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Genetically based latitudinal variation in *Artemisia californica* secondary chemistry

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Steep climatic gradients may select for clinal adaptation in plant functional traits with implications for interspecific interactions and response to future climate change. Terpenes are common in Mediterranean environments and mediate plant interactions with both the abiotic and biotic environment, including herbivores. Clines in traits such as terpenes have received much attention because they are linked to plant fitness and experience strong selection from the abiotic and biotic environment. In this study, we tested for intraspecific variation in *Artemisia californica* terpene chemistry in a common garden of plants sourced from populations spanning a large precipitation gradient (6° latitude) and grown in treatments of high and low precipitation. We found genetic variation in terpene richness, diversity, concentration and composition among *A. californica* populations spanning this species' range. Of these traits, terpene composition and monoterpene concentration varied clinally with respect to source site latitude. Regarding terpene composition, pairwise dissimilarity among populations increased in parallel with geographic distance between source sites. At the same time, monoterpene concentration decreased monotonically from plants of southern origin (source sites with high temperature, aridity, and precipitation variability) to plants of northern origin. Our precipitation manipulation suggests that phenotypic selection by precipitation may underlie this clinal variation in monoterpene concentration, and that monoterpene concentration and other aspects of terpene chemistry are not phenotypically plastic. In summary, this study provides novel evidence for a genetically based latitudinal cline in plant secondary chemistry and suggests that adaptation to a key aspect of the abiotic environment may contribute to this intraspecific variation. Accordingly, changes in terpene chemistry under projected future climates will likely occur solely through the relatively slow process of adaptation, with important consequences for plant interactions with the abiotic environment and a diverse community of associates.

The study of clines in ecologically important plant traits has taken on a new urgency because of anticipated global climate change. Evidence of clinal adaptation demonstrates the importance of past evolutionary processes for contemporary ecological dynamics, and suggests a key role for adaptation in plant responses to a changing environment (Clausen et al. 1948, Woods et al. 2012, Pratt and Mooney 2013). In addition, geographical gradients in the abiotic environment and interspecific interactions often result in clinal variation or ecotypic differentiation in plant traits (Linhart and Grant 1996, Thompson et al. 2007). Studying plant adaptation to gradients using common environment approaches can elucidate whether trait clines are genetically based or due to phenotypic plasticity (Clausen et al. 1940, Woods et al. 2012, Holeski et al. 2013). Such common environment studies, when done in conjunction with environmental manipulations, provide an especially powerful approach to pinpoint the underlying causes of clinal variation in plant traits and determine how this intraspecific variation relates to large-scale ecological variation (Mitchell-Olds and Shaw 1987).

Plant adaptation to latitudinal clines is likely driven by the response to variation in both the abiotic and biotic environment (Woods et al. 2012, Thompson et al. 2013). Latitudinal clines in herbivore resistance have received much attention because resistance traits are linked to plant fitness (Marquis 1992). Predictions that intense biotic interactions at lower latitudes select for investment in plant defenses (Dobzhansky 1950, Schemske et al. 2009) have mixed support (Coley and Aide 1991, Pennings et al. 2009, Schemske et al. 2009, Moles et al. 2011, Rasmann and Agrawal 2011, Woods et al. 2012). Because herbivore defense is costly (Gershenzon 1994, Langenheim 1994) and can tradeoff with plant growth (Mooney et al. 2010), plant adaptations to the abiotic and biotic environment are likely to be deeply intertwined. Accordingly, gradients in traits conferring herbivore resistance may be driven by the interactive effects of variation in both herbivory and the abiotic

environment (Woods et al. 2012), including precipitation (Said et al. 2011, Pearse and Hipp 2012). Gradients in precipitation are common (Pratt and Mooney 2013) and are of particular interest in arid environments where water availability plays a central role in shaping plant evolution (Niklas 1997). Thus, accounting for climatic variables may be necessary to explain large-scale geographical gradients in plant defense (Pearse and Hipp 2012).

Terpenes – one of the most diverse groups of plant secondary compounds – are important in providing defense against herbivores and directly influence plant interactions with the abiotic environment. They are particularly common in arid Mediterranean environments, which routinely experience water stress during seasonal (summer) droughts and as such, may be particularly sensitive to altered precipitation (Thompson et al. 2007). Several studies have documented intraspecific variation in foliage terpene composition and concentration among naturally occurring populations (Yani et al. 1993, Thompson et al. 2013). Those conducted along aridity gradients show contrasting results with terpenes either increasing (Gershenzon 1994) or decreasing (Said et al. 2011) with water availability. Similarly, in manipulative experiments, imposed droughts have been shown to increase foliar terpene concentrations (Kainulainen et al. 1992), decrease terpene emissions (Lavoir et al. 2009) and concentrations (Yani et al. 1993) or have no effect (Penuelas and Llusia 1997). Moreover, responses to decreased water availability can vary based on terpene identity (Leicach et al. 2010). Both terpene concentration and composition (e.g. diversity, identity) contribute to a plant's resistance to herbivores (Firn and Jones 2000, Thoss and Byers 2006, Iason et al. 2011), and plant investment in herbivore defense is likely due to an interaction between herbivore pressure and resource availability. In addition to plant defense, terpenes play several additional roles in the community (Langenheim 1994). They are involved in plant-plant communication (Kirby and Keasling 2009), drought and thermal tolerance (Yani et al. 1993, Penuelas et al. 2005) and adaptation to fire (e.g. flammability; White 1994), and can influence plant relationships with nearby conspecific and heterospecific plants (e.g. via allelopathy; Barney et al. 2005), animals, and microorganisms (Langenheim 1994).

In this study, we examine intraspecific variation in terpene chemistry in *Artemisia californica* (Asteraceae), a foundation species in California's coastal sage scrub community, from a common garden of source populations distributed along a four-fold precipitation gradient (6° latitude). Our past work in this system indicates genetically based clinal variation among populations in functional traits, including growth rate, leaf nitrogen content, and relative total terpene content (Pratt and Mooney 2013). As host to a diverse arthropod community of more than 250 species (Pratt unpubl.), *A. californica* provides important nesting and foraging habitat for several species of concern (e.g. coastal cactus wren *Campylorhynchus brunneicapillus*), and is therefore a target of conservation and restoration efforts (Spencer et al. 2001). Thus, clinal adaptation in *A. californica* terpene chemistry and patterns of selection by precipitation on this important phenotype are likely to have community-wide effects.

Here we test for genetically based clinal variation in foliar terpene richness, diversity, concentration, and composition among *A. californica* from five source populations grown in a common garden with experimentally altered precipitation, mimicking conditions of the southern and northern range margins. We further examine whether precipitation is a key selective force on terpene chemistry. Specifically, we document whether patterns of selection on terpene chemistry differ under high and low precipitation treatments in a manner consistent with patterns of terpene chemistry observed in the common garden for plants from the relatively wet (northern) and dry (southern) extremes of this species' distribution. This work thus documents population variation in this key functional trait, and investigates whether such variation is driven by corresponding geographic variation in the abiotic environment.

Material and methods

Study system

California sagebrush *Artemisia californica* (Asteraceae) ranges approximately 1000 km along the California coast (< 800 m elevation), spanning a five-fold precipitation gradient from northern Baja, Mexico (average annual precipitation: 20 cm) to Mendocino County, California (average annual precipitation: 103 cm). The coastal sage scrub community type within which *A. californica* occurs is highly fragmented throughout this range, has been reduced to 10–15% of its historical distribution as a result of land-use change, and is considered a critically threatened ecosystem (Spencer et al. 2001). The present work, based upon five populations of *A. californica* distributed over 700 km in southern and central California (32.5° to 37.5° latitude), represents 70% of its range and includes 85% of the precipitation gradient across which it occurs (Pratt and Mooney 2013). This gradient is characterized (from south-to-north) by ~ 3°C decrease in temperature, a four-fold increase in precipitation, a 61% decrease in interannual precipitation variability, and no detectable pattern for temperature variability (Pratt and Mooney 2013).

Experimental protocols

Common garden design

The design of the common garden used for this experiment is described in detail in Pratt and Mooney (2013). Briefly, in spring 2008 we collected 20 cuttings from each of 20 *A. californica* plants in each of five source populations distributed along the gradient described above. All source plants within a population were at least 20–100 m apart to help ensure that they represented separate genotypes. The minimum distance between populations was 100 km. We collected 2000 total plant cuttings because this species is difficult to grow from cuttings, with < 20% of cuttings forming roots and a majority lost to fungal attack at very early stages. From south to north the source population sites were Scripps Coastal Reserve, San Diego, CA (32°52′N, 117°14′W; 95 m a.s.l.; 25 cm average annual precipitation [avg. precip.]; hereafter, 'SD33'), Santa Monica Mountains

National Recreation Area, Santa Monica, CA (34°03′N, 118°59′W; 80 m a.s.l.; 32 cm avg. precip.; hereafter, 'SM34'), Kenneth S. Norris Rancho Marino Reserve, Cambria, CA (35°31′N, 121°04′W; 20 m a.s.l.; 50 cm avg. precip.; hereafter, 'CAM35′), Wilder Ranch State Park, Santa Cruz, CA (36°58′N, 122°07′W; 20 m a.s.l.; 74 cm avg. precip.; hereafter, 'SC37'), and Rodeo Beach, Golden Gate National Recreation Area, San Francisco, CA (37°50′N, 122°32′W; 57 m a.s.l.; 95 cm avg. precip.; hereafter, 'GG38'). The source population name abbreviations contain a number corresponding to the population's latitude. In addition, SD33 and SM34 are discussed as southern populations, and CAM35, SC37 and GG38 as northern populations based upon the climatic and biogeographic boundary recognized to separate southern and northern coastal sage scrub community types (Schoenherr 1992). To minimize non-genetic (maternal) effects associated with plants cloned from cuttings (Roach and Wulff 1987), rooted cuttings were grown in a greenhouse and common garden for a total of 24 months before traits were measured. In December 2008 surviving plants (n = 152; SD33 = 17, SM34 = 43, CAM35 = 33, SC37 = 31, GG38 = 28) were planted into common garden plots at a site in Newport Beach, CA (33°39′N, 117°53′W; 16 m a.s.l.; 28 cm avg. precip.). This site, part of the Upper Newport Bay Ecological Preserve, is a degraded patch of upland habitat approximately 100 m from Newport Bay and 6 km inland from the ocean coastline. Intact coastal sage scrub habitat is found in patches throughout the areas adjacent to the common garden. The common garden consisted of three blocks, each containing a pair of 5 × 6 m plots, with a minimum of 2 m between plots and 4 m between blocks. The total sample size for each source population was evenly distributed among plots and randomized within each plot. In December 2009, we implemented a precipitation manipulation at the plot level using overhead sprinklers to supply supplemental water to one plot within a block (hereafter high precipitation plots); the remaining plot received ambient precipitation (low precipitation plots). Sprinklers were located at all four corners of the high precipitation plots and were adjusted to ensure that all water was applied strictly within the high precipitation plot at a slow enough rate to prevent run-off of water onto adjacent plots (2–8 m away), and we did not water on windy days. We applied water equivalent to the precipitation difference between the southern and northern extremes of the species' range (70 cm annually), mimicking the seasonal cycles of precipitation in our Mediterranean climate with 56% applied in winter (December–February, 13 cm month^{-1}), 22% applied in spring (March–May, 5 cm month^{-1}), 1.5% applied in summer (June–August, 0.75 cm month^{-1}), and 20.5% applied in fall (September–November, 4.5 cm month^{-1}; Pratt and Mooney 2013).

Terpene chemistry

On 28 April 2010, during peak growing season, we collected 10 fully expanded leaves from a subset of plants from each population in our common garden (n = 121 plants; SD33 = 15, SM34 = 33, CAM35 = 26, SC37 = 24, GG38 = 23). Healthy appearing, actively growing plants were randomly selected for sampling from each population with the goal of achieving relatively even sample sizes among populations because sample sizes varied from n = 15 (SD33) to n = 40 (SM34) in the experiment at the time of collection. To assess terpene concentrations, collected leaves were immediately placed in 2 ml *n*-hexane (99.9% purity), sonicated for 10 min and soaked at room temperature. After seven days, extracts were poured off and stored at −80°C until analysis by gas chromatography and mass spectrometry (GC-MS) and leaf material was dried at 60°C for 72 h and weighed. For terpene analysis, 10 µl of an internal standard solution (0.13 µl ml^{-1} *m*-xylene in *n*-hexane) was added to 90 µl of each sample extract. Samples were injected (4 µl) onto a GC-MS fitted with a 30 m × 0.25 mm × 0.25 µ film thickness DB-5 fused silica column. The GC was operated in splitless mode with helium as the carrier gas (flow rate 1 ml min^{-1}). The GC oven temperature program was: 1 min hold at 50°C, 5°C min^{-1} ramp to 180°C, 20°C min^{-1} ramp to 290°C and 1 min hold at 290°C. The mass spectrometer was operated in electron ionization mode at 70.0 eV and data were collected between 50–650 *m/z*. Terpenes were identified by comparing their mass spectra and retention indices with those of pure standards (for six compounds) and published values (for remaining compounds; Adams 2007). Terpene concentrations are reported as µg mg^{-1} dry weight for those quantified with standard calibration curves (monoterpenes: β-pinene, 1,8-cineole (eucalyptol), α-thujone, camphor, borneol; sesquiterpene: β-carophyllene). The concentrations of other monoterpenes were calculated by taking the average of the five monoterpene standard calibration curves and are thus presented as micrograms 'monoterpene equivalents'; similarly, sesquiterpenes are reported as micrograms β-carophyllene equivalents.

We recorded the total number of terpene compounds in each plant (richness) and the total amount of each compound (concentration). To assess the relative distribution of terpenes across source populations and treatments, we calculated terpene diversity for each plant using the Shannon–Weiner index: $H' = -\Sigma(P_i \log[P_i])$, where P_i is the relative amount of a given terpene divided by the total terpene amount in each plant.

Plant fitness

We assessed total flower production as a component of plant fitness as described by Pratt and Mooney (2013). We first counted inflorescences monthly on all plants from April–December 2011. Then, in October 2011 we collected ten inflorescences from each of 96 flowering plants representing the five source populations in both precipitation treatments and counted the number of flowers per inflorescence under a dissecting scope. We multiplied inflorescence counts by the source population mean within each precipitation treatment to calculate flower production each month and total overall flower production for 2011.

Statistical analyses

Compound richness, diversity, and concentration

We used two-way ANOVAs to test for main effects of source population, precipitation treatment and their interaction on the richness, diversity (Shannon–Weiner, *H′*),

and concentration of monoterpenes and sesquiterpenes, as well as the sum of the two (total terpenes). A significant source population effect indicates genetic differences among populations, a significant precipitation effect indicates trait plasticity, and a significant source population-by-precipitation interaction (i.e. a G × E effect) indicates differences among populations in the degree of plasticity and that genetic differences among populations are dependent upon precipitation environment. All analyses were conducted using the MIXED procedure in SAS ver. 9.2 specifying the block and precipitation-by-plot interaction as random effects. To meet ANOVA assumptions of normally distributed residuals and homogeneity of variances, terpene concentrations were log-transformed while all other variables were untransformed. Where two-way ANOVAs showed a significant main effect of population, we tested for a clinal pattern across the latitudinal gradient for all traits by conducting linear regressions between the population means across treatments for that trait and latitude using PROC REG.

Terpene composition

Differences in terpene composition, independent of differences in overall concentration, were assessed with data on the relative concentration of individual compounds. To examine the main and interactive effects of source population and precipitation treatment on terpene composition, we conducted a permutational multivariate ANOVA (PerMANOVA) on the Bray–Curtis dissimilarity matrix for total, monoterpene, and sesquiterpene composition using PRIMER 6 + PerMANOVA (Anderson et al. 2008). A PerMANOVA is analogous to an ANOVA, but partitions similarity matrices between treatments and uses permutation tests with pseudo F-ratios. All PerMANOVA tests were based upon 9999 permutations of raw data (relative concentrations of compounds; Anderson et al. 2008). We then used the similarity percentages (SIMPER) routine in PRIMER (Clarke and Gorley 2006) to examine which compounds contribute to differences observed between source populations and treatments. SIMPER analysis calculates the average contribution of individual compounds to the average dissimilarity between samples or groups that are known a priori to differ (from the PerMANOVA results). To further test the null hypothesis of no difference in terpene composition among source populations, we performed a canonical analysis of principal coordinates (CAP; Anderson et al. 2008), which provides a constrained ordination on a subset of axes produced within the CAP analysis (here, $m = 12$) that maximizes the differences among a priori groups. CAP analysis calculates a misclassification error of samples to a priori groups by comparing known and allocated groups, with a higher percentage of correct classifications indicating a greater degree of group discrimination by canonical axes. CAP calculates a trace statistic of canonical discriminant analysis and obtains a p-value based upon 9999 permutations (Anderson et al. 2008).

To test for clinal patterns in terpene composition, we tested whether chemical dissimilarity between populations increased in accordance with the geographic distance separating those populations. We performed a Mantel test on the association between pairwise source population geographic distance (measured in decimal degrees latitude) and the pairwise dissimilarity between those populations in total, monoterpene, and sesquiterpene composition (using Bray–Curtis dissimilarity matrices). In other words, we tested the hypothesis that the geographic distances between source populations should lead to increasing terpene dissimilarity. Because the converse – increasing similarity with distance – would not occur, we report p-values based on a one-tailed test. A Mantel statistic (r) was calculated to measure the relationship between the Bray–Curtis dissimilarity matrices and geographic distance matrices, and Monte Carlo randomization (9999 permutations) was used to test the null hypothesis that there was no relationship between the matrices using the 'ade4' package in R (2013). This test for clinal variation in multivariate terpene composition data is the parallel analysis to the linear regressions we conducted on the univariate data (e.g. concentration) described above.

Phenotypic selection on terpene chemistry

We sought to determine whether variation in terpene chemistry observed amongst populations in the common garden is driven by variation in precipitation regime from population source sites. To do so, we tested for phenotypic selection on all chemical traits that varied clinally within the common garden, and whether such selection gradients vary between high and low precipitation treatments. This approach is based upon the assumption that clinal variation observed in the common garden is indicative of the phenotypic variation at population source sites. If precipitation is a selective force on plant terpene chemistry, then we expect selection to vary between treatments, with selection in the high and low precipitation treatments favoring trait values observed in the garden from populations sourced from the north and south, respectively. We regressed relative fitness (individual flower production/mean flower production) onto plant trait values, including precipitation treatment and the trait-by-precipitation treatment interaction using PROC GLM in SAS ver. 9.2. In this analysis, a significant effect of a trait (e.g. monoterpene concentration) indicates phenotypic selection on that trait, while a significant trait-by-precipitation treatment interaction indicates that phenotypic selection is influenced by precipitation. When we observed a significant trait-by-precipitation treatment effect on fitness, we then conducted selection analyses within each precipitation level in order to inspect the separate patterns of selection under high and low precipitation.

Results

Overall, we detected 42 terpenoid compounds in *Artemisia californica* leaf tissue, including 27 monoterpenes and 17 sesquiterpenes, of which 33 were positively identified (Supplementary material Appendix 1). On average, terpenoid compounds comprised 14.2 ± 0.7% SE of leaf weight (range: 2.6–48.4%) with monoterpenes representing 58.1% of this total. The five compounds at highest concentrations, which together accounted for an average of

47.9 ± 1.4% SE (range: 10.2–77.0%) of total terpene concentration, were the monoterpenes α-thujone and artemisia ketone, unknown terpene I, and the sesquiterpenes germacrene D and bicyclogermacrene. The compounds α-thujone, unknown terpene I, and germacrene D were detected in more than 95% of plants sampled (n = 121) and, based on concentration, were among the five most abundant compounds in each of the five source populations. While occurring at lower concentrations overall, the monoterpene 1,8-cineole was the most common, detected in 98% of sampled plants (Supplementary material Appendix 1). Figure 1 shows the percent of the total terpene composition for all compounds that represented more than five percent of the terpene composition for one or more populations.

Compound richness, diversity, and concentration

We did not find a precipitation treatment main effect or a source population-by-precipitation treatment interaction on total, monoterpene, or sesquiterpene richness, diversity (Shannon–Weiner, *H′*), or concentration (Table 1A), and therefore only report main effects of source population below.

Source populations varied in total and monoterpene richness, but not sesquiterpene richness (Table 1A, Fig. 2A). This variation was not clinal based on regressions between source population means and latitude (for total compounds: $F_{1,4} = 0.74$, $p = 0.45$, $R^2 = 0.19$; for monoterpenes: $F_{1,4} = 0.73$, $p = 0.46$, $R^2 = 0.19$). Similarly, source populations varied in total and monoterpene diversity, but not sesquiterpene diversity (Table 1A, Fig. 2B). This variation was also not clinal based on regressions between source population means and latitude (for total compounds: $F_{1,4} = 0.33$, $p = 0.60$, $R^2 = 0.09$; for monoterpenes: $F_{1,4} = 0.38$, $p = 0.58$, $R^2 = 0.11$). Source populations varied in total, monoterpene, and sesquiterpene concentration (μg compound mg^{-1} dry weight; Table 1A, Fig. 2C). Monoterpene concentration decreased with latitude ($F_{1,4} = 11.34$, $p = 0.04$, $R^2 = 0.79$); the northernmost population (GG38) had half the monoterpene concentration of the southernmost population (SD33; Fig. 2A). Total terpene concentration showed a negative trend with latitude ($F_{1,4} = 6.16$, $p = 0.08$, $R^2 = 0.67$), and for sesquiterpene concentration there was no pattern ($F_{1,4} = 1.92$, $p = 0.26$, $R^2 = 0.39$).

Terpene composition

We did not find a source population-by-precipitation treatment interaction for total, monoterpene, or sesquiterpene composition (Table 1B), and therefore only report main effects below. Source populations differed in total, monoterpene, and sesquiterpene composition (PerMANOVA analysis; Table 1B); these differences were independent of differences in overall concentration among populations because data were analyzed as relative concentrations. Sesquiterpene, but not total or monoterpene composition differed between precipitation treatments (Table 1B). Mantel tests for the association between source population pairwise dissimilarity in latitude and pairwise dissimilarity in total, monoterpene, and sesquiterpene composition indicated marginally significant positive relationships in each case (all tests one-tailed, $0.05 < p < 0.10$; Table 1C). The similarity percentage analysis (SIMPER), which calculates the average contribution of individual compounds to the average pairwise dissimilarity between groups known a priori to differ (via PerMANOVA), revealed that α-thujone and artemisia ketone are the primary compounds explaining

Table 1. Statistics for main and interactive effects of source population (source site) and precipitation treatment on *A. californica* foliar terpene (A) richness, diversity (*H′*), concentration, and (B) composition (with perMANOVA). Where ANOVAs indicated significant differences among source populations (but no source population × precipitation treatment interactions), we tested for clinal patterns by regressing population trait means on latitude (L). To test for clinal patterns in terpene composition, we tested whether chemical dissimilarity between pairs of populations was positively correlated with the distance separating populations with a Mantel test (C).

Variable (A)	Source site F_{DF} (p)	Precipitation F_{DF} (p)	Source × Precip F_{DF} (p)
Richness – Total	$5.86_{4,107}$ **(0.0003)**	$0.02_{1,2}$ (0.8982)	$0.38_{4,107}$ (0.8251)
Richness – Mono	$18.61_{4,107}$ **(<0.0001)**	$0.23_{1,2}$ (0.6777)	$0.19_{4,107}$ (0.9447)
Richness – Sesqui	$1.36_{4,107}$ (0.2528)	$0.29_{1,2}$ (0.6444)	$0.4_{4,107}$ (0.8096)
H′ – Total	$7.11_{4,107}$ **(<0.0001)**	$0.25_{1,2}$ (0.6673)	$0.82_{4,107}$ (0.5174)
H′ – Mono	$22.0_{4,107}$ **(<0.0001)**	$0.01_{1,2}$ (0.9210)	$0.18_{4,107}$ (0.9476)
H′ – Sesqui	$2.11_{4,107}$ (0.0840)	$0.08_{1,2}$ (0.8084)	$0.32_{4,107}$ (0.8668)
Concentration – Total	$3.96_{4,107}$ **(0.0049)**, L†	$1.40_{1,2}$ (0.3587)	$0.45_{4,107}$ (0.7722)
Concentration – Mono	$3.91_{4,107}$ **(0.0053)**, L*	$0.10_{1,2}$ (0.7831)	$0.49_{4,107}$ (0.7426)
Concentration – Sesqui	$3.61_{4,107}$ **(0.0084)**	$6.81_{1,2}$ (0.1208)	$0.29_{4,107}$ (0.8866)
(B) Permanova statistics – based on relative abundance			
All compounds	$8.69_{4,107}$ **(0.001)**	$1.53_{1,2}$ (0.150)	$0.68_{4,107}$ (0.910)
Monoterpenes	$14.56_{4,107}$ **(0.001)**	$1.22_{1,2}$ (0.260)	$0.62_{4,107}$ (0.904)
Sesquiterpenes	$3.08_{4,107}$ **(0.001)**	$2.58_{1,2}$ **(0.013)**	$0.62_{4,107}$ (0.904)
(C) Mantel-test statistics	*r* (p)		
All compounds	0.403 (0.0551)		
Monoterpenes	0.364 (0.0652)		
Sesquiterpenes	0.311 (0.0757)		

*significant regression between source population means and latitude, $p < 0.05$.
†marginally significant regression between source population means and latitude, $0.05 < p < 0.10$.

Table 2. Results of SIMPER analysis showing contributions of individual compounds to the pairwise dissimilarity between source population sites. Each pairwise comparison required 3–4 compounds to explain > 40% of the dissimilarity.

	SM34		CAM35		SC37		GG38	
Source site	Compound	Cumm. % explained	Compound	Cumm. % explained	Compound	Cumm. % explained	Compound	Cumm. % explained
SD33	α-thujone	14.52	α-thujone	25.49	α-thujone	21.48	α-thujone	18.69
	artemisia ketone	13.59	artemisia ketone	8.69	artemisia ketone	8.49	artemisia ketone	10.67
	camphor	8.30	camphor	5.72	camphor	5.55	camphor	6.18
	unknown I	4.21	germacrene D	4.25	sabinyl acetate	5.53	unknown I	4.47
SM34			α-thujone	26.89	α-thujone	23.24	α-thujone	20.45
			artemisia ketone	10.15	artemisia ketone	9.82	artemisia ketone	11.05
			camphor	6.30	camphor	6.10	camphor	6.73
			cis-thujone	4.99	sabinyl acetate	5.37	unknown I	4.24
CAM35					α-thujone	24.19	α-thujone	27.31
					sabinyl acetate	8.47	artemisia ketone	6.87
					bicyclogermacrene	5.25	unknown I	4.65
					germacrene D	4.88	germacrene D	3.81
SC37							α-thujone	23.94
							sabinyl acetate	7.10
							artemisia ketone	6.39
							unknown I	4.50

the pairwise dissimilarity among all populations for both total (on average 20.6% and 8.7%, respectively) and monoterpene composition (on average 29.5% and 12.7%, respectively). Likewise, α-bisabolol and unknown terpene F explained 10.7% and 9.2%, respectively, of the pairwise dissimilarity among all populations for sesquiterpene composition and 9.8% and 9.0%, respectively, of the sesquiterpene dissimilarity among treatments. The results of the SIMPER analysis for total terpene composition (Table 2) showed that seven of the 42 detected compounds explain > 40% of the pairwise dissimilarities among all populations; these represented seven of the nine compounds that individually constituted > 5% of the total terpene concentration for one or more populations (Fig. 1).

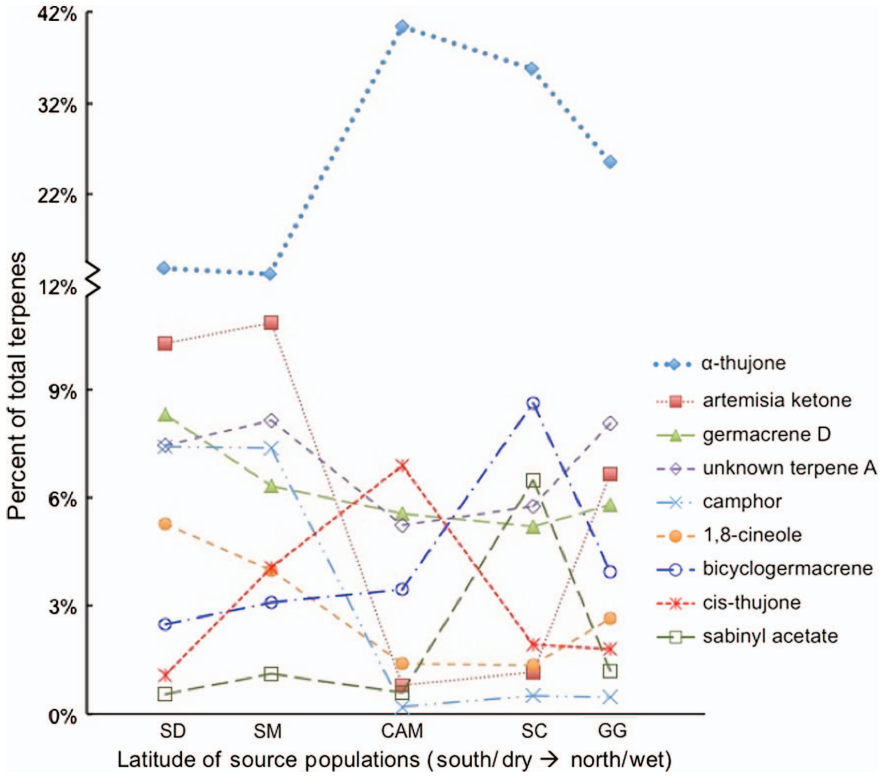

Figure 1. Percentage of total foliar terpenes for all compounds representing more than 5% of the total terpene composition for one or more populations of *Artemisia californica* grown within a common garden. Letters on the x-axis represent source population codes (n = 5; SD, San Diego; SM, Santa Monica; CAM, Cambria; SC, Santa Cruz; GG, Golden Gate National Recreation Area). Note broken y-axis separating α-thujone from all other compounds.

Canonical analysis of principal coordinates (CAP) results confirmed those of the PerMANOVA analysis, indicating differences in terpene composition among source populations ($p = 0.001$) but not between precipitation treatments ($p = 0.138$). The assignment of plants into the correct source populations by CAP was better than random chance for all populations: SD33 = 67%, SM34 = 64%, CAM35 = 88%, SC37 = 63%, GG38 = 70% (average = 70.2%), indicating that a high degree of group discrimination was achieved by the canonical axes (Fig. 3). There were 11 individual terpenes that had a Pearson correlation $> \pm 0.5$ with either CAP1 or CAP2 (shown as vectors; Fig. 3; Supplementary material Appendix 2). The monoterpenes α-thujone, 1,8-cineole, and camphor most strongly correlated with CAP1, and primarily distinguished southern populations from northern populations. Verbenyl acetate and unknown monoterpene B most strongly correlated with CAP2 and primarily distinguished populations within regions (southern or northern).

Phenotypic selection on terpene chemistry

We performed phenotypic selection analysis only for total monoterpene concentration because this was the one chemical trait that exhibited significant clinal variation among populations (Fig. 2C). While tests for directional selection on monoterpene concentration across both precipitation treatments were inconclusive ($F_{1,111} = 15.88$, $p = 0.06$), a significant monoterpene concentration-by-precipitation treatment interaction showed that selection varies between treatments ($F_{1,111} = 4.51$, $p = 0.03$). Separate selection analyses within each precipitation treatment showed patterns of selection consistent with decreasing monoterpene concentration for populations from relatively wet, northern source sites; selection favored lower monoterpenes in the high precipitation treatment (relative fitness $= -0.0046 \times$ monoterpene concentration $+ 1.629$) and higher monoterpenes in the low precipitation treatment (relative fitness $= 0.00071 \times$ monoterpene concentration $+ 0.630$), with the former being marginally

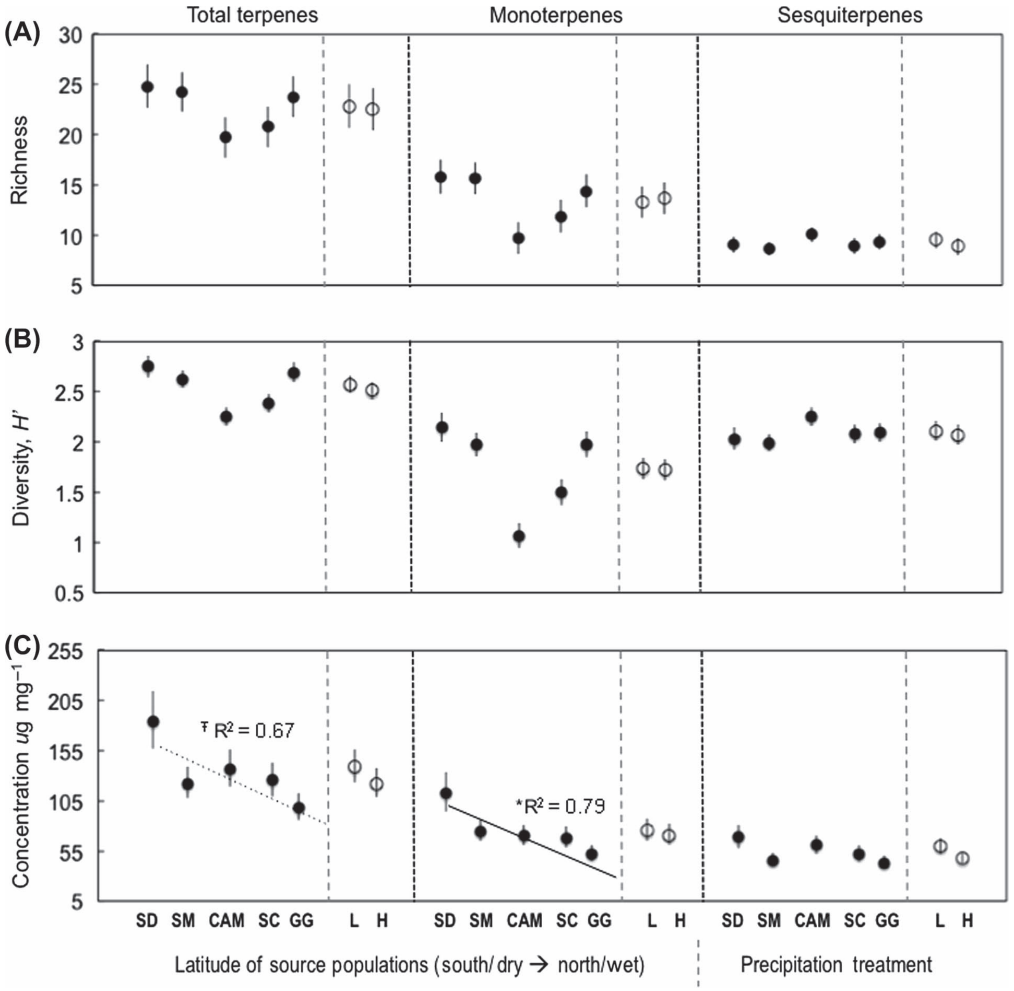

Figure 2. Source population (n = 5; filled circles; SD, San Diego; SM, Santa Monica; CAM, Cambria; SC, Santa Cruz; GG, Golden Gate National Recreation Area) and treatment (n = 2; open circles; L, low precipitation; H, high precipitation) means ± SE for foliar terpene richness (A), Shannon–Weiner diversity, *H′* (B), and concentration (C) of total terpenes (left panels), monoterpenes (center panels), and sesquiterpenes (right panels) from plants grown within a common garden. Where ANOVA results indicated significant differentiation among source populations, a significant or marginally significant test for clinal variation amongst the five populations is indicated with a regression line. Symbols next to R^2 values indicate the level of significance of the regression: Ŧ = $0.05 < p < 0.10$, * = $p < 0.05$. We did not find a significant main effect of treatment or source population-by-precipitation treatment interaction for any traits shown (Table 1).

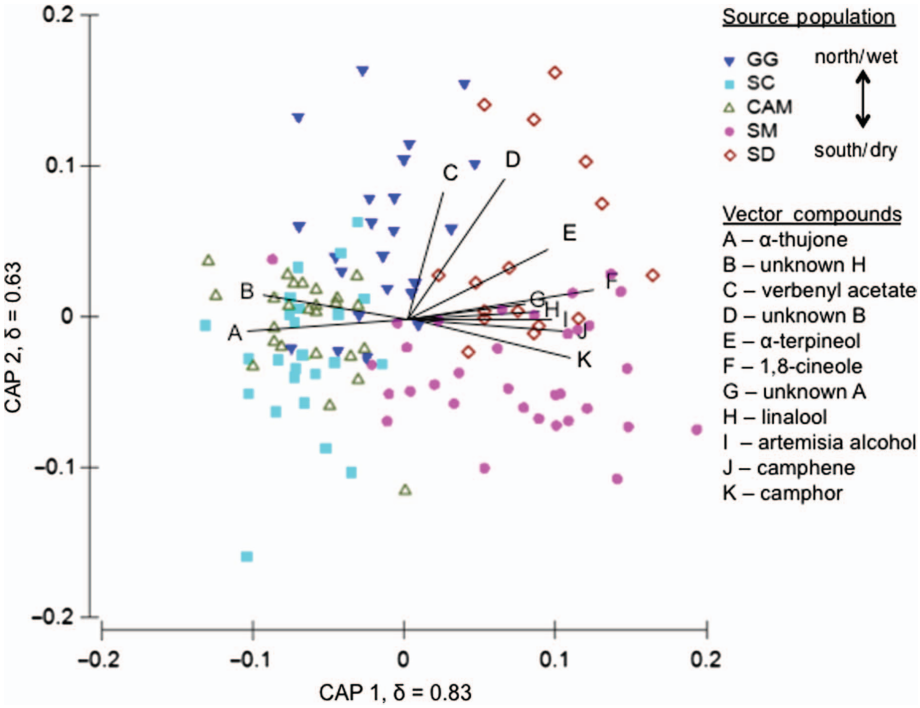

Figure 3. Canonical analysis of principal coordinates (constrained to $m = 12$ PCO axes; see Methods) of *A. californica* foliar terpenes ($n = 42$) sampled from five source populations grown in a common environment. Vectors indicate all compounds with a Pearson correlation $> \pm 0.5$ with CAP 1 or CAP 2. The length of the vector is proportional to the strength of correlation (range: |0.504| to |0.727|; Supplementary material Appendix 2). Canonical correlations (δ) indicate the strength of association between the multivariate foliar terpene data and the discrimination of a priori groups (source populations).

significant ($p = 0.07$), but the latter not significant ($p = 0.56$).

Discussion

Genetically based chemical phenotypes of *Artemisia californica*, including compound richness, diversity, concentration and composition, varied among populations within the common garden sourced from across this species' range. For two traits, monoterpene concentration and overall terpene composition, variation among populations was parallel to abiotic clines of southwardly increasing temperature, aridity, and precipitation variability, suggesting clinal adaptation for these components of terpene chemistry. Our manipulation of precipitation showed that phenotypic selection by precipitation likely contributes to this clinal variation in monoterpenes. However, monoterpene concentration and other aspects of terpene chemistry were not phenotypically plastic in response to four-fold variation in precipitation, as has been shown for other genetically based traits in this species (Pratt and Mooney 2013), and we found no evidence for genotype-by-environment interactions. Predicted changes in precipitation regimes along the California coast (Hayhoe et al. 2004) – and the resultant changes in the selective environment – may alter terpene chemistry across this species' range, with important consequences for biotic interactions between *A. californica* and its associates. Our results indicate that such changes are likely to occur without phenotypic plasticity, and instead through the relatively slow process of adaptation.

This research adds to a growing body of literature documenting intraspecific variation in foliar terpene chemistry among naturally occurring populations (Yani et al. 1993, Said et al. 2011, Thompson et al. 2013). Past studies on pine have shown that feeding by different invertebrate and vertebrate herbivores is inhibited by the presence of different terpenes such that greater chemical diversity confers resistance to a broader array of herbivores (Thoss and Byers 2006, Iason et al. 2011), and that terpene diversity corresponds to diversity at other biological scales, e.g. for Scots pine (Iason et al. 2005). The intraspecific variation in mono- and total terpene richness and diversity we observed here may result in some populations being resistant to a greater diversity of invertebrate and vertebrate herbivores.

Although we found significant clinal variation in terpene composition across latitude, we did not find distinct 'chemotypes' (i.e. populations that possess chemical profiles with unique dominant compounds), as observed in previous studies of Mediterranean shrubs (Thompson et al. 2013). Instead, we showed that clinal variation in terpene composition was mostly due to changes in the relative concentrations of the same major compounds. In terms of terpene composition, the three multivariate approaches we used (PerMANOVA, SIMPER and CAP) gave complementary results, consistently highlighting a small subset of compounds responsible for observed population differences in composition; most were compounds that represented $> 5\%$ of total terpenes for at least one population (Fig. 1). For example, α-thujone was the most abundant compound in all five source populations, but it differed strongly in relative abundance, representing ~ 14% of total

terpene composition in the two southernmost populations (SD33 and SM34) and ~34% (on average) in the three northern populations. This monoterpene is presumed to be under strong selection by arthropod herbivores, as previous studies have shown significant decreases in α-thujone following experimental defaunation (in *Artemisia tridentata*; Wiens et al. 1991), and is a known feeding deterrent to deer (Burney and Jacobs 2011). Similarly, camphor was one of the most abundant compounds in southern populations, where it represented > 10% of total terpene composition, but was only present in trace amounts in the three northern populations. Camphor can inhibit germination of seeds of annual plants (Muller and Delmoral 1966) and has been shown to be a specific antifeedant to rabbits (in *Picea glauca*; Sinclair et al. 1988). Artemisia ketone, which has antioxidant and antimicrobial properties (Cacuteavar et al. 2012), was the second or third most abundant compound for SD33 (10%), SM34 (11%), and GG38 (7%), but was only found in trace amounts in CAM35 and SC37. Similarly, the monoterpenes *cis*-thujone and sabinyl acetate were each only present in high abundance in one population, CAM35 (6.9%) and SC37 (6.5%), respectively. This observed variation in the specific identities and relative abundances of terpenes present (i.e. composition) can influence the outcome of species' interactions (Thoss and Byers 2006, Iason et al. 2011). The above patterns demonstrate strong population variation with respect to multiple compounds. To our knowledge, this study is the first to use a multivariate approach to examine clinal variation in terpene composition.

Using flower production as a fitness correlate, we show that the pattern of phenotypic selection by precipitation on monoterpene concentration matches the clinal variation we observed in this trait amongst plants grown under both high and low precipitation within the common garden. This indicates that precipitation is likely a key selective influence on *A. californica* terpene chemistry. Clinal patterns in plant traits suggest local adaptation to clinally varying abiotic factors across the species' range, but do not reveal which abiotic factors drive that variation. Our selection analysis tests for the contribution of precipitation in particular, but we cannot conclude that other abiotic factors don't also select for clinal variation in traits. While we did not measure terpene chemistry in plants at population source sites as a complement to the clinal patterns observed in our common garden setting, our experimental design allowed us to directly assess the effects of broad phenotypic variation in monoterpenes on plant fitness, facilitating our ability to detect patterns of selection specifically from precipitation (Mitchell-Olds and Shaw 1987). The lack of phenotypic plasticity and genotype-by-environment interactions in terpene chemistry in response to four-fold variation in precipitation, coupled with the results of our selection analysis, indicate that adaptation to this gradient in precipitation has shaped terpene chemistry across this species' range.

Studies that have examined the effects of precipitation or water availability on intraspecific terpene variation show contrasting results. While Gershenzon et al. (1978) found monoterpene concentrations decreased in the perennial herb *Clinopodium douglasii* at more arid sites, a study of the Mediterranean shrub *Pistacia atlantica* found that terpene concentrations increased clinally along a gradient of increasing aridity (Said et al. 2011). Our results are in parallel with the latter, as monoterpene (but not sesquiterpene) concentrations increased clinally with increasing aridity of *A. californica* source population sites. Experimentally imposed droughts have also been shown to produce contrasting results, increasing foliar terpene concentrations in temperate trees (Kainulainen et al. 1992), decreasing terpene emissions (Lavoir et al. 2009) and concentrations (Yani et al. 1993) in Mediterranean trees, or causing no change in terpene concentrations (in *Rosamarinus officinalis*; Penuelas and Llusia 1997). Yani et al. (1993) found that changes in terpene concentrations were due both to increased terpene emission and metabolism, hypothesizing that high concentrations of terpenes in plants that experience seasonal drought, like *A. californica*, may increase drought tolerance because terpenes can be metabolized during periods of intense water stress. Consistent with this hypothesis, *A. californica* from arid (southern) source sites had relatively high concentrations of terpenes, but we did not observe plasticity in terpene concentration in response to experimentally altered water availability. Decreased water availability can also result in more complex changes in terpene chemistry that are dependent upon individual species and season (Llusia et al. 2006), or the individual terpenes studied (Leicach et al. 2010). For example, Leicach et al. (2010) found variation in the response of individual terpenes to experimentally imposed drought in *Eucalyptus camaldulensis* with increases in some and decreases in others by up to 50%, with the resulting changes in composition conferring higher resistance to herbivory. Given this body of literature demonstrating plasticity in terpene chemistry, it is surprising that we did not observe changes in most aspects of *A. californica* terpene chemistry in response to increased water availability. In fact, only sesquiterpene composition responded plastically to our precipitation manipulation and this was due to varying increases and decreases in the relative concentrations of several individual sesquiterpenes in response to increased water availability (according to SIMPER analysis).

Plant adaptation to the abiotic and biotic environment may be deeply intertwined, and selection on terpene chemistry is likely due to an interaction between the biotic (e.g. herbivore pressure) and abiotic environment (Pearse and Hipp 2012, Woods et al. 2012). While we found evidence for selection by precipitation on *A. californica* terpene chemistry, this may be mediated by interactions with herbivores occurring at our study site. Mean arthropod density within this experiment was 549 ± 62 individuals per m^3 canopy volume at peak plant growth (May) across two years (Pratt unpubl.). More arid conditions could lower tolerance to herbivory, thus leading to positive selection for terpenes. Predicted tradeoffs between plant growth and defense are unlikely to underlie this selection by precipitation; our previous work shows that highly defended plants from southern populations are also faster growing (Pratt and Mooney 2013). The clinal variation we observed here could be due in part to variable herbivory (with northern populations experiencing less herbivore

pressure) or a gradient in tolerance to herbivory across *A. californica* populations. Studies documenting clinal variation in plant populations along latitudinal gradients lend mixed support to the hypothesis of increasing investment in plant defense at lower latitudes (Dobzhansky 1950, Pennings et al. 2009, Rasmann and Agrawal 2011, Woods et al. 2012). Our results are consistent with predictions for southward increases in herbivore defense and decreases in plant quality, with southern populations having higher concentrations of terpenes, and our previous work shows this is coupled with decreases in nitrogen content (Pratt and Mooney 2013). Latitudinal patterns are likely not driven by variation in herbivore pressure alone, and an explicit consideration of variation in the abiotic environment will likely be necessary to more fully explain geographic variation in plant defensive traits.

Understanding clinal variation in ecologically important traits and patterns of selection across environmental gradients can shed light on the potential ecological and evolutionary consequences of environmental change. California's Mediterranean climate is predicted to become substantially warmer, drier and more variable in the future (Hayhoe et al. 2004). Accordingly, patterns of selection from the abiotic environment may be altered. Moreover, asynchronous shifts in phenology and distribution across multiple trophic levels indicates that plants and animals differ in their sensitivity to climate and their ability to move to areas of more favorable climate (Parmesan et al. 1999). Herbivores are likely to respond more quickly to climate change than their plant hosts (Parmesan et al. 1999); as such, species interactions and patterns of selection from the biotic environment could also be significantly modified. Thus, if the herbivory environment changes in conjunction with climate, these combined stresses may decrease the ability for plants to respond appropriately to either change. Our results indicate that for *A. californica*, plastic responses in secondary chemistry (Fig. 2) and other functional traits (Pratt and Mooney 2013) are unlikely; rather, changes in such phenotypes will likely occur through relatively slow, evolutionary responses. For long-lived species like *A. californica* with highly fragmented populations across the species' range, rates of migration and adaptation may not keep pace with the rate of current and predicted environmental change (Parmesan et al. 1999, Hayhoe et al. 2004). The response of ecologically important traits in foundational species like *A. californica* may have community-wide effects; our work highlights the need to consider past evolutionary processes in predicting responses to environmental change.

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Supplementary material (available online as Appendix oik-01156 at <www.oikosjournal.org/readers/appendix>). Appendix 1–2.