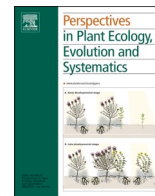


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Perspectives in Plant Ecology, Evolution and Systematics

journal homepage: www.elsevier.com/locate/ppees

Research article

Does insular adaptation to subtropical conditions promote loss of plasticity over time?

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ARTICLE INFO

Key words:

Environmental canalization
Genetic accommodation
Island colonization
Olea europaea
Phenotypic plasticity
Subtropical habitats

ABSTRACT

Phenotypic plasticity (i.e. the ability to express different phenotypes under changing environmental conditions) is thought to play a key role in habitat adaptation, but little is known about how trait plasticity evolves following dispersal into novel island habitats. We hypothesize that shifts from seasonal Mediterranean climates to more stable (subtropical) island conditions would promote a net reduction in trait plasticity over time. To test this hypothesis, we set two common gardens with contrasting environmental (low resource vs. mesic) conditions, where we grew seedlings of wild olive (*Olea europaea* var. *sylvestris*) populations that represented two Canary Island lineages with different colonization times (old vs. young) and their Mediterranean ancestral lineage (N = 275 individuals). Plasticity was assessed for 12 morphological, photosynthetic and chemical traits by (i) subjecting half of the seedlings to simulated herbivore browsing (50% of aerial biomass removal) and (ii) comparing phenotypic values between both common garden settings. Simulated herbivore browsing induced few plastic responses, mostly restricted to photosynthetic traits, but these were similarly displayed by all lineages. Comparisons between common gardens revealed a contrasting response between the Mediterranean and both subtropical island lineages in leaf phenotypes. Furthermore, the older island lineage showed an overall lack of plasticity (i.e. environmental canalization) in morphological and chemical traits. These results suggest that, unlike photosynthetic traits that are fundamental for fast acclimation to environmental shifts, some developmental traits may lose plasticity over time as a result of phenotypic adjustment to subtropical insular conditions.

1. Introduction

Phenotypic adjustment to environmental conditions plays a key role in successful colonization of novel habitats (Yeh and Price, 2004; Ghalambor et al., 2007; Wang and Althoff, 2019). The identification of mechanisms involved in this process is often challenging, since phenotypic traits are commonly modulated by complex interactions among genotypic and environmental factors (Fox et al., 2019). Local adaptation (i.e. genetically-based expression of phenotypes) and phenotypic plasticity (i.e. the ability of a given genotype to generate different phenotypes under changing environmental conditions) are thought to be the main mechanisms allowing species' acclimation to shifting conditions.

Traditionally, both mechanisms have been confronted (e.g. Kawecki and Ebert, 2004; Anderson et al., 2012). A growing body of evidence, however, suggests that environmental and genetic factors are interdependent components of phenotypic variation and, ultimately, species adaptation (Pigliucci et al., 2006; Valladares et al., 2014; Fox et al., 2019). Thus, under certain circumstances, trait plasticity may drive local adaptation to the point of being lost through a process termed “genetic assimilation”, which results in constitutive trait expression (Waddington, 1942; Pigliucci et al., 2006; Ghalambor et al., 2007). The theoretical framework to understand the evolution of plasticity has been clearly defined, yet empirical data supporting this issue still remain scarce (Lande, 2015; Kelly, 2019).

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<https://doi.org/10.1016/j.ppees.2022.125713>

Received 6 October 2022; Received in revised form 26 October 2022; Accepted 29 November 2022

Available online 1 December 2022

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Island lineages (i.e. groups of island populations that result from a dispersal event from a known mainland area) offer excellent opportunities to understand the mechanisms underlying phenotypic evolution after colonization of new habitats (Patiño et al., 2017). Recent island colonizers typically experience environmental conditions that were not previously experienced by their mainland ancestors, such as climatic stability or reduced herbivore pressures, which cause selective pressures towards new phenotypic optima (Burns, 2019). To achieve such new phenotypes, studies based on mainland-island comparisons suggest that defensive and behavioral traits are plastically induced on islands, whereas other anatomical traits (e.g. body or leaf size) may be genetically fixed (Burns, 2019; Baier and Hoekstra, 2019). In sum, we are still far from clearly understand the role of phenotypic plasticity in the process of island colonization and whether plasticity itself may be modified over time as an evolutionary outcome of island adaptation (c.f. Aubret, 2015; Hendry, 2016).

Quantitative genetic theory has put forward clear hypothesis on how trait plasticity could evolve in scenarios such as those associated with dispersal to subtropical islands. Thus, colonization of the new island habitat from a climatically contrasting mainland source should promote an initial increase in plasticity, followed by slow genetic assimilation of the plant phenotype that would lead to a reduction of plasticity over time (Masel et al., 2007; Lande, 2015). Island habitats are typically characterized by more stable climatic conditions than temperate continental areas (Weigelt et al., 2013) and seasonality is thought to be a factor that strongly influences the evolution of trait plasticity (Williams et al., 2017). Since plasticity is favored under heterogeneous, less predictable environmental conditions (Lind et al., 2011; Hendry, 2016), comparisons between closely related populations occurring on mainland and subtropical islands may be particularly suitable to test the prediction of loss of plasticity under less climatically seasonal conditions. To our knowledge, however, there is no information to date on how trait plasticity may be affected by transitions from temperate to subtropical climatic conditions in plants.

Previous studies comparing island-mainland species pairs have reported increases in leaf size, loss of chemical or mechanical defences and lower photosynthetic rates on islands (Burns et al., 2012; García-Verdugo et al., 2019a, 2020; reviewed in Burns, 2019 and Schrader et al., 2021), but, to our knowledge, how trait plasticity is affected by insular colonization has never been addressed in plants. A seminal study on tiger snakes (*Notechis scutatus*) showed that older island colonizers have lost phenotypic plasticity in favor of environmental canalization, whereas young colonizers still displayed substantial phenotypic plasticity (Aubret and Shine, 2009). Considering these results and theoretical expectations on the evolution of plasticity in novel environments (e.g. Lande, 2015), we hypothesize that shifts from seasonal continental areas to subtropical island habitats should have selected for leaf phenotypes commonly described under insular conditions (e.g. increased leaf size, reduced levels of defences; see Burns, 2019), but also for a net reduction in trait plasticity over time.

In this study, we analyze three closely related plant lineages of the wild olive (*Olea europaea* var. *sylvestris*) tree to gain some insights into the underlying mechanisms generating insular-specific traits. There is extensive genetic and morphological evidence supporting the idea that Canary Island populations of *O. europaea* are clearly differentiated from domesticated cultivars and mainland counterparts of wild origin (Green, 2002; Besnard et al., 2009; García-Verdugo et al., 2009, 2010; Goudet, 2014). Furthermore, since extant Canary populations are the result of two independent waves of colonization (García-Verdugo et al., 2009a, Goudet, 2014), we can address whether phenotypic plasticity is related to colonization time by comparing phenotypic traits between both island lineages using the mainland source as a reference (Ottaviani et al., 2020). Several biochemical and physiological traits are known to vary plastically in *O. europaea* (e.g. Rubio de Casas et al., 2011), including responses to herbivore damage (Massey and Hartley, 2000). If evolution under Canary Island conditions (i.e. subtropical climate or low browsing

pressure by herbivores until the arrival of human colonists; e.g. Castilla-Beltrán et al., 2021) tends to reduce plasticity over time, we would expect plasticity in these traits to be correlated with colonization time: i.e. older lineages would be less plastic than younger lineages when exposed to different environmental conditions (Aubret and Shine, 2009). In the present study, we established two common garden experiments with contrasting growing conditions and reared replicated sets of populations that represented Canary and Mediterranean lineages to examine trait plasticity between both environments. In addition, we subjected common garden plants to treatments of simulated herbivore browsing to analyze potential differences in plasticity to damage among lineages. Specifically, we analyze leaf trait variation in this study system to answer these questions: (1) Does simulated herbivore browsing trigger different plastic responses between old and young island colonizing lineages? (2) Do the experimental conditions of the common gardens result in different patterns of trait plasticity between these two lineages? (3) Do leaf phenotypes (i.e. multivariate trait variation) show contrasting patterns between island and mainland lineages?

2. Materials and methods

2.1. Study system and sampling

The genus *Olea* comprises long-lived trees and shrubs mostly restricted to warm temperate and tropical regions of the Old World. In the Mediterranean basin, wild olive (*Olea europaea* var. *sylvestris*) trees are one of the most representative elements of the region, which is characterized by unpredictable resource availability in space and time (e.g. Valladares et al., 2002). Mediterranean *O. europaea* var. *sylvestris* (MDT, hereafter) plants are evergreen, heteroblastic (i.e. leaf traits display abrupt changes throughout ontogenetic development) and sclerophyllous (Moreno-Alias et al., 2009). In the Canary Islands, *O. europaea* is represented by two lineages that likely colonized the archipelago at different times (García-Verdugo et al., 2009a, 2019b; Goudet, 2014). Biogeographical analyses suggest that the eastern islands may have been severely impacted by Pleistocene extinction, which was followed by a second wave of colonization of these islands in more recent times (García-Verdugo et al., 2019b). According to molecular estimates, the older Canary lineage (CAN-O) occupied the western islands between 0.2 and 1.2 Mya, while the eastern island populations (CAN-Y) are the result of a much younger expansion (8000–280,000 yr. BP) (García-Verdugo et al., 2009a; Goudet, 2014).

In order to grow plants of CAN-O, CAN-Y and MDT lineages under common garden conditions, we collected fruits from 10 to 12 mother plants in six of the largest wild populations of the two Canary lineages plus four representative populations of the MDT lineage (Table 1; Fig. S1 Supplementary Information). We sampled MDT populations in two regions of high genetic diversity within the western Mediterranean (Southern Iberian Peninsula and Balearic Islands), and we focused on those that belong to the ancestral haplotype groups of both Canary lineages (Besnard et al., 2013; Díaz-Rueda et al., 2020). Within each sampled lineage, we also sought to represent source populations that experience contrasting conditions because if lineages are environmentally canalized, climatic conditions of source areas should have a lesser impact on the phenotypic expression between common gardens. Climatic conditions in CAN-Y and CAN-O populations are subtropical (low seasonal variability in temperature; see Temperature range values in Table 1), but the former occur in areas that are also close to conditions found in MDT populations (they mostly experience temperate climates with hot, dry summers: Csa, according to Köppen-Geiger classifications; Table 1).

2.2. Common garden experiments

In September 2019, fruits were measured with a digital caliper and the endocarp of each fruit was carefully broken with a tube cutter to

Table 1

Populations sampled in this study with coordinates, haplotype group(s) represented, general climatic conditions (Köppen-Geiger climate classification and Temperature annual range) and number of seedlings considered in each common garden (N_{LOW} , N_{MES}) along with data for the common garden locations where *Olea europaea* seedlings were grown.

Population/Common garden	Coord (Long/Lat)	Hap group ⁽¹⁾	Lineage	K-G Climate ⁽²⁾	Temp range ⁽³⁾	N_{LOW}	N_{MES}
Tarifa	36.062 / - 5.760	E1, E2	MDT	Csa	20.6	15	18
Sierra de Ubrique	36.645 / - 5.432	E1, E2	MDT	Csa	24.5	15	10
Sierra de Na Burguesa	39.568 / 2.576	E1, E2	MDT	Csa	22.4	11	22
Santanyi	39.350 / 3.166	E2	MDT	Csa	21.7	13	17
Barranco Guiniguada	28.065 / - 15.464	MG-2	CAN-Y	BWh	14.6	13	19
Barranco Cernícalos	27.968 / - 15.493	MG-2	CAN-Y	Csa	16.1	19	20
Barranco Guayadeque	27.936 / - 15.506	MG-2	CAN-Y	Csa	15.4	12	18
Anaga	28.545 / - 16.158	MG-1	CAN-O	Csb	15.0	10	12
Valle de Hermigua	28.147 / - 17.198	MG-1	CAN-O	Csb	15.6	12	19
LOW	39.641 / 2.644	All	All	Csa	22.4	120	-
MES	42.406 / - 8.6429	All	All	Csb	16.4	-	155

(1) Following Besnard et al. 2013 and Díaz-Rueda et al. (2020); (2) Following <http://koeppen-geiger.vu-wien.ac.at/present.htm>; (3) Extracted from WorldClim data (<https://www.worldclim.org>)

release the seed. Approximately 40 seeds from each population (i.e. 3–4 seeds per mother plant) were transported to each of the two common garden settings used in this study, totaling 400 seeds in each common garden. Seeds were planted in germination trays filled with commercial substrate and watered regularly. Three months later, germination success was 38.2% (± 9.4 SD) among populations; fewer than three seedlings could be obtained from one population of the CAN-O lineage from the island of La Palma, and this population was therefore removed from the experiment (for final sample sizes, see Table 1).

In order to compare trait expression among *Olea* lineages, we set two common garden experiments that broadly represented a low resource (LOW, hereafter) and a mesic environment (MES, hereafter). The LOW common garden was set in the facilities of the University of the Balearic Islands – Institut Mediterrani d'Estudis Avançats, whereas the MES common garden was set in the facilities of the Misión Biológica de Galicia – Consejo Superior de Investigaciones Científicas in NW mainland Spain. Climatic conditions were measured throughout the duration of the experiments using RH/T H8 HOBO and S-LIA-M003 PAR sensors coupled with a U12 datalogger (Onset Computer Corp., Massachusetts, USA) as in previous studies (García-Verdugo et al., 2009b) (Table S1). To some extent, the two experimental sites broadly approach climatic conditions of seed source areas (Table 1), but our main goal was to ensure that each replicated set of plants were grown under markedly different environments. To this end, plants in the MES common garden were grown in a greenhouse that was characterized by narrower daily variation in microclimatic conditions (Fig. S2) and higher availability of nutrients and water. Thus, mesic conditions were provided by the geographical location of the experiment (mild winter and summer temperatures, moderate light exposure) and other growing conditions (nutrients, water, temperature thresholds) that were experimentally controlled in the greenhouse. In MES, seedlings were grown in large (7.5-L) pots filled with a substrate composed of peat (85%) and perlite (15%) and a controlled release fertilizer (Osmocote bloom NPK 12–7–18 + microelements) was supplemented from the beginning of the experiment. Pots were irrigated every 3–4 days to ensure that water availability was not a limiting factor. Furthermore, drastic fluctuations in temperature were controlled with a cooler that was set to be activated when air temperature reached 30 °C (see Fig. S2). Contrarily, LOW seedlings were grown in small (1.5-L) pots containing a commercial substrate (90% peat moss, 10% perlite) and watered every 10 days. Nutrients were supplemented with a liquid fertilizer (NPK 7–5–6 + microelements) once every month throughout the duration of the experiment. To avoid systematic effects of microenvironmental variation within the common gardens, pots were arranged in several lines composed of seedlings randomly assigned from each population, and positions were randomized within lines approximately every month. Since the growing conditions in each common garden resulted in disparate plant sizes (Plant height_{LOW} = 18.8 \pm 6.1 cm vs. Plant height

MES = 82.4 \pm 25.7 cm; Fig. S1), we measured plant height and crown diameter of each individual. Plant height was strongly correlated with crown diameter in both common gardens ($r^2_{\text{LOW}} = 0.90$, $n = 120$; $r^2_{\text{MES}} = 0.91$, $n = 155$), and we therefore used the former variable alone as an estimate of plant size.

2.3. Simulated browsing treatment

In June 2020, we subjected half of the seedlings of each population in each common garden to a treatment of simulated herbivore browsing. We expected Canary Island lineages to show differential responses as compared to MDT due to varying levels of browsing pressure between regions during their evolutionary history, while different island colonization times may be associated with different responses between Canarian lineages. Our treatment simulated the level of plant damage exerted by large mammals (e.g. goats, rabbits) in young trees and consisted in the removal of approximately 50% of the aerial biomass, in addition to clipping the apical part of each seedling (see Barton, 2016). All browsed seedlings induced the activation of axillary buds within the next two days (C. G-V, personal observation), and trait measurements were performed 5–7 days in the most apical leaves that remained following the application of the treatment.

2.4. Study traits

To analyze the effect of lineage, common garden conditions and the browsing treatment, we focused on a set of 12 traits related to leaf anatomy, biochemistry and photosynthetic performance.

2.4.1. Leaf anatomical traits

Two fully expanded leaves were sampled from each seedling. Leaves were scanned, dried at 65 °C for 48 h and weighed using a precision balance (AB54, Mettler-Toledo AG, Greifensee, Switzerland). Image J software (Abramoff et al., 2004) was used to obtain leaf area (as a proxy of leaf size) and specific leaf area (SLA) was obtained by dividing leaf area by its corresponding dry mass.

2.4.2. Biochemical traits

Leaves used to calculate SLA were also used to determine the leaf carbon isotope composition ($\delta^{13}\text{C}$) and nitrogen content (% leaf N) (Fullana-Pericàs et al., 2019; see Supplementary Text 1).

Leaf phenolic compounds were identified using ultra-performance liquid chromatography coupled with electrospray ionization quadrupole (Thermo Dionex Ultimate 3000 LC) time-of-flight mass spectrometry (UPLC-Q-TOF-MS/MS) (Bruker Compact™) following previous studies of our group (e.g. Moreira et al., 2020). The identified compounds were grouped into four classes: rutin, apigenin (and derivatives), oleuropein (and derivatives) and luteolin (and derivatives). For

quantification, 10 μ L of each sample were injected in an UHPLC (Nexera LC-30 CE; Shimadzu) equipped with a Nexera SIL-30AC injector and one SPD-M20A UV/VIS photodiode array detector (for a detailed description on phenolic characterization and quantification, see Supplementary Text 1).

2.4.3. Photosynthetic traits

Photosynthetic traits included CO₂ assimilation rate (A), stomatal conductance (g_s) and intrinsic water use efficiency (iWUE) and were measured using a LI-6400 Portable Photosynthesis System (LI-COR, Lincoln, NE, USA) as in [Granado-Yela et al. \(2011\)](#) (for particular details, see Supplementary Text 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 vapor pressure deficit within limited ranges of variation among measurements. Due to time constraints, a subset of 10–15 seedlings per population were considered for these trait measurements.

2.5. Statistical analysis

We performed two types of analysis: one considering the effect of common garden conditions and simulated browsing treatments on single traits and another considering phenotypic changes between common gardens using all traits together. All analyses were performed using R v4.1.1 ([R Core Team, 2021](#)).

For single trait analyses, we built linear mixed effects models with ‘Setting’ (two levels: MES and LOW), ‘Lineage’ (three levels: CAN-O, CAN-Y and MDT), and ‘Browsing’ (two levels: damaged and control plants) as fixed factors and each trait as a dependent variable. We also included ‘Population’ nested within ‘Lineage’ as a random factor. Interaction terms (e.g. ‘Setting \times Lineage’) were included to examine potential differences in trait plasticity among lineages. Linear models were run using the ‘car’ package, normal distribution of residuals was checked with Kolmogorov-Smirnov tests and homoscedasticity with Bartlett tests using the ‘olsrr’ package. Following these tests, SLA, oleuropein and luteolin were log-transformed to achieve homoscedasticity. When significant “Setting \times Lineage” interactions were detected, parallelisms tests were run to examine potential differences in the slope of reaction norms among lineages, using plant size as a covariate ([Gianoli and González-Teuber, 2005](#)) and the ‘multi-comp’ package for pairwise comparisons. To examine the potential contribution of maternal effects in our results, we correlated fruit size and one proxy for plant fitness (i.e. plant size) across populations in each common garden. In a second step, we compare the size (MD; magnitude of change) and the direction (θ) of the phenotypic trajectories of each lineage between common garden settings in a multidimensional trait space (i.e. phenotypic change vector analysis), following the method and R script developed by [Adams and Collyer \(2009\)](#). This method allows statistical comparisons of the multivariate characterization of phenotypes considering two or more levels (i.e. MES and LOW common gardens in our study) across two or more lineages (see [Adams and Collyer, 2009](#); [Mitchell et al., 2022](#)). Since browsing treatments did not result in significant differences among lineages (see Results), all samples were pooled for this analysis.

3. Results

3.1. Single trait analysis

The environmental conditions of the common gardens promoted consistent phenotypic changes in most plant traits with the exception of total phenolics, oleuropein, % leaf N and g_s ([Table 2](#)). Four traits were found to be significantly different among lineages ([Table 2](#)). In particular, leaf size, SLA and rutin were the highest in CAN-O, intermediate in CAN-Y and the lowest in MDT, whereas A was the lowest in CAN-O, intermediate in CAN-Y and the highest in MDT ([Fig. 1](#)). Significant

Table 2

Results of the linear mixed effects models (F-ratios) testing for the fixed effects of two common garden environments (Setting), a simulated browsing treatment (Browsing) and their interaction in 12 leaf traits of the three *Olea europaea* lineages considered (Lineage). Population was included as a random factor nested in Lineage. Degrees of freedom = Setting: F_{1,258}; Lineage: F_{2,6}; Setting \times Lineage: F_{2,258}; Browsing: F_{1,258}; Setting \times Browsing: F_{1,258}; Lineage \times Browsing: F_{2,258}. Significant differences for each factor are indicated with asterisks (* P < 0.05, ** P < 0.01, *** P < 0.001).

Trait	Setting (S)	Lineage (L)	S \times L	Browsing (B)	S \times B	L \times B
Leaf size	10.61***	12.90**	9.27***	0.58	0.02	0.08
SLA (log)	19.10***	13.50**	4.10*	15.00***	2.80	0.20
Total phenolics	0.01	0.73	16.60***	3.40	0.01	0.08
Oleuropein (log)	0.49	0.28	11.03***	2.70	0.16	0.11
Luteolin (log)	8.35**	0.29	13.43***	0.01	2.65	0.19
Apigenin	6.18*	0.09	24.43***	0.35	0.86	0.31
Rutin	7.43**	12.35**	11.71***	2.04	0.36	0.55
% leaf N ⁽¹⁾	0.06	2.93	1.31	0.01	0.06	1.41
A ⁽¹⁾	67.40***	50.12***	0.89	2.54	1.36	0.37
g _s ⁽¹⁾	1.47	3.58	2.04	5.93*	0.01	0.33
iWUE ⁽¹⁾	89.51***	0.66	5.26**	5.14*	3.19	0.98
$\delta^{13}C$ ⁽¹⁾	272.78***	0.83	1.60	0.17	0.93	2.13

(1) Setting: F_{1,203}; Lineage: F_{2,6}; Setting \times Lineage: F_{2,203}; Browsing: F_{1,203}; Setting \times Browsing: F_{1,203}; Lineage \times Browsing: F_{2,203}

‘Setting \times Lineage’ interactions were found in eight traits ([Table 2](#)). Remarkably, 9 out of 12 traits, including all anatomical and biochemical traits, were not significantly plastic in CAN-O (i.e. flat reaction norms), but they were in CAN-Y and/or MDT lineages ([Fig. 2A, B, C, D, E, F, G, H, K](#)). Leaf nitrogen was not significantly plastic in any lineage ([Fig. 2H](#)), whereas photosynthetic traits (A, iWUE and $\delta^{13}C$) were plastic in all lineages ([Fig. 2I, J, L](#)). With the exception of some biochemical traits (total phenolics, oleuropein, luteolin and apigenin) populations typically displayed consistent reaction norms within lineages ([Fig. S3](#)).

For those traits showing significant ‘Setting \times Lineage’ interactions, parallelisms tests revealed significant differences in the slope of the reaction norms between both Canarian lineages and MDT for leaf size, total phenolics, oleuropein and g_s ([Fig. 2A, C, D, K](#)), whereas different slopes between CAN-Y and the other two lineages were observed for SLA and rutin ([Fig. 2B, E](#)). Plant size had a significant effect on leaf size, SLA and apigenin, but all ‘Setting \times Lineage’ interactions remained significant when controlling for this trait ([Table S2, Supplementary Material](#)). We did not find a significant relationship between seed and plant size in either of the two common garden settings ($r_{LOW} = 0.28$, $P > 0.05$; $r_{MES} = -0.49$, $P > 0.05$).

The simulated browsing treatment induced very few phenotypic responses and no interactions between this treatment and the main factors were observed (i.e. lack of significant ‘Lineage \times Browsing’ or ‘Setting \times Browsing’ interactions for any trait; [Table 2](#)). All treatment effects were similar across lineages: browsing consistently induced a decrease in SLA and iWUE, and an increase in g_s in plants of both common gardens ([Table 2; Fig. S4](#)).

3.2. Phenotypic trajectories

When all traits were analyzed together in a multidimensional trait space, the AMOVA showed that leaf phenotypes were significantly affected by the main factors “Lineage” (F₂₃₇₈ = 8.64, P < 0.001), “Setting” (F₁₁₈₈ = 22.49, P < 0.001) and their interaction (F₂₃₇₈ = 3.29, P < 0.001). Permutations tests did not reveal statistically significant differences among lineages in trajectory size (i.e. the amount of change between common garden settings was not different considering the multivariate phenotypes), but MDT plants showed a direction that was significantly different to that displayed by both Canarian lineages

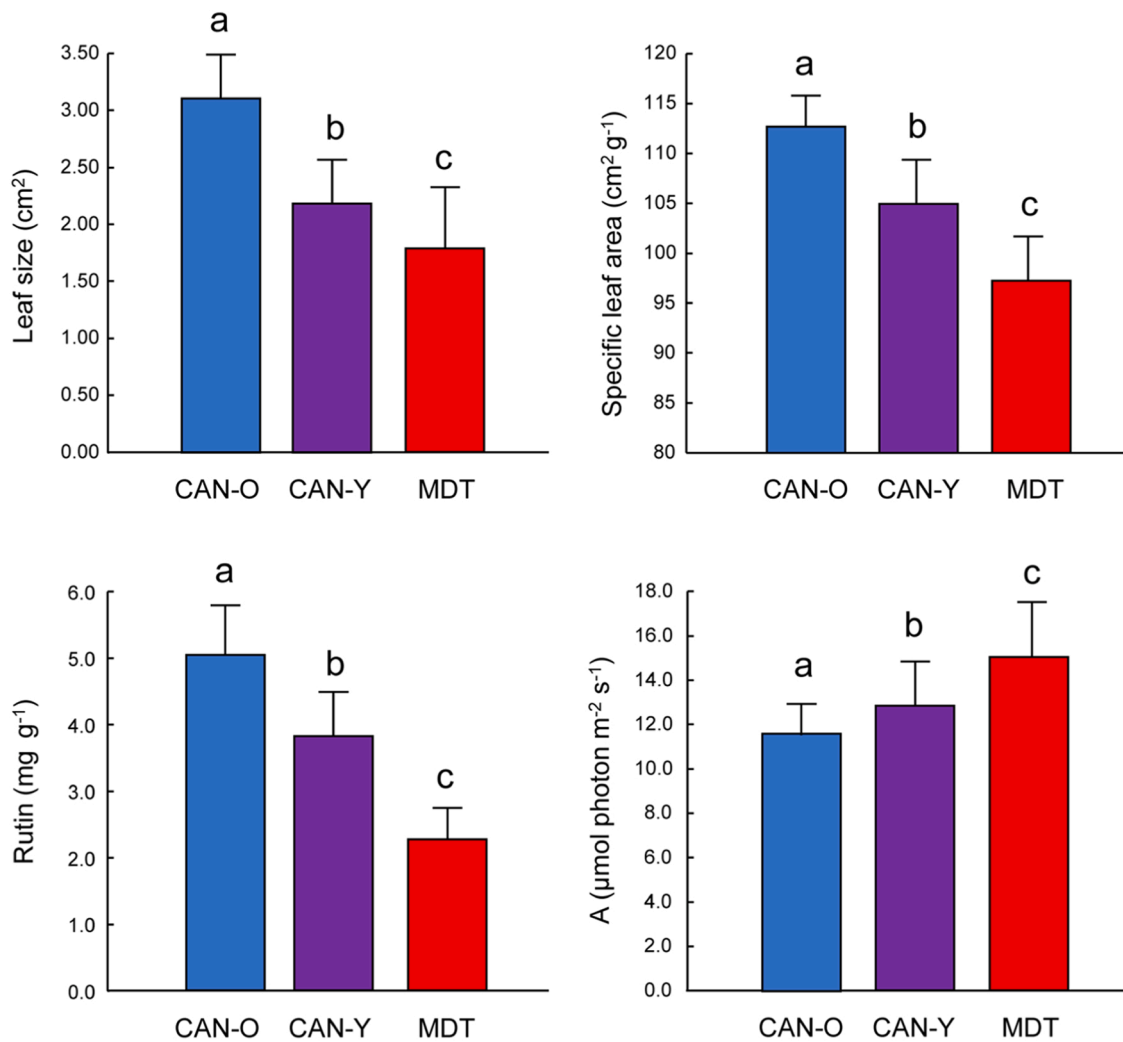


Fig. 1. Mean (\pm SD) values for traits that were statistically significant among the three *Olea europaea* lineages considered (CAN-O, CAN-Y, MDT; see text for descriptions). Different letters indicate significant differences ($P < 0.05$) among lineages according to Tukey's post-hoc tests.

(Table 3). Thus, while leaf phenotypes tended to converge under MES conditions, MDT plants clearly diverged from CAN-Y and CAN-O lineages under LOW conditions, mostly due to the contribution of traits such as SLA, all phenolic compounds and leaf size (Fig. 3).

4. Discussion

In this study, we analyzed patterns of phenotypic variation in a set of leaf traits within the comparative evolutionary framework provided by three closely related plant lineages that span Mediterranean and subtropical island climatic conditions. By growing plants in two common gardens representing contrasting environmental conditions, we found evidence that (i) some phenotypic differences between lineages (e.g. leaf size, SLA, photosynthetic rate, rutin content) are genetically-based, (ii) plasticity is remarkably low in the oldest island lineage and (iii) phenotypic trajectories in leaf phenotypes are similar between both subtropical island lineages, but different to that displayed by the Mediterranean lineage.

We observed remarkably different patterns in leaf plasticity among *O. europaea* lineages reared under contrasting environmental conditions. Considering the biogeographical context of the study system, our results are consistent with the process of adaptation to a sudden change in the mean environment predicted by theory, in this case the shift from Mediterranean to seasonally stable island habitats. The predominant role attributed to phenotypic plasticity upon colonization of a new

habitat (Ghalambor et al., 2007; Lande, 2015) could be exemplified by the young Canary lineage, which showed significant levels of trait plasticity for most traits. In evolutionary terms, phenotypic plasticity may be the fastest solution to approach an optimal phenotype in traits subject to strong directional selection on subtropical islands (Aubret and Shine, 2009), such as large leaves (Burns et al., 2012), low photosynthetic rates (García-Verdugo et al., 2020) or high contents of leaf phenolic compounds (Monroy and García-Verdugo, 2019; Moreira et al., 2019). All these traits were consistently displayed by the old Canary lineage (Fig. 1), thus supporting the idea that their expression is genetically based as a result of directional selection upon island colonization.

Substantial levels of phenotypic plasticity in the young Canary lineage may be maintained by climatic heterogeneity (see Hendry, 2016), since it occupies areas that approach certain Mediterranean conditions (Table 1). Alternatively, the observed levels of plasticity may be explained by its recent island colonization (i.e. selection against plasticity has not occurred yet). It is interesting to note that leaf phenotypes of both Canary lineages followed similar multivariate patterns (Table 3, Fig. 3), which suggests a common phenotypic trend associated with common island conditions. Temperate climates have been implicated as major factors in the evolution of convergent island traits such as shifts from herbaceous to woody growth forms or increases in leaf size (Burns, 2019; Zizka et al., 2022). Since the Canary Islands provide a more climatically stable environment than the Mediterranean source

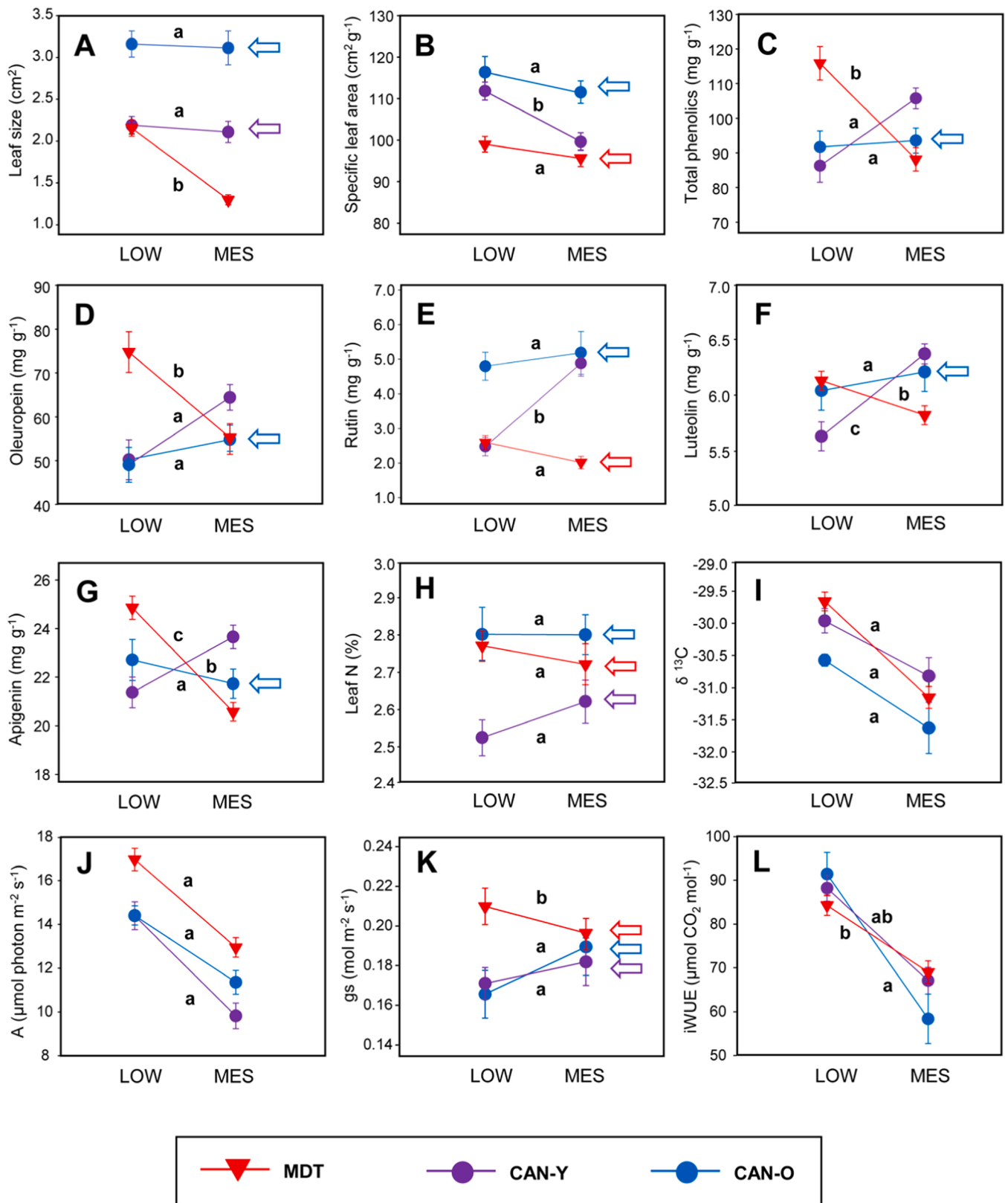


Fig. 2. Reaction norms of 12 leaf traits (mean \pm SD) in three *Olea europaea* lineages (MDT, CAN-Y, CAN-O) reared in two common garden settings (LOW, MES; see text for descriptions). Arrows indicate lack of trait plasticity in a given lineage (i.e. flat reaction norm) following Tukey's post-hoc tests comparing LOW and MES trait values ($P > 0.05$). Different letters indicate significant differences ($P < 0.05$) in the slope of reaction norms following parallelism tests.

Table 3

Statistical comparisons of phenotypic trajectory size (below diagonal) and direction (above diagonal) among *O. europaea* lineages (CAN-O, CAN-Y, MDT; see main text for details). Significant differences based on 10,000 random permutations are indicated with asterisks (* $P < 0.05$, *** $P < 0.001$).

	CAN-O	CAN-Y	MDT
CAN-O	–	49.7	53.1 *
CAN-Y	0.09	–	85.2 ***
MDT	0.12	0.02	–

area (Valladares et al., 2002; Cropper and Hanna, 2014), sustained generations of stabilizing selection may have selected against trait plasticity in favor of canalization (Debat and David, 2001; Aubret and Shine 2009). In keeping with this expectation, the older Canarian lineage displayed flat reaction norms in most traits (Fig. 2), particularly those linked to high developmental plasticity (Sultan, 2000).

Our results point to an association between long-term residence on climatically stable islands and substantial canalization of leaf phenotypes, but the mechanistic factor(s) responsible for this pattern are still unclear. We can hypothesize that some non-mutually exclusive factors (genetic, ontogenetic, maternal effects) may account for such a loss of plasticity. First, it has been recently suggested that retention of ancestral polymorphism following island colonization may fuel ecological adaptation (Choi et al., 2021). It follows that species like *O. europaea*, which tend to track similar Canarian habitats as those occupied in the Mediterranean source area (García-Verdugo, 2014), may have limited genomic potential to expand their ecological range on islands, being therefore more sensitive to the effect of stabilizing selection (and subsequent canalization) in a given environment (Ackerly, 2003; Flatt, 2005). Second, loss of plasticity in *O. europaea* seedlings may be explained by ontogenetic factors (i.e. loss of heteroblasty). Reversals from hetero- to homoblasty have been reported in the flora of other subtropical islands (Burns and Dawson, 2009), which may be due to selection for homoblasty in predictable environments (Zotz et al., 2011). To test this possibility, we extracted common garden data published in a previous study on *O. europaea* adult individuals for the same CAN-O populations (García-Verdugo et al., 2009b), and found that leaf size, one trait indicative of heteroblasty in this species (Moreno-Alias et al., 2009), was similar between adult plants and seedlings (ANOVA “Population × Setting” term: $F_{2,51} = 1.33$, $P = 0.27$; Fig. S5 Supplementary information). This preliminary test would support the idea of homoblasty for leaf size in the older island lineage, but this topic deserves deeper investigation. Lastly, maternal effects (i.e. transgenerational plasticity) may have contributed to some of the observed patterns, since the magnitude and sign of this phenomenon on phenotypes may vary depending on the environmental conditions where parents evolved

(Kuijper and Hoyle, 2015). However, our analysis did not reveal a significant association between seed and plant size, which may indicate a lesser role of maternal effects in the observed patterns. Such effects are expected to be stronger at early stages of development (i.e. germination) (Bischoff and Muller-Scharer, 2010) and are unlikely to have promoted phenotypic canalization in two common garden experiments conducted under such contrasting environmental conditions. It would be, however, interesting to explore in future studies the effect of transgenerational plasticity at early stages of island colonization, when plasticity is thought to play a major role (Lande, 2015).

Our simulated browsing treatment induced very few plastic responses and did not support the hypothesis that island lineages are more susceptible to herbivore damage (Cubas et al., 2019). Notably, all lineages consistently displayed the same array of responses to simulated browsing, i.e. reduced SLA and intrinsic water use efficiency and increased stomatal conductance. Decreases in SLA following substantial damage have been attributed to accumulation of non-structural carbohydrates in leaves necessary for bud activation and, ultimately, quick recovery of photosynthetic tissue in small plants (Volin et al., 2002; Pinkard et al., 2007; Wang et al., 2020) or leaf-level modifications in cell wall composition in the short term (Flexas et al., 2021). Increased stomatal conductance did not result in significant increases in CO_2 assimilation, probably because plants had already reached maximum photosynthetic rates (Flexas et al., 2014), but this is also a typical compensatory response following plant damage (Volin et al., 2002; Wang et al., 2020). Our results suggest that *O. europaea* seedlings deploy physiological responses to quickly ameliorate the loss of photosynthetic tissue, and therefore plasticity in these traits either following substantial damage or contrasting environmental conditions (Fig. 2) does not appear to be significantly affected by colonization of subtropical habitats.

5. Limitations of the study and future prospects

Our results are based on the analysis of a set of traits measured from a few populations that aim to represent plant lineages spanning broad geographical areas. While the contrasting levels of trait plasticity observed in both Canarian lineages can be put in context by the comparison with their Mediterranean ancestral lineage, a broader sampling of both the old Canarian lineage (represented by two populations in the present study) and the Mediterranean closest haplotypes (four populations) would be needed to reinforce our observations. We detect a pattern of canalization (flat reaction norms) for anatomical and biochemical traits in the old Canarian lineage, but it would be interesting to check if other key plant traits (i.e. seed size, plant hydraulics) follow a similar pattern, and whether exposure to other environmental

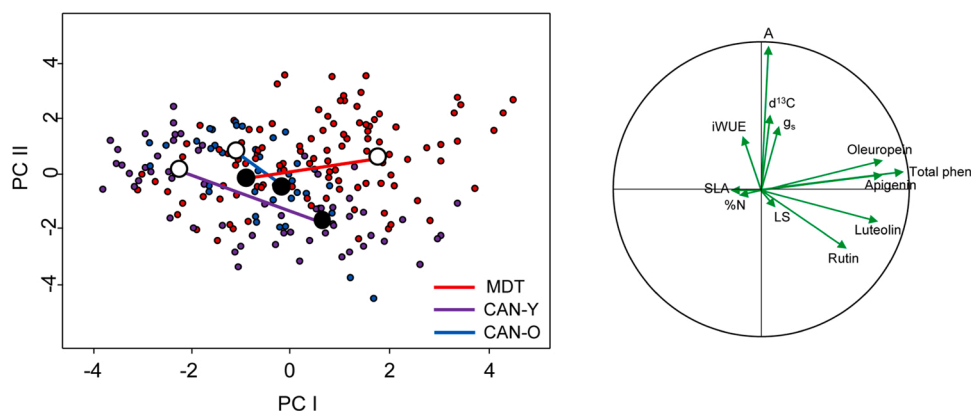


Fig. 3. Representation of phenotypic trajectories between two common garden settings (LOW = open circles, MES = closed circles) for three *O. europaea* lineages (CAN-O, CAN-Y, MDT). Each seedling is represented by a small circle with a color associated with the lineage where it belongs. The contribution of each trait to the multidimensional space is depicted next to the chart showing phenotypic trajectories (see text for description of traits).

conditions such as drought or warming render similar results (e.g. considering non-linear reaction norms and potential conditions for decanalization; Flatt, 2005). To this end, apart from common garden experiments, field measurements could help us understand phenotypic expression under local conditions. Although our results should be taken with caution, this study provides a further step in our understanding of phenotypic canalization in subtropical islands, a process that may be crucial for us to foresee if island biotas experience limitations to adjust their phenotypes to the shifting conditions induced by global change.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We dedicate this work to the memory of Dr. Luis Balaguer for his pioneer studies on the evolution of phenotypic plasticity in *Olea europaea*. We appreciate the assistance provided by B. Oliver, M. Truyols, B. Lago and L. Álvarez at different stages of the common garden experiments, and the thorough comments provided by two anonymous reviewers and the subject editor (J. Alexander). This research was financially supported by a Vincenç Mut fellowship (Conselleria d'Innovació, Recerca i Turisme, Govern de les Illes Balears and the European Social Fund) to CGV, a grant from the Spanish Ministry of Science, Innovation and Universities (RTI2018-099322-B-I00) to XM and the Ramón y Cajal Research Programme (RYC-2013-13230) to XM. Funding for the open access charge was kindly provided by the Universidad de Granada / CBUA.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ppees.2022.125713](https://doi.org/10.1016/j.ppees.2022.125713).

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