



# Leaf functional traits and insular colonization: Subtropical islands as a melting pot of trait diversity in a widespread plant lineage

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## Abstract

**Aim:** One of the main goals of functional biogeography is to examine distribution patterns of trait diversity, and islands provide excellent study cases for this emerging field. We tested the hypothesis that multiple dispersals from a common mainland pool would promote functional similarity among island systems when environmental conditions are similar, but also novel phenotypic traits related to colonization history and exploitation of new habitats.

**Location:** Mediterranean Basin and Macaronesian islands.

**Taxon:** Wolfbane (*Periploca laevigata*)

**Methods:** We used the well-known biogeographical history of a woody plant complex (*P. laevigata* s.l.) to examine trait variation and how it relates to climatic conditions of mainland and subtropical island settings. In a common garden experiment, we measured a suite of leaf physiological and anatomical traits tightly related to plant performance in 320 seedlings representing 21 populations of five sublineages—the oldest (2.6 my) island colonization (western Canary Islands) as a reference, three sublineages stemming from independent events of island colonization in the last 0.5 my from NW Africa (Cape Verde, Fuerteventura, Lanzarote) and their widespread Mediterranean mainland counterpart.

**Results:** We observed strong phenotypic divergence between island and mainland sublineages linked to contrasting climatic conditions. Mediterranean mainland populations displayed a very specialized leaf phenotype characteristic of arid plants (i.e. small leaves, amphistomaty, isobilateral mesophyll, high photosynthetic rates). In turn, low seasonality on islands was linked to the recurrent expression of a phenotype characterized by larger leaves and lower photosynthetic rates. Our analyses showed that the high investment in secondary compounds (i.e. tannins) on islands decouples photosynthesis from growth rates. Despite this pattern of parallel differentiation, each island sublineage displayed a distinctive phenotype, with some traits related to colonization time, which resulted in a mosaic of functional variation across island systems.

**Main conclusions:** Our data suggest that the studied subtropical islands promote expression of traits specific to certain sublineages and other common traits that are no longer adaptive in the original mainland pool due to Pleistocene climatic shifts. These



findings ultimately extend the role of islands as biodiversity refugia and hotspots of plant functional diversity.

#### KEY WORDS

common garden experiment, functional biogeography, island syndrome, leaf size, Mediterranean plants, parallel evolution, *Periploca laevigata*, photosynthesis

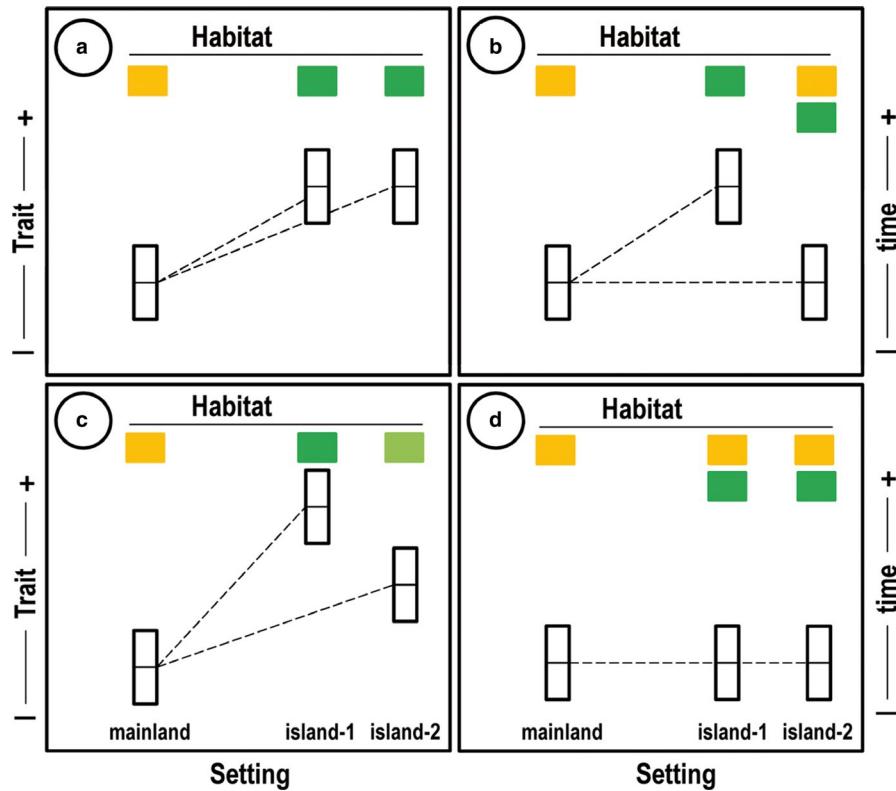
## 1 | INTRODUCTION

Plant physiology has been long considered a key factor in explaining species distributions (Mason & Stout, 1954; Walter, 1964), but only recently plant functional traits have been conceptually integrated into plant biogeography. In a seminal paper, Violette, Reich, Pacala, Enquist, and Kattge (2014) defined functional biogeography as 'the analysis of the patterns, causes, and consequences of the geographic distribution of the diversity of form and function—namely, trait diversity'. Since then, trait-based approaches (e.g. Farneda et al., 2019; Whittaker et al., 2014) have allowed a better understanding of the link between diversity and spatial distributions, although experimental studies on this topic considering an evolutionary framework are particularly scarce yet (for recent reviews see Cavender-Bares, Ackerly, Hobbie, & Townsend, 2016; Ottaviani et al., 2020).

Island settings have traditionally inspired biogeographical research (Humboldt, 1814; MacArthur & Wilson, 1967; Whittaker, Triantis, & Ladle, 2008) and could provide an excellent model system to address questions relating to the emerging field of functional biogeography. One of the most popular island topics to which functional biogeography could bring a fresh perspective is the process of convergent evolution. According to this concept, insular colonizers tend to evolve similar functional phenotypes as a response to similar adaptive pressures across islands (e.g. Burns, 2019; Burns, Herold, & Wallace, 2012; Mahler, Ingram, Revell, & Losos, 2013). As a result, the evolution of functional traits upon island colonization would be largely predictable, and documented cases of recurrent shifts towards insular woodiness or loss of plant physical defences (and other traits collectively referred to as the 'island syndrome'; Carlquist, 1974; reviewed in Burns, 2019) give support to such an idea. Nevertheless, environmental conditions and biotic interactions are recognized as main factors in the field of functional biogeography (Taylor, Weigelt, König, Zottz, & Kreft, 2019; Violette et al., 2014). Climatic and soil factors, in particular, are the basic components of the so-called environmental filter, which is the one acting after dispersal filters to set the capability of species to adapt to particular habitats (de Bello et al., 2012; Cornwell & Ackerly, 2009; Lambers, Chapin, & Pons, 2008). Consequently, climate (and other environmental conditions) could interact with insularity itself producing observable biases in the expected pattern of convergent evolution across islands. Research on how plant traits are shaped at multiple island scales is thus needed to gain insights into the role of insular regions as global biodiversity refugia of functional variation (Keppel et al., 2018).

Island–mainland comparisons can help us identifying the factors governing functional traits across spatial scales. Lineages distributed at both insular and continental regions are valuable in that they allow for straightforward tests of phenotypic differentiation associated with colonization of novel (insular) environments (e.g. Martén-Rodríguez, Quesada, Castro, Lopezaraiza-Mikel, & Fenster, 2015; Moreira et al., 2019; Ottaviani et al., 2020). Furthermore, by shifting the typical focus of functional biogeography on communities to intra-specific variation, we could test predictions at finer biodiversity scales (Taylor et al., 2019; Violette et al., 2007), while controlling for the stochastic biogeographical patterns of island colonization (Clegg et al., 2002; García-Verdugo, Mairal, Monroy, Sajeva, & Caujapé-Castells, 2017). Thus, if pathways and timing of colonization are known, a set of islands colonized from a recognizable mainland source would be a valuable case study to examine the pattern of parallel phenotypic evolution, which we use herein as a concept similar to convergent evolution, but applied to closely related lineages (see Conte, Arnegard, Peichel, & Schlüter, 2012). Assuming that selection upon island colonization has a predominant effect on phenotypes (e.g. Clegg et al., 2002), parallel evolution would promote functional similarity among colonizing lineages, but only if environmental pressures in the recipient habitats are similar across islands (Figure 1a; Burns et al., 2012; Frankiewicz et al., 2020). Contrarily, time constraints (Figure 1b) or different selective pressures between island habitats (Figure 1c) may enhance functional variation across islands (Biddick, Hendriks, & Burns, 2019; García-Verdugo, Caujapé-Castells, Mairal, & Monroy, 2019).

Functional similarity across islands associated with multiple colonization events has been often reported (Baldwin, 2007; Foster, McKinnon, Steane, Potts, & Vaillancourt, 2007; Frankiewicz et al., 2020; García-Verdugo, Forrest, Balaguer, Fay, & Vargas, 2010), but the contribution of biogeographical factors such as colonization time or climatic shifts is still poorly understood in island plant lineages. Having estimates about divergence times from mainland counterparts is crucial to assess the effect of colonization time on phenotypic diversity (Clegg et al., 2002). In addition, leaf phenotypes may not respond in a coordinated fashion, since different traits may show discordant patterns of variation following island colonization (Biddick et al., 2019; García-Verdugo, Baldwin, Fay, & Caujapé-Castells, 2014; García-Verdugo, Méndez, Velázquez-Rosas, & Balaguer, 2010). In order to integrally assess the outcomes of island colonization on phenotypic diversity, it follows that trait-based studies would benefit from implementing information derived from the biogeographical history of the study system and examining a comprehensive set of plant functional traits (Cavender-Bares et al., 2016).



**FIGURE 1** Schematic representation of the phenotypic trajectories expected under different biogeographical scenarios of two island lineages stemming from a common mainland pool: (a) Parallel phenotypic similarity due to adaption to similar island habitats over time; (b) Phenotypic divergence (i.e. novelty on one island versus phenotypic stasis on another island) either due to recent colonization or habitat similarity between one island and the mainland source ('orange' habitat); (c) Parallel phenotypic diversification due to differences between island habitats; (d) Phenotypic stasis on both islands, either due to habitat similarity among islands and mainland or recent colonization of both islands ('green' habitat). Bars in each setting represent mean and (an arbitrary) variance for a given trait, whereas different colours represent contrasting habitats [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

In this study, we used a plant complex (*Periploca laevigata* s.l., Apocynaceae) with a distribution range encompassing several Macaronesian islands (i.e. Canary and Cape Verde archipelagos) and the Mediterranean Basin to examine patterns of leaf functional variation. Stable climatic conditions in the Macaronesian islands are thought to have provided a refugium from the environmental shifts experienced in neighbour mainland areas, such as the onset of the Mediterranean climate c. 3.2–2.6 mya (Fernández-Palacios et al., 2011; Suc, 1984). Under this scenario of climatic stability, we could hypothesize that multiple colonizations from a common mainland source would have promoted similar leaf phenotypes among island systems (Burns et al., 2012). However, recent studies suggest that those islands geographically closer to mainland areas may have been more impacted by climatic shifts than other islands within Macaronesia (García-Verdugo, Caujapé-Castells, Illera, et al., 2019; Neto et al., 2020), which may have triggered disparate patterns of phenotypic adaptation at the regional scale. Considering this alternative scenario, conditions specific to each island lineage (i.e. colonization time, climatic filters) may have triggered phenotypic novelty, at least at the island scale. Here we analyzed the outcome of the pattern of multiple island colonization described in *P. laevigata* (García-Verdugo, Caujapé-Castells, Mairal, et al., 2019; García-Verdugo et al.,

2017) on its geographical pattern of trait diversity. In particular, we asked the following questions: (a) Do island systems show a pattern of differentiation congruent with parallel evolution? (b) Are phenotypic patterns similar among plant traits? (c) Do climatic factors play a key role in plant functional variation and performance? To address these questions, we measured a suite of physiological, anatomical and growth traits in seedlings grown under common garden conditions and compiled climatic information for each geographical area represented in the experiment. Overall, this work builds towards a better understanding on the factors affecting the spatial distribution of plant variation, and particularly, the impact of island colonization on trait diversity at regional scales.

## 2 | MATERIALS AND METHODS

### 2.1 | Study system

*Periploca laevigata* s.l. is a complex of woody shrubs that extends from the Mediterranean Basin to the Atlantic Macaronesian islands (Arista & Ortiz, 2012; Figure 2; see Figure S1, Appendix S1). Phylogenetic reconstructions and taxonomic data support



*P. somaliense* Browicz, a species currently restricted to mountain areas of the Horn of Africa and the Arabian Peninsula, as sister to the common ancestor of all *P. laevigata* sublineages identified thus far (García-Verdugo et al., 2017; Ionta, 2009). Twenty-one populations representing the five sublineages identified in previous biogeographical analyses (García-Verdugo, Caujapé-Castells, Mairal, et al., 2019; García-Verdugo et al., 2017) were considered for this study (see Table S1, Appendix S1 for specific sample sizes) as follows: the central and western Canaries (CAN1), the Mediterranean mainland area (MED), and the islands of Cape Verde (CAV2), Lanzarote (CAN3) and Fuerteventura (CAN4; note that numbers in sublineage codes refer to the order of island colonization; Figure 2a,b, see Appendix S2, for a detailed biogeographical framework and sampling design).

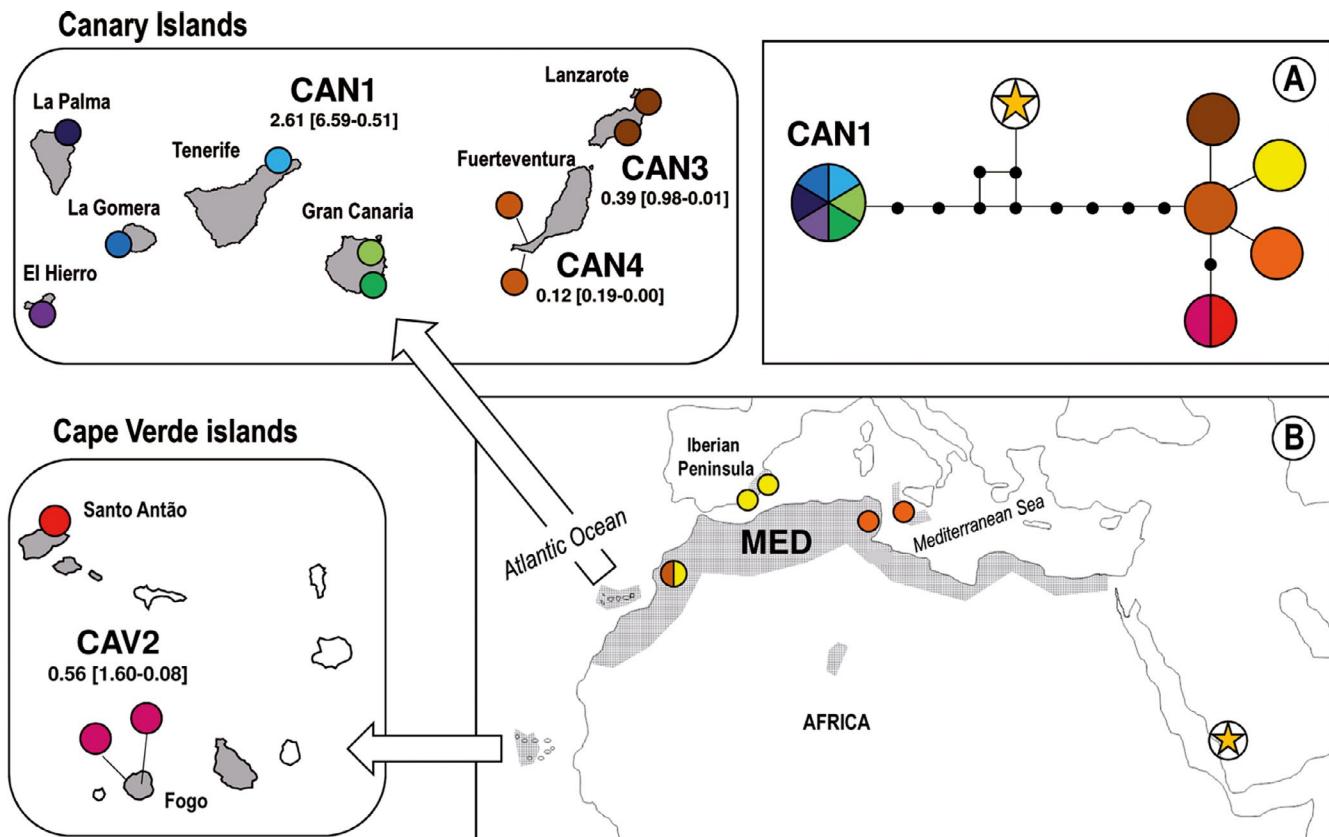
## 2.2 | Experimental setting

A common garden experiment using seeds collected from each population was set up in the facilities of the 'Viera y Clavijo' botanic garden (coordinates: 28.065278 latitude, -15.461944 longitude) on

the island of Gran Canaria. Briefly, in June 2014, one seedling from each mother plant was transplanted into a 6-L pot and grown under sun-exposed conditions. Pots were regularly watered to avoid water limitations throughout the 2-year duration of the experiment, that is, once a week in winter and twice a week in summer (Monroy & García-Verdugo, 2019). A complete description of the experimental setting can be found elsewhere (García-Verdugo et al., 2017; Monroy & García-Verdugo, 2019).

## 2.3 | Study traits

Plant form and function are believed to be governed by factors of variation chiefly related to (a) size (of whole plants and their organs) and (b) the so-called 'leaf economics spectrum', which includes traits balancing growth and construction costs (Díaz et al., 2016; Wright et al., 2004). Because insular colonization implies acclimation to new habitats, we aimed at characterizing a set of functional traits tightly related to both axes of plant form and function. Between May and June, 2016, we conducted phenotypic



**FIGURE 2** Haplotype network of the *Periploca laevigata* complex (a) showing the genetic relationship among the most frequent haplotypes detected in the populations considered in the present study (simplified from García-Verdugo et al., 2017), and geographical location of the areas sampled for representation in the common garden experiment (b). Codes refer to each of the island sublineages described in the main text, and numbers below each code are molecular estimates of island colonization (mean and 95% intervals in parentheses) as inferred in previous studies (García-Verdugo et al., 2017; García-Verdugo, Caujapé-Castells, Mairal, et al., 2019). The sampling point in the Mediterranean sea represents four populations in the strait of Sicily. The star indicates the genetic (a) and geographical (b) position of *P. somaliense*, the closest living relative of all *P. laevigata* sublineages. Areas in grey indicate the complete distribution area of the *P. laevigata* complex [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



measurements of 10 morpho-functional traits in 15–17 seedlings per population ( $N = 5$  in the two smallest populations; see Table S1 in Appendix S1). First, we measured three size-related traits: leaf size (LS,  $\text{cm}^2$ ), total number of leaves (TNL) and total leaf area (TLA,  $\text{cm}^2$ ) of seedlings. To characterize a subset of traits related to the leaf economics spectrum, we measured specific leaf area (SLA,  $\text{cm}^2 \text{g}^{-1}$ ), photosynthetic rate ( $A$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and leaf tannin content (% of tannin content on a dry mass basis). Among these functional traits, photosynthetic-related traits are particularly relevant in our study because they allow characterization of carbon gain versus costs imposed by water loss, which is a fundamental selective force in Mediterranean-type ecosystems like those herein considered (Flexas et al., 2014). We therefore also estimated stomatal conductance ( $g_s$ ,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) and water use efficiency (WUE,  $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ ). Two stomatal traits with strong implications in photosynthetic performance (stomatal ratio and total stomata density; Drake, de Boer, Schymanski, & Veneklaas, 2019; Dunbar-Co, Sporck, & Sack, 2009) were also included in the set of leaf morpho-physiological traits.

Additionally, we measured a set of leaf anatomical traits using a subsample of individuals ( $N = 7$  per sublineage,  $N_{\text{total}} = 35$ ), which included, cuticle thickness and epidermis cells thickness on adaxial and abaxial leaf sides, palisade mesophyll thickness and total leaf thickness. We expected anatomical and morpho-physiological traits to co-vary, although trade-offs (e.g. associated with colonization of contrasting habitats) may have resulted in different responses among traits and sublineages (Díaz et al., 2016; Gutschick, 1999).

### 2.3.1 | Measurement of leaf morpho-physiological traits

The number of leaves of each seedling was counted twice and averaged, thus giving the TNL. Then, two or three fully expanded leaves were sampled from the third to fourth internode of shoots located at different positions of the canopy and exposed to sun conditions. Leaves were scanned, dried at 65°C for 48 hr and weighed using a precision balance (AB54; Mettler-Toledo AG, Greifensee, Switzerland). LS was estimated from scanned images using Image J software (Abramoff, Magalhaes, & Ram, 2004) and SLA was obtained by dividing LS by its corresponding dry mass. Since our plants were relatively small and displayed leaves very similar in appearance along the shoots, TLA was estimated by multiplying the number of leaves on each individual by its mean LS. In order to compare LS of the *P. laevigata* complex with that of its closest living relative, 3–4 leaves from 12 herbarium specimens of *P. somalicum* deposited in K, RBGE and KACST herbaria were additionally measured.

Stomatal traits were measured from imprints of the adaxial and abaxial surfaces from one leaf per individual. Nail varnish peels were taken between the midrib and the margin of each leaf for both surfaces, placed on a microscope slide and examined with an Olympus BX41 microscope coupled with an Olympus DP47 camera (Figure

S2 in Appendix S1). Photographs ( $\times 100$  magnification) were taken at three randomly selected portions per peel. Stomatal counts of each portion were averaged to calculate the number of stomata per  $\text{mm}^2$  on each surface. From these counts, two traits at the leaf level were calculated: stomatal ratio (adaxial stomatal density/abaxial stomatal density) and total stomatal density (sum of adaxial and abaxial stomatal density; Dunbar-Co et al., 2009).

Photosynthetic traits were measured in one leaf per individual using a LI-6400 Portable Photosynthesis System (LI-COR, Lincoln, NE, USA) equipped with a standard  $2 \times 3$  cm chamber using natural light. Leaves were kept in the chamber for 2–3 min before steady-state leaf gas exchange was recorded at saturating irradiance (typically  $>1,200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). Gas exchange measurements were taken within a similar time interval (09.00–12.00 a.m.) every day to keep air temperature and leaf-to-air vapour pressure deficit within limited ranges of variation among measurements (26.3–31.2°C and 2.24–3.54 kPa respectively, for the whole dataset). In addition, measurements were randomized among populations and sublineages throughout the day to avoid potential biases associated with temporal variation in these parameters. The portion of the leaf kept in the LI-6400 cuvette during gas-exchange trials was scanned and its area estimated for further calculations. Photosynthetic rate on an area basis ( $A$ ),  $g_s$  and intrinsic WUE (i.e.  $A/g_s$ ) were obtained for each individual after correcting the data by the area enclosed in the cuvette and the stomatal ratio (i.e. the value previously obtained for a given individual; see above), following manufacturer's instructions.

### 2.3.2 | Measurement of leaf anatomical traits

Leaf anatomical traits were measured following standard protocols (Liu et al., 2015). Transverse slices that included the midrib and a portion of the lamina were cut from the middle parts of the leaf blade, fixed in an ethanol-glycerol mixture (1:1 v/v), dehydrated with ethanol series (50%–100%), and embedded in Epon-resin, as described in Meek (1976). Next, the embedded samples were cross-sectioned at 3.5  $\mu\text{m}$  on a Tesla 490A microtome, stained with PAS (Periodic Acid-Schiff) and toluidine blue, and mounted in Euparal on a microscope slide. All sections were examined with an Olympus BX41 microscope equipped with a Canon EOS 70D camera. Measurements were taken using OptaView 7 software (Opta-Tech, Warsaw, Poland). The contribution of each anatomical parameter to total leaf thickness (% of the total) was calculated for further analysis.

### 2.3.3 | Estimates of growth rates and tannin content

We obtained data for two plant traits used as surrogates of individual investment in primary metabolism (i.e. estimates of growth rate on a mass basis) versus secondary metabolism (i.e. tannin content; see below; Herms & Mattson, 1992). Individual growth rates were based on estimates of above-ground biomass production within a given timeframe (Monroy & García-Verdugo, 2019). Five growing



**TABLE 1** Kruskall–Wallis results for the comparison among *Periploca laevigata* sublineages based on data for climatic descriptors obtained from different databases for each population ( $N = 21$ )

| K-W      | Climatic variable |                                  |                |                                  |                  |                                    |                  |                  |                 |
|----------|-------------------|----------------------------------|----------------|----------------------------------|------------------|------------------------------------|------------------|------------------|-----------------|
|          | T <sup>a</sup>    | T <sub>season</sub> <sup>a</sup> | P <sup>a</sup> | P <sub>season</sub> <sup>a</sup> | PET <sup>b</sup> | PET <sub>season</sub> <sup>b</sup> | Rad <sup>a</sup> | Vpd <sup>c</sup> | WS <sup>c</sup> |
| $\chi^2$ | 1.45              | 15.6                             | 4.79           | 12.1                             | 2.79             | 11.4                               | 13.7             | 3.46             | 12.9            |
| $p$      | 0.83              | 0.003                            | 0.04           | 0.02                             | 0.59             | 0.02                               | 0.008            | 0.48             | 0.01            |

Abbreviations: P, annual precipitation; PET, annual potential evapotranspiration; PET<sub>season</sub>, potential evapotranspiration seasonality; P<sub>season</sub>, precipitation seasonality; Rad, solar radiation; T, mean annual temperature; T<sub>season</sub>, temperature seasonality; vpd, vapour pressure deficit; WS, wind speed.

<sup>a</sup>Obtained from WorldClim 2.1 (Fick & Hijmans, 2017).

<sup>b</sup>Obtained from TerraClimate (Abatzoglou et al., 2018).

<sup>c</sup>Obtained from ENVIREM (Title & Bemmels, 2017).

shoots of similar size were chosen from different canopy areas of 5–10 plants per population ( $N_{\text{total}} = 200$ ), and the third internode of each shoot was double-tagged with a loose knot of floss and a small mark of white paint. The portion of the shoot developed after 40 days was excised, dried at 65°C in a paper bag for a minimum of 48 hr and weighed using a precision balance. Growth rates on a mass basis (mg/day) were estimated for each individual as mean biomass measurements across shoots divided by the monitoring time (number of days). In addition, data extracted from Monroy and García-Verdugo (2019) on leaf tannin content were used here combined with additional measurements to include a larger number of populations ( $N = 5$  individuals, for new populations;  $N_{\text{total}} = 286$ ). Total leaf tannin pools were estimated using the protocol described in Hagerman (1987) with the modifications detailed in Monroy and García-Verdugo (2019).

## 2.4 | Climatic data

To examine climatic differences among sublineages, we extracted nine climatic variables from the WorldClim v 2.1 (Fick & Hijmans, 2017), TerraClimate (Abatzoglou, Dobrowski, Parks, & Hegewisch, 2018) and ENVIREM (Title & Bemmels, 2017) databases at a resolution of 30-arc-second (~1 km<sup>2</sup>). Our climatic descriptors (Table S2) included both mean annual values and estimates of seasonality (i.e. within-year variation in temperature and precipitation) following previous studies comparing island and mainland habitats (García-Verdugo, 2014; Weigelt, Jetz, & Kreft, 2013).

## 2.5 | Statistical analyses

For datasets with low replication at the population level (i.e. climatic descriptors and leaf anatomical traits), individual values were grouped by sublineage and analysed with a nonparametric test using the *kruskal.test* function in R v. 3.6.0 (R Core Team, 2019). All other leaf traits were grouped by sublineage and population, and analysed in a nested ANOVA with two factors: 'sublineage' (fixed) and 'population' nested within 'sublineage' (random). Depending

on the trait, data were square-root (i.e. 'Stomatal ratio', 'Tannin content') or log-transformed (all other traits) to achieve normality and homoscedasticity. Differences in trait values among sublineages were tested in a linear mixed-effects model using the 'nlme' package (Pinheiro, Bates, DebRoy, & Sarkar, 2017). Correlations between variables of interest (i.e. divergence times and phenotypic variation; Conte et al., 2012) were explored with Spearman's rank correlations.

In addition to previous analyses of single traits, we examined the pattern of multi-trait variation across sublineages. To this end, we conducted a principal component analysis (PCA) using the 'ade4' package based on the correlation matrix of leaf traits. Two analyses were run—one including the dataset of 17 leaf anatomical and morpho-functional traits ( $N = 35$  individuals) and a following PCA based on the data for the 10 morpho-functional traits ( $N = 286$ ). Because both results were similar (see Results), we plotted the first two axes of the second PCA (54.5% of variance explained) to visualize the structure of functional variation in a bidimensional space.

We ran a piece-wise structural equation model (SEM) using data at the population level to investigate direct associations among climatic factors, leaf functional traits, secondary metabolism (i.e. tannins) and primary metabolism (i.e. growth rates), as well as the indirect associations between climate and functional traits. To reduce the number of independent variables in the model (see Geber & Griffen, 2003), we chose two uncorrelated traits (i.e. A and SLA) with the highest loading factors in the two axes of the previous PCA (see Section 3.4). To avoid multiple testing of individual variables, 21 descriptors obtained from WorClim v2.1 were summarized running a principal component analysis on this climatic dataset. The standardized z-scores of these PCs were used for the SEM. Direct associations in the SEM were estimated as standardized partial regression coefficients, whereas indirect associations were calculated by multiplying the specified coefficients for direct relationships between both the predictor and the response (Lefcheck, 2016). We assessed the significance of direct and indirect coefficients with t tests. We used the *psem* function to obtain SEM fit parameters and the *partialResid* function to extract the partial effects of significant predictors.



## 3 | RESULTS

### 3.1 | Climatic descriptors of source populations

Source populations of *Periploca* sublineages did not significantly differ in climatic conditions for most variables based on mean annual values (Table 1, Figure S3 Appendix S1). By contrast, source populations of sublineages significantly differed in all climatic descriptors of seasonality, particularly for temperature ( $p = 0.003$ ; Table 1). A deeper analysis of the pattern of intra-annual variation revealed that MED displayed typical Mediterranean conditions (i.e. summer drought, lowest temperatures in winter; Figure S4). The pattern in the Canary Islands (i.e. CAN3-CAN4-CAN1) was similar to MED, but inter-annual variation in temperature was remarkably lower and summer drought tended to be less pronounced. Finally, CAV2 showed a non-Mediterranean pattern, with minimum variation of temperatures between months and abundant precipitation in summer and autumn (Figure S4).

The PCA conducted on climatic variables rendered two PCs that accounted for 71% of the variance: PC1 ('Clim PC1' hereafter) was positively related to temperature variability (i.e. seasonality, annual range, maximum temperature of the warmest month and minimum temperature of the coldest month), and negatively related to solar radiation and isothermality. PC2 ('Clim PC2' hereafter) was positively related to precipitation regime (annual precipitation, precipitation of the wettest month, precipitation of the wettest quarter and precipitation of the warmest quarter).

### 3.2 | Patterns of leaf morpho-physiological traits

All sublineages in our common garden displayed similar TLA and total stomatal density as revealed by nested ANOVA results (Table 2). All other functional traits were significantly different among sublineages and, with the exception of WUE, among populations within sublineages (Table 2). Most notably, MED showed a strong phenotypic divergence in most traits with regard to island sublineages. Thus, island plants consistently displayed a small number of larger leaves with lower photosynthetic rate and stomatal conductance than MED (Figure 3a,b,d,e). The ratio between photosynthetic rate and stomatal conductance, that is, WUE, showed an unclear pattern except that values were significantly higher in CAV2 than in the other sublineages (Figure 3f). In addition, analysis of stomatal ratios revealed that mainland populations were clearly amphistomatic (i.e. stomata equally distributed among adaxial and abaxial surfaces), while island plants tended to hypostomaty (stomata mostly concentrated on the abaxial surface; Figure 3g).

For several traits (Figure 3a,b,d,e), the three island sublineages closely related to the mainland pool (CAV2, CAN3, CAN4) tended to exhibit the phenotype displayed by the oldest island colonizer (CAN1). In the case of LS, the phenotype of older island colonizers was similar to that of the mainland relative closest to the *P. laevigata* complex ancestor (grey area in Figure 3b). Correlation tests showed that stomatal ratio was the only trait significantly associated with divergence times across island sublineages ( $R = -0.95$ ,  $p = 0.05$ ), although LS and A also displayed relatively high correlation

**TABLE 2** Nested ANOVA results for analyses of 10 leaf functional traits measured in 320 *P. laevigata* seedlings under common garden conditions. Mean of squares (MS) and F-ratios (F) values are based on square-root (stomatal ratio, %Tannins) or log-transformed (all other traits) measurements

| Factor           | Leaf patterns  |                |       |                   | Photosynthetic |                |                     |                   |                      |                   |
|------------------|----------------|----------------|-------|-------------------|----------------|----------------|---------------------|-------------------|----------------------|-------------------|
|                  | Num. of leaves |                | TLA   |                   | A              |                | $g_s$               |                   | WUE                  |                   |
|                  | MS             | F <sup>a</sup> | MS    | F <sup>a</sup>    | MS             | F <sup>a</sup> | MS                  | F <sup>a</sup>    | MS                   | F <sup>a</sup>    |
| Sublineage       | 5.648          | 55.7***        | 0.06  | 2.4 <sup>NS</sup> | 0.452          | 23.6***        | 0.69                | 22.9***           | 0.114                | 7.6***            |
| Pop (sublineage) | 0.101          | 13.0***        | 0.07  | 4.3***            | 0.019          | 2.4**          | 0.03                | 1.98**            | 0.015                | 1.6 <sup>NS</sup> |
| Error            | 0.008          |                | 0.02  |                   | 0.008          |                | 0.02                |                   | 0.009                |                   |
| Leaf morphology  |                |                |       |                   |                |                |                     |                   |                      |                   |
| Factor           | Leaf size      |                | SLA   |                   | Stomatal       |                |                     |                   | Secondary metabolism |                   |
|                  | MS             | F <sup>a</sup> | MS    | F <sup>a</sup>    | Stomatal ratio |                | Total stom. density |                   | %Tannins             |                   |
|                  | 4.995          | 64.7***        | 0.070 | 5.8**             | 1.763          | 11.6***        | 0.094               | 2.3 <sup>NS</sup> | 22.42                | 47.7***           |
| Sublineage       | 0.077          | 9.1***         | 0.012 | 2.7***            | 0.152          | 17.4***        | 0.040               | 6.4***            | 0.47                 | 2.92**            |
| Error            | 0.008          |                | 0.004 |                   | 0.009          |                | 0.006               |                   | 0.16                 |                   |

Abbreviations: % Tannins (% of tannin content on a dry mass basis); A, photosynthetic rate on an area basis;  $g_s$ , stomatal conductance; NS, non-significant differences; SLA, specific leaf area; WUE, intrinsic water use efficiency.

<sup>a</sup>df Sublineage:  $F_{4,16}$ /df Pop (Sublineage):  $F_{16,299}$ .

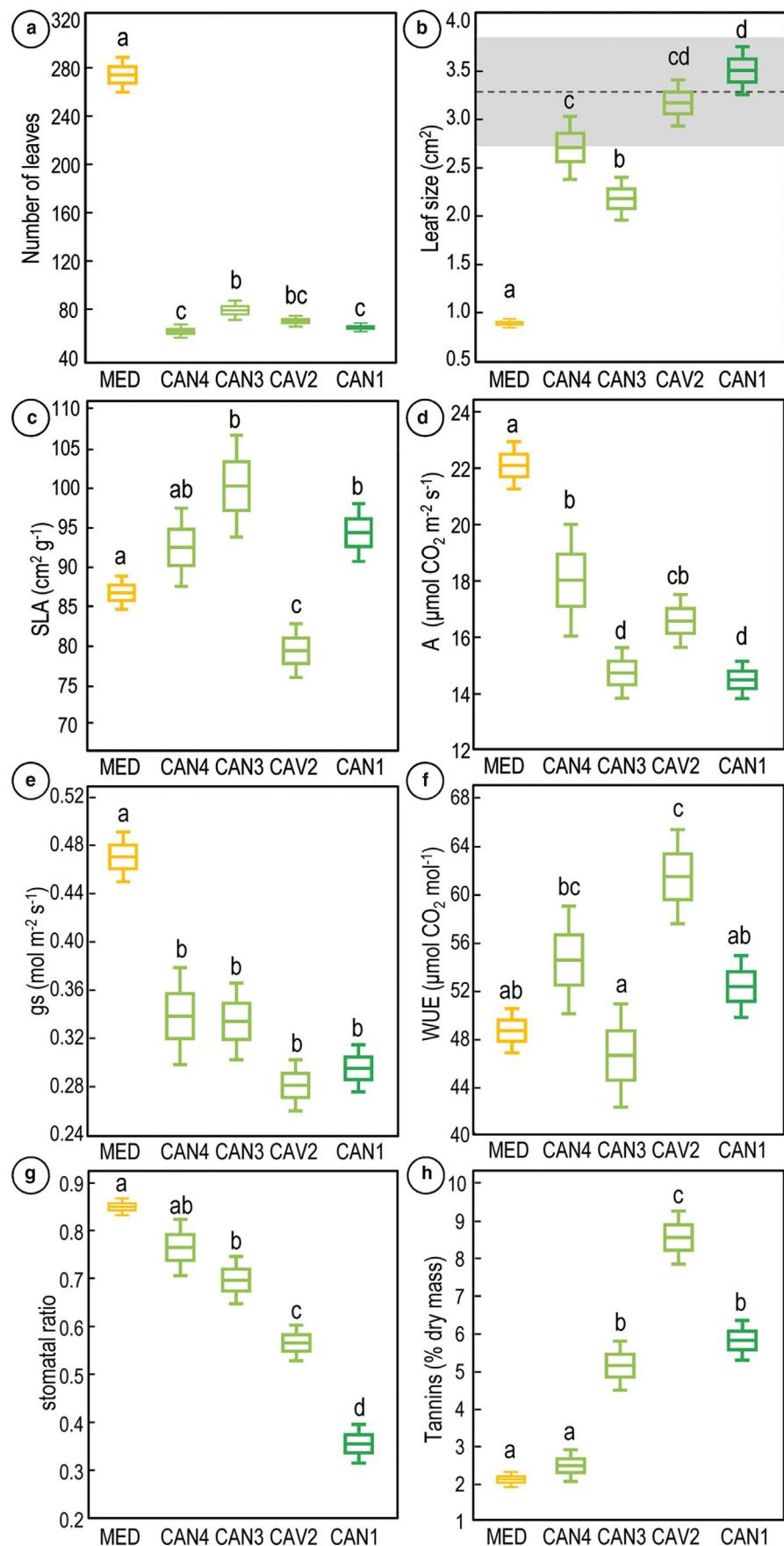
<sup>b</sup>df Sublineage:  $F_{4,16}$ /df Pop (Sublineage):  $F_{16,285}$ .

\*\*\* $p < 0.001$ ;

\*\* $p < 0.01$ .



**FIGURE 3** Box plots of common garden data for eight functional leaf traits measured in 320 *P. laevigata* seedlings and grouped by sublineage (see Figure 2 for codes). In the chart displaying 'leaf size' values, the data obtained from herbarium specimens of the sister species *P. somaliense* is indicated by the dotted line (mean) and grey area (*SD*). Different letters indicate significantly different groups according to post hoc tests [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]





coefficients ( $R > 0.6$  in both cases; Table 3). Despite parallel mainland–island patterns in certain traits, phenotypic similarity across all island sublineages (i.e. Figure 1a) was only observed for stomatal conductance (Figure 3e).

### 3.3 | Patterns of leaf anatomical traits

Leaf cuticle and epidermis thickness were similar among sublineages, but the proportion of palisade mesophyll was significantly larger in MED (c. 60%), followed by CAN4 (54%) and the rest of island sublineages (51%–44%; Table 4). Such differences between island and mainland anatomical traits were mediated by the contrasting structure of the mesophyll–mainland populations exhibited a clear isobilateral arrangement of the palisade layer, whereas three out of four island sublineages displayed a dorsiventral arrangement (Figure 4). The sublineage representing the most recent island colonization (CAN4), displayed a somewhat intermediate arrangement between these two patterns (Figure 4).

### 3.4 | Functional trait PCA

The results of the PCA showed that the functional space defined by 10 leaf traits was clearly structured into two main groups of individuals (MED versus CAN1 + CAV2) scattered along the PC1 (c. 41% of explained variance), with some intermediate phenotypes related to the more recent island colonizers (CAN3 and CAN4; Figure 5). Thus, PC1 separated leaf phenotypes characterized by high photosynthetic rate, stomatal conductance and stomatal ratio, and small leaf size and tannin content, from those (island) individuals displaying the opposite traits. PC2 (c. 14% of explained variance) also depicted leaf variation mostly mediated by two leaf traits (SLA and WUE), but functional diversification was more evident between the island sublineages than within the space defined by mainland individuals (Figure 5). The same pattern was observed with the dataset using 17 leaf traits, with the exception that leaf thickness (instead of SLA) had a major contribution to PC2 (Figure S5).

### 3.5 | Direct and indirect climatic effects on leaf functional traits and plant performance

Results from the piece-wise SEM indicated significant direct associations between climate and SLA, photosynthesis and secondary

metabolism, but not between climate and primary metabolism (Figure 6). Specifically, climatic conditions related to temperature and solar radiation (Clim PC1) were positively associated with photosynthesis and secondary metabolism, and climatic conditions related to precipitation (Clim PC2) were positively associated with secondary metabolism and negatively associated with SLA (Figure 6). Results also indicated significant direct negative associations between SLA and photosynthesis, between photosynthesis and secondary metabolism and between primary and secondary metabolism (Figure 6). Our model found significant indirect associations between climate and both primary and secondary metabolism (via SLA and photosynthesis, Figure 6). Specifically, we found a negative indirect association between Clim PC1 and primary metabolism, and a positive indirect association between Clim PC2 and secondary metabolism.

## 4 | DISCUSSION

### 4.1 | Functional trait differentiation between island and mainland settings

Our results revealed strong phenotypic divergence between the Mediterranean mainland sublineage of the *P. laevigata* complex and all the sublineages that independently colonized the Macaronesian islands. The multi-trait approach adopted in our study, either on a trait-by-trait basis (Figures 3 and 4) or considering functional variation in a bidimensional space (Figure 5), clearly indicated such a remarkable differentiation between island and mainland settings. Two main lines of evidence suggest that leaf traits in our study system have predominantly evolved in a coordinated, adaptive fashion—climate-trait associations and the parallel patterns of phenotypic variation observed across islands.

Island and mainland sublineages displayed contrasting suites of phenotypic traits (i.e. leaf size, SLA, A) that are known to have a strong implication in plant form and function (Díaz et al., 2016; Reich, 2014). For instance, when compared to island sublineages, mainland phenotypes were characterized by remarkably smaller leaves (Figures 3b and 5). Evolution towards reduced leaf size has often been viewed as an optimal strategy to cope with the strong seasonality of Mediterranean-type habitats (Ackerly, Knight, Weiss, Barton, & Starmer, 2002). In particular, small leaves allow high transpiration (i.e. high photosynthetic rates) when evaporative demand is low and water is not a limiting factor (e.g. our common garden conditions), and efficient heat loss when

**TABLE 3** Correlation coefficients ( $R$ ) and significance ( $p$ ) for tests examining associations between divergence time and functional traits among island sublineages

|     | Num. of leaves | Leaf size | SLA  | A     | $g_s$ | WUE   | Stom rat | %tannins |
|-----|----------------|-----------|------|-------|-------|-------|----------|----------|
| $R$ | -0.27          | 0.75      | 0.12 | -0.65 | -0.51 | -0.10 | -0.95    | 0.24     |
| $p$ | 0.73           | 0.25      | 0.87 | 0.35  | 0.48  | 0.90  | 0.05     | 0.76     |

Abbreviations: % tannins (% of tannin content on a dry mass basis); A, photosynthetic rate on an area basis;  $g_s$ , stomatal conductance; SLA, specific leaf area; Stom rat, stomatal ratio; WUE, intrinsic water use efficiency.



**TABLE 4** Mean ( $\pm$ SE) values for anatomical traits measured in leaves collected from seven individuals of each *P. laevigata* sublineage (N = 35) grown under common garden conditions. Results of the Kruskal–Wallis (K-W) analysis testing for differences among lineages are shown

| Sublineage | Leaf thickness ( $\mu\text{m}$ ) | %cuticule                | %epidermis               | %palisade                  | %spongy                    |
|------------|----------------------------------|--------------------------|--------------------------|----------------------------|----------------------------|
| MED        | 460 $\pm$ 25                     | 1.9 $\pm$ 0.1            | 10.6 $\pm$ 0.5           | 59.6 $\pm$ 2.1             | 27.8 $\pm$ 2.5             |
| CAN4       | 442 $\pm$ 15                     | 1.9 $\pm$ 0.2            | 8.9 $\pm$ 0.5            | 54.3 $\pm$ 2.2             | 34.8 $\pm$ 2.2             |
| CAN3       | 448 $\pm$ 14                     | 1.9 $\pm$ 0.1            | 10.8 $\pm$ 0.8           | 51.8 $\pm$ 3.2             | 35.5 $\pm$ 2.9             |
| CAV2       | 537 $\pm$ 27                     | 1.8 $\pm$ 0.2            | 9.0 $\pm$ 0.9            | 43.9 $\pm$ 1.0             | 45.3 $\pm$ 1.6             |
| CAN1       | 426 $\pm$ 32                     | 2.0 $\pm$ 0.2            | 9.9 $\pm$ 0.9            | 47.9 $\pm$ 3.6             | 40.1 $\pm$ 3.9             |
| K-W test   | $\chi^2 = 8.2, p = 0.08$         | $\chi^2 = 1.8, p = 0.76$ | $\chi^2 = 6.4, p = 0.18$ | $\chi^2 = 16.7, p = 0.002$ | $\chi^2 = 15.5, p = 0.003$ |

temperatures are high and water scarcity forces stomatal closure (i.e. Mediterranean summer conditions; Lambers et al., 2008; Yates, Verboom, Rebelo, & Cramer, 2010). Other suites of correlated traits displayed in our experiment by mainland plants, such as amphistomaty and the isobilateral organization of the palisade mesophyll are typically described as leaf adaptations to maximize the temporal window of favourable conditions in arid zones (Drake et al., 2019; Gibson, 1996). Our observations, coupled with the results obtained in other physiological studies (Dghim, Abdellaoui, Boukhris, Neffati, & Chaieb, 2018), support the idea that mainland plants have evolved a specialized phenotype as a response to seasonal Mediterranean environments.

Conversely, island sublineages showed a recurrent pattern of variation towards a contrasting phenotype, commonly characterized by large leaves. Our data indicated that larger leaves on islands are intrinsically associated with high investments in secondary metabolism (i.e. tannin content; Figures 3b,h and 5). A previous study suggested that geographical variation in tannins across the distribution range of *P. laevigata* was associated with latitudinal variation in light conditions (Monroy & García-Verdugo, 2019). Findings from our SEM support this view, as tannin production was negatively associated with climatic factors (including temperature and solar radiation), while positively related to precipitation. Tannins are large, carbon-based molecules with high constructions costs (Chapin, Matson, & Mooney, 2002). This may explain why photosynthetic rates in our plants were more tightly linked to the carbon sink represented by the secondary metabolism than to growth rate (Figure 6). Large leaves, indeed, most probably have additional costs not considered in our study (e.g. investment in support tissues; Niinemets et al., 2007). In *Periploca*, larger leaves were also associated with lower photosynthetic rates, which imply longer payback times from an economic point of view (Poorter et al., 2006; Wright et al., 2004). Taken together, we hypothesize that island populations can sustain larger leaves with high construction costs (and smaller photosynthetic rates) because they can assume longer payback times. Climatic stability on islands, chiefly represented by limited temperature oscillation throughout the year (PC Clim 1, in our model; Figure 6; see also Weigelt et al., 2013; Burns, 2019), apparently promote the expression of large leaves, but when costs associated with secondary metabolism are implemented, such a strategy is linked to

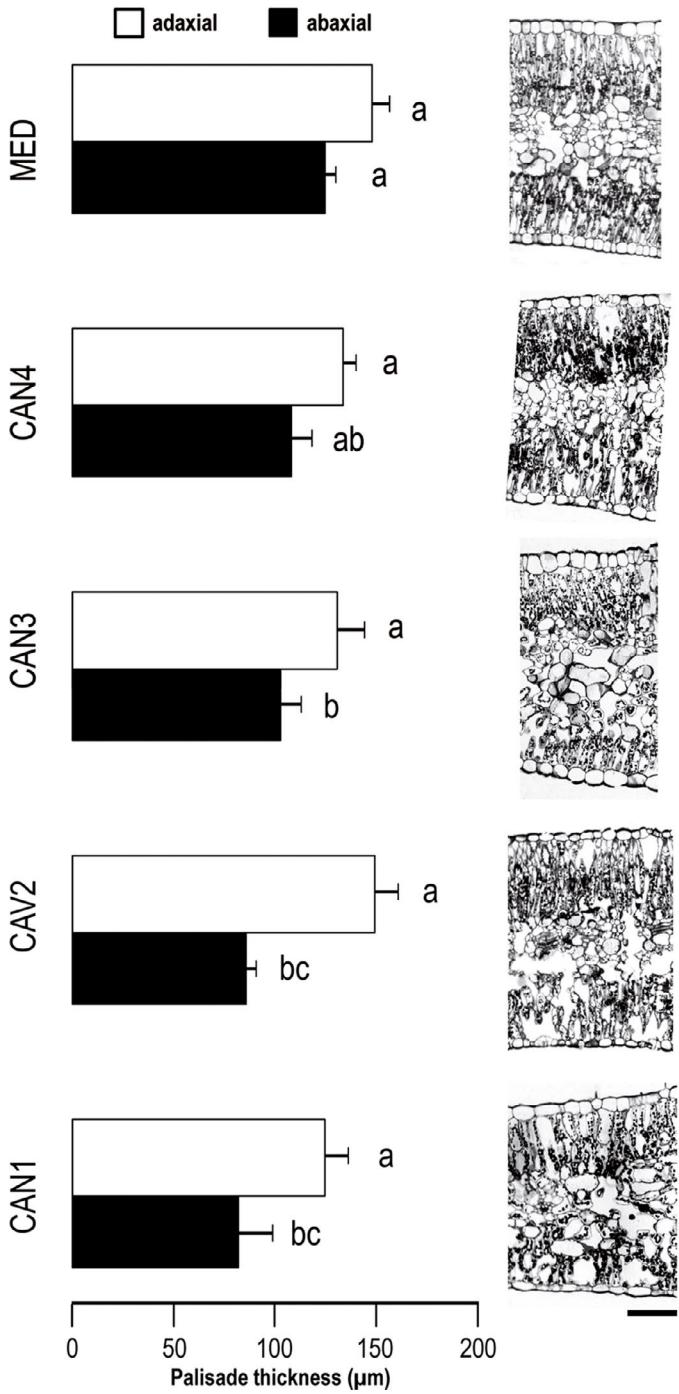
slow growth rates. However, because climatic stability in subtropical islands typically translates into longer growing seasons (Figure S4; see also Carlquist, 1974), large payback times could be compensated despite slow growth rates.

A second line of support for the adaptive scenario in our study system is that the direction of the phenotypic shifts observed (e.g. increases in leaf size or tannin content) was consistent across island sublineages. Such parallel patterns are often taken as strong evidence of selective processes driven by island conditions (Baldwin, 2007; Frankiewicz et al., 2020; Givnish, 2010), and may represent a new suite of traits supporting the ‘island syndrome’ (sensu Carlquist, 1974; reviewed in Burns, 2019), at least under conditions similar to those observed in our study system (Mediterranean-type versus subtropical insular habitats). In summary, we postulate that phenotypic divergence between mainland and island settings is the result of both adaptation to arid conditions in the mainland and an adaptive parallel pattern towards a markedly different phenotype on islands. An open question is, however, whether extant island traits have been exclusively shaped by habitat selection or, alternatively, other factors attributable to biogeographical history are involved.

#### 4.2 | Functional trait variation among island sublineages: climatic and biogeographical constraints

In contrast to previous studies focusing on leaf and seed traits across lineages (Burns et al., 2012; García-Verdugo, Caujapé-Castells, Mairal, et al., 2019; García-Verdugo, Forrest, et al., 2010; Kavanagh & Burns, 2014), we found that the apparent pattern of parallel evolution between island and mainland populations did not result in functional similarity among island populations. We discuss below how climatic and biogeographical information can be crucial to understand geographical patterns of extant trait variation, as well as to gain insights into the evolution of particular functional traits.

Considering variation among islands at the trait level, we observed that the most recent colonizer (CAN4) exhibited values of stomatal ratio, tannin content and photosynthetic rate similar to those displayed by the mainland sublineage. In this case, our climatic descriptors of source populations could hardly explain this pattern



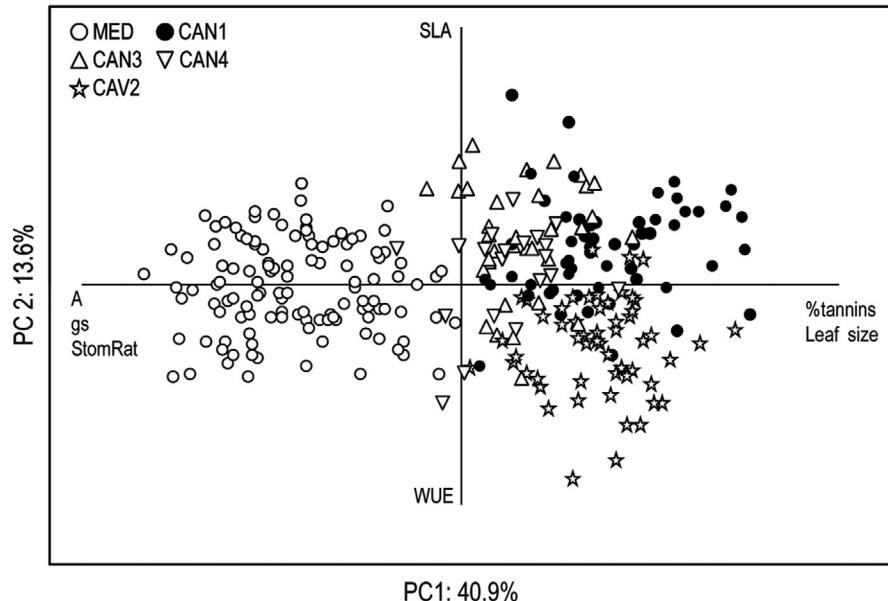
**FIGURE 4** Mean (+SE) adaxial and abaxial palisade thickness values, leaf cross sections and twig phenotypes representative of each *P. laevigata* sublineage ( $N = 7$  per group; see main text for codes). Letters indicate traits that are significantly different according to multiple comparisons tests. Scale bar = 100 µm [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

because the CAN4 and CAN3 sublineages apparently experience identical climatic conditions (see Results), despite remarkable phenotypic differentiation between them (Figure 3). Our biogeographical framework suggests that CAN4, being the most recent insular colonizer (0.19–0.0 mya, 95% confidence intervals for estimates of colonization time; Figure 2), may be particularly affected by evolutionary constraints related to colonization history. The pattern of incomplete lineage sorting (i.e. haplotype sharing) for CAN4 and the neighbouring mainland populations documented elsewhere (García-Verdugo et al., 2015, 2017; Figure 2) further supports potential genetic constraints in trait evolution following a relatively recent colonization of the island setting.

Contrary to the most recent island colonizer, we found that CAV2 displayed the most contrasting functional phenotype in traits such as SLA, WUE or tannin content (Figures 3 and 5). Phenotypic novelty in the Cape Verde sublineage is accompanied by several climatic peculiarities of these islands, such as a subtropical climate with high summer precipitations (see also Neto et al., 2020). According to our SEM (Figure 6), climatic factors such as precipitation and solar radiation were positively associated with higher investments in secondary metabolism, that is, compounds with high construction costs, as discussed above. Indeed, low SLA (due to thicker leaves; see anatomical results) and high tannin content (on average, c. 9% on a leaf dry mass basis) in CAV2 likely translates into the highest leaf construction



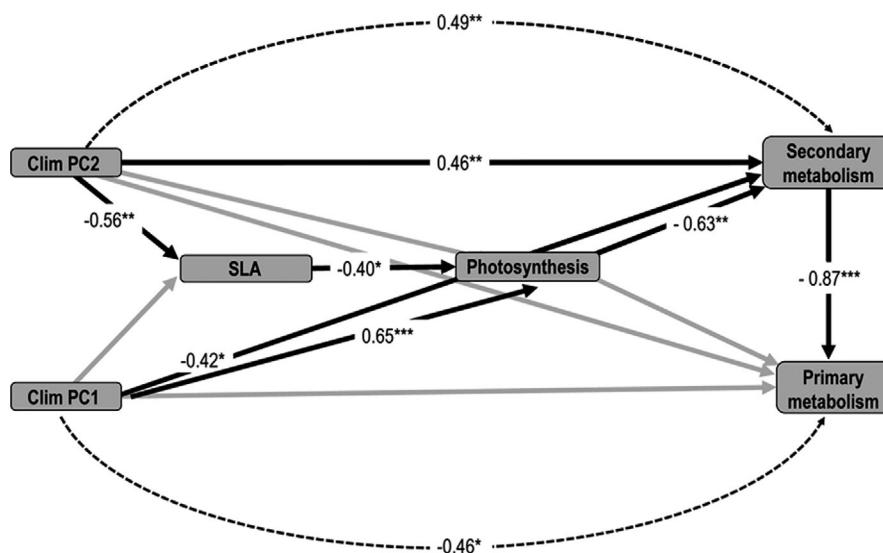
**FIGURE 5** Plot of the PCA based on the correlation matrix among 10 leaf traits (see Statistical analysis for details) measured in 286 individuals of the five *P. laevigata* sublineages analysed. Traits with highest loading factors ( $>0.7$ ) are shown next to each axis, whereas the percentage of variance of the bidimensional space explained by each axis is indicated outside the plot



costs in our study system (Chapin et al., 2002; Poorter et al., 2006; Villar & Merino, 2001). However, this sublineage illustrates how mild climatic conditions on islands may compensate for such costs. While hot, arid Mediterranean summer conditions induce leaf shedding in mainland plants (Dghim et al., 2018), the Cape Verde islands are being particularly subject to the trade winds and the rainy season, which extends *Periploca* growing period (M. Romeiras, personal communication; see also Neto et al., 2020). Thus, longer leaf life spans favoured by mild conditions probably ensures payback times in these island plants (cf. Villar & Merino, 2001). By contrast, leaf size in Cape Verde populations is not characterized by phenotypic

novelty; trait values are similar to those displayed by both the oldest island colonizer (CAN1) and the closest mainland relative of the *P. laevigata* common ancestor (*P. somaliense*; Figure 3). Considering that *Periploca* colonized the Cape Verde islands when climatic conditions in NW Africa were very different than those observed nowadays (Suc, 1984; Fernández-Palacios et al., 2011), our results suggest that leaf size in CAV2 represents the ancestral state of (extinct) mainland source populations. This scenario, however, needs to be further examined within a solid phylogenetic framework.

Our findings also highlight the importance of analysing intraspecific variation in a wide biogeographical island context for gaining



**FIGURE 6** Diagram showing the results from a piece-wise structural equation model testing for associations among climatic factors (Clim PC1, Clim PC2) and leaf functional traits (SLA, photosynthetic rate) on the estimates of primary metabolism (i.e. growth rate) and secondary metabolism (i.e. tannin content) of 21 *P. laevigata* populations. Climatic factors represent z-score values from a principal component analysis summarizing a suite of variables associated with temperature (Clim PC1) and precipitation (Clim PC2). Values associated with each arrow are path coefficients (i.e. standardized regression coefficients). Continuous arrows indicate direct associations, whereas broken arrows indicate indirect associations. Only significant (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ) path coefficients are shown for the ease of interpretation



insights into ecological and evolutionary aspects of functional plant traits. Specifically, the continuous variation in stomatal ratio observed across islands in our common garden experiment (Figure 3g) challenges the global pattern of nearly discrete distributions (i.e. hypostomaty versus amphistomaty) inferred from among-species observations (Muir, 2015). In keeping with previous studies (Moore, Schlichting, Aiello-Lammens, Mocko, & Jones, 2018), our results show that within-species variation follows island–mainland patterns not detectable at other biological (i.e. across species or communities) or biogeographical (i.e. within-island or archipelago) scales. Stomatal ratio was the only trait that showed a pattern of variation among island systems associated with divergence time (Table 3), but considering traits together, it appears that colonization history may have had an impact on island *Periploca* phenotypes (i.e. most recent island colonizers displaying leaf syndromes closer to mainland plants; Figure 6). Although the number of observations (i.e. sublineages) in our study is rather limited to draw stronger conclusions, such an association appears to indicate a differential impact of evolutionary constraints following island colonization on multi-trait phenotypes, as previously discussed.

## 5 | CONCLUSIONS

The currently available literature on functional island biogeography has employed community-level analyses to test predictions derived from classical island biogeography (i.e. the relationship between functional diversity and island area or species richness; Farneda et al., 2019; Ottaviani et al., 2020; Whittaker et al., 2014). By examining the pattern of functional variation in a recently diversified plant complex, our study shifts the focus to other geographical scales and organizational levels that fit the definition of this cross-disciplinary field (Violle et al., 2014). We showed that common garden experiments shed light on pre-existing biogeographical data for testing predictions on the spatial distribution of phenotypic diversity. We infer that factors such as climatic shifts and different island colonization events have driven strong phenotypic divergence between islands and mainland source populations. These two biogeographical factors appear to have promoted the coexistence of island-specific traits and other traits derived from (recent or extinct) mainland ancestral populations. While this pattern qualifies subtropical islands as refugia of functional trait diversity (Keppel et al., 2018), it adds another layer of concern when assessing the effects of global change on biodiversity.

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## DATA AVAILABILITY STATEMENT

Data files with information at the individual level are available at the Dryad repository (<https://doi.org/10.5061/dryad.9zw3r22bs>)

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## BIOSKETCH

The research of all the authors focuses on understanding the spatial patterns of plant functional variation. In particular, they share an interest in applying complementary approaches (molecular techniques, common garden and field measurements) to address research questions relating to plant ecology and evolution.

**Author Contributions:** C.G.-V., P. M. and J.F. conceived the ideas; C.G.-V., P. M., F.I.P. and J.J.-M. collected the data; C.G.-V. and X.M. analysed the data; and C.G.-V. and J.F. led the writing.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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