



Parallel evolution of insular *Olea europaea* subspecies based on geographical structuring of plastid DNA variation and phenotypic similarity in leaf traits

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Received 2 August 2009; accepted for publication 29 October 2009

Strong geographical isolation within the distribution of a species may result in differentiated lineages exhibiting conspicuous phenotypic differences. In the present paper, we investigate whether plastid and phenotypic variation is geographically structured within the *Olea europaea* complex in Macaronesia, which comprises three subspecies separated by oceanic barriers: *maroccana* (south-west Morocco), *guanchica* (Canary Islands) and *cerasiformis* (Madeira archipelago). Plastid variation showed a significant pattern of geographical structure ($N_{ST} > G_{ST} = 0.56$), because of the lack of shared haplotypes among subspecies and the presence of a single and private haplotype in the eastern Canary Islands. Such a clear molecular structure, however, was not reflected in a congruent pattern of phenotypic differentiation among taxa in leaf morpho-functional traits. Despite the substantial genetic differentiation observed between the subspecies from Madeira and the Canary Islands, they displayed both higher leaf size (leaf area) and specific leaf area (leaf surface area-to-mass ratio) than their continental counterparts, probably as a result of oceanic conditions in subtropical environments. Unlike most of the plant groups previously studied in the Macaronesian region, the lineages of *Olea* illustrate how low phenotypic differentiation can be also related to a clear molecular differentiation in oceanic island enclaves. © 2010 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2010, **162**, 54–63.

ADDITIONAL KEYWORDS: archipelagos – geographical isolation – leaf size – lineage differentiation – plastid variation – specific leaf area.

INTRODUCTION

Geographical races (including subspecies) provide challenging opportunities for studying the mechanisms by which molecular and phenotypic diversity are distributed across geographical areas (Brochmann *et al.*, 1995; Chiang *et al.*, 2006; Phillimore & Owens, 2006). Phenotypic differentiation may have little relationship to the degree of genetic divergence between closely related taxa. Extensive morphological variation can be achieved through a modest number of

genetic changes, whereas marked genetic differentiation may be obscured by similar phenotypic expression (Mayr, 2004). Limits to dispersal and selective environmental pressures, however, relate both molecular and phenotypic variation to geographical patterns, even when subtle differentiation exists at the infraspecific level (Brochmann *et al.*, 1995; Jørgensen, Richardson & Andersson, 2006; Rubio de Casas *et al.*, 2009).

The representatives of the *Olea europaea* L. (Oleaceae) complex in the Macaronesian region (*sensu* Sunding, 1979) constitute an interesting study case for analysing the distribution of genetic and phenotypic variation on a limited geographical scale. Three

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subspecies of anemophilous and bird-dispersed plants coexist in an area comprising a small location in north-west Morocco (subsp. *maroccana* (Greuter & Burdet) P.Vargas *et al.*) and two oceanic archipelagos: Madeira (subsp. *cerasiformis* (Webb & Berth.) Kunk. & Sund.) and the Canary Islands (subsp. *guanchica* P.Vargas *et al.*). Despite the climatic differences between the mainland and the archipelagos of this area (Médail & Quézel, 1999; Médail, Quézel & Besnard, 2001), little morphological differentiation has been reported among these three taxa (Médail *et al.*, 2001; Vargas *et al.*, 2001). Lack of conspicuous morphological differentiation and unknown reproductive barriers have led researchers to suggest a sub-specific treatment for these taxa (Médail *et al.*, 2001; Vargas *et al.*, 2001) and geographical location is recognized as the main criterion for taxon distinction (for a taxonomic revision, see Green, 2002). Molecular data showed that these subspecies share an exclusive plastid sublineage which is closely related to other mainland subspecies, namely *europaea* (Mediterranean Basin) and *laperrinei* (Batt. & Trab.) Cif. (Central Sahara) (Besnard, Rubio de Casas & Vargas, 2007). Indeed, a recent study conducted with fingerprinting markers showed that populations of subsp. *guanchica* sampled on the eastern Canarian islands were more related to subsp. *europaea* than to western subsp. *guanchica* (García-Verdugo *et al.*, 2009a). Nevertheless, specific tests for the structure of plastid variation in this area have not yet been attempted and might result in a complex pattern because of hybridization events among these subspecies (Besnard *et al.*, 2007; García-Verdugo *et al.*, 2009a).

In the present study, we focus on the Macaronesian subspecies in order to gain more insight into the patterns of plastid and phenotypic variation within the *O. europaea* complex. Most of the taxonomic traits that have been used for taxon delimitation in this complex are related to leaf biometrics (e.g. leaf length, width or length/width ratio; Médail *et al.*, 2001; Vargas *et al.*, 2001). Leaf biometric measurements are easy to perform, but they may show poor resolution for taxa discrimination (Barone *et al.*, 1994) and their relevance in plant performance is not always clear (e.g. Rubio de Casas *et al.*, 2009). Leaf functional traits, in contrast, are more suitable for investigating the phenotypic acclimation of plants to environmental conditions, because they are strongly linked to plant performance and, consequently, are thought to be of a marked evolutionary relevance (Geber & Griffen, 2003). In particular, leaf size and specific leaf area (leaf surface area-to-mass ratio) are two attributes of great importance for plant performance because variation in these traits allows adaptation to the

range of environmental conditions that occur in contrasting habitats (Reich, Walters & Ellsworth, 1997; Ackerly *et al.*, 2002). We could therefore expect that subtropical conditions in different Macaronesian archipelagos may have promoted a similar phenotypic expression between insular taxa (Brochmann *et al.*, 1995). Indeed, it is expected that they are more closely related to the lauroid leaf phenotype typically described under subtropical environments (e.g. larger leaves; Pérez Latorre & Cabezudo, 2006) than to the sclerophyllous leaf phenotypes displayed under Mediterranean environments (Rubio de Casas *et al.*, 2009). We specifically test (1) whether the strong geographical isolation associated with these subspecies is reflected in a marked phylogeographical structure of plastid DNA, (2) whether similar oceanic environments have led to a similar expression in two relevant functional traits (leaf size and specific leaf area) and (3) to what extent differentiation in plastid lineages is associated with differentiation at the phenotypic level.

MATERIAL AND METHODS

DISTRIBUTION AND SAMPLING

The *O. europaea* complex in Macaronesia comprises three subspecies with a clear geographical delimitation (Vargas *et al.*, 2001; Green, 2002): *O. europaea* subsp. *guanchica* is a diploid occurring only in the Canary Islands, *O. europaea* subsp. *cerasiformis* is a tetraploid endemic to the two Madeiran islands and *O. europaea* subsp. *maroccana* is a hexaploid occupying a total area of a few hundred hectares in the western High Atlas. Seed dispersal in this group is mainly mediated by birds, a feature that may allow long-distance connection among taxa. In this study, we analysed a set of populations throughout the geographical range of each Macaronesian subspecies (*guanchica*, *cerasiformis* and *maroccana*, hereafter). As *cerasiformis* and *maroccana* are narrow endemics, only three populations were considered for each of these two subspecies, whereas one to six populations were sampled on each Canarian island for *guanchica*. A previous study detected a genetic split within *guanchica* (García-Verdugo *et al.*, 2009a) and we therefore considered two different areas within the Canary archipelago: populations on the eastern islands (GUA_E) and those on the western ones (GUA_W). The islands of El Hierro and Lanzarote were not included in this study, given the rarity of wild olive trees on these two islands.

To represent the closest relative (*O. europaea* subsp. *europaea*) of the Macaronesian subspecies, individuals from 17 locations distributed throughout the western Mediterranean (Morocco, the Iberian

Peninsula and the Balearic Islands) were also included. Thus, a total of 98 individuals were analysed for plastid variation, including *guanchica* (GUA_E, $N = 26$; GUA_W, $N = 32$), *cerasiformis* ($N = 12$), *maroccana* ($N = 11$) and *europaea* ($N = 17$). In addition, two or three shoots were collected from upper, south-facing parts of some of the trees included in the genetic analysis ($N = 100$) in order to analyse the phenotypic expression of leaf traits (for a detailed list of the sample sizes considered for each analysis, see Supporting Information, Table S1). Some individuals of subspecies *laperrinei* were also included in this dataset.

MOLECULAR DATA

Total genomic DNA was extracted from c. 30 mg silica dried leaf material using the DNAeasy Plant Minikit (Qiagen Inc.) following the manufacturer's protocol. In a first step, molecular variation in a subset of 16 samples was screened for 17 plastid markers based either on previously published markers (Weising & Gardner 1999; Besnard, Rubio de Casas & Vargas, 2003) or on new markers isolated through sequencing additional plastid regions as part of a wider study in the *O. europaea* complex in Spain (A. Forrest & P. Vargas, unpubl. data). Five of these 16 markers were selected for the present study as they were polymorphic (indels and/or length variation in microsatellite motifs): three have been published previously (*trnSG*-indel1 and *trnSG*-indel2 in the *trnS-trnG* spacer, (Besnard *et al.*, 2003); *ccmp5* in the *atpB-rbcL* spacer, Weising & Gardner, 1999) and two were newly developed in *Olea*. Primers and PCR conditions can be consulted for the published primers in the respective publications; those for the new markers are given in the Supporting Information (Table S2). One of each pair of primers was labelled with a fluorescent dye and fragments were visualized on an Applied Biosystems 377 DNA Sequencer and scored manually using GeneMapper software. Each size variant allele had been previously sequenced in *O. europaea* to confirm correspondence between polymorphism and fragment size and some additional sequences were generated for *guanchica* for this study. Polymorphisms were scored as multi-state characters (length of microsatellites and indels) and used to define a plastid haplotype for each sample (see also Supporting Information, Table S3).

PHENOTYPIC DATA

A total of 661 leaves collected from 100 individuals were examined for phenotypic traits (see also Supporting Information, Table S1). For each leaf, we measured three traits related to leaf biometrics that were used in earlier studies of the *O. europaea* complex: leaf length, width and length/width ratio (Médail *et al.*, 2001; Vargas *et al.*, 2001; Green, 2002). In addition, two

functional traits, leaf size (area) and specific leaf area (leaf area-to-mass ratio) were measured to investigate the phenotypic response of plants to environmental conditions. Measurements were only taken from current-year, fully expanded leaves. Two or three leaves were randomly chosen from each shoot and scanned. Leaves were then oven-dried at 65 °C for 48 h and weighed. Images of scanned leaves were analysed with Scion Image software (Scion Corp., MD, USA) and leaf length, leaf width and area were calculated. Specific leaf area (SLA, hereafter) was calculated as the ratio between leaf area and mass.

DATA ANALYSIS

Haplotype relatedness was investigated by constructing a median joining network (Bandelt, Forster & Röhl, 1999) with the software NETWORK 4.5 (<http://www.fluxus-engineering.com>). As the input file for this software only allows two digits per allele, each character state was coded by two digits but keeping the differences in length between multi-state characters. Characters were not weighted, assuming the same relevance for each marker (see NETWORK 4.5, user guide). In addition, the degree of geographical structuring of plastid variation was investigated using two approaches. First, N_{ST} was compared with G_{ST} to determine whether related haplotypes were clustered by geographical distribution, following the procedure described in Pons & Petit (1996) and implemented in PERMUT-CPSSR 2.0 (<http://www.pierroton.inra.fr/genetics/labo/Software>). Second, populations were grouped by geographical region in a hierarchical AMOVA performed using ARLEQUIN 3.0 (Excoffier *et al.*, 1992).

All the phenotypic traits were analysed with a principal component analysis (PCA) based on the correlation data matrix, to control for any correlation between the variables. In addition, Mahalanobis distances were calculated between all possible pairs of populations and subspecies using the software XLSTAT (Addinsoft, France).

To investigate the similarity in phenotypic expression between geographical areas, each functional trait was analysed in an ANOVA with the factor 'Subspecies' nested within 'Environment'. 'Environment' grouped those subspecies occurring on oceanic (*guanchica*, east and west, and *cerasiformis*) or mainland (*maroccana*, *europaea* and *laperrinei*) locations. When significant differences ($P < 0.05$) were detected for a factor, Tukey tests were used to determine them.

Lastly, Mantel tests were performed in order to assess the association between genetic or phenotypic variation and geographical distance and to explore the association between genetic and phenotypic variation. Four matrices were constructed, one based on

phenotypic Mahalanobis distances among all the Macaronesian populations (PHE; see Nielsen *et al.*, 2003 for a similar approach), another based on plastid genetic distances (PLAST) as calculated in ARLEQUIN 3.0, a third based on AFLP (nuclear, NUCL) distances from a previous study on the same populations (García-Verdugo *et al.*, 2009a) and, lastly, another based on relative geographical distances (GEO). Comparison of plastid and nuclear results of Mantel tests allowed us to determine whether plastid or nuclear differentiation is more related to phenotypic expression (see Nielsen *et al.*, 2003; Rubio de Casas *et al.*, 2009). The level of significance was assigned after 1000 permutation tests, as implemented in GENALEX 6 (Peakall & Smouse, 2006).

RESULTS

PLASTID DNA VARIATION

Molecular variation in the five plastid regions yielded a total of 19 haplotypes (see also Supporting Infor-

mation, Table S3). Five haplotypes were found in samples from the western Mediterranean region and the other 14 within the Macaronesian region, which extends the number of haplotypes (10) previously identified (Besnard *et al.*, 2007). Four haplotypes were detected in south-west Morocco, three in Madeira and seven in the Canarian archipelago (Fig. 1). None of the haplotypes was present in more than one geographical area. The haplotype network showed a close relationship among the Macaronesian subspecies and missing haplotypes were virtually absent in internal positions of the network (Fig. 1). Haplotypes C, D, E, F and G were exclusively found in the western Canaries, whereas a single haplotype (B) was the only one found in all those individuals sampled on the eastern islands. In addition, all haplotypes found in *maroccana* and *cerasiformis* constituted a separate clade, that was closely related to haplotype B (eastern Canaries).

The N_{ST} (0.67) was higher than G_{ST} . Permutation analysis computed a mean N_{ST} value of 0.55, almost

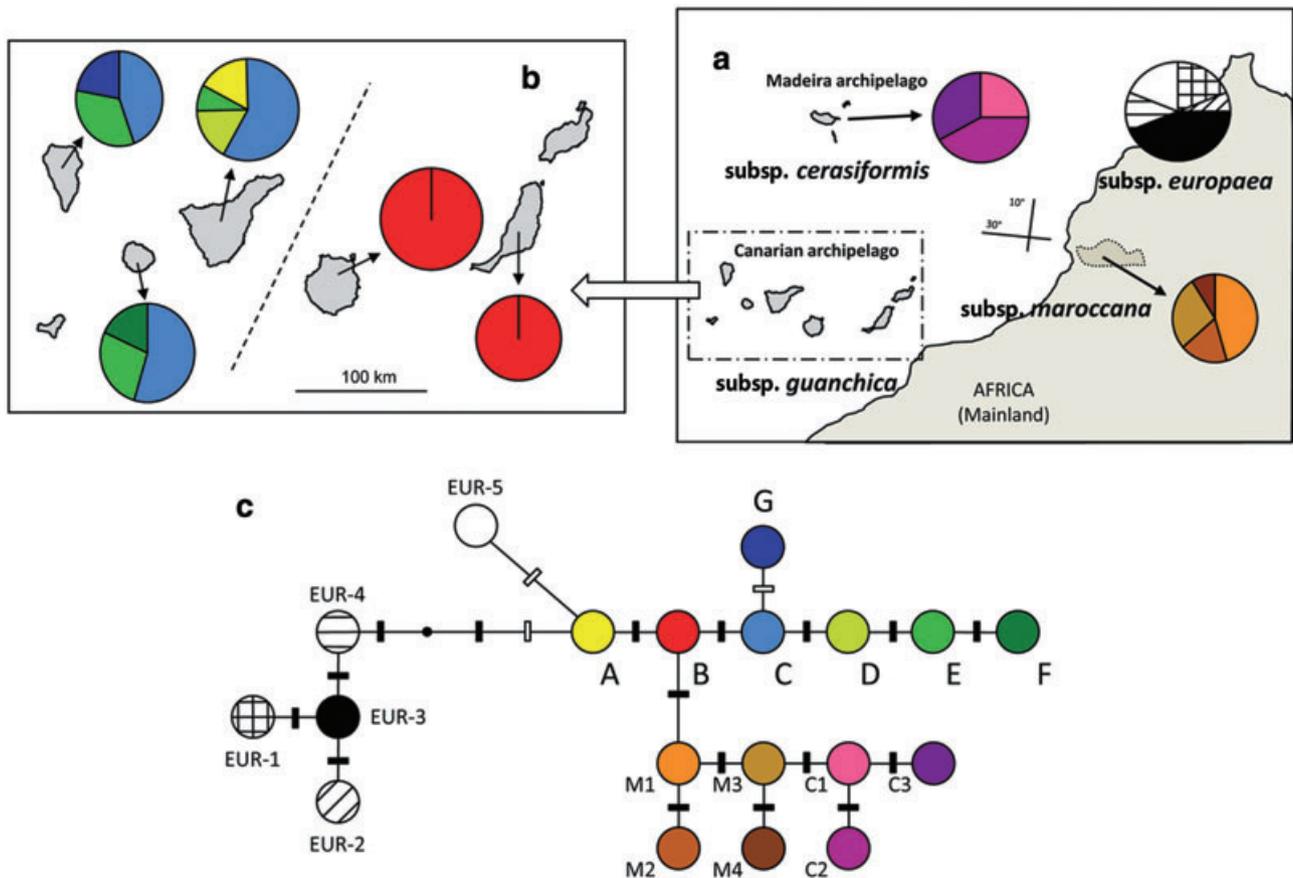
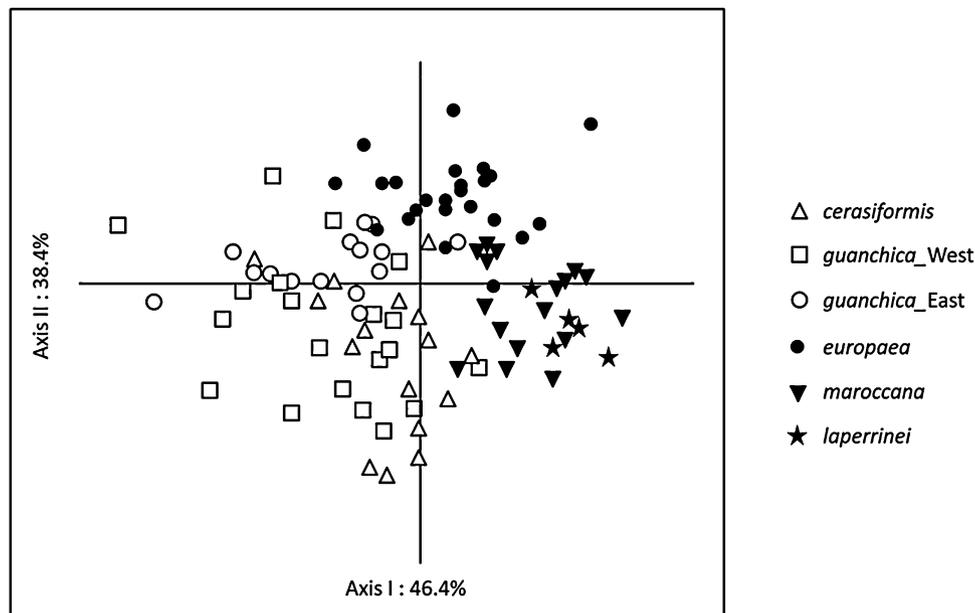


Figure 1. Geographical location and sampling of the Macaronesian *Olea* subspecies, showing the haplotype frequencies of each subspecies/island considered (a, b). Below, haplotype network based on a median joining procedure (c) in which each haplotype is represented by a code and a colour/pattern. Closed bars between haplotypes represent single step mutations, open bars represent indels and the small black dot represents a missing haplotype.

Table 1. Hierarchical analysis of molecular variance based on plastid DNA markers among geographical areas (Madeira/Western Canaries/Eastern Canaries/south-west Morocco), among populations within areas and among individuals within populations

Source of variation	d.f.	Sum of squares	Variance components	%Var	<i>P</i>	Φ
Among areas	3	30.37	0.46	51%	< 0.001	$\Phi_{CT} = 0.51$
Among populations within areas	7	8.74	0.13	15%	< 0.001	$\Phi_{SC} = 0.30$
Within populations	70	21.25	0.30	34%	< 0.001	

Each island of the Canary archipelago was considered as a population. d.f., degrees of freedom; % Var, percentage of variation.

**Figure 2.** Principal components analysis of five morpho-functional traits in insular (open symbols) and mainland (closed symbols) groups. The percentage of variance explained by each component is shown next to the axis.

identical to the observed G_{ST} value (0.56), with less than 5% of permuted N_{ST} values greater than the observed N_{ST} value. This result suggested that groups of related haplotypes were restricted to particular geographical areas in the Macaronesian region. Furthermore, AMOVA revealed that a considerable proportion of the genetic variance was partitioned among geographical areas ($\Phi_{CT} = 0.51$, $P < 0.001$; Table 1) and, to a lesser extent, among populations within each area ($\Phi_{SC} = 0.30$, $P < 0.001$).

PHENOTYPIC VARIATION

Principal component analysis showed that insular and mainland subspecies displayed leaf phenotypes that are partially distinguishable when a combination of morpho-functional traits is used (Fig. 2). Axis 1 accounted for 46.4% of the variance and separated

most of the insular samples from the mainland ones because of the contribution of variables such as leaf area (loading factor: -0.95) and SLA (-0.64). Axis 2 explained 38.4% of the variance and related *cerasiformis* to *maroccana* and *laperrinei*, because of the contribution of variables such as leaf length/width ratio (loading factor: -0.96) and leaf length (-0.83).

When functional traits were considered individually, nested ANOVA showed that both leaf size and SLA were significantly higher in the insular environments than those displayed by the mainland subspecies (Fig. 3). Differences within environments were only because of differences in phenotypic expression among mainland subspecies, but not among insular subspecies. Thus, *laperrinei* had a smaller leaf size than *maroccana* and *europaea*, whereas *maroccana* displayed smaller SLA values than the other subspecies. There were no differences in these traits between

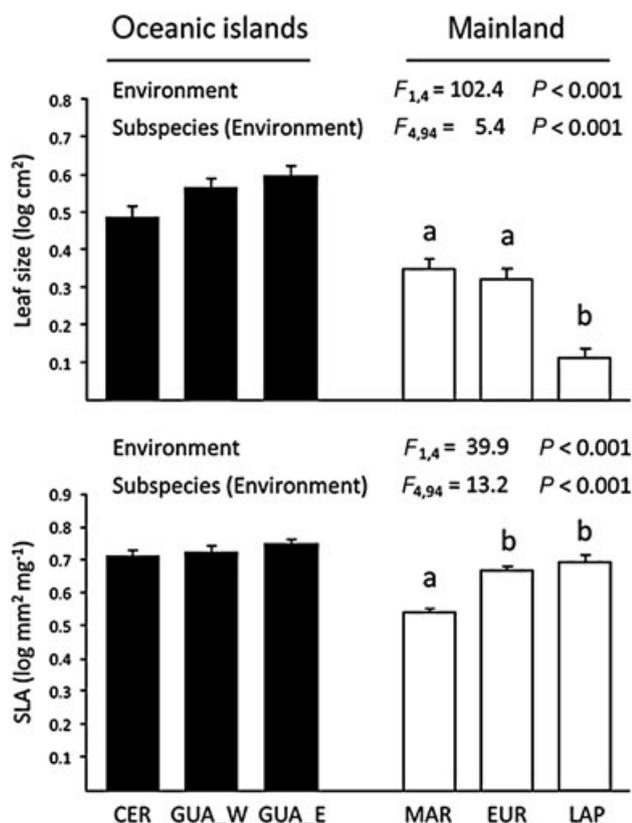


Figure 3. Results for the nested ANOVA of differences in two functional leaf traits between environments (insular vs. mainland) and subspecies within environments. Different letters over the bars denote significant differences among mainland subspecies according to Tukey test. Codes for each group are as in Table 2.

individuals sampled on the eastern and the western Canary Islands, nor was there a significant difference between plants from the Madeiran and Canarian archipelagos. This result was also reflected when Mahalanobis distances were calculated. Those groups sampled on the oceanic islands (GUA_E, GUA_W and CER) showed the minimum phenotypic distance (minimum Mahalanobis distance = 1.50; maximum Mahalanobis distance = 3.95, Table 2). The most similar mainland subspecies to the island populations was *europaea*, with a minimum distance of 5.60 to eastern populations of *guanchica*.

GEOGRAPHICAL, MOLECULAR AND PHENOTYPIC DISTANCES

Mantel tests revealed that genetic distance based on either plastid DNA or AFLP variation among populations was significantly related to geographical distances (Table 3). In contrast, phenotypic distances were not significantly correlated with geographical

Table 2. Pairwise Mahalanobis distances between groups based on five phenotypic leaf traits

	CER	GUA_E	GUA_W	MAR	EUR
CER	0	–	–	–	–
GUA_E	3.95	0	–	–	–
GUA_W	1.69	1.50	0	–	–
MAR	9.41	13.03	15.58	0	–
EUR	9.47	5.60	9.41	8.43	0

Distances between insular groups are in bold type. CER, *cerasiformis*; EUR, *europaea*; GUA_E, *guanchica* (eastern islands); GUA_W, *guanchica* (western islands); MAR, *maroccana*.

distances ($P = 0.17$). When genetic and phenotypic distances were compared, plastid and phenotypic distances were not significantly correlated ($P = 0.22$), whereas nuclear distances were correlated marginally with phenotypic distances ($P = 0.03$, Table 3).

DISCUSSION

GEOGRAPHICAL STRUCTURE OF PLASTID DNA IN MACARONESIAN OLIVE TREES

Our analyses showed that plastid DNA variation is structured within the taxa of *O. europaea* endemic to the Macaronesian region. Structuring of haplotypic diversity in particular geographical areas, as supported by the present study, indicates a high differentiation among taxa (Schaal *et al.*, 1998). In the case of the Macaronesian *O. europaea* complex, both G_{ST} (0.55) and AMOVA ($\Phi_{CT} = 0.51$) indices revealed substantial differentiation among groups for plastid variation (Petit *et al.*, 2005) and comparison of N_{ST} and G_{ST} confirmed the geographical structuring of related haplotypes. As expected, the level of genetic differentiation for maternally inherited markers in the present study clearly exceeded that revealed by biparentally inherited markers in these populations ($F_{ST} = 0.24$; García-Verdugo *et al.*, 2009a) because of the lower effective population size associated with plastid DNA (Birky, Maruyama and Fuerst, 1983; Petit *et al.*, 2005).

Genetic structuring of plastid variation in angiosperms has typically been explained by the contemporary factors determining seed dispersal and the historical patterns of relationship among the groups studied (Schaal *et al.*, 1998; Avise, 2009). As inferred from our results, oceanic barriers and geographical distance have probably hindered seed dispersal between the subspecies of *O. europaea*. Lack of gene flow between *cerasiformis* and *guanchica* had been previously suggested (Hess *et al.* 2000; García-Verdugo *et al.*, 2009a) and was strongly supported in the present study. In contrast, the haplotype distribu-

Table 3. Correlations among plastid (PLAST), geographical (GEO), phenotypic (PHE) and nuclear (NUCL) distances of Macaronesian populations of *Olea*, as obtained from pairwise Mantel tests

	PLAST	GEO	PHEN	NUCL
PLAST	–	–	–	–
GEO	$R = 0.37$ $P < 0.01$	–	–	–
PHEN	$R = 0.14$ $P = 0.22$	$R = 0.12$ $P = 0.17$	–	–
NUCL	$R = 0.19$ $P = 0.10$	$R = 0.76$ $P < 0.001$	$R = 0.32$ $P = 0.03$	–

Significant correlations ($P < 0.05$) are shown in bold type.

tion found within the Canarian archipelago could be explained by recurrent seed dispersal between islands, given the smaller oceanic barriers at this scale. A single and private haplotype was detected in the eastern islands and, according to the haplotype network, this haplotype was closely related to mainland subspecies (*europaea* and *maroccana*). Genetic affinities between eastern Canarian populations and north-west Africa in *Olea* are in agreement with the botanical and ecological similarities between these two areas (Sunding, 1979) and reflect the fact that insularity itself does not represent a strong barrier to seed dispersal in certain plant groups. In addition, a close relationship between eastern Canarian and mainland populations is also suggested by the haplotype network, in which all the haplotypes detected in the hexaploid *maroccana* (north-west Africa) were positioned between those of tetraploid *cerasiformis* and the eastern diploid *guanchica*. This result suggests that historical patterns of hybridization between these taxa (allopolyploidization) may also explain the genetic affinities detected between mainland and insular populations (see also García-Verdugo *et al.*, 2009a).

PHENOTYPIC SIMILARITY IN OCEANIC ENVIRONMENTS

The analysis of phenotypic variation revealed a high similarity between the subspecies of *O. europaea* occurring on oceanic islands, despite the genetic differences between them, i.e. ploidy and both nuclear and plastid diversity (Besnard *et al.*, 2007; García-Verdugo *et al.*, 2009a, and current results). Similar environmental pressures, however, lead populations to exhibit a common phenotype, although they display different ploidy (Lumaret & Barrientos, 1990) or unrelated haplotypes (Foster *et al.*, 2007). Presumably, populations in Madeira and the Canary Islands occur in similar habitats (Médail *et al.*, 2001; Terral *et al.*, 2004), favouring the expression of a similar leaf phenotype.

Leaf size and SLA are functional traits driving plant acclimation to multiple environmental factors such as water availability, light exposure or pressures from herbivory (Bowen & van Vuren, 1997; Reich *et al.*, 1997; Ackerly *et al.*, 2002). In the present study,

phenotypic similarity between archipelagos for these two traits was associated with significant differentiation from the mainland taxa. Oceanic environments preserve plants from exposure to unfavourable conditions experienced in mainland areas, such as higher temperature oscillation, variation in relative humidity or pressure from ungulates (Bowen & van Vuren, 1997; Cronk, 1997). In addition, subtropical conditions in the Madeiran and Canarian archipelagos are also characterized by higher water availability and shorter dry periods than those experienced in mainland areas (Médail *et al.*, 2001; Terral *et al.*, 2004). Accordingly, higher leaf size and SLA in oceanic taxa probably reflect higher site productivity and the occurrence of fewer stress factors.

The present study cannot unambiguously identify the evolutionary mechanisms responsible for the observed pattern in leaf functional traits. Levels of genetic variation found in the plastid genome and those reported with fingerprinting markers (Lumaret *et al.*, 2004; García-Verdugo *et al.*, 2009a) rule out genetic drift as a plausible explanation. Thus, selection and/or environmentally induced (phenotypic plasticity) mechanisms might be involved in such phenotypic expression. A recent study, however, showed that four of six *guanchica* populations displayed a similar leaf area under greenhouse and field conditions (García-Verdugo *et al.*, 2009b), suggesting a genetic basis for a larger leaf size and an apparently moderate implication of phenotypic plasticity. Most functional traits are thought to be under selection (Geber & Griffen, 2003) and hence parallel patterns of selection in similar environments might be responsible for phenotypic similarity between insular taxa (see also Brochmann *et al.*, 1995; Foster *et al.*, 2007).

LINEAGE DIFFERENTIATION IN *OLEA*: BIOGEOGRAPHICAL ASPECTS

The observed pattern of genetic and phenotypic differentiation among the Macaronesian subspecies may have been promoted by Tertiary climatic events. The split of the lineages considered here from the lineage formed by subsp. *cuspidata* (Wall. ex G. Don) Cif.

(primarily distributed in subtropical areas of Africa) appears to have occurred approximately 6 Myr ago, coinciding with the aridification of African midlatitudes (Besnard *et al.*, 2009). Most of the Macaronesian islands had already emerged at that period, offering populations of *Olea* an oceanic climate buffered against long-term climatic extremes and close to the original subtropical environments experienced by the ancestors of *O. europaea* (Terral *et al.*, 2004). Insular isolation and increased aridification on the mainland probably enhanced fragmentation and restricted gene flow among *Olea* populations (Besnard *et al.*, 2007). Thus, lack of genetic connectivity and contrasting climatic conditions led populations to diverge both genetically and phenotypically. Contemporary expression of functional traits such as leaf size, SLA (analysed in the present study), leaf scales (Besnard *et al.*, 2009) and vessel conductivity (Terral *et al.*, 2004) support the hypothesis that mainland subspecies adapted progressively to environmental conditions less favourable than those experienced by insular taxa. Island plants, in turn, acquired leaf phenotypes more appropriate to subtropical conditions, probably resembling those expressed by *Olea* ancestors in their original subtropical habitats. Nevertheless, phenotypic differentiation across geographical areas is typically interpreted in terms of adaptation (Brochmann *et al.*, 1995; Jorgensen *et al.*, 2006), but differentiation may also be the result of maladaptive patterns. Restricted movement of genes would prevent the expression of adequate phenotypic solutions (reviewed in Dlugosch & Parker, 2008), resulting in phenotypic differentiation among populations. In the case of *Olea*, however, a maladaptive scenario appears less likely than an adaptive scenario: restricted gene flow is supported among subspecies, but appears to be substantial within geographical areas (García-Verdugo *et al.*, 2009a), and the pattern of phenotypic differentiation is congruent with an adaptive response to the environmental conditions of each area, as discussed above.

In summary, our results suggest that strong isolation in *Olea* led to genetic structuring of populations. Lineage differentiation as inferred from plastid variation allows the study of phylogeographical patterns, but appears to be a poor predictor of phenotypic differentiation (see also Percy *et al.*, 2008). Thus, phenotypic similarity between genetically differentiated taxa can be achieved when they experience similar environmental conditions. This pattern of parallel evolution in *Olea* is contrary to that described in most of the Macaronesian lineages previously studied, in which high phenotypic diversification is often related to moderate to low levels of genetic differentiation in neutral markers (e.g. Carine *et al.*, 2004 and references therein). Further studies should be conducted in a larger number of Macaronesian lineages showing

low diversification and widespread distributions (e.g. *Periploca* L., *Rubia* L., *Pistacia* L., *Juniperus* L.) for a non-biased understanding of the pattern of phenotypic and molecular evolution in oceanic biotas.

ACKNOWLEDGEMENTS

We thank R. Domínguez, M. I. Blanch, C. Granado and R. Rubio for field assistance, and E. Cano for laboratory assistance. We sincerely thank G. Besnard for DNA samples of *O. europaea* and for valuable information for detecting plastid polymorphisms. Two anonymous reviewers provided insightful comments for data interpretation and discussion. E. Simó (Parque Rural de Teno) and A. Fernández (Parque Nacional de Garajonay) provided useful information and facilities for population sampling. This work was financially supported by the Spanish Ministerio de Educación y Ciencia (project CGL2005-03062/BOS: 'Procesos y mecanismos evolutivos en *Olea europaea* L.'), by the Comunidad de Madrid (project REMEDINAL, S-0505/AMB/0335) and a FPU grant to CG-V (AP-2004-3448).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Populations sampled in this study and sample sizes for genetic (pAnalysis) and phenotypic (pheAnalysis) data. The haplotypes found in each population are also detailed.

Table S2. Primer sequences and PCR conditions for amplification of plastid regions *psbE-petL* and *rps16-trnQ*. PCR conditions: initial denaturation at 94 °C for 5 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 30 min followed by storage at 4 °C.

Table S3. List of haplotypes.

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