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Genetic and phenotypic variation, dispersal limitation and reproductive success in the invasive herb *Eschscholzia californica* along an elevation gradient in central Chile

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Background: Reductions of genetic diversity and phenotypic changes in invasive plants are often observed to occur at high elevations. Genetic/phenotypic changes of invasive plants along elevation help to understand mechanisms of the presumed resistance of mountain ecosystems to invasion.
Aims: To assess genetic variability and phenotypic plasticity along an elevation gradient of *Eschscholzia californica* in the Andes, central Chile.
Methods: Eleven microsatellites were used to describe the genetic structure and the allelic diversity individuals, distributed at three elevations and two sites. We assessed the number of flowers per plant, floral biomass, leaf area, number of leaves, vegetative biomass and plant height of plants at each elevation.
Results: Genetic diversity as genetic structure did not decrease with elevations. Plant height and flower numbers decreased while leaf number and vegetative biomass increased with elevation. The ratio of the number of flowers to vegetative biomass, decreased significantly with elevation.
Conclusions: Strong genetic differences among elevations and similar genetic diversity along elevation do not suggest dispersal limitation to higher elevation. Reduction of reproductive and vegetative traits concomitantly with an increase of the reproductive cost suggests reproductive stress with increasing elevation, reducing the invasiveness of this species to higher elevation.

Keywords: Andean mountains; central Chile; elevation gradient; genetic diversity; plasticity

Introduction

Plant invasions constitute a key topic in conservation biology research, as invasions can pose a serious threat to biodiversity (Vitousek et al. 1996; Dukes and Mooney 1999; Pyšek et al. 2010). During colonisation, exotic species can be limited by native biota (D'Antonio 1993; Siemann and Rogers 2001) or simply by abiotic conditions (Mihulka and Pyšek 2001; Seipel et al. 2016). A successful invader can have morphological, physiological and life history traits pre-adapted from its native environment (Montague et al. 2008), or characters selected by microevolution in the invaded range (Maron et al. 2004; Leger et al. 2009; Monty and Mahy 2009; Phillips et al. 2010; Chevin and Lande 2011; Clements and DiTomaso 2011). Phenotypic/genotypic differentiation among populations may occur as an ecological or microevolutionary response at new sites (Monty and Mahy 2009; Chevin and Lande 2011; Pahl et al. 2013).

Montane ecosystems are particularly interesting when it comes to investigating the factors that limit species invasion (Dietz and Edwars 2006), as abrupt environmental variations occur across short distances. Atmospheric pressure, CO_2 content, air temperature and length of the growing season, decrease with elevation, while annual precipitation, frost frequency and solar radiation increase during the growing season at temperate regions (Pyrke and Kirkpatrick 1994; Peterson et al. 1997; Hemborg and Karlsson 1998; Körner 2003).

High elevation environments have traditionally been considered as ecological barriers for invasive plants (Alpert et al. 2000; Pauchard et al. 2009). However, plant invasions have recently been reported from high mountain ecosystems from the Arctic to the Tropics, including the United States (Weaver et al. 2001), Argentina (Petryna et al. 2002), South Africa (Carbutt and Edwards 2003), Switzerland (Becker et al. 2005), Hawaii (Daehler 2005), Japan (Tachibana 1968) and Australia (McDougall et al. 2005).

The probability of establishment of exotic species generally decreases with elevation (Alexander et al. 2011; Haider et al. 2012; Seipel et al. 2016). Low propagule pressure with increasing elevation appears to be the most parsimonious explanation. This explanation has emerged as a determining factor regarding successful invasions at both local (Von Holle and Simberloff 2005) and regional spatial scales (Rouget and Richardson 2003; Colautti et al. 2006).

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The magnitude of dispersal limitation in invasive plants may be assessed by comparing the genetic diversity among populations at different elevations (Cain et al. 2000). Low propagule pressure to higher elevations (Pauchard et al. 2009) will convey a reduction of effective population size and an increase in the effect of genetic drift in populations located at higher altitudes. The result of such dispersal limitation and associated reduction in gene flow will be a decrease in the genetic diversity of populations with increasing elevation (Ohsawa and Ide 2008; Alexander et al. 2009). In spite of the importance of plant genetic variability in understanding plant invasiveness (Lee 2002), studies that address genetic differentiation among populations are still scarce (Parker et al. 2003; Alexander et al. 2012).

One way plants can respond to the abiotic stress is through phenotypic changes, a fact well documented in exotic plants (Arroyo et al. 1981; Callaway et al. 2002; Fabbro and Körner 2004; Schneller and Liebst 2007). In native plants leaf area, or plant height tend to decrease with elevation, while root thickness, leaf and bud hardness tend to increase (Diggle 1997; Körner 1999; Cavieres 2000; Craine and Lee 2003). A reduction in plant height in turn, decreases the potential impact of wind exposure while an increase in leaf hardness and a reduction of stomata protect leaves against solar radiation and water loss due to high levels of evapotranspiration (Maron et al. 2007). Such phenotypic changes observed in native plants are likely to occur in exotic plants too.

To discern whether phenotypic changes constitute adaptations they need to be related with reproductive success (RS), defined as the number of flowers produced per plant (Bazzaz et al. 2000; Obeso 2002). In stressful mountain environments, plant reproduction should be costly (Obeso 2002), and an estimation of this cost could be calculated comparing the RS produced by a unit of vegetative biomass at different elevations, increasing elevation representing increasing abiotic stress. If the reproductive cost increases with elevation, we expect the RS per vegetative biomass ratio (RS:VB) to decrease significantly with elevation (Jolls 1980; Ladinig and Wagner 2005; Milla et al. 2009).

In this study, we used an observational approach, to examine genetic/phenotypic responses of the exotic plant *Eschscholzia californica* along an elevation gradient. *E. californica* is a perennial herb, native to the USA and invasive in Chile, New Zealand, Australia and South Africa, among other Mediterranean countries. In Chile, this species has a broad elevation/latitude distribution. To evaluate the response, we compared populations located at the invasion front (ca. 2000 m a.s.l.) with others located at lower elevations (1500 and 1000 m) at two independent sites (Farellones and Lagunillas). We tested the following

predictions (a) due to dispersal limitation (low propagule pressure), we expected a reduction in genetic diversity with elevation and a strong genetic differentiation (structure) among elevations; (b) plant traits such as height and reproduction would decrease with elevation; and (c) since reproduction is costly in stressful environments, the ratio RS:VB would decrease with elevation.

Materials and methods

E. californica is endemic to north-western California and is an invasive species in many Mediterranean ecosystems (Stebbins 1965). It is a perennial plant, self-incompatible and pollinated by beetles (Cook 1962; Nakamura 1994) and bees, including species from the genera *Apis* and *Bombus*. Halictinae and Melittidae are the most frequent flower visitors (Timberlake 1956; Cook 1962). Seed dispersal is explosive but is limited to up to 2 m from the parent plants. This plant is a successful coloniser across a wide range of environmental conditions in both its native and introduced range, often occupying both natural and anthropogenically disturbed open landscapes (Cook 1962; Leger and Rice 2007). In Chile, *E. californica* is distributed from 18–38° S and from sea level to 2200 m (Arroyo et al. 2000; Peña-Gómez et al. 2014). The introduction probably occurred during the mid-1800s to early 1900s, and inform escapees from botanic gardens of coastal and inland cities (Frias et al. 1975; Arroyo et al. 2000). It is also likely that accidental introductions occurred via the importation of lucerne (*Medicago sativa* L.) seed (Gillis 1885; Hillman and Henry 1928).

The study was carried out in two localities: Farellones (33º 22′ S, 70º 26′ W) and Lagunillas (33º 39′ S, 70º 20′ W), 32 km apart from each other, at elevations of 1000 m, 1500 m and 2000 m. Although *E. californica* occurs between sea level and 1000 m our study region, this elevation zone is intensively affected by agriculture. Therefore we focused our study on the more natural environment between 1000 m to 2000 m, representing an adiabatic cooling rate 6° K km^{-1} (Cavieres and Arroyo 1999).

Farellones and Lagunillas are located relatively close to one another and have similar climatic conditions; however they differ in topography and vegetation. At Farellones the slope is steep, annual mean temperature is 10°C, annual mean precipitation is 509 mm and the vegetation is dominated by *Acacia caven, Baccahris linearis, Colliguaja odorifera* (1000–1500 m) and *Kagenekia oblonga* (2000 m). In comparison, at Lagunillas the slope is more gentle, annual mean temperature is 10.4°C, mean annual precipitation is 616 mm and the vegetation is dominated by *B. linearis, Peumus boldus, Quillaja saponaria* (1000–1500 m) and *B. linearis* (2000 m).

Population genetic structure

To estimate genetic diversity and genetic differentiation among populations, a population genetics analysis was made using 27 individuals per elevation (1000, 1500 and 2000 m) and site (Farellones and Lagunillas). The total number of individuals for genetic analysis was 27×3 (elevation) $\times 2$ (sites) = 162. Individuals were selected along linear transects, separated 15 m each, to reduce spatial dependence of data, as it has been documented for this species in similar studies (Anic et al. 2015).

Total genomic DNA was extracted from leaf tissue preserved in silica gel, using the CetylTrimethylammonium Bromide (CTAB) extraction method (Tel-Zur et al. 1999). Six microsatellites (Ecalifdi1, Ecaldi9, Ecalifdi11, Ecalifdi16, Ecalifdi22 and Ecaltet1) described by Veliz et al. (2012) and five new microsatellites (Appendix) were used for genetic analysis.

The number of alleles per locus, linkage disequilibrium, expected (H_E) and observed (H_O) heterozygosity were estimated with the GENETIX software (Belkhir et al. 1996). To test for departures from Hardy Weinberg Equilibrium (HWE), 5,000 allele permutations were used. In order to test the spatial genetic structure, two different indices were used. First, the F_{ST} was estimated between pairs of studied sites using the GENETIX software and the statistical significance was tested using 5,000 permutations. Second, we used the G''_{ST}, an estimator not influenced by the heterozygosity of samples. G''_{ST} was estimated using GenAlEx software (Peakall and Smouse 2012) and the statistical significance was evaluated with 9,999 permutations. Further, to evaluate the level of population structure, we used the Bayesian method described by Pritchard et al. (2000) and implemented it in STRUCTURE (Falush et al. 2007). Without considering the geographical origin of the samples, STRUCTURE estimates the most probable number of populations to which the individuals analysed belong to. The procedure was run from $k = 1$ to $k = 7$, repeating each analysis three times to control for potential variations among runs. For each K-analysis, we used a burn-in of 100,000 and an after burn-in with 300,000 iterations each and the log probability (Ln P(X|K)) to infer the number of populations.

Reproductive and vegetative traits

Sampling was carried out on the basis of previous knowledge on plant phenology of the area. Reproductive and vegetative traits were recorded during the peak of the flowering phase (late September at 1000 m a.s.l. and late December at 1500 and 2000 m a.s.l., respectively) at Farellones and Lagunillas. For 30 plants at each elevation and at the two sites ($n = 180$), the following reproductive traits were also measured: (i) number of flowers per plant, (ii) total floral biomass (g of flowers and floral twigs) (iii) leaf area (cm^2), (iv) number of leaves, (v) vegetative biomass (g) and (vi) plant height (cm). For leaf area, five leaves per plant were selected and photographed. The images were processed using the software Sigma Scan 5.0 (SPPS, Chicago, IL), thus obtaining an average value. For reproductive and vegetative biomass, the material was dried at 80°C for 72 h.

To compare phenotypic differences between elevations, we used Generalised Lineal Mixed Models (GLMM), with elevation as a fixed factor (three levels). This analysis aimed to detect the main effect of elevation. Therefore locality was included as a random factor. Poisson error distribution was used for count data (e.g. number of leafs and number of flowers) and a Gaussian error distribution for continuous data (e.g. plant height, vegetative biomass, floral biomass, leaf area and RS:VB). All statistical analyses were conducted in R version 3.2.5 (R Core Team 2017), using the *lmer* function implemented in the library *lme4* (Bates et al. 2015).

Results

Population genetic structure

All analysed loci were polymorphic, at five of the six population of study. The mean number of alleles varied from 3 (Ecalif27WV) to 10.2 (Ecaltet1) with a mean of 6.4 alleles per locus (Table 1). Departures from the HWE were detected in all loci, but deviations were not associated with any specific locus. Also, null alleles do not appear to be responsible for the observed departures from HWE. Eight out of the 330 comparisons showed evidence for linkage disequilibrium; however this disequilibrium was not observed in the same pair of alleles from all sites.

In the case of the genetic structure, both F_{ST} and G''_{ST} revealed statistical differences between all pairs of sites analysed (Table 2). Further, Bayesian analysis showed that $k = 6$ was the number of clusters from this data set (Figure 1), representing the two sites and three elevations. The genetic diversity, measured as the mean number of alleles per loci and site, did not decrease with elevation. In Farellones, the mean number of alleles per loci was 6.6 (SD = 2.84) at 1000 m, 5.4 (SD = 2.71) at 1500 m and 6.1 (SD = 1.78) at 2000 m. In the case of Lagunillas, these values were: 6.4 (SD = 2.74) at 1000 m, 6.6 (SD = 2.53) at 1500 m and 7.2 (SD = 3.16) at 2000 m. Pairwise comparisons detected no significant differences in any pair of comparisons (Wilcoxon paired test, $P > 0.05$).

Reproductive and vegetative traits

There was a significant elevation effect on five of the plant traits selected in this study (Table 3; Figure 2 A-E). Individuals at 1000 m had a greater number of flowers per plant compared with those at 1500 m and 2000 m

Table 1.   Summary of genetic variability of the 11 microsatellites used in this study at three elevations in Farellones and Lagunillas, central Chile. N: sample size; Na: number of alleles; H_E: expected heterozygosity; H_O: observed heterozygosity; F_{IS}: according to Weir and Cockerham (1984). * indicates samples showing statistical departures from HWE ($P < 0.01$).

	Farellones			Lagunillas		
	1000 m	1500 m	2000 m	1000 m	1500 m	2000 m
Ecaldi1						
N	29	27	25	29	28	28
Na	5	7	4	8	5	4
He	0.685	0.615	0.545	0.742	0.434	0.6333
Ho	0.931	0.778	0.240	0.862	0.464	0.9286
Fis	−0.344*	−0.248*	0.573*	−0.145	−0.052	−0.4519*
Ecaldi16						
N	28	16	28	29	29	28
Na	4	3	5	6	7	6
He	0.635	0.461	0.756	0.652	0.699	0.678
Ho	0.214	0.000	0.500	0.621	0.655	0.893
Fis	0.673*	0.999*	0.354*	0.065	0.080	−0.301*
Ecaldi22						
N	29	21	28	17	29	27
Na	8	6	5	6	8	5
He	0.813	0.628	0.727	0.720	0.660	0.676
Ho	0.172	0.191	0.679	0.118	0.138	0.472
Fis	0.795*	0.714*	0.085	0.845*	0.797*	0.305*
Ecalif90V9						
N	29	16	20	18	14	28
Na	6	4	7	6	5	7
He	0.499	0.713	0.733	0.787	0.735	0.724
Ho	0.379	0.813	0.900	0.778	0.571	0.929
Fis	0.257	−0.108	−0.204	0.040	0.257	−0.266*
Ecaltet9						
N	29	22	26	29	25	28
Na	8	4	7	4	5	8
He	0.583	0.645	0.670	0.536	0.658	0.756
Ho	0.483	0.773	0.615	0.517	0.680	0.821
Fis	0.188	−0.176	0.101	0.052	−0.014	−0.069
EcalifG72Q						
N	17	14	20	21	18	28
Na	2	1	6	3	5	6
He	0.360	0.000	0.506	0.135	0.543	0.576
Ho	0.000	0.000	0.150	0.048	0.333	0.143
Fis	0.999*		0.716*	0.661*	0.410*	0.760*
EcalifUHK8						
N	29	24	28	27	24	28
Na	11	8	9	5	7	9
He	0.734	0.760	0.677	0.686	0.746	0.763
Ho	0.586	0.625	0.643	0.852	0.708	0.929
Fis	0.218*	0.199	0.069	−0.224	0.071	−0.199
Ecaltet1						
N	28	17	25	24	26	27
Na	8	9	7	10	12	15
He	0.759	0.741	0.550	0.799	0.837	0.851
Ho	0.286	0.353	0.120	0.458	0.462	0.852
Fis	0.635*	0.545*	0.789*	0.444*	0.464*	0.018
Ecalif27WV						
N	29	27	28	23	28	28
Na	6	3	4	2	2	3
He	0.555	0.517	0.529	0.496	0.499	0.460
Ho	0.621	0.963	0.750	0.565	0–821	0.679
Fis	−0.100	−0.860*	−0.403	−0.117	−0.634	−0.462
Ecadi11						
N	29	27	28	28	28	28
Na	11	10	9	11	9	10
He	0.784	0.740	0.692	0.687	0.854	0.835
Ho	0.931	0.815	0.643	0.679	0.607	0.893
Fis	−0.171	−0.082	0.089	0.030	0.306*	−0.051
EcalifOTIS						
N	14	21	10	24	24	28
Na	3	4	4	9	8	6

(*Continued*)

Table 1. (*Continued*).

	Farellones			Lagunillas		
	1000 m	1500 m	2000 m	1000 m	1500 m	2000 m
He	0.602	0.729	0.415	0.788	0.819	0.690
Ho	0.643	0.667	0.200	0.667	0.542	0.964
Fis	−0.031	0.110	0.556	0.175	0.357*	−0.382*

Table 2. F_{ST} (above diagonal) and G''_{ST} (below diagonal) values of the comparison of pairs of sites and elevations (1000, 1500, and 200 m a.s.l.) for *E. californica*. * represents values $P < 0.05$.

	Farellones 1000	Farellones 1500	Farellones 2000	Lagunillas 1000	Lagunillas 1500	Lagunillas 2000
Farellones 1000		0.065*	0.067*	0.085*	0.104*	0.131*
Farellones 1500	0.183*		0.104*	0.105*	0.106*	0.166*
Farellones 1500	0.195*	0.286*		0.087*	0.115*	0.184*
Lagunillas 1000	0.248*	0.291*	0.249*		0.087*	0.144*
Lagunillas 1500	0.321*	0.313*	0.356*	0.270*		0.112*
Lagunillas 2000	0.413*	0.497*	0.567*	0.452*	0.377*	
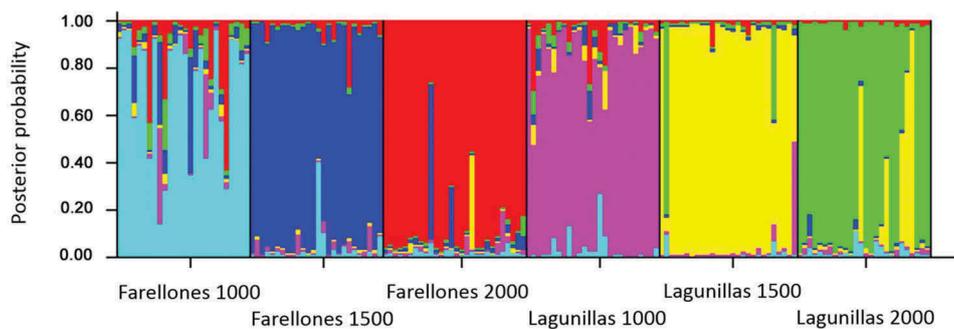
Figure 1. Posterior probability of assignment for 162 individuals of *Eschscholzia californica* (vertical lines) to six genetic clusters based on Bayesian analysis of variation at 11 microsatellite loci. Colours represent the six sampling sites 1000, 1500 and 2000 m a.s.l. at two localities: Farellones and Lagunillas, central Chile.

($p < 0.001$, Figure 2A); the numbers also differed between 1500 and 2000 m ($p < 0.001$). Individuals located at 1000 m presented a lower number of leaves (Figure 2B) compared with individuals at 1500 m ($p < 0.001$) and 2000 m ($p < 0.001$). Additionally, individuals at 1500 m had lower number of leaves than individuals at 2000 m ($p < 0.001$). Individuals at 1000 m had lower floral biomass than those at 1500 m ($p < 0.001$) and 2000 m; there were no significant differences between 1500 and 2000 m ($p = 0.709$, Figure 2C). There were significantly differences among populations at different elevations. No differences were detected between 1000 and 1500 m ($p = 0.426$) for vegetative biomass (Figure 2D) and it was significantly higher at 2000 m ($p < 0.01$ for both comparisons). Plant height differed statistically with elevation (Figure 2E), with the plants at the lowest elevation being significantly taller than the plants at 1500 m and 2000 m ($p < 0.05$ for both comparisons). Plants at 1500 m did not differ from those at 2000 m ($p = 0.155$). Leaf area

showed not statistical differences among elevations. Finally, the RS:VB ratio (Table 3, Figure 2F) for plants at 1000 m was higher than at either 1500 ($p = 0.039$) or 2000 m ($p < 0.007$); no statistical differences were detected between 1500 and 2000 m ($p = 0.087$).

Discussion

Population genetic structure

Our results do not provide evidence of a decrease in genetic diversity with elevation, thus rejecting dispersal restriction from lower to higher elevations. We hypothesise that colonisers at 1500 and 2000 m came from distant populations as a product of human activity. This activity would compensate frontal diffusive dispersal that would lead to genetic impoverishment with dispersal, which could restore genetic diversity. We also hypothesise that propagules could arrive through construction material (soil, sand), vehicles or

Table 3.   Results for generalised linear mixed models (GLMMs) used to explain the seven variables of *E. californica*. Elevation (1000, 1500, 2000 m a.s.l.) was included as explanatory variable (fixed factor) and site was treated as random sources of variation. * represents values $P < 0.05$.

	Estimate	std. error	z value	Pr(>|t|)
Number of flowers				
1000 vs. 1500	−1.02	0.06	−18.26	<0.0001*
1000 vs. 2000	−0.78	0.05	−15.57	<0.0001*
1500 vs. 2000	0.24	0.06	3.80	0.0001*
Number of leaves				
1000 vs. 1500	0.38	0.02	22.13	<0.0001*
1000 vs. 2000	0.58	0.02	35.14	<0.0001*
1500 vs. 2000	0.19	0.01	13.17	<0.0001*

	Estimate	std. error	t value	Pr(>|t|)
Total floral biomass				
1000 vs 1500	0.09	0.01	11.53	0.0008*
1000 vs 2000	0.09	0.01	11.87	0.0007*
1500 vs 2000	0.01	0.01	0.14	0.7087
Leaf area				
1000 vs 1500	−65.34	51.41	−1.27	0.2748
1000 vs 2000	171.80	50.53	3.40	0.0669
1500 vs 2000	237.14	50.99	4.65	0.0424
Vegetative biomass				
1000 vs 1500	−1.48	2.32	−0.64	0.4263
1000 vs 2000	15.73	2.28	6.89	0.0094*
1500 vs 2000	17.20	2.30	7.47	0.0069*
Plant height				
1000 vs 1500	−12.37	1.54	−8.01	0.0052*
1000 vs 2000	−9.24	1.52	−6.09	0.0146*
1500 vs 2000	3.13	1.53	2.04	0.1550
RS/VB ratio				
1000 vs 1500	−1.14	0.26	−4.33	0.0390*
1000 vs 2000	−1.91	0.26	−7.40	0.0072*
1500 vs 2000	−078	0.26	−2.97	0.0866

peoples' clothing (Cain et al. 2000; Mack and Londsale 2001).

The strong population structure observed at different elevations could be explained by the low local dispersal potential and a high longevity of individuals that arrived to a new area. In the case of *E. californica*, explosive dispersal is limited to up to 2 m away from parent plants and adults can live for more than 4 years (Peña-Gómez and Bustamante 2012). This reduced dispersal ability is a common trait in plants and has been responsible for spatial genetic structure observed in other plant species (Williams and Waser 1999; Schmidt and Jensen 2000; Chung et al. 2011).

Given that we used neutral genetic markers, which are not related to the phenotypic traits examined in our study, we cannot connect these genetic changes with the adaptive value of phenotypic expression of plants (Linhart and Grant 1996). We need to go further in population genetic studies to examine adaptive gene expression along elevation gradients (Whitehead and Crawford 2006). Adaptive genetic expression should occur at the edge of distribution ranges (Hoffman and

Blows 1994; Eckert et al. 2010). Recent advances using a genomic approach, combining molecular data, phenotypic traits and fitness across distribution ranges seem to present a promising avenue of research in the examination of the adaptive basis of gene expression in the field (Sexton et al. 2009).

Reproductive and vegetative traits

Individuals of *E. californica* at higher elevations showed significant differences in both reproductive and vegetative traits when compared with populations located at lower elevations. Ideally, we should have assessed changes along more than three points along the full elevation gradient to obtain a complete measure of variation of traits with elevation. Nonetheless, our results suggest that the three selected elevations were sufficient to detect phenotypic changes and showed that these changes varied in a nonlinear way, perhaps as a response to abrupt change in climatic conditions (Van Kleunen and Fisher 2005; Valladares et al. 2007). We detected abrupt phenotypic changes between 1000 and 1500 m (decrease of plant height, increase of number of flowers per plant, increase of total floral biomass, increase of number of leaves per plant) or between 1500 and 2000 m (increase of vegetative biomass) presumably as a consequence of the increase of precipitations. Interestingly, the two traits that are more related with reproduction were more sensitive and plastic to change in the abiotic environment (Sultan 2000), varying significantly from 1000 and 1500 m. From theory, we know that life history traits that are more related with fitness are more sensitive to changes in environmental variables (Nylin and Gotthard 1998) while others, not directly related with reproduction are less plastic (Nicotra et al. 2010).

Plant height reduction, observed along elevation gradients, is one of the major documented phenotypic changes for plants; plant height reduction is regarded as an adaptive strategy to optimise the capture of thermal energy at soil level (Körner 1989). These phenotypic responses observed in populations of *E. californica* are similar to those of native plants (Körner 2003), thus suggesting convergent responses among native and exotic plants due to a strong abiotic filter.

In our study, floral biomass, along with flower size, increased and the number of flowers decreased with elevation. This result suggests a compensation between floral size and flower number. For plants with availability to a limited amount of resources, it is usual to produce few but large flowers especially if pollinators are scarce, a fact largely documented in mountain ecosystems (Herrera 2005; Arroyo et al. 2013). Interestingly, another reproductive trade-off between the number of flowers and vegetative biomass was also reflected in the RS:VB ratio. We found that RS:VB significantly decreased with increasing elevation. Where individuals experience lower temperature, they will proportionally allocate more energy to vegetative biomass rather than to reproduction as a response to abiotic severity (Coleman et al. 1994; Hiraga and Sakai 2007).
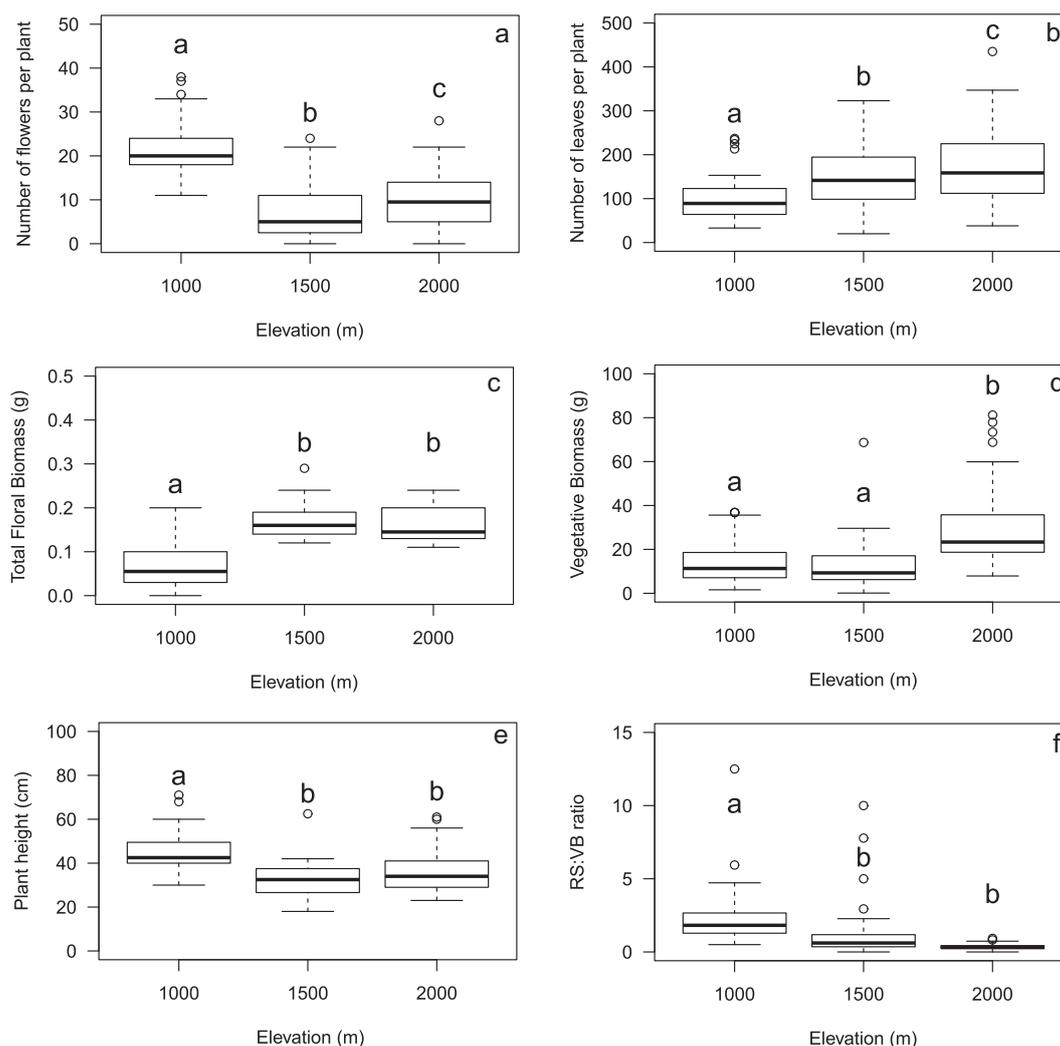
Figure 2. Phenotypic trait changes of *Eschscholzia californica* along elevation (1000, 1500 and 2000 m a.s.l.): A) Number of flowers per plant, B) Number of leaves per plant, C) Total Floral Biomass, D) Vegetative Biomass, E) Plant height and F) RS:VB ratio. Box differing in letters represent statistical differences ($P < 0.05$).

Conclusions

In this research, we have used a novel approach to elucidate whether there is dispersal limitation and phenotypic responses in the invasive plant *E. californica* along altitudinal gradient. Genetic information suggests that at higher elevation there is no dispersal limitation; however phenotypic changes observed in this plant suggest reproductive limitation. Further studies to elucidate the mechanisms which confer adaptive value of the observed phenotypic variation should be a fruitful research avenue in the future. In the same line, further studies to link adaptive traits with genetic variation are an interesting research line, rarely addressed in plant invasion studies.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Notes on contributors

Ramiro O. Bustamante, Professor, is interested in plant population ecology, geographical ecology, conservation biology and invasion ecology.

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Appendix   Microsatellite development

An individual of *Eschscholzia californica* was collected from Farellones in 2013 and stored in Silica Gel. Total genomic DNA was extracted with the CetylTrimethylammonium Bromide (CTAB) extraction method (Tel-Zur et al. 1999). DNA quality and quantity was checked using a Bioanalyzer Agilent Model 2100 and the library was constructed using the GS Rapid library Preparation kit in OMICS-Solutions (http://omics-solutions.cl). In order to maximize sequencing, four different species were bar coded in the same run using a 454 GS Junior system (Roche, Penzberg, Germany), thus one-fourth of the reads were for *E. californica*. After sequencing, repeated motifs were sought as described by Zeng et al. (2013). Five primer pairs tested in our laboratory showed reliable amplifications in the agarose gel electrophoresis. To evaluate polymorphism in an automatic sequen-

cer, reverse primers of each locus were marked with a fluorescent dye.

Genetic variability was assessed in all individuals used in this study. Polymerase chain reaction (PCR) amplification mixtures (12 µL) contained template DNA (100 ng), 0.25 µM of each primer and 10 µL of the Type-it Microsatellite PCR Kit. Cycling conditions consisted of an initial denaturing step of 3 min at 95º C, followed by 35 cycles consisting of 30 s at 95ºC, 30 s at 55ºC and 90 s at 72º C, and a final elongation step at 72º C for 5 min. PCR products with fluorescent primers were genotyped in the sequencing service at Pontificia Universidad Católica de Chile. Peaks were analysed with Peak Scanner software (Applied Biosystems). Sequences were published in Genbank with accession numbers from KX608528 through to KX608532. Table A1 shown the primer sequences and number of alleles observed.

Table A1.   Primer sequence and trait for five microsatellite loci for *Eschscholzia californica*. N: number of individuals analysed; NA: number of alleles obtained from polled data from this study.

Locus	Primer sequence (5′-3′)	Repeat motif	GenBank accession no.	N	N_A	Size range (bp)
Ecalif_90V9	F: GTGTAGTAGACAATCAGACA R: CAGATGATGTGTCTTAATAT	$(TC)_{12}$	KX608528	125	10	172-190
Ecalif_G72Q	F: GGAGAGCTTATTATATCAC R: ACTAGGATATTAGTAGGAAAC	$(AG)_{10}$	KX608529	118	9	208-260
Ecalif_UHK8	F: GAGATTAGTGTAATGGTTAA R: GTCTTACCTGATATTATCAA	$(TA)_{10}T\ (GT)_5$	KX608530	160	19	104-146
Ecalif_27WV	F: CAATAAGTGAGATATACTAGG R: GTATCTTTTATTCTAGCCT	$(AG)_{11}$	KX608531	163	9	132-150
Ecalif_OTIS	F: CAAGATGTCAATAACTAACT R: AGTTTTGAGTTATACATGAG	$(AC)_{11}$	KX608532	121	14	129-135