collected leaf samples from each genetically distinct individual—one cutting per each of the 15 inland and 15 coastal individuals, and 46 seedlings (n = 76)—into racked cluster tubes (Corning), froze them in liquid nitrogen, and maintained them at –80°C until DNA extraction. We then disrupted the frozen leaf tissue with beads in a 1600 MiniG Automated Cell Lyser

TABLE 2. *ERIOGONUM* MICROSATELLITE MARKERS DEVELOPED BY RILEY ET AL. 2011. Forward (Fwd) and reverse (Rev) primer sequences. Lower case bases indicate appended M13 dye primer overlap sequence. An asterisk (*) indicates loci with poor amplification that were excluded from downstream analysis.

Primer set	Direction	Sequence	M13 dye
Erar_85	Fwd	cacgacgttgtaaaacgacAGTGGCACGTGTTGAAACC	VIC
	Rev	GTTTCTTGTTGGGTGTCTTAGTGGCG	
Egic_82	Fwd	cacgacgttgtaaaacgacCAGCTGGGTTTGCATGTCC	NED
	Rev	GTTTCTTAAAGCAGCAAGACCTGTTATC	
Egic_110	Fwd	cacgacgttgtaaaacgacGTGTCACAAATGGGAAAGCAC	FAM
	Rev	GTTTCTTTGGCAGATAGTTTGGTGGAA	
Ergr_43	Fwd	cacgacgttgtaaaacgacCCAACACTAACATCCAAATTCTATC	PET*
	Rev	GTTTCTTAGCGCTTCAAAAGATGGTGG	
Ergr_308	Fwd	cacgacgttgtaaaacgacCCCACACTCTCCAAACCAAT	PET*
	Rev	GTTTCTTGGCAAAGAGGGTGAAAGAGA	
Egic_96	Fwd	cacgacgttgtaaaacgacTGACACGGCCTTTTCTTTGC	VIC
	Rev	GTTTCTTAGAAGGCACATCCGTAGCG	
Ergi_99	Fwd	cacgacgttgtaaaacgacAGCTCCCCATCTCTCTCTTC	FAM
	Rev	GTTTCTTCTCTCTTCACGCTCTCTTGC	

(SPEX, Metuchen, NJ) and extracted DNA using a CTAB protocol modified for high-tannin content (Doyle and Doyle 1990; Porebski et al. 1997). For each DNA sample, we then amplified seven microsatellite loci using primer pairs following reaction conditions previously successful for *E. fasciculatum* (30 cycles with 57°C annealing temperature, 8 cycles with 53°C annealing temperature; Riley et al. 2011; Table 2). Each marker was amplified by PCR with GoTaq (Promega) on a thermocycler (Applied Biosystems) following an M13-labelling protocol (Boutine-Ganache et al. 2001; Table 2). Each 10 uL PCR reaction included 2 uL template DNA, 0.1 uL GoTaq, 2 uL manufacturer supplied 5× buffer, 0.9 uL 25 mM $MgCl_2$, 0.025 uL 10 mM forward primer stock, 0.2 uL 10 mM reverse primer stock, 0.2 uL labelled M13 primer, and 0.2 uL 10 mM dNTPs in ddH_2O. The resulting seven marker PCR products were pooled (2 μL/marker/sample) by individual with Hi-Di Formamide and LIZ 500 ladder and then denatured. After sample denaturation, we conducted fragment analysis on a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) in the UC Berkeley Museum of Vertebrate Zoology Evolutionary Genomics Lab. Fragment read lengths were called using Geneious v. 2019.1 (19 February 2019; https://www.geneious.com). Fragments were scored as diploid. We rarely observed more than two alleles in one individual, and thus the allopolyploid nature of the genome likely did not significantly impact marker amplification or our findings. Because our analysis revealed that the two markers labeled with PET did not amplify well, we excluded those two markers from downstream analyses.

Data Analysis

Average trichome density and leaf length for each plant were calculated in Microsoft Excel. The data were then analyzed in R (version 3.5.1) and R Studio (version 1.1.456) using the *ggplot2* package (Wickham 2016). Various two-way ANOVAs were performed to test for significant impact(s) of population, time, treatment condition, or interaction terms on trichome density and leaf length. Residuals and Normal Quantile-Quantile plots were generated, and a Shapiro-Wilk test was run for each dependent variable to ensure the assumption of normality was met. Tukey's Honest Significant Difference post-hoc tests were performed to determine which pairwise contrasts significantly differed. One outlier individual was excluded, as it had been collected from an inland location with different abiotic conditions and was quite divergent from the rest. A Chi-square Contingency Test was performed on seedling data to ascertain whether germination success rate in each population was independent of temperature. The microsatellite data from five markers were analyzed using STRUCTURE version 2.3.4 to determine the number of genetically distinct populations (Pritchard et al. 2000). STRUCTURE output files were analyzed and visualized using the Cluster Markov Packager Across K algorithm (CLUMPAK; Kopelman et al. 2015). Using the GenAlEx (Peakall and Smouse 2012) software package, we summarized patterns of allelic variation and heterozygosity by marker and population, and we calculated two metrics of genetic differentiation based on heterozygosity (G''_{ST}) or the effective number of alleles (D_{est}). The former metric may not be fully robust because *E. fasciculatum* subsp. *foliolosum,* a polyploid, violates the assumption of diploidy, but the latter metric is independent of population size and ploidy (Miermans et al. 2018).

RESULTS

Ecotypic Trait Variation in Field and Controlled Conditions

Leaf traits differ between coastal and inland populations in the field. Cole (1967) had qualitatively observed greater trichome densities on leaves of

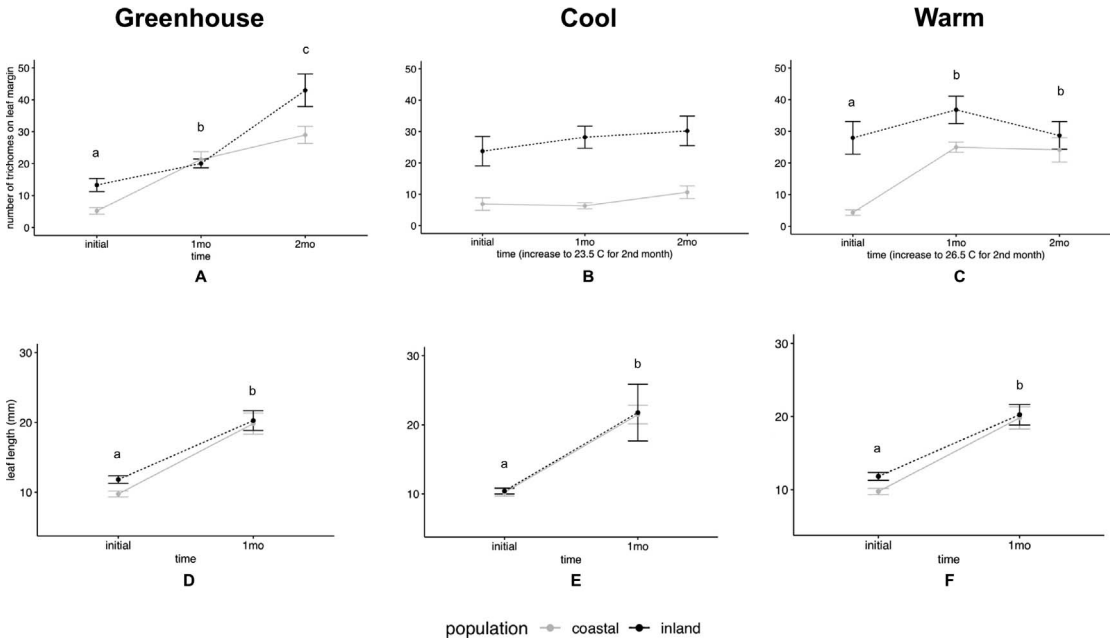

FIG. 2. Leaf traits of *E. fasciculatum* cuttings by treatment. Trichome densities over 2 mo of growth in the (A) greenhouse (B) cool growth chamber and (C) warm growth chamber. Leaf length after 1 month of growth in each treatment (D–F). Temperature increased from 1st–2nd month from 22–23.5°C in the cool chamber, and from 25–26.5°C in the warm chamber. Different letters indicate a significant difference in cross-population means between timepoints based on Tukey post-hoc tests. Solid circles with error bars show the means and standard errors for each population.

inland *E. f.* subsp. *fasciculatum* individuals relative to coastal individuals. To confirm this observation quantitatively, we measured trichome density and leaf length for samples obtained from inland and coastal populations at the field site (Fig. 1B, C). Leaves of inland plants had significantly more trichomes (Welch's T-test, $t[16.93] = 3.0941$, $P = 0.00667$, $n = 30$) and were longer (Welch's T-test, $t[21.584] = 3.1828$, $P = 0.0044$, $n = 30$) than leaves of coastal plants (Fig. 1D, E).

Plasticity within greenhouse and growth chambers by time. In the greenhouse, where plants experienced ambient temperature fluctuations, inland cuttings initially started with higher trichome densities and longer leaves on average than the coastal cuttings (Fig. 1D, E). After 1 mo of growth in the greenhouse, new leaves of both populations had increased trichome densities, converging to a similar phenotype (Fig. 2A). Following a second month of growth, trichome densities were even further elevated for leaves on cuttings of both populations, but this increase was more drastic for the inland cuttings relative to the coastal, demonstrating a significant population-by-environment interaction (Fig. 2A, Table 3). We observed that leaves were also longer after the cuttings were grown for a month, in the greenhouse and in both growth chambers as well, but we detected no significant differentiation between inland and coastal samples for this trait (Fig 2D, E, F, Table 3).

For the cuttings in the cool chamber, no significant plasticity to temperature or population-by-environment interaction for trichome density was detected; the populations maintained their initial differentiation in this trait across time and temperature conditions (Fig 2B, Table 3). In contrast, we

TABLE 3. ANOVA RESULTS FOR EACH TREATMENT BY TIME. GH = Greenhouse, Warm = Chamber set to 25°C and 26.5°C, Cool = Chamber set to 22°C and 23.5°C, Initial, after 1 mo, and/or After 2 Mo of Growth. Significant P-values ($\alpha = 0.05$) are indicated in bold.

Trait (treatment)	Population	Time	Pop. × time
Trichomes (GH)	$F_{1, 48} = 8.214$; $P = \mathbf{0.006}$	$F_{2, 48} = 47.653$; $P < \mathbf{0.001}$	$F_{2, 48} = 4.240$; $P = \mathbf{0.020}$
Leaf length (GH)	$F_{1, 32} = 1.496$; $P = 0.230$	$F_{2, 32} = 33.309$; $P < \mathbf{0.001}$	$F_{2, 48} = 0.499$; $P = 0.485$
Trichomes (warm)	$F_{1, 44} = 21.615$; $P < \mathbf{0.001}$	$F_{2, 44} = 9.575$; $p < \mathbf{0.001}$	$F_{2, 32} = 3.606$; $P = \mathbf{0.035}$
Leaf length (warm)	$F_{1, 30} = 0.111$; $P = 0.741$	$F_{1, 30} = 48.719$; $P < \mathbf{0.001}$	$F_{1, 30} = 1.024$; $P = 0.320$
Trichomes (cool)	$F_{1, 45} = 58.429$; $P < \mathbf{0.001}$	$F_{2, 45} = 1.456$; $P = 0.244$	$F_{2, 45} = 0.315$; $P = 0.731$
Leaf length (cool)	$F_{1, 29} = 0.244$; $P = 0.625$	$F_{1, 29} = 43.263$; $P < \mathbf{0.001}$	$F_{1, 29} = 0.001$; $P = 0.981$

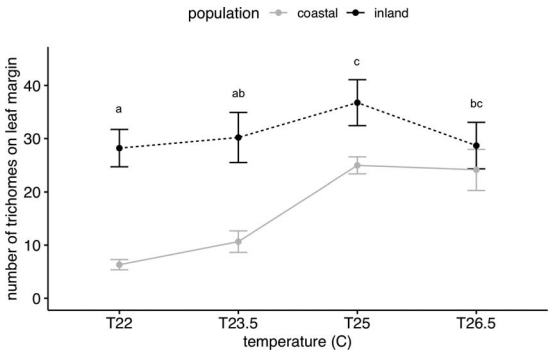

FIG. 3. Trichome density of *E. fasciculatum* cuttings grown at different temperatures. X-axis labeled with T followed by the temperature in Celsius (T22 = 22°C, etc.). 22°C and 23.5°C were the temperatures to which the cool chamber was set during the first and second month, respectively. 25°C and 26.5°C were the temperatures in the warm chamber during the first and second month, respectively. Different letters indicate a significant difference in cross-population means between treatments based on Tukey post-hoc tests. Solid circles with error bars show the means and standard errors for each population.

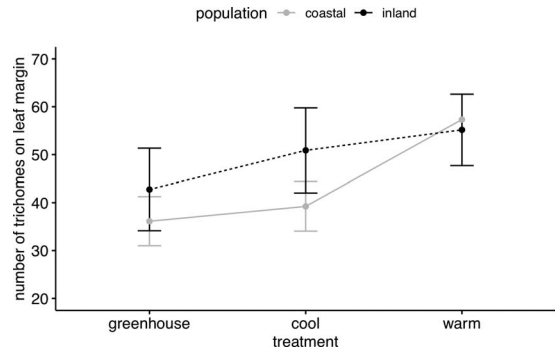

FIG. 4. Trichome densities of *E. fasciculatum* seedlings. Seedlings were germinated in three treatments: greenhouse, cool growth chamber (22°C for 1 mo, 23.5°C for the second), and warm growth chambers (25°C for 1 mo, 26.5°C for the second). Solid circles with error bars show the means and standard errors for each population.

observed a significant increase in leaf trichome density after the first month of growth in the warm growth chamber, but the trichome densities of new leaves did not increase further after the temperature was raised an additional 1.5°C during the second month (Fig. 2C). We also detected a significant population-by-environment interaction for trichome density within this chamber (Table 3). The coastal cuttings produced leaves with more trichomes in response to the increased temperature, while the inland cuttings continued to produce leaves already at consistently high trichome densities.

Plasticity in trichome density by temperature. To examine phenotypic plasticity in response to temperature, we analyzed trichome density reaction norms for cuttings from each source population based on the leaves that developed entirely in each temperature condition. This analysis revealed that both inland and coastal populations produced leaves with elevated trichome densities after 1 mo of growth in the warm chamber at 25°C (Fig. 3, Table 4). But there was no significant change in either chamber when the temperatures were increased by 1.5°C after another month. Notably, this analysis revealed a significant population-by-environment interaction, indicating differentiation in the plasticity of this trait to temperature between the populations. In contrast to the leaves on inland cuttings for which trichome density increased only marginally with temperature, leaves of coastal cuttings exhibited stronger plasticity for the trait. The leaves of coastal cuttings had lower mean trichome densities at 23.5°C and below than at 25°C and above.

Seedling phenotypes and germination. To determine if the divergence in trichome density and its plasticity to temperature is observable in new seedlings, we scored trichome densities of seedlings that had newly germinated in each growth condition. We did not detect any significant effects of population, treatment condition, or their interaction on seedling trichome density (Fig. 4, Table 5). However, compared to our other experiments, our power was more limited in this experiment due to low and variable germination rates (Table 1), and thus the marginally significant effect of population on trichome density ($P = 0.106$) may be a false negative result. Additionally, we observed more germination in the cool growth chamber (22–23.5°C) than the warm growth chamber (25–26.5°C), especially for the coastal seedlings. A Chi-square Contingency Test revealed that germination rate was independent of temperature for inland seeds, but was associated significantly ($P < 0.005$) for coastal seeds (Table 1). These results may indicate genetic divergence for germination requirements has evolved between the populations.

Population Genetics

To examine whether the coastal and inland plant populations are distinguished by significant population structure or if phenotypic differentiation has

TABLE 4. ANOVA RESULTS FOR GROWTH OF TRICHOMES BY TEMPERATURE (22°C, 23.5°C, 25°C, 26.5°C). Significant P-values ($\alpha = 0.05$) are indicated in bold.

	Population	Temperature	Pop. × temperature
Trichomes	$F_{1,\ 59} = 43.818$; $P < \mathbf{0.001}$	$F_{3,\ 59} = 7.896$; $P < \mathbf{0.001}$	$F_{3,\ 59} = 2.815$; $P = \mathbf{0.047}$

TABLE 5. AVOVA RESULTS FOR NEW GROWTH, TRICHOMES, LEAF LENGTHS, AND SEEDLING TRICHOMES BY TREATMENT (GREENHOUSE, WARM, OR COOL GROWTH CHAMBER). Significant P-values ($\alpha = 0.05$) are indicated in bold.

Trait	Population	Treatment	Pop. × treatment
Growth (1st month)	$F_{1,\ 43} = 2.161$; $P = 0.149$	$F_{2,\ 43} = 5.972$; $P =$ **0.005**	$F_{2,\ 43} = 1.177$; $P = 0.318$
Growth (2nd month)	$F_{1,\ 43} = 2.545$; $P = 0.118$	$F_{2,\ 43} = 0.123$; $P = 0.885$	$F_{2,\ 43} = 0.056$; $P = 0.945$
Trichomes	$F_{1,\ 63} = 31.75$; $P <$ **0.001**	$F_{3,\ 63} = 13.964$; $P <$**0.001**	$F_{3,\ 63} = 5.832$; $P =$ **0.001**
Leaf length	$F_{1,\ 63} = 2.922$; $P = 0.092$	$F_{3,\ 63} = 22.514$; $P <$**0.001**	$F_{3,\ 63} = 3.702$; $P =$ **0.016**
Seedling trichomes	$F_{1,\ 24} = 2.827$; $P = 0.106$	$F_{2,\ 24} = 0.982$; $P = 0.389$	$F_{2,\ 24} = 0.239$; $P = 0.789$

occurred despite gene flow, we genotyped cuttings and seedling samples. All markers segregated for two to nine alleles in both populations, with the exception of one marker fixed for one allele in the inland population, and the majority of the alleles found were shared between populations (Table 6). This level of polymorphism is consistent with previous work on a sample of smaller size (Riley et al. 2011). Population structure analysis with STRUCTURE and CLUMPAK yielded $K = 2$ as the optimal number of genotypic clusters (Evanno et al. 2005; Fig. 5A). However, the coastal and inland sample groups are not differentiated by cluster; all coastal and inland plants show mixed ancestry from the two genotypic clusters (Fig. 5B). Consistent with a lack of population structure associated with habitat, G''_{ST} (G_{ST} adjusted for small population size) was low on average across the five markers (0.048; Table 7). Notably, D_{est}, a metric for genetic diversity that is robust to ploidy, was also quite low (0.020; Table 7). Thus, we infer that the differences in phenotypic plasticity that we observe between the coastal and inland samples likely persist despite ample gene flow between the populations.

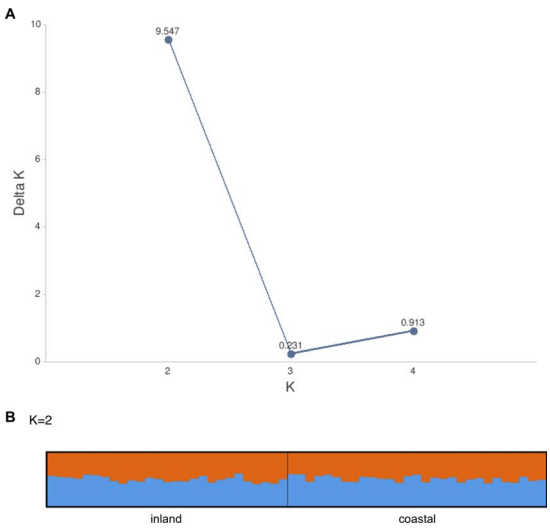

FIG. 5. (A) Plot of ΔK (Evanno et al. 2005), which quantifies the second-order rate of change in the likelihood function with respect to the number of clusters, K, and (B) plot of STRUCTURE results for microsatellite polymorphism data at $K = 2$ genotypic clusters. Proportion assignment to each genotypic cluster is shown for each individual with contribution from each of the clusters visualized as a different color.

DISCUSSION

Differential Plasticity between Coastal and Inland Populations

Consistent with past reports (Cole 1967), we observed that inland plants of *E. fasciculatum* subsp. *foliolosum* had longer leaves with greater densities of trichomes than coastal plants in the field. We then sought to determine how genetic differences, phenotypic plasticity in response to temperature, and/or genetic differentiation of phenotypic plasticity to temperature contribute to these phenotypic differences by conducting common garden experiments that also simulated a range of temperature regimes predicted to result from future climate change in California.

For the cuttings we propagated, we found significant evidence for plasticity to temperature in inland and coastal populations for both leaf traits. Leaf lengths of inland and coastal cuttings largely converged to a common value after 1 mo of growth in all three growth environments, indicating that this trait is plastic to temperature for both populations, and the mean length in each case was notably longer than that observed in the field. We expect that this added length is explained by the relatively water- and nutrient-rich growth conditions the plants experienced in the greenhouse relative to the field.

We observed significant plasticity of trichome density to temperature in our experiments. As in the field, inland plants produced new leaves with greater trichome densities than coastal plants in the greenhouse, though this difference was only apparent after 2 mo of growth. Like with leaf lengths, trichome densities were greater for both populations in the greenhouse than in the field, although for this trait we also detected a population-by-environment interaction. The plastic rise in trichome density was greater for coastal plants during the first month but greater for the inland plants during the second month. The elevated trichome densities observed in the greenhouse over time may be explained by increasing temperatures in the greenhouse as spring progressed. Although the outside temperatures did not change significantly on average from the first to

TABLE 6. ALLELIC PATTERNS FOR EACH MICROSATELLITE MARKER BY POPULATION. Sample size (n), number of alleles (n_a), expected number of alleles (n_e), number of unique alleles (n_u), observed heterozygosity (H_o), expected heterozygosity (H_e), and fixation index (F).

Population	Locus	n	n_a	n_e	n_u	H_o	H_e	F
Inland	Erar_85	23	1	1.000	0	0.000	0.000	-
	Egic_82	20	6	3.433	1	0.400	0.709	0.436
	Egic_110	25	2	1.220	0	0.040	0.180	0.778
	Egic_96	22	9	6.205	3	0.182	0.839	0.783
	Ergi_99	24	3	1.237	2	0.042	0.192	0.783
Coastal	Erar_85	26	2	1.080	1	0.000	0.074	1.000
	Egic_82	25	5	4.019	0	0.520	0.751	0.308
	Egic_110	29	3	1.515	1	0.000	0.340	1.000
	Egic_96	23	6	3.992	0	0.130	0.750	0.826
	Ergi_99	20	2	1.105	1	0.000	0.095	1.000

second month (see Methods), other seasonal changes that would affect the uncontrolled ambient greenhouse environment (e.g., solar radiation, cloud cover, day length, etc.) typically change in ways expected to lead to higher greenhouse temperatures during the transition from winter to spring in Berkeley, CA.

Our findings from the growth chambers provide evidence supporting this hypothesis. In the cool growth chamber, the inland cuttings produced leaves with much higher trichome densities than the coastal cuttings under both temperature conditions (22°C and 23.5°C). Within the warm growth chamber, however, the new leaves produced by coastal plants had significantly greater trichome densities after growth at 25°C for 1 mo, though still not as great as the inland cuttings. But following another month at 26.5°C, both sets of cuttings produced leaves of equivalently high trichome densities. We suggest these results indicate that there has been adaptive differentiation in the phenotypic plasticity of trichome density to temperature between coastal and inland populations. After cuttings from both populations initially established in common conditions, inland cuttings consistently produced new leaves with high trichome densities following transfer to all temperature treatment conditions, potentially reflecting adaptation to a site where greater seasonality leads warmer temperature extremes to be experienced more consistently. Although annual mean temperatures of the sites are equivalent (inland: 16.58°C, coastal: 16.55°C), temperature seasonality is greater on average at the inland site (standard deviation of mean monthly temperature = 3.482°C) than at the coastal site (standard deviation of mean monthly temperature = 2.773°C), but the difference between sites in average maximum temperature (see above) is larger than the difference in average minimum temperature (inland 7.3°C; coastal 8.8°C; Fick and Hijmans 2017). In contrast, the coastal cuttings produced leaves with high trichome densities in only the warmest growth regimes, potentially reflecting a more predictable relationship between elevated temperature and stresses experienced during the active growth season.

Phenotypic Differentiation by Habitat not Detected in Seedlings

Because the cuttings were taken from plants growing in the field and thus could potentially confound environmental effects experienced earlier in development with genetic differences, we also assessed trichome densities and their plasticity to temperature in inland and coastal plants grown from seed. Notably, we detected no significant effects of population of origin, temperature treatment, or their interaction. Germination rates were quite low in most conditions (n = 30 seedlings germinated total across populations and treatments), and consequently our analysis may lack the statistical power to detect such effects, particularly if subtle at this growth stage. If our observations do adequately reflect the biology of the system, however, additional considerations besides lack of past acclimation to distinct field conditions could explain the absence of differentiation for trichome density between the coastal and inland seedlings that we did observe for the cuttings. For instance, differences in trichome

TABLE 7. SUMMARY OF GENETIC DIFFERENTIATION STATISTICS FROM GENALEX ANALYSIS OF MICROSATELLITE MARKERS. N = 56, Loci = 5, Populations = 2, Permutations = 999.

Locus	G''_{st}	P-value of G''_{st}	D_{est}	P-value of D_{est}
Erar	−0.003	0.770	0.000	0.770
Egic_82	−0.063	0.207	0.049	0.207
Egic_110	−0.004	0.412	−0.001	0.412
Egic_96	0.330	0.028	0.290	0.028
Ergi_99	−0.007	0.506	−0.001	0.506
Total	0.048	0.031	0.020	0.032

density may only be observed for leaves of plants that have passed through a juvenile-to-adult phase change (Poethig 2010), and thus the coastal and inland seedlings may simply have been too young to express genetically determined differences in this phenotype. Likewise, there may be ontogenetic contingency for the plasticity of this trait to temperature such that coastal plants may produce leaves with fewer trichomes only after reaching a particular stage or age. Such developmental differences in the genetic or environmental expression of trichome density between young and older leaves is observed in other systems and may reflect adaptation to differences in the intensity of herbivory experienced by seedlings compared to larger, more mature plants (Telfer et al. 1997; Holeski 2007).

Differentiation Likely Persists Despite Gene Flow Between Populations

Assessment of population structure with microsatellite markers indicated that the coastal and inland *E. f.* subsp. *foliolosum* samples lack significant structure by habitat. All individuals regardless of location were assigned ancestry from both genotypic clusters defined by STRUCTURE at $K = 2$. Moreover, two metrics of genetic differentiation based on heterozygosity (G''_{ST}) or the effective number of alleles (D_{est}), the latter of which is independent of population size and ploidy (Miermans et al. 2018), were near zero. This lack of genetic structure is consistent with unpublished results from a survey of eight populations (A. Montalvo, University of California, Riverside, personal communication). Thus, we conclude that background levels of genetic differentiation are very low on local scales in this system. If the observed differences in plasticity to environmental cues that distinguish coastal and inland samples in common garden conditions are due to genetic divergence, the primary possibility discussed above, then the persistence of this differentiation despite the ongoing homogenizing impact of gene flow suggests the differentiation is maintained by natural selection.

New Protocol for Vegetative Propagation of a Woody Shrub

California native plants, including *Eriogonum fasciculatum,* are often propagated from seed for conservation efforts. However, cuttings may provide an alternate means to preserve genetic variation or disseminate it to new areas, particularly for species recalcitrant to germination (e.g., Ramos-Palacios et al. 2012). This method can also be advantageous if the seasonal timing of seed production is limited, if seed production only occurs after woody plants have matured vegetatively for several years, or if there is habitat-associated variation in germination parameters (as observed here for *E. fasciculatum* subsp. *foliolosum*). Alternate means of plant conservation, such as removal of entire plants for transplantation to other sites, may also be more likely to negatively impact the persistence of the source population, particularly for threatened or endangered plants.

There have been reports of vegetative propagation of California buckwheat in horticultural settings (Montalvo and Beyers 2010), though we could not obtain any published protocols. Generally, propagation of woody plant species from cuttings is primarily performed with samples taken during periods of active growth in the spring. Despite the fact that the cuttings sampled for this study were collected from wild populations during the fall dry season, we achieved an establishment rate of 61.33% and 68.0% (coastal and inland, respectively). Notably, these propagation rates were higher than the germination rates (ranging from 6.67% to 66.67%). If transferrable to other species, our propagation procedure could therefore be employed as a less invasive sampling technique for conservation efforts of rare California native woody shrubs.

Leaf Trait Plasticity and Climate Change Adaptation

Our findings (1) that leaf traits differ between inland and coastal genotypes that experience different microclimates, (2) that these traits respond to ambient temperature, and (3) that populations from different habitats differ in their plasticity are consistent with previous studies of plasticity and the ecophysiological roles of leaf phenotypes. For instance, Zunzunegui et al. (2010) reported that there was more plasticity for multiple traits in Mediterranean plants observed in xeric sites during the hot, dry season. Multiple studies on a variety of taxa have reported plasticity in trichome density for various environmental cues, especially temperature and solar radiation (e.g., Holeski 2007; Gainoli and Gonzalez-Teuber 2005). Ehleringer et al. (1976) found that trichome density decreases incoming solar radiation into leaves by reflecting visible light, which has the consequent advantage of decreasing transpiration losses at high temperatures. However, they found that this advantage comes with a tradeoff, as decreases in incoming solar radiation may also decrease photosynthetic rates. Although recent work has shown that trichomes do affect photosynthesis by filtering which wavelengths of radiation are absorbed or reflected and affecting gas exchange, significant effects of trichomes on the boundary layer and transpiration have come into question (Bickford 2016).

Both populations exhibited leaf trait plasticity in response to more stressful environmental conditions, including those intended to simulate future climates, and this plasticity may facilitate persistence and subsequent adaptation to changing environments. Also known as the Baldwin effect, it has been hypothesized that plasticity can allow populations to adjust and maintain sufficient population sizes upon experiencing novel conditions such that natural

selection can act over generations to further improve population fitness in the new environment (Crispo 2007). Such an adaptive response may include a process known as genetic accommodation, where the plastic response that permits persistence is itself altered by selection by changing the mean trait value across conditions and/or parameters of the reaction norm (West-Eberhard 2005). Our findings suggest that genetic accommodation has likely occurred as *E. fasciculatum* subsp. *foliolosum* has adapted to inland and coastal habitats, as the coastal and inland populations differ in the plasticity of leaf trichome density to temperature. While inland populations maintained high trichome densities across all temperature treatments, coastal populations only produced leaves with high trichome densities at high temperatures. If further experimentation confirms this differentiation in phenotypic plasticity is genetically based, then its persistence despite gene flow between populations would indicate that these differences in plasticity are locally adaptive. If other traits show similar levels of plasticity and local adaptation through genetic accommodation, this capacity for adaptive flexibility may explain in part why the *Eriogonum* genus is so diverse, with over 250 species adapted to specific habitats throughout North America (Sanchez and Kron 2008).

Our observations of how *Eriogonum fasciculatum* subsp. *foliolosum* leaf traits respond to temperature changes and how selection has likely maintained differences in this plasticity across short distances also inform our understanding of how chaparral shrubs may adapt to climate change. For instance, the coastal population reaction norm for leaf trichome density indicates there may be a minimum threshold between 23.5°C and 25°C above which ecophysiological adaptations to temperature are favored. This pattern could indicate that should climate change proceed in accord with a low carbon emissions scenario, the projected temperature increase may indeed not seriously challenge plants in this California ecosystem in certain areas. Moreover, natural variation in the developmental plasticity of ecophysiological traits may facilitate adaptation through genetic accommodation. However, in species with longer generation times or with populations connected by limited gene flow, such adaptation may not occur apace with climate change, and evolutionary rescue may require assisted gene flow (Aitkin and Whitlock 2013).

Climate change is also projected to greatly affect precipitation, fire frequency and severity, and sea level rise. For chaparral plants adapted to specific seasonality patterns, increasingly erratic weather fluctuations associated with climate change could be of greater concern than rising average temperatures, perhaps particularly for the coastal populations that have been less apt to experience high temperature extremes historically. Nonetheless, our results indicate that these plants are able to adjust the traits of newly produced leaves in response to temperature. If this plasticity or similar responses in buckwheat and other chapparal plants do enhance survival or reproduction when faced with such challenges, then plasticity could be an important factor fostering the resiliency of this unique ecosystem to future climate change.

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