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RESEARCH ARTICLE

Testing the hypothesis of loss of defenses on islands across a wide latitudinal gradient of *Periploca laevigata* populations

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PREMISE OF THE STUDY: We tested a hypothesis that predicts loss of chemical defenses on island plant populations (LCDIH) as an evolutionary response to limited herbivore pressures.

METHODS: Using a common garden approach, we grew 16 populations ($N = 286$ seedlings) of *Periploca laevigata*, a Mediterranean shrub for which previous studies suggested that animal browsing elicits defensive responses mediated by tannins. Our experimental setting represented a wide latitudinal gradient (37–15°N) encompassing three island systems, virtually free of large herbivores, and three mainland areas. Putative chemical defenses were estimated from tannin–protein precipitation assays, and inducible responses in growth and chemical traits were assessed between seasons and by subjecting plants to a pruning treatment.

KEY RESULTS: We failed to find support for the LCDIH, since island populations (Canary Islands, Cape Verde) had increasingly higher constitutive levels of tannins at lower latitudes. Seasonality, but not experimental pruning, induced variation in levels of tannins in a consistent pattern across populations. Thus, net differences in leaf tannin concentration remained similar among geographical areas regardless of the factor considered, with latitude being the best explanatory factor for this trait over seasonal growth patterns.

CONCLUSIONS: Geographical variation in total tannin pools appears to be mediated by factors other than herbivore pressure in *P. laevigata*. We hypothesize that abiotic correlates of latitude not considered in our study have promoted high constitutive levels of leaf tannins across Macaronesian populations, which ultimately may explain the pattern of seasonal variation and latitudinal increase from Mediterranean to subtropical Cape Verde populations.

KEY WORDS Apocynaceae; chemical defense; common garden experiment; growth–differentiation balance; inducible responses; island–mainland comparisons; latitudinal patterns; pruning treatment; UV exposure.

Environmental pressures on islands often differ markedly from those driving phenotypic responses in neighboring continental areas (Carlquist, 1974; Burns et al., 2012). Dispersal limitations, for instance, have precluded the colonization of remote islands by mainland terrestrial herbivores (Wallace, 1880), which results in reduced pressures for plants evolving in these systems (Grant, 1998; Vourc'h et al., 2001; Burns, 2014). Lack of large browsers, coupled with evidence that anti-herbivore defenses are costly and labile (Coley et al., 1985; Kursar et al., 2009), support the prominent idea that plant evolution on islands may be characterized by a generalized pattern toward loss of defenses (Carlquist, 1974; Bowen and Van Vuren, 1997; Vourc'h et al., 2001).

Plants respond to herbivore attack by deploying structural and chemical responses (Herms and Mattson, 1992; Hanley et al., 2007). While the relaxed expression of mechanical traits associated with anti-herbivory defense has been frequently documented (Bowen and Van Vuren, 1997; Burns, 2014; Kavanagh, 2015), empirical support for the hypothesis of loss of chemical defenses on islands (LCDIH, hereafter) is equivocal (cf. Adsersen and Adsersen, 1993; Bowen and Van Vuren, 1997; Vourc'h et al., 2001; Pardo et al., 2018).

The mixed results obtained in chemical studies might be explained by different untested factors. For instance, if the levels of defensive compounds vary depending upon plant damage (i.e., plant defenses are inducible; Karban and Baldwin, 1997), time-point measurements of chemical compounds could not capture plastically induced responses (Anstett et al., 2016). Assuming a cost to defense, low herbivory risk on oceanic islands should favor induced over constitutive responses: environments with low herbivore pressure (e.g., islands) should select for low constitutive defenses and high inducibility because the costs of continuously producing constitutive defenses are high when damage is low (i.e., costs of constitutive defenses outweigh their benefits) (Karban and Baldwin, 1997; Moreira et al., 2014; Bixenmann et al., 2016).

At broad geographical scales, several studies suggest that production of constitutive defenses may be driven by environmental covariates of latitudinal variation (Hallam and Read, 2006; Pearse and Hipp, 2012; Wang et al., 2016; but see Moles et al., 2011). Physiological theories, in turn, predict that the amount of resources invested in defense trades off with growth patterns (Coley et al., 1985; Herms and Mattson, 1992). Because plant defense may therefore be governed by a combination of intrinsic (e.g., growth rates) and extrinsic (environmental) factors, formal testing of the LCDIH should consider such explanatory factors. A multifactorial approach is particularly relevant when sets of undetermined chemical compounds are used as proxies of plant defense. Tannins, for instance, are among the most popular secondary compounds measured in studies of plant defense, but due to their mixed composition, variation in total tannin pools has been linked to several factors that are not strictly related to antiherbivore responses, including seasonality or exposure to solar radiation (Close and McArthur, 2002; Salminen and Karonen, 2011). Despite this functional diversification, broad-scale correlations between levels of herbivory pressure and plant investment in tannins (Wang et al., 2016; Moreira et al., 2018) are suggestive of strong biogeographical patterns in plant defense modulated by these phenolic compounds. Therefore, if herbivore pressure is the primary driver of tannin investment, we would expect lower levels of tannins in oceanic island populations than those observed in mainland populations (Bowen and Van Vuren, 1997) or, assuming a cost-effective induced response (Moreira et al., 2014; Bixenmann et al., 2016), that defenses on islands are preferentially released following plant damage.

In this paper, we conducted a common garden experiment to test the prediction that populations on island settings should display low levels of chemical defense. We used the shrub *Periploca laevigata* Aiton (Apocynaceae) as a study case because its distributional range, coupled with its well-documented biogeographical history and chemical responses, provide an ideal framework for testing ideas relating to the evolution of putatively defensive compounds on islands. Previous field studies at mainland locations suggest that tannin production in *P. laevigata* is a plastic trait that varies seasonally and is elicited by mammal browsing (Alados et al., 2002; Barroso et al., 2003). Based on this information, we anticipated that islands with low herbivore risk should favor induced over constitutive chemical defenses. The species' widespread distribution, spanning from the Mediterranean Basin to the Cape Verde Islands, also allows investigation of the major drivers of latitudinal trait variation. Recent phylogeographical studies add a valuable layer of interest: *P. laevigata* comprises four sublineages that have been identified as independent cases of mainland-to-island colonizations (García-Verdugo et al., 2017, 2018). Each island sublineage can therefore be considered as a replicated experiment, and similar patterns of trait variation would be expected among sublineages if the LCDIH was a generalized phenomenon. Based on these premises, we addressed the following questions regarding geographical variation and inducible production of tannins: (1) Do island populations have lower levels of tannins than mainland populations when plants are grown in similar environments? (2) Do extrinsic factors typically related to tannin investment, such as growth patterns or plant damage, account for patterns of chemical variation across latitudinal gradients? Our study aims at gaining some insight into how chemical variation of putatively defensive compounds can be influenced by geographical variation and environmental cues, with a particular focus on the biogeographical background provided by oceanic islands.

MATERIALS AND METHODS

Study system and species

Periploca laevigata typically occurs in open, semi-arid areas with poor-nutrient soils (Castro et al., 2003; Arista and Ortiz, 2012). It comprises four sublineages of wind-dispersed shrubs that have colonized different island settings, including two Macaronesian archipelagos (Cape Verde and Canary Islands), and several Mediterranean islands in the strait of Sicily (Arista and Ortiz, 2012; García-Verdugo et al., 2017). As a thermophyllous element, it is generally restricted to low elevation (<500 m a.s.l.) habitats across its mainland distribution. However, island colonization in *Periploca* is associated with niche shifts, and populations on the western Canarian islands often reach higher elevation in south-exposed areas (above 700 m a.s.l.), whereas most populations on the Cape Verde islands are found above 1200 m a.s.l. (Chevalier, 1935; García-Verdugo, 2014; Table 1).

Due to its ecological and medical importance in semiarid regions, *P. laevigata* has been subject to extensive chemical

TABLE 1. Number of populations (pops.) sampled for each of the recognized *Periploca laevigata* sublineages, estimated times of island colonization inferred in previous phylogeographical studies, and latitudinal (Lat.) and elevation (Elev.) ranges represented in this study.

Sublineage	Sampling region (no. pops.)	Island colonization time (Ma)[a]	Lat. range (°)/Zone	Elev. range (m a.s.l.)
Mediterranean (MED)	mainland (4) + island (3)	<0.20	37–30/temperate	40–250
Eastern Canaries (ECA)	island (3)	0.39-0.20	29–28/subtropical	50–140
West + central Canaries (WCA)	island (3)	2.10	27–28/subtropical	100–700
Cape Verde (CAV)	island (3)	0.56	17–15/tropical	1200–1800

[a] Million years ago (Ma), from García-Verdugo et al., 2017, 2018.

characterization (Barroso et al., 2003; Dghim et al., 2013; Mahmoud et al., 2016; Neffati et al., 2017). All these studies have detected substantial amounts of tannins in different plant parts, while other secondary compounds with suspected anti-herbivore activity, such as alkaloids or cardiac glycosides, are absent (Dghim et al., 2013; Mahmoud et al., 2016). In *P. laevigata*, tannins have been identified as the chemical defense involved in the response to browsers (Alados et al., 2002; Barroso et al., 2003), and thus this plant species provides an interesting study system for analyzing how chemical compounds suspected to act as anti-herbivore defenses may have evolved following repeated introduction into new island habitats.

Recent genetic studies in *Periploca* have provided a well-supported biogeographical scenario of island colonization that may have started in the Plio-Pleistocene (García-Verdugo et al., 2017). Populations of the western and central Canary Islands constitute the oldest sublineage, with an estimated divergence time from mainland counterparts of ca. 2 Myr ago (Ma). Two other Macaronesian island sublineages diverged more recently from mainland populations, namely, Cape Verde (560,000 years ago) and the easternmost Canarian islands of Lanzarote and Fuerteventura (390,000 years ago; García-Verdugo et al., 2017). According to phylogeographical reconstructions, the Mediterranean islands probably represent the most recent event of colonization, although Fuerteventura may have experienced another recent episode of colonization from mainland areas in the last 200,000 years (García-Verdugo et al., 2017, 2018).

Similar to other oceanic islands, one common feature to all the Macaronesian islands where *Periploca* occurs is that large mammal browsers have been absent until human colonization. The only native vertebrates that might have acted as browsers are the giant tortoises in the genus *Geochelone*, but the fossil record suggests that they went extinct before the arrival of *Periploca* (Hutterer et al., 1997). The introduction of domestic ungulates on both archipelagos probably started some centuries ago, but the widespread distribution of *Periploca*, spanning all major islands, coupled with the historic concentration of introduced goats on the easternmost Cape Verde and Canary islands (Gangoso et al., 2006; Hazevoet and Masseti, 2011), strongly support the idea that the evolutionary impact of large herbivores has been limited in space and time. Furthermore, our field observations on *P. laevigata* and examination of large numbers of herbarium specimens (García-Verdugo et al., 2017) indicate that Macaronesian plants rarely show evidence of ungulate or insect attack. The only known endemic insect for which *P. laevigata* acts as an obligate host plant is the microlepidoteran *Trifurcula nigrifasciata* (Walsingham, 1908), that exclusively occurs on two of the central Canarian islands (Klimesch, 1977). Other types of herbivores (e.g., belowground nematodes) have not been documented. Thus, the biogeographical framework for *Periploca* illustrates that island populations are undergoing a process of relatively recent allopatric divergence, yet sufficiently old to safely assume that extant phenotypes have evolved in the absence of significant herbivore pressures at broad island scales (see Bowen and Van Vuren [1997] and Vourc'h et al. [2001] for similar empirical backgrounds).

Field sampling

To represent all the recognized island sublineages and compare these with extant mainland counterparts, we sampled one or two fruits from 20 mother plants from three island populations of each sublineage (Fig. 1, Table 1). For the western Canarian sublineage ("WCA", hereafter), populations were sampled on three islands. In the case of the eastern Canarian ("ECA") and Cape Verde ("CAV") sublineages, sampling was restricted to two islands with the largest populations (Gomes et al., 1999; García-Verdugo et al., 2017). Although Lanzarote and Fuerteventura populations likely arose from multiple colonization events, they were treated as a single sublineage because both islands experience very similar environmental conditions and represent a similar temporal window of colonization in the context of the study (Table 1). Four mainland populations ("MED-main"), representing the general distribution of *P. laevigata* in the continent (Arista and Ortiz, 2012; García-Verdugo et al., 2017), were considered. Three Mediterranean island populations ("MED-isl": Lampedusa, Linosa and Pantelleria) were additionally sampled to represent the former sublineage under island conditions (Table 1).

Common garden setting

A common garden experiment was set up in the facilities of the Viera y Clavijo Botanic Garden (28°03′55″N, 15°27′43″W, Canary Islands). Four or five seeds of each mother plant collected in the natural populations were sown in germination trays filled with commercial substrate and watered to field capacity every 4 days. Approximately 10 days after sowing, germination rates were already high (55–90% among populations), and 2 weeks after emergence, seedlings were transplanted to small pots and kept under greenhouse conditions. Once the plants had reached approximately 20 cm in height, one seedling originating from the material collected in the field from each mother plant was randomly selected to maximize the representation of genetic variability at the population level ($N = 20$ per population). Seedlings were transplanted into 6-L pots with a substrate composed of 50% of mineral soil, 25% of commercial peat moss, and 25% of lava cinder and were transferred to the common garden setting.

In June 2014, pots were assigned to each of three experimental blocks set on an open area of the research facilities under sun-exposed conditions (Appendix S1). Temperature was monitored with a meteorological station (Appendix S2), and plants were regularly watered to avoid drought stress (typically, twice a week in summer and once a week in winter). To minimize potential bias due to microclimatic effects within the experimental setting, each population of the same sublineage was assigned to a different block, with the exception of two mainland Mediterranean populations that co-occurred in the central block. Individuals of the same population were arranged in the same row, but the position of pots was randomized within rows every 20–30 days and populations randomized within blocks every 6 months. To examine the chemical composition of the substrate, samples from three pots were analyzed 1 year after transplantation (see García-Verdugo et al., 2017, for details). On average, phosphorus concentration in our experiment (111 ± 35 mg/kg) was ca. 2 times higher than that reported in mainland field populations, and levels of nitrogen were similar (0.15 ± 0.02%; Castro et al., 2003). Commercial fertilizers were not added at any stage of the experiment, because plants did not show any symptom of nutrient deficiency throughout the course of the study. Approximately 1 year after germination, all plants were woody and attained leaf phenotypes similar to those observed under field conditions.

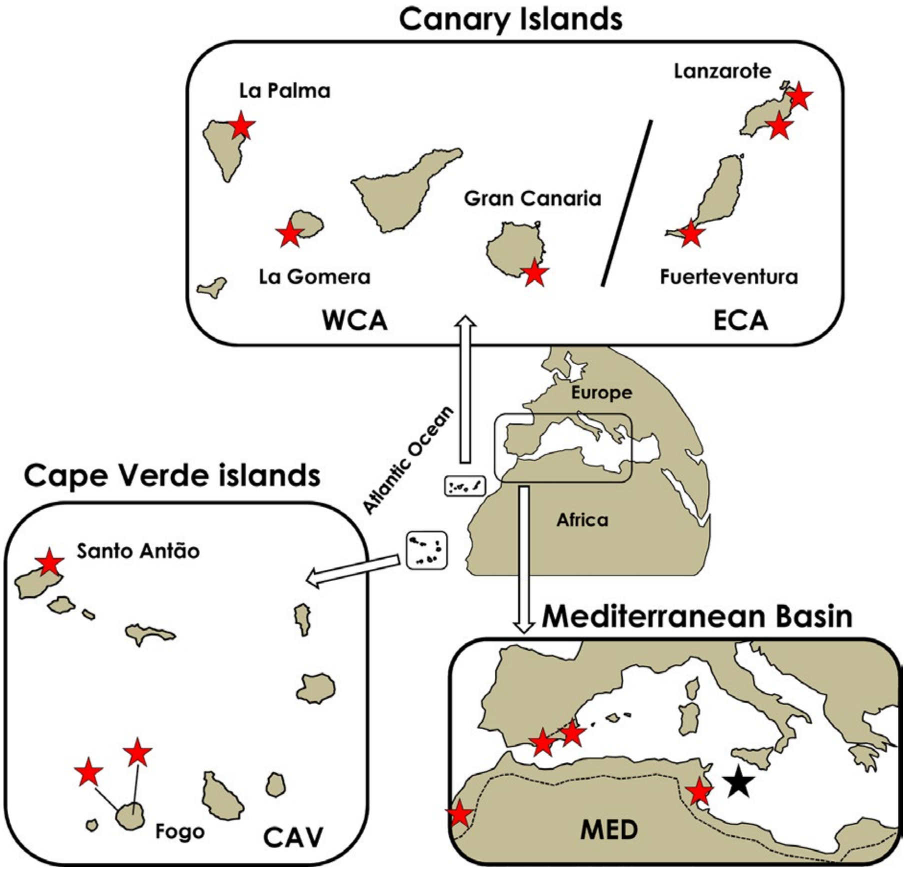

FIGURE 1. Locations of the *Periploca* sublineages (abbreviations as in Table 1) and populations represented in the common garden experiment. Dotted lines show the distribution of mainland Mediterranean populations; the black star indicates the location of three Mediterranean island populations that were represented in the experiment.

All plants were inspected twice a week throughout the duration of the experiment to correct potential issues. Close visual inspection suggested that damage by insect herbivores was only sporadic. We rarely observed cases of terminal leaves of plants being wrapped and partially consumed by the caterpillars of one undetermined species of Lepidoptera, but the extent of damage at the individual level was very local and widely dispersed throughout the year and experimental setting. Mortality of *Periploca* plants was also markedly low, affecting only four individuals during the experiment.

Experimental approach

Tannin pool quantification—Sampling was performed at two contrasting times of year (early autumn 2016 and winter 2017; Appendix S2), based on our observation in preceding years that shoot growing in common garden plants was highly conditioned by seasonality. Thus, at the end of summer 2016, 18 individuals per population were sampled for quantification of leaf tannins. Sampling was conducted over 3 weeks, but it was stratified across sublineages and populations to dilute the potential effect of collection time on tannin quantification. Between 10 and 20 fully expanded, sun-exposed leaves were collected from the third to fourth internode of different shoots. Leaves of each individual were immediately put in a paper bag that was immersed in silica gel and left in the dark for 2 days to ensure complete leaf dehydration. Preliminary trials revealed that this drying technique provides proportional results than those obtained from fresh leaf material (Appendix S3). Quantification of tannin concentration was therefore made from dried leaf material, following the radial-diffusion assay (Hagerman, 1987) and the protocol described for *Periploca* mainland populations in Barroso et al. (2003), with small modifications. This method allows estimation of the concentration of both hydrolysable and condensed tannins with protein-binding capacity (Hagerman, 1987), which should be the target of chemical assays if ungulate browsing, rather than insect consumption, is the suspected type of herbivory driving plant responses (Salminen and Karonen, 2011). Briefly, 200 mg of leaves were weighed and transferred to a 2-mL Eppendorf tube with two 5-mm stainless steel grinding balls. Then, the leaf material was ground into fine powder in a MM200 mixer mill (Retsch, Haan, Germany). Tannins were extracted by adding 1.5 mL of a methanol-distilled water (1:1) solution to the leaf powder with constantly shaking of the mixture at 20°C. After 3 h, the mixture was centrifuged at 15,000 × *g* for 10 min, and the supernatant was recovered with a pipette and transferred to another tube. Leaf extracts were stored at −20°C until tannin quantification using the method of Barroso et al. (2003). To examine the consistency of the radial-diffusion method, we assayed each sample twice. The results for replicates were identical in most cases, and means over the two replicates were used for calculations of tannin concentration. Tannin concentration was expressed as a percentage (w/w) of tannic acid on a dry leaf basis (Barroso et al., 2003).

Assessment of growth patterns—Following previous studies in *Periploca* (Ennajeh et al., 2010) and other Mediterranean multi-branched plants (e.g. Llorens et al., 2004), mean elongation rates across a subsample of shoots per individual were measured to estimate individual growth rates. First, to determine the number of replicates that may be representative at the plant level, all the shoots showing active apical growth were counted in 15 individuals from each population in early autumn 2016. Although CAV individuals tended to have fewer active shoots (mean = 8.5), this trait was generally similar across sublineages (mean = 11.4; results not shown). Thus, five lateral shoots with similar sizes were chosen from different areas of each plant, and the third internode of each shoot was double-tagged with a loose knot of floss and a small mark of white paint. Based on the growth patterns described for *Periploca* by Ennajeh et al. (2010), shoot elongation was measured 40 days after tagging. Elongation rates (mm day^{-1}) were estimated for each individual as the average of measurements across shoots divided by the monitoring time (number of days).

To assess the pattern of biomass allocation to different plant organs (stems or leaves), we cut the portion of shoots developed during the monitoring of elongation rates from each of the five tagged shoots and transported them to the laboratory. Ten individuals per population, corresponding with those subsequently subject to the pruning experiment (see below) were used for biomass allocation measurements (N = 160 individuals). Leaves from 7–9 individuals per population were additionally collected for leaf size characterization. Once in the laboratory, a minimum of two leaves randomly chosen per individual were scanned, and leaf area was measured using Image J software (https://imagej.nih.gov/ij). Leaves and stems were carefully separated from each collected shoot segment, stored in paper bags, dried at 65°C for a minimum of 48 h, and weighed using an AB54 Mettler Toledo (Mettler-Toledo AG, Greifensee, Switzerland) precision balance.

Experimental pruning—At the beginning of December 2016, the previous 10 individuals per population used in analyses of biomass allocation were subjected to a pruning treatment. To minimize potential defense induction via airbone volatiles between treated and untreated plants (Karban et al., 2014), we allocated all individuals in the pruning treatment to one of the peripheral experimental blocks. Browsing was simulated by manually removing 75% of the foliage and clipping the distal part of the shoots with hand shears (Appendix S1). The intensity of this treatment was planned to ensure the levels of damage suspected to elicit chemical responses in *Periploca* following artificial and ungulate browsing (Barroso et al., 2003). The remaining eight plants per population previously characterized for leaf tannin concentration were kept as a control treatment. The pruning treatment induced strong activation of vegetative buds approximately 2 weeks after application, resulting in an 8× increase of active growing areas in MED populations, 6× in ECA populations, and 4× in WCA and CAV populations with respect to the number of active shoots documented in early autumn (results not shown). All pruned plants resprouted vigorously, although newly developed shoots were particularly small and congested, and attempts to analyze patterns of biomass allocation as previously done in early autumn measurements were unsuccessful due to the very limited development of internodes (Appendix S1). We therefore characterized biomass allocation in winter by comparing the number and size of leaves and stems (internode length) developed by the plants during the winter trial. In January 2017, shoot elongation and leaf tannin concentration were characterized in pruned and control plants following the previously described methods.

Statistical analyses—We constructed a data matrix including the following traits measured in early autumn (t1) and/or in winter (t2): leaf size (t1, t2), shoot elongation rate (t1, t2), percentage of biomass allocation to leaves (t1), leaf tannin concentration (t1, t2), number of leaves (t2), and internode length (t2). Data were averaged at the population level, log-transformed when needed to achieve homoscedasticity (i.e., elongation rates, biomass allocation to leaves), and analyzed using a hierarchical ANOVA model: Trait (t1) = setting + sublineage (setting), where setting indicates whether the population was sampled from an island or mainland area.

Next, the effect of abiotic factors in trait variation was examined by adding a set of covariates to the model. Thus, population-level data for 19 bioclimatic variables related to temperature and precipitation were obtained from Worldclim (http://www.worldclim.org) at a 2.5-min resolution (Hijmans et al., 2005). To summarize the potential contribution of these variables, we used a principal coordinate analysis. The first component (PC1) explained 53.2% of the variance, with temperature and precipitation seasonality displaying the highest loadings (>0.92, in both cases) in positive and negative directions, respectively. Standardized scores for PC1 were used as a covariate ("climPC1"), in addition to two physical factors associated with plant investment in defense (population latitude and elevation; Moreira et al., 2015; Abdala-Roberts et al., 2016) in the following model: Trait (t1) = setting + sublineage (setting) + covariate.

Comparing the results of both models, shifts from significant to nonsignificant effects of categorical factors allow identification of the covariates accounting for geographical patterns of variation in particular traits.

The effects of seasonality (t1 vs. t2 measurements) and the pruning treatment (control vs. pruned plants) were tested using two separate mixed-model ANOVAs, with "season" or "pruning" as the within-subject factor. An interaction term considering the within-subject factor and "sublineage (setting)" was included to identify potential differences among geographical areas in their response to these two factors. Regression analyses were used to examine seasonal patterns of variation between tannins and intrinsic and extrinsic factors.

RESULTS

Phenotypic measurements in early autumn

Island and mainland settings did not display significant differences in early autumn, because (1) most of the variation was found among sublineages and (2) Mediterranean island and mainland populations were similar in all traits (Table 2). Differences among sublineages revealed that, contrary to the predictions of the LCDIH, all the Macaronesian islands showed higher investment in constitutive tannins than the mainland populations. Thus, CAV had the highest concentration of leaf tannins, followed by WCA, then ECA. In contrast, leaf tannin concentration did not significantly vary between Mediterranean island and mainland populations, both showing the smallest values (Table 2). Morphological traits roughly followed a correlated pattern of variation with that observed for tannins; Macaronesian sublineages had larger leaves, lower shoot elongation rates and a higher percentage of investment in leaf biomass than did Mediterranean plants (Table 2). Inclusion of covariates in the model suggested that latitude was the only factor significantly driving differences in leaf tannins among sublineages, whereas climatic correlates of latitude (climPC1) and latitude accounted for differences in growth patterns (Appendix S4). Thus, populations sampled at lower latitudes had higher tannin investment and slower elongation than those originating from higher latitudes.

Effect of seasonality and pruning treatment on leaf tannin concentration

Winter measurements of control plants showed that elongation rates were significantly slower than those measured in early autumn for the same subset of plants ($F_{1,4}$ = 38.3, P < 0.001), thus resulting in convergent winter rates across sublineages (Fig. 2A). Leaf tannin concentration was also significantly lower than in early autumn in all sublineages ($F_{1,4}$ = 13.7, P < 0.01), but, contrary to elongation rates, net differences among sublineages remained similar to those

TABLE 2. ANOVA results (*F*-ratios and significance) for the analysis of phenotypic traits of 286 *Periploca* individuals measured in a common garden in early autumn. Independent factors include setting (island vs. mainland) and sublineage (nested in setting); below, mean (±SE) trait values for each sublineage are indicated. Letters for a given trait represent significant differences following post-hoc tests. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, ns = nonsignificant.

Factor/Sublineage	Leaf tannins (w/w)	Leaf size (cm²)	Leaf biomass (%)	Shoot elongation (mm day⁻¹)
Setting	$F_{1,3} = 0.84^{ns}$	$F_{1,3} = 1.4^{ns}$	$F_{1,3} = 2.7^{ns}$	$F_{1,3} = 1.3^{ns}$
Sublineage (setting)	$F_{3,11} = 26.0^{***}$	$F_{3,11} = 16.6^{***}$	$F_{3,11} = 3.8^{*}$	$F_{3,11} = 6.2^{**}$
MED_main	2.25 ± 0.33^{A}	0.90 ± 0.09^{A}	74.8 ± 1.2^{A}	0.91 ± 0.10^{A}
MED_isl	1.82 ± 0.12^{A}	0.87 ± 0.06^{A}	77.4 ± 2.9^{A}	0.93 ± 0.03^{A}
ECA	4.23 ± 0.89^{B}	2.33 ± 0.20^{B}	81.9 ± 4.6^{B}	0.58 ± 0.13^{B}
WCA	5.95 ± 0.57^{C}	3.53 ± 0.45^{C}	87.8 ± 0.6^{C}	0.33 ± 0.07^{BC}
CAV	8.53 ± 0.59^{D}	3.00 ± 0.32^{C}	87.3 ± 1.3^{C}	0.24 ± 0.04^{C}

in autumn, with CAV populations having the highest values followed by WCA, then ECA (Fig. 2B).

Pruning induced resprouting of shoots with higher elongation rates than those measured in control plants ($F_{1,4} = 16.25$; $P = 0.016$), but patterns of biomass allocation were different between control and pruned plants. Pruned plants produced shorter internodes, resulting in larger numbers of smaller leaves than on control plants (Fig. 3A–C). However, leaf tannin concentration was not significantly different between control and pruned plants, with the exception of the Cape Verde populations (Fig. 3D). Again, differences in tannin concentration among sublineages of pruned plants remained similar to those in the early autumn and control winter plants (Fig. 3D).

Regression analyses revealed a significant, positive association between leaf tannin concentration and elongation rates in early autumn across *Periploca* populations (Fig. 4A), but such an association was not significant for winter measurements (Fig. 4B). Latitude had, in turn, a consistently significant positive association with leaf tannin concentration (Fig. 4C), thus suggesting that island populations at lower latitudes have high leaf tannin content regardless of the season.

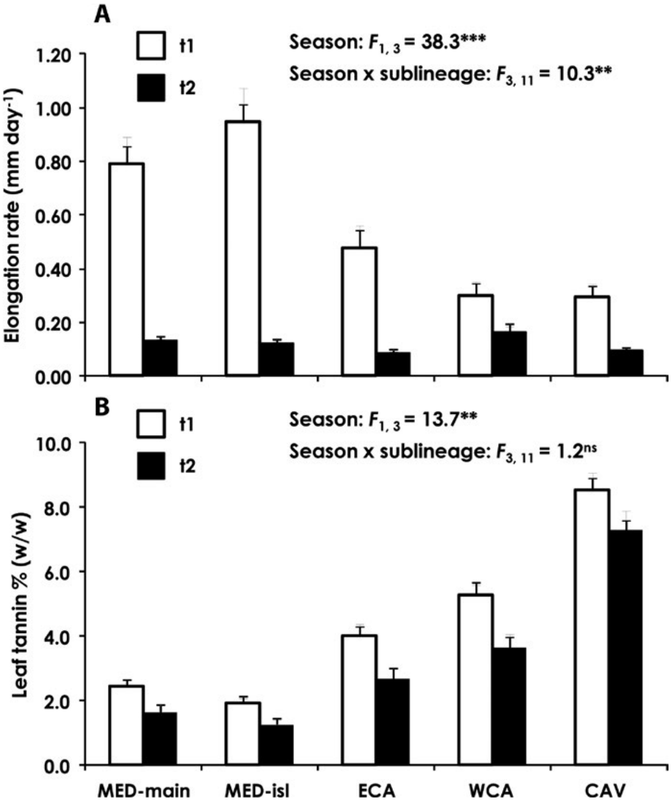

FIGURE 2. Mean (+SE) of shoot elongation rates (A) and leaf tannin concentration (B) between two contrasting seasons (t1 = early autumn; t2 = winter) measured in *Periploca* control plants reared under common garden conditions. Results of the ANOVA testing for differences between seasons and sublineage interactions are detailed for each trait. All comparisons between seasons within sublineages were significantly different ($P < 0.05$). Sublineages are abbreviated as in Table 1. $^{***}P < 0.001$, $^{**}P < 0.01$.

DISCUSSION

Island-mainland contrasts: lack of support for the LCDIH based on tannin pools

The results from our common garden experiment did not support the idea that island populations invest less in putative defensive compounds than their mainland counterparts (cf. Bowen and Van Vuren, 1997). In our study, such a prediction was indeed rejected three times; populations representative of independent episodes of island colonization (Cape Verde, West Canaries and East Canaries; García-Verdugo et al., 2017) all had substantially larger concentrations of leaf tannins than those of putatively ancestral mainland populations. Repeated evolution toward increased tannin concentrations further suggests that this trait may have been positively selected across Macaronesian sublineages (Givnish, 1994; Burns, 2014), while phenotypic similarity between Mediterranean island and mainland populations may be explained by recent island colonization and niche conservatism (Blondel, 1985; García-Verdugo, 2014).

The observed pattern in tannin investment does not necessarily imply that Mediterranean populations are more vulnerable to large herbivores than Macaronesian populations. Our results suggest that Mediterranean plants deploy morphological rather than chemical responses to cope with eventual browsing damage (Turley et al., 2013). We did not find evidence that plant damage elicits a significant increase of leaf tannins in mainland *Periploca* populations, most probably because such a response could be restricted to recurrent episodes of very intense biomass removal (Barroso et al., 2003). Even when a clear chemical response to potential browsing damage was not detected, our experiment showed that mainland populations grow fast in nonstressful conditions (Llorens et al., 2004; Ennajeh et al., 2010). Following herbivore attack, fast-growing species preferentially develop resilience responses by replacing leaf area lost to damage more quickly than slow-growing plants do (Coley et al., 1985; Turley et al.,

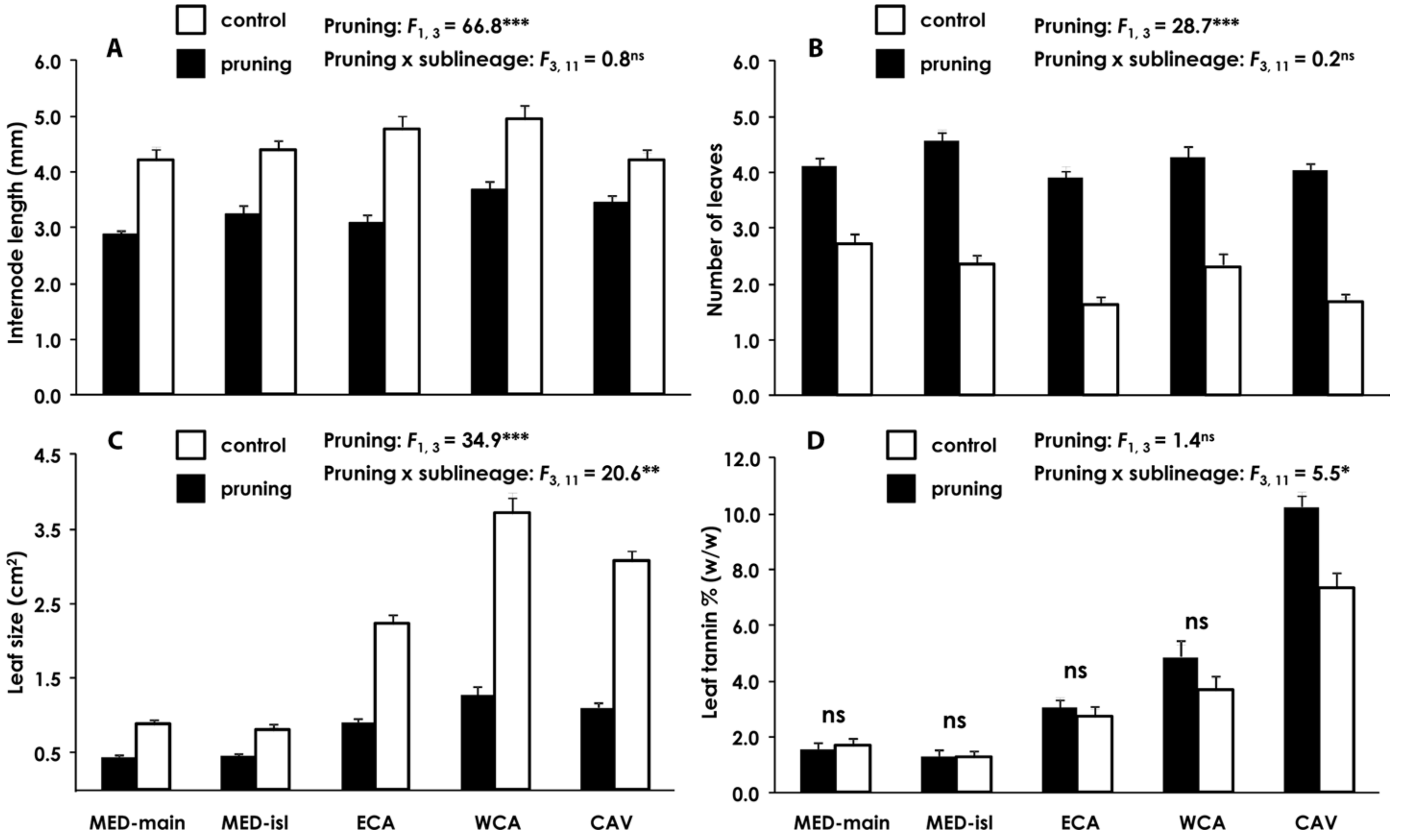

FIGURE 3. Means (+SE) of winter measurements of control and pruned *Periploca* plants grown in the common garden experiment. Results of the ANOVA testing for treatment (control vs. pruned plants) effects and sublineage interactions are detailed for each trait: internode length (A), number of leaves (B), leaf size (C) and tannin concentration (D). If not marked "ns" (nonsignificant), differences between control and pruned plants within each sublineage were statistically significant ($P < 0.05$) according to post-hoc tests. Sublineages are abbreviated as in Table 1. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

2013). Experimental pruning in Mediterranean *Periploca* plants induced fast development of large numbers of small leaves, which may originally have evolved as a strategy to cope with great biomass loss in drought-prone ecosystems (Paula and Pausas, 2006).

Plant damage and risk of herbivory on islands: Are tannins actual anti-herbivore defenses in *Periploca*?

The subtropical conditions experienced by oceanic island lineages are thought to have promoted acquisition of plant traits related to slow growth rates as compared to mainland congeners, such as larger leaf sizes and increased woodiness (Carlquist, 1974; Burns et al., 2012; García-Verdugo et al., 2014). In agreement with this notion, climatic correlates of latitude (most notably, precipitation and temperature seasonality) accounted for differences in growth rates among *Periploca* sublineages. Our study species conformed to expectations of the growth-differentiation hypothesis (Herms and Mattson, 1992) in that slow-growing Macaronesian populations invested in larger leaves with higher levels of secondary compounds than in fast-growing Mediterranean populations. Nevertheless, while higher constitutive investment in secondary compounds (tannins) appears to be tightly associated with slower growth in *Periploca*, the potential selection of these metabolites due to their anti-herbivore role is not evidenced by our study.

Both the high constitutive levels of tannins in Macaronesian populations (Table 2) and the very limited effect of our pruning treatment on this trait reject the hypothesis that low herbivore risk on islands may have favored inducible defensive responses (see Moreira et al., 2014; Bixenmann et al., 2016). Following experimental damage, only those populations with the highest constitutive levels of tannins (Cape Verde) had a significant increase in tannin concentration, but this chemical response was coupled with shifts in biomass allocation within shoots. Taken together, increased tannin concentration in Cape Verde resprouting tissues can be interpreted as a passive chemical alteration between plant organs, rather than an active response against herbivores (Stock et al., 1993; Häring et al., 2007). As an alternative explanation for the island-mainland pattern detected, herbivore guilds known to occur on the islands (i.e., insects), instead of large mammalian browsers, may be responsible for high levels of tannins among insular populations. This possibility, however, is not supported by the available information. First, our chemical essays provided estimates of leaf tannins with protein-precipitation capacity (Hagerman, 1987), which, as opposed to tannins with oxidative activity, are thought to have a very limited effect on insect performance (Salminen and Karonen, 2011). Thus, selection of tannins with protein-binding activity in island populations could have hardly evolved as a direct response to insect herbivory. In addition, unlike in other systems (Kursar et al., 2009; Moreira et al., 2018), field observations over a decade (C. García-Verdugo, personal observation) suggest that insect damage

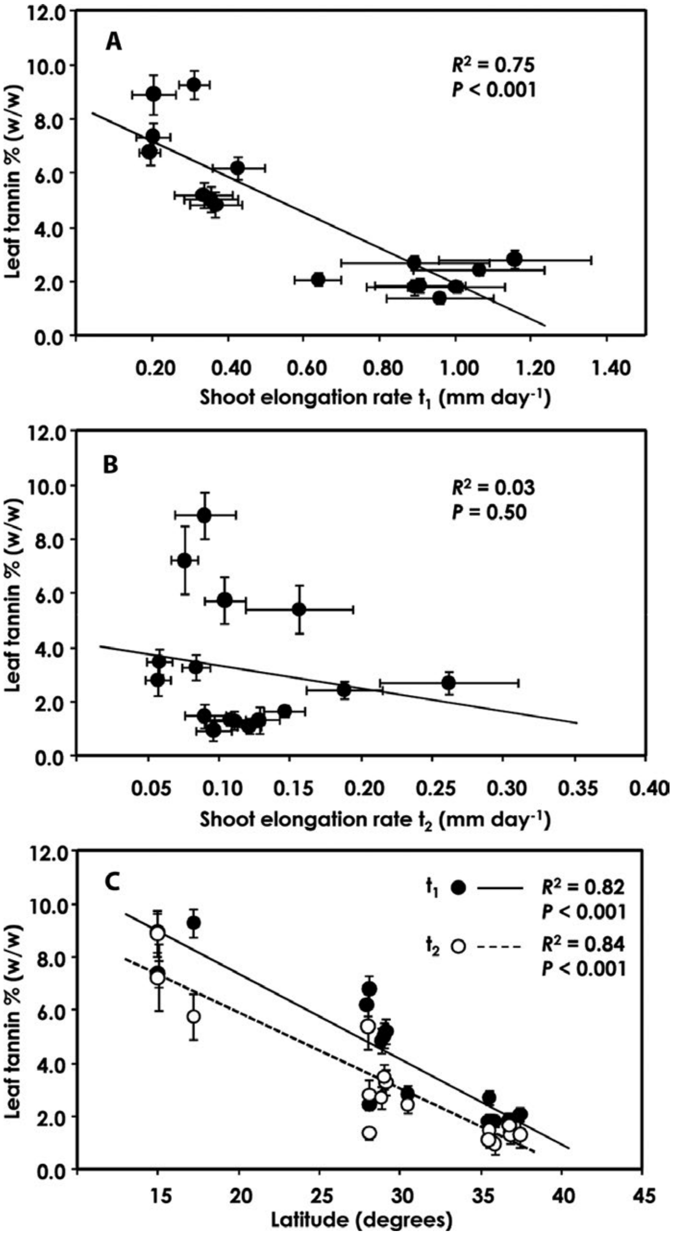

FIGURE 4. Relationship between (A) leaf tannin concentration and shoot elongation rate in early autumn (t1), (B) shoot elongation rate in winter (t2), and (C) latitudinal location for both seasons across 16 *Periploca* populations reared in the common garden. Regression lines and correlation coefficients are detailed for each test.

does not seem to be as frequent or intense as to represent a strong selective factor in *Periploca*, at least among extant populations. Rather, we hypothesize that factors other than herbivory are responsible for the described pattern of geographical variation in leaf tannins.

Environmental factors accounting for latitudinal gradients of tannin variation

Colonization of the Macaronesian islands from continental areas by *Periploca* implied (1) expansion of the mainland distribution range to lower latitudes and (2) niche shifts to higher elevation areas. The extreme of this niche expansion is represented by Cape Verde populations, which occur in mountain areas above 1200 m a.s.l. at tropical latitudes (Chevalier, 1935). Furthermore, because *Periploca* occurs in open habitats, individuals are subject to intense solar exposition, particularly in summer. The pattern of geographical variation in leaf tannin concentration detected in our study correlates with population latitudinal location and seasonality (i.e., smaller concentration at higher latitudinal areas and winter). Our analysis did not find particular bioclimatic variables that may be responsible for such a strong pattern, likely because many environmental factors that covary with latitude remain unexplored (Anstett et al., 2016). For instance, exposure to UV-B radiation positively correlates with higher altitude and lower latitude, and seasonally increases from winter to summer in the northern hemisphere (Rozema et al., 1997; Comont et al., 2012). This pattern encouraged us to explore the relationship between latitudinal variation in leaf tannins and UV exposure by extracting solar UV irradiation data from broad-scale climate data sources (www.weather-atlas.com; www.temis.nl). Based on a subset of *Periploca* populations for which monthly UV data were available from nearby stations (N = 11, excluding the small Mediterranean islands and two Macaronesian locations), we found strong correlations for both early autumn ($R = 0.94$, $P < 0.001$) and winter ($R = 0.90$, $P < 0.001$) measurements. Although this relationship should be further addressed by using a more thorough approach (see for instance, Comont et al., 2012), our tentative findings point toward a potential role of *Periploca* tannins in photodamage protection (Close and McArthur, 2002).

Increased tannin concentrations with lower latitude are commonly found when the spatial scale of the study spans from temperate to subtropical/tropical areas (e.g., Hallam and Read, 2006; Pearse and Hipp, 2012). This coincident pattern qualifies increasing UV exposure toward the tropics as a candidate driver of leaf tannin concentration. However, while the potential contribution of UV radiation to the observed latitudinal patterns in tannins is widely acknowledged (e.g., Hallam and Read, 2006; Wang et al., 2016), attempts to examine its effect have been seldom considered in correlatives studies (but see Comont et al., 2012). Because both temperature and UV irradiance covary with latitude from temperate to tropical regions, the most frequent abiotic factor associated with tannin variation (i.e., temperature; Pearse and Hipp, 2012; Wang et al., 2016) should be jointly analyzed with UV data. Taking all these results together, we hypothesize that *Periploca* plants evolved higher UV resistance as a response to population expansion toward lower latitudinal areas and that this set the template for successful altitudinal habitat shifts on subtropical oceanic islands.

Limitations and future prospects

Our study is based on the examination of a single chemical trait (total tannin pool) and therefore our results should not be taken as strong evidence against the LCDIH, since other unmeasured traits in *Periploca* (e.g., trichomes, terpenoids) may be involved in herbivore resistance or defense (Anstett et al., 2016). In addition, the analysis of trait variation in a single common garden setting limits our ability to underscore local adaptation as the mechanism responsible for the observed plant responses (e.g., Williams et al., 2008). Our results revealed that leaf tannins respond plastically to seasonality, and therefore plasticity may be involved in population differentiation. However, similar leaf tannin concentrations of mainland populations characterized in the field (Alados et al., 2002; Barroso et al., 2003) and in a common garden (this study) suggest that phenotypic plasticity in this trait is limited; i.e., plasticity alone could hardly explain the substantial differences observed among *Periploca* sublineages, which were maintained regardless of seasonality or plant damage. Notwithstanding these limitations, our study suggests that correlative studies investigating the main drivers of plant tannin variation across latitudinal gradients may greatly benefit from implementing measurements of light environmental conditions, in addition to the biotic (e.g., herbivore rates) and abiotic (e.g., soil nutrients, temperature) variables typically considered. It also highlights that future tests of the LCDIH should be based on screening chemical traits with known anti-herbivore roles, in addition to considering constitutive and induced plant responses.

CONCLUSIONS

We did not find support for the idea that total pools of plant secondary compounds suspected to act as herbivore deterrents are low on islands. Rather, we found the opposite pattern. Our findings thus add to the growing body of evidence that variation in total leaf tannins should not be exclusively interpreted in the context of anti-herbivore defense (Close and McArthur, 2002; Anstett et al., 2016), even though the patterns of variation may fit well in the predictions of classic evolutionary theories of secondary chemistry (e.g., Herms and Mattson, 1992). Our study further suggests that latitudinal trait variation in tannins can be associated with abiotic factors related to light conditions, which may be a common trend for distributions spanning from temperate to tropical regions.

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AUTHOR CONTRIBUTIONS

C.G-V. conceived the ideas; P. M. and C. G.-V. collected the data; C.G-V. analyzed the data and wrote the manuscript.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.35n1163 (Monroy and García-Verdugo, 2018).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. General view of the common garden design.

APPENDIX S2. Mean temperatures recorded at the common garden area throughout the 3-year period of the study.

APPENDIX S3. Effect of sample manipulation before tannin extraction on estimates of leaf tannin concentration.

APPENDIX S4. Results of the model testing the effect of abiotic variables on patterns of trait variation.

LITERATURE CITED

Abdala-Roberts, L., S. Rasmann, J. C. Berny-Mier y Terán, F. Covelo, G. Glauser, and X. Moreira. 2016. Biotic and abiotic factors associated with altitudinal variation in plant traits and herbivory in a dominant oak species. *American Journal of Botany* 103: 2070–2078.

Adsersen, A., and H. Adsersen. 1993. Cyanogenic plants in the Galapagos Islands: ecological and evolutionarya spects. *Oikos* 66: 511–520.

Alados, C. L., M. L. Giner, L. Dehesa, J. Escos, F. G. Barroso, J. M. Emlen, and D. C. Freeman. 2002. Developmental instability and fitness in *Periploca laevigata* experiencing grazing disturbance. *International Journal of Plant Sciences* 163: 969–978.

Anstett, D. N., K. A. Nunes, C. Baskett, and P. M. Kotanen. 2016. Sources of controversy surrounding latitudinal patterns in herbivory and defense. *Trends in Ecology and Evolution* 31: 789–802.

Arista, M., P. L. Ortiz. 2012. *Periploca* L. *In* S. Talavera, C. Andrés, M. Arista, M. P. Fernández Piedra, M. J. Gallego, P. L. Ortiz, C. Romero Zarco, F. J. Salguerio, et al. [eds.], Flora ibérica, vol. XI: Gentianaceae–Boraginaceae, 130–133. Servicio de Publicaciones del CSIC, Madrid, Spain.

Barroso, F., T. F. Martínez, T. Paz, C. L. Alados, and J. Escós. 2003. Relationship of *Periploca laevigata* (Asclepediaceae) tannins to livestock herbivory. *Journal of Arid Environments* 53: 125–135.

Bixenmann, R. J., P. D. Coley, A. Weinhold, and T. A. Kursar. 2016. High herbivore pressure favors constitutive over induced defense. *Ecology and Evolution* 6: 6037–6049.

Blondel, J. 1985. Habitat selection in island versus mainland birds. *In* M. L. Cody [ed.], Habitat selection in birds, 477–516. Academic Press, London, UK.

Bowen, L., and D. Van Vuren. 1997. Insular endemic plants lack defenses against herbivores. *Conservation Biology* 11: 1249–1254.

Burns, K. C. 2014. Are there general patterns in plant defence against megaherbivores? *Biological Journal of the Linnean Society* 111: 38–48.

Burns, K. C., N. Herold, and B. Wallace. 2012. Evolutionary size changes in plants of the south-west Pacific. *Global Ecology and Biogeography* 21: 819–828.

Carlquist, S. 1974. Island biology. Columbia University Press, NY, NY, USA.

Castro, H., C. Nabais, C. L. Alados, and H. Freitas. 2003. Effects of cessation of grazing on leaf-level photosynthesis of *Periploca laevigata*. *Applied Vegetation Science* 6: 255–260.

Chevalier, A. 1935. Les iles du Cap Vert. Flore de l'archipel. *Revue de Botanique Appliquée* 15: 733–1090.

Close, D. C., and C. McArthur. 2002. Rethinking the role of many plant phenolics—protection from photodamage not herbivores? *Oikos* 99: 166–172.

Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. *Science* 230: 895–899.

Comont, D., J. Martinez-Abaigar, A. Albert, P. Aphalo, D. R. Causton, F. L. Figueroa, A. Gaberscik, et al. 2012. UV responses of *Lolium perenne* raised along a latitudinal gradient across Europe: a filtration study. *Physiologia Plantarum* 145: 604–618.

Dghim, F., S. Zouari, M. Boukhris, and M. Neffati. 2013. Chemical composition of root bark of *Periploca angustifolia* growing wild in Saharian Tunisia. *Journal of Essential Oil Bearing Plants* 16: 338–345.

Ennajeh, M., H. Cochard, and H. Khemira. 2010. Re-introduction success of an autochthonous plant species, *Periploca angustifolia*, in the Natural Reserve of Oued Dekouk, Tunisia. *Spanish Journal of Agricultural Research* 8: 1005–1011.

Gangoso, L., J. A. Donázar, S. Scholz, C. J. Palacios, and F. Hiraldo. 2006. Contradiction in conservation of island ecosystems: plants, introduced herbivores and avian scavengers in the Canary Islands. *Biodiversity and Conservation* 15: 2231–2248.

García-Verdugo, C. 2014. Character shift and habitat colonization in widespread island taxa. *Botanical Journal of the Linnean Society* 174: 399–411.

García-Verdugo, C., B. G. Baldwin, M. F. Fay, and J. Caujapé-Castells. 2014. Life history traits and patterns of diversification in oceanic archipelagos: a meta-analysis. *Botanical Journal of the Linnean Society* 174: 334–348.

García-Verdugo, C., M. Mairal, P. Monroy, M. Sajeva, and J. Caujapé-Castells. 2017. The loss of dispersal on islands hypothesis revisited: implementing phylogeography to investigate evolution of dispersal traits in *Periploca* (Apocynaceae). *Journal of Biogeography* 44: 2595–2606.

García-Verdugo, C., J. Caujapé-Castells, M. Mairal, and P. Monroy. 2018. How repeatable is microevolution on islands? Patterns of dispersal and colonization-related plant traits in a phylogeographical context. *Annals of Botany* https://doi.org/10.1093/aob/mcy191.

Givnish, T. J. 1994. Thorn-like prickles and heterophylly in *Cyanea*: adaptations to extinct avian browsers on Hawaii? *Proceedings of the National Academy of Sciences, USA* 91: 2810–2814.

Gomes, I., T. Leyens, B. Luz, J. da Costa, and F. Gonçalves. 1999. New data on the distribution and conservation status of some angiosperms of the Cape Verde Islands, W Africa. *Willdenowia* 29: 105–114.

Grant, P. R. 1998. Patterns on islands and microevolution. *In* P. R. Grant [ed.], Evolution on islands, 1–17. Oxford University Press, Oxford, UK.

Hagerman, A. E. 1987. Radial diffusion method for determining tannin in plant extracts. *Journal of Chemical Ecology* 13: 437–449.

Hallam, A., and J. Read. 2006. Do tropical species invest more in anti-herbivore defence than temperate species? A test in *Eucryphia* (Cunoniaceae) in eastern Australia. *Journal of Tropical Ecology* 22: 41–51.

Hanley, M. E., B. B. Lamont, M. M. Fairbanks, and C. M. Rafferty. 2007. Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics* 8: 157–178.

Häring, D. A., D. Suter, N. Amrhein, and A. Lüscher. 2007. Biomass allocation is an important determinant of the tannin concentration in growing plants. *Annals of Botany* 99: 111–120.

Hazevoet, C. J., and M. Masseti. 2011. On the history of the green monkey *Chlorocebus sabaeus* (L., 1766) in the Cape Verde Islands, with notes on other introduced mammals. *Zoologia Caboverdiana* 2: 12–24.

Herms, D. A., and W. J. Mattson. 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* 67: 283–335.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.

Hutterer, R., F. García-Talavera, N. López-Martínez, and J. Michaux. 1997. New chelonian eggs from the Tertiary of Lanzarote and Fuerteventura, and a review of fossil tortoises of the Canary Islands (Reptilia, Testudinidae). *Vieraea* 26: 139–161.

Karban, R., and I. T. Baldwin. 1997. Induced responses to herbivory. University Chicago Press, Chicago, IL, USA.

Karban, R., L. H. Yang, and K. F. Edwards. 2014. Volatile communication between plants that affects herbivory: a meta-analysis. *Ecology Letters* 17: 44–52.

Kavanagh, P. H. 2015. Herbivory and the evolution of divaricate plants: structural defences lost on an offshore island. *Austral Ecology* 40: 206–211.

Klimesch, J. 1977. Beiträge zur Kenntnis der Microlepidopteren-Fauna des Kanarischen Archipels. I. Teil: Nepticulidae, Tischeriidae. *Vieraea* 6: 191–218.

Kursar, T. A., K. G. Dexter, J. Lokvam, R. T. Pennington, J. E. Richardson, M. G. Weber, E. T. Murakami, et al. 2009. The evolution of anti-herbivore defenses and their contribution to species coexistence in the tropical tree genus *Inga*. *Proceedings of the National Academy of Sciences, USA* 106: 18073–18078.

Llorens, L., J. Peñuelas, M. Estiarte, and P. Bruna. 2004. Contrasting growth changes in two dominant species of a Mediterranean shrubland submitted to experimental drought and warming. *Annals of Botany* 94: 843–853.

Mahmoud, M. R., F. A. Ahmed, K. Abo-El-Sooud, S. A. El-Toumy, H. S. Yaecob, and H. M. El-Tantawy. 2016. Preliminary phytochemical screening and diuretic activity of *Periploca angustifolia* L. *International Journal of Pharma Sciences* 6: 1454–1458.

Moles, A. T., S. P. Bonser, A. G. B. Poore, I. R. Wallis, and W. J. Foley. 2011. Assessing the evidence for latitudinal gradients in plant defence and herbivory. *Functional Ecology* 25: 380–388.

Monroy, P., and C. García-Verdugo. 2018. Data from: Testing the hypothesis of loss of defences on islands across a wide latitudinal gradient of *Periploca laevigata* populations. Dryad Digital Repository. https://doi.org/10.5061/dryad.35n1163.

Moreira, X., K. A. Mooney, S. Rasmann, W. K. Petry, A. Carrillo-Gavilán, R. Zas, and L. Sampedro. 2014. Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. *Ecology Letters* 17: 537–546.

Moreira, X., L. Abdala-Roberts, V. Parra-Tabla, and K. A. Mooney. 2015. Latitudinal variation in herbivory: influences of climatic drivers, herbivore identity and natural enemies. *Oikos* 124: 1444–1452.

Moreira, X., B. Castagneyrol, L. Abdala-Roberts, J. C. Berny-Mier y Teran, B. G. H. Timmermans, H. H. Bruun, F. Covelo, et al. 2018. Latitudinal variation in plant chemical defences drives latitudinal patterns of leaf herbivory. *Ecography* 41: 1124–1134.

Neffati, N., Z. Aloui, H. Karoui, I. Guizani, M. Boussaid, and Y. Zaouali. 2017. Phytochemical composition and antioxidant activity of medicinal plants collected from the Tunisian flora. *Natural Product Research* 31: 1583–1588.

Pardo, A., Y. Caceres, and F. Pulido. 2018. Intraspecific variation in heritable secondary metabolites and defensive strategies in a relict tree. *Journal of Plant Ecology* 11: 256–265.

Paula, S., and J. G. Pausas. 2006. Leaf traits and resprouting ability in the Mediterranean basin. *Functional Ecology* 20: 941–947.

Pearse, I. S., and A. L. Hipp. 2012. Global patterns of leaf defenses in oak species. *Evolution* 66: 2272–2286.

Rozema, J., J. van de Staaij, L. Björn, and M. Caldwell. 1997. UV-B as an environmental factor in plant life: stress and regulation. *Trends in Ecology and Evolution* 12: 22–28.

Salminen, J. P., and M. Karonen. 2011. Chemical ecology of tannins and other phenolics: We need a change in approach. *Functional Ecology* 25: 325–338.

Stock, W. D., D. Le Rouxm, and F. Van der Heyden. 1993. Regrowth and tannin production in woody and succulent karoo shrubs in response to simulated browsing. *Oecologia* 96: 562–568.

Turley, N. E., W. C. Odell, H. Schaefer, G. Everwand, M. J. Crawley, and M. T. J. Johnson. 2013. Contemporary evolution of plant growth rate following experimental removal of herbivores. *American Naturalist* 181: S21–S34.

Vourc'h, G., J. L. Martin, P. Duncan, J. Escarre, and T. P. Clausen. 2001. Defensive adaptations of *Thuja plicata* to ungulate browsing: a comparative study between mainland and island populations. *Oecologia* 126: 84–93.

Wallace, A. R. 1880. Island life: or, the phenomena and causes of insular faunas and floras, including a revision and attempted solution of the problem of geological climates. Macmillan & Co, London, UK.

Wang, X. F., J. F. Liu, W. Q. Gao, Y. P. Deng, Y. Y. Ni, Y. H. Xiao, F.-F. Kang, et al. 2016. Defense pattern of Chinese cork oak across latitudinal gradients: influences of ontogeny, herbivory, climate and soil nutrients. *Scientific Reports* 6: 27269.

Williams, J. L., H. Auge, and J. L. Maron. 2008. Different gardens, different results: native and introduced populations exhibit contrasting phenotypes across common gardens. *Oecologia* 157: 239–248.