

How repeatable is microevolution on islands? Patterns of dispersal and colonization-related plant traits in a phylogeographical context

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- **Background and Aims** Archipelagos provide a valuable framework for investigating phenotypic evolution under different levels of geographical isolation. Here, we analysed two co-distributed, widespread plant lineages to examine if incipient island differentiation follows parallel patterns of variation in traits related to dispersal and colonization.
- **Methods** Twenty-one populations of two anemochorous Canarian endemics, *Kleinia neriifolia* and *Periploca laevigata*, were sampled to represent mainland congeners and two contrasting exposures across all the main islands. Leaf size, seed size and dispersability (estimated as diaspore terminal velocity) were characterized in each population. For comparison, dispersability was also measured in four additional anemochorous island species. Plastid DNA data were used to infer genetic structure and to reconstruct the phylogeographical pattern of our focal species.
- **Key Results** In both lineages, mainland–island phenotypic divergence probably started within a similar time frame (i.e. Plio-Pleistocene). Island colonization implied parallel increases in leaf size and dispersability, but seed size showed opposite patterns of variation between *Kleinia* and *Periploca* species pairs. Furthermore, dispersability in our focal species was low when compared with other island plants, mostly due to large diaspore sizes. At the archipelago scale, island exposure explained a significant variation in leaf size across islands, but not in dispersability or seed size. Combined analyses of genetic and phenotypic data revealed two consistent patterns: (1) extensive within-island but very limited among-island dispersal, and (2) recurrent phenotypic differentiation between older (central) and younger (peripheral) island populations.
- **Conclusions** Leaf size follows a more predictable pattern than dispersability, which is affected by stochastic shifts in seed size. Increased dispersability is associated with high population connectivity at the island scale, but does not preclude allopatric divergence among islands. In sum, phenotypic convergent patterns between species suggest a major role of selection, but deviating traits also indicate the potential contribution of random processes, particularly on peripheral islands.

Key words: Allopatric differentiation, Canary Islands, genetic drift, island phylogeography, *Kleinia neriifolia*, leaf size, *Periploca laevigata*, seed size, wind-dispersal traits.

INTRODUCTION

Island systems have provided an exceptional arena for testing hypotheses on the spatial and temporal patterns that shape biodiversity (Whittaker and Fernández-Palacios, 2007; Gillespie and Clague, 2009). Studies on phenotypic evolution of island plants have traditionally focused on lineages representing examples of rampant speciation (e.g. Santiago and Kim, 2009; Scoffoni *et al.*, 2015). Island biotas, however, also contain a significant proportion of lineages composed of single endemic species with no apparent taxonomic diversification (Stuessy *et al.*, 2006). Among these, widespread island species are clearly exposed to strong evolutionary forces, due to the varying levels of geographical isolation and ecological gradients inherent in multi-island distributions (Gillespie and Clague, 2009; Irwin, 2012). Indeed, genetic studies have shown that

widespread island distributions are generally associated with regional phylogeographical structure (García-Verdugo *et al.*, 2013a; Spurgin *et al.*, 2014; Betzin *et al.*, 2016). Widespread monotypic endemics could therefore represent valuable study cases for investigating the relationship between phenotypic traits and incipient lineage differentiation (Clegg *et al.*, 2002; Spurgin *et al.*, 2014), with comparative phylogeography providing the spatio-temporal background for hypothesis-testing (Papadopoulou and Knowles, 2016).

Island–mainland comparisons have generated well-supported predictions on the phenotypic evolution of traits associated with dispersal to, and subsequent colonization of, island habitats (e.g. Burns *et al.*, 2012; Patiño *et al.*, 2013). In the case of dispersal-related traits, islands impose strong selective barriers for terrestrial plants, and expectations for evolutionary trends in these traits have been typically formulated in the

context of dispersal costs (e.g. [Cody and McOverton, 1996](#)). Thus, the classic view of the evolution of dispersability posits that recently established anemochorous island populations are expected to display higher dispersal potential than their mainland counterparts, but, within a few generations, a substantial reduction in this trait should occur due to the negative selective pressure exerted by mortality at sea ([Carlquist, 1974](#); [Cody and McOverton, 1996](#); [Knobe et al., 2012](#)). In recent years, however, the 'loss of dispersal on islands' hypothesis has been critically evaluated in plants (for discussions see [García-Verdugo et al., 2017](#); [Burns, 2018](#)). Some studies thus suggest that dispersability of anemochorous species with high colonization abilities is not selected against on islands, provided that habitat availability is not a limiting factor ([Gravuer et al., 2003](#); [García-Verdugo et al., 2017](#)). Other studies have documented phylogenetic relationships that are fully compatible with events of dispersal between distant archipelagos ([Whittaker and Fernández-Palacios, 2007](#)), which is evidence that some island plant groups have not been dramatically impacted by reduced dispersability. In addition, dispersability may evolve due to factors more related to post-dispersal competitive advantage than to dispersal distance itself, e.g. as a by-product of selection for larger seed sizes ([Greene and Johnson, 1993](#); [Burns, 2018](#)). Because diaspores are composed of morphological structures involved in different ecological processes, evolution of plant dispersal on islands could be shaped by different environmental pressures, yet we have limited data to identify the mechanisms behind the mixed phenotypic patterns documented to date ([Burns, 2018](#)).

Nevertheless, not all the phenotypic novelties displayed by island endemics are the result of selection, and random processes associated with colonization have been highlighted as relevant drivers of insular phenotypic evolution ([Rundell and Price, 2009](#)). In highly fragmented systems such as archipelagos, we have evidence that drift alone may strongly determine the phenotypic make-up of isolated island populations ([Jordan and Snell, 2008](#)). Thus, under a scenario of restricted gene flow, older island populations may show phenotypic deviations hardly attributable to adaptive patterns ([Jordan and Snell, 2008](#); [Spurgin et al., 2014](#)). In contrast, if selection plays a predominant role in the evolution of phenotypic traits, one would expect recurrent patterns of phenotypic variation across taxa ([Clegg et al., 2002](#); [Santiago and Kim, 2009](#)). In line with this prediction, island studies have reported convergent evolutionary responses across archipelagos and colonizing lineages in some relevant plant traits, including woodiness ([Carlquist, 1974](#); [García-Verdugo et al., 2014](#)), investment in mechanical and chemical defences ([Bowen and Van Buren, 1997](#)), and seed and leaf size ([Burns et al., 2012](#); [Kavanagh and Burns, 2014](#)). Among these, evolution of leaf size represents a key aspect in the process of colonization, as this trait is tightly related to plant acclimation to environmental variation ([Carins Murphy et al., 2014](#); [Wright et al., 2017](#)), and may thus be crucial for population establishment following dispersal to novel island habitats ([Santiago and Kim, 2009](#); [García-Verdugo et al., 2013b](#)).

In this study, we extended the patterns documented at the island–mainland scale to test the hypothesis that selective pressures promote similar patterns of phenotypic differentiation in co-occurring lineages at the archipelago scale ([Baldwin, 2007](#); [Mahler et al., 2013](#)). Hence, we predicted that multi-island

distributions may result in convergent patterns of phenotypic differentiation, assuming that selective forces have a predominant role in the evolution of plant traits associated with colonization. To address our research questions, we focused on two island species that (1) have well-established sister species in adjacent mainland areas, (2) do not show clear patterns of taxonomic diversification, and (3) share a number of biological features, such as dispersal mode and largely overlapping island distributions, meaning that they may have experienced similar environmental pressures. By combining a comparative phylogeographical approach with extensive sampling of phenotypic data, we aimed at gaining some insight into the factors affecting the process of incipient lineage differentiation. Specifically, we addressed some questions related to phenotypic differentiation at two (i.e. island–mainland and within-archipelago) hierarchical spatial scales, with a focus on dispersal-related traits: (1) Are patterns of variation between island and mainland congeneric species consistent between lineages? (2) How do population age and gene flow affect phenotypic patterns across islands? (3) Does seed size promote similar patterns of dispersability at both geographical scales?

MATERIALS AND METHODS

Study species and traits

Kleinia neriifolia (Asteraceae) and *Periploca laevigata* (Apocynaceae) are two common shrubby species of the semi-arid, open habitats of all the main Canarian islands. Both plants occupy a nearly continuous distribution belt around each island, although their populations are much more abundant on the central and western islands compared with those on the easternmost ones ([Bramwell and Bramwell, 2001](#)) ([Supplementary Data Fig. S1](#)). Populations of these two taxa also occur on the north-facing areas of each island, where exposure to North Atlantic trade winds is often associated with more mesic conditions than those found on the drier, southern areas ([del Arco et al., 2002](#)). In addition to similar habitat preferences and island distribution, both species produce diaspores with morphological traits typically related to wind dispersal: while *K. neriifolia* achenes are attached to a plumose pappus, seeds of *P. laevigata* are comose ([Figure 1](#)). Previous population-level studies have reported significant levels of population structure within these island taxa ([García-Verdugo et al., 2015, 2017](#); [Sun and Vargas-Mendoza, 2017](#)), which is suggestive of ongoing processes of within-species differentiation. *Kleinia anteuphorbium* and *Periploca angustifolia* are the sister mainland species of the two island taxa, and their populations are abundant in (and restricted to, in the former case) south-west Morocco ([Halliday, 1986](#); [Venter, 1997](#)).

In our study, characterization of *Kleinia* and *Periploca* populations was focused on three plant traits (leaf size, seed size and dispersability) that allowed us to test predictions about phenotypic patterns associated with island colonization. Variation in leaf size was expected to follow the general pattern of increase from mainland to island areas ([Bowen and Van Buren, 1997](#); [Burns et al., 2012](#)), and, at the archipelago scale, populations were expected to attain larger leaf sizes under more mesic conditions (i.e. on western islands and at northern-island exposures;

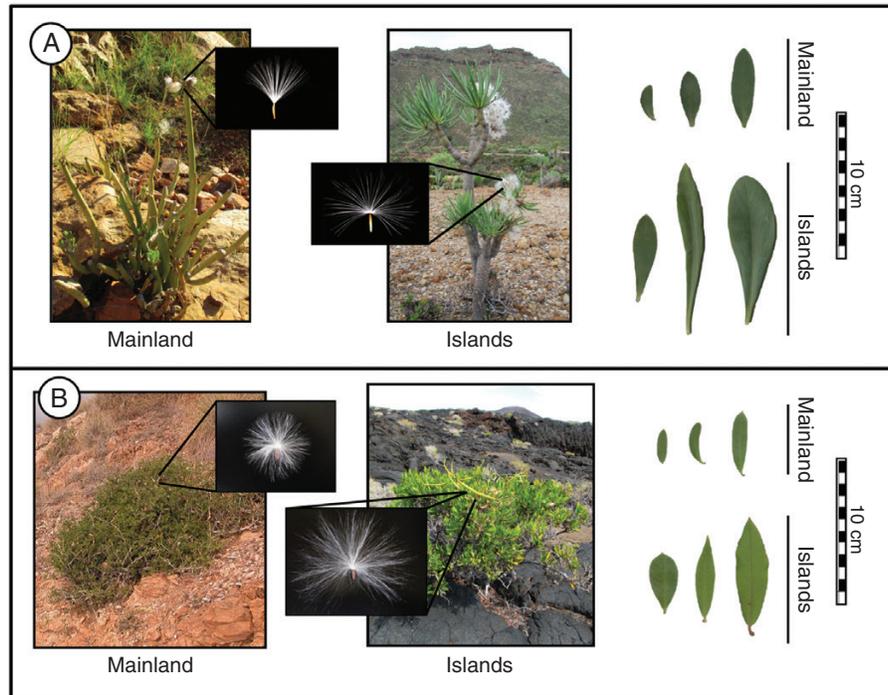


FIG. 1. Overall plant appearance, representative diaspores and range of leaf size variation observed under field conditions of the mainland–island species pairs *Kleinia anteuphorbium*–*K. neriifolia* (A) and *Periploca angustifolia*–*P. laevigata* (B) analysed in this study. Photograph credits: *P. angustifolia*, J. A. López Espinosa; others, C. García-Verdugo.

Humphries, 1976; Hamann, 1979; García-Verdugo, 2014). Predictions for patterns of dispersal ability were, however, more challenging due to the limited information available to date (Burns, 2018). Based on the large extension of island habitats occupied by both species (Supplementary Data Fig. S1), we expected *Kleinia* to follow the pattern previously described for *Periploca* (García-Verdugo et al., 2017), i.e. island populations produce more dispersible diaspores than mainland congeneric populations. However, dispersal potential may be constrained by factors such as seed size (Kavanagh and Burns, 2014; Burns, 2018) or colonization age (Cody and McOverton, 1996; García-Verdugo et al., 2017). The implication of seed size in the patterns of dispersability was assessed at two levels: first, we compared estimates of dispersability in our focal species with those obtained for other Canarian island anemochorous species (across-species comparison); second, seed size was studied across *Kleinia* and *Periploca* populations (within-lineage comparison). In addition, we generated a comparative phylogeographical framework to analyse how trait variation in our focal species relates to population genetic structure and colonization age (old versus recently established populations).

Population sampling

Twenty-one populations of each lineage were sampled, including 18 island plus three mainland *Kleinia* populations, and 17 island plus four mainland *Periploca* populations (Supplementary Data Table S1). Whenever possible, the sampling locations were chosen to represent co-occurring populations of both species (17 cases). For island taxa, population

sampling was stratified by exposure, in order to represent the contrasting environmental conditions of ‘north-exposure’ and ‘south-exposure’ habitats (Hamann, 1979; García-Verdugo et al., 2010). One or two populations per contrasting exposure, depending on island size, were considered in order to analyse phenotypic variation at the island level.

In total, 472 and 333 individuals of *Kleinia* and *Periploca*, respectively, were characterized for leaf size. From May to July 2016, 15–25 adult plants were randomly sampled in each island population, but individuals were separated by a minimum distance of 20 m from each other. Ten fully expanded leaves were collected from the third internode of different twigs of each plant: two or three of these were used for leaf size measurements, and the remaining leaves were either kept in silica gel (*Periploca*) or transported to the laboratory (*Kleinia*) for DNA extraction (see below). Seed data for *Periploca* populations were obtained from García-Verdugo et al. (2017). Fruits of *Kleinia* island populations were sampled later in the year (September–November), as the peak of fruit production for this species is concentrated within that period. Between ten and 20 capitula were collected per individual and kept in paper bags until morphological characterization. Samples from mainland *Kleinia* and *Periploca* populations were obtained in field collections conducted in 2012 and 2014, following the same methodology previously described. A total of 1073 fruits from 360 *Kleinia* individuals and 1736 seeds from 438 *Periploca* individuals were considered for analyses of dispersability. Due to its succulence and rich composition in secondary metabolites, attempts to preserve *Kleinia* specimens in good condition for herbarium vouchers were unsuccessful during our field trips. Voucher individuals were therefore not collected, but photographs from a

minimum of two representative individuals of each population were taken for identification purposes (e.g. [Supplementary Data Fig. S2](#)). Voucher individuals for *Periploca* populations can be found in previous studies ([García-Verdugo et al., 2015, 2017](#)).

Four anemochorous species representative of contrasting island distribution ranges were additionally sampled for characterization of dispersability in a broader taxonomic context: two endemics ascribed to highly diversified lineages (*Pericallis webbii* and *Sonchus acaulis*), and two native, non-endemic species that occur on all of the main islands (*Launaea arborescens* and *Phagnalon saxatile*). Each of these species develops a plumose pappus attached to the fruit that allows dispersal by wind, although fruit morphology and size are markedly different among species ([Supplementary Data Table S2](#)). All four species occur in the same habitats as those occupied by the two focal species, but sampling of both endemic and non-endemic species was limited to three populations per species. Between ten and 30 fruits from 18–21 individuals of each population were sampled and kept in paper bags for subsequent estimates of diaspore dispersability.

Phenotypic measurements

Leaf size was calculated as the area measured from scanned images of each leaf using ImageJ software ([Abramoff et al., 2004](#)). Dispersability in wind-dispersed plants is generally estimated by measuring diaspore terminal velocity (V_T); the smaller the value of V_T , the longer the diaspores are sustained in the air ([Sheldon and Burrows, 1973](#)). Terminal velocity has been shown to be a key trait for population spread and long-distance dispersal ([Heydel et al., 2014](#)), and thus it may play a crucial role in connecting fragmented distribution areas such as those displayed by widespread island plants. Estimates of dispersal ability for *Periploca* seeds were obtained from [García-Verdugo et al. \(2017\)](#). For *Kleinia*, three intact diaspores (fruits) with a well-developed pappus were taken from each individual for estimation of V_T . In order to generate V_T data for the total sample of 1073 diaspores, we followed the same approach as that previously adopted for *Periploca* and other anemochorous species ([García-Verdugo et al., 2017](#), and references therein) ([Supplementary Data Notes S1](#) and [Fig. S3](#)). Seed size was estimated using seed mass (*Periploca*) and achene mass (*Kleinia*) measurements.

To compare estimates of dispersability across island species and thus analyse the relationship of this trait with diaspore size and distribution range, two or three diaspores from each sampled individual of the extended set of anemochorous species were characterized for V_T . In addition, 20 diaspores randomly selected from each species were used for measurements of pappus size (i.e. length of the pappus bristles) with a stereo microscope (Olympus SZX12, Tokyo, Japan) and of seed (achene) mass with a precision balance (AB54 Mettler Toledo). Because individual achenes of *P. saxatile* and *P. webbii* were too light to be accurately measured, 20 groups of 20 achenes were weighed to infer mean and variance values of seed mass.

All the phenotypic measurements were averaged per individual, and a phenotypic data matrix was constructed, including codes for 'species', 'island' (seven levels corresponding to each Canarian island), 'exposure' (two levels: north versus

south) and 'population' as categorical variables. Differences between pairs of congeneric species in traits were analysed by ANOVA, with 'population' (random factor) nested in 'region' (fixed factor). Leaf size, dispersability and seed size values were log-transformed when needed to meet the ANOVA requirements of homoscedasticity. Components of variance were then calculated using a restricted maximum likelihood procedure to analyse the distribution of phenotypic variation at the archipelago scale (i.e. within populations, between exposures within islands and among islands). Differences in dispersability across the extended set of anemochorous species were analysed in a nested ANOVA design, using V_T values as a dependent factor and 'population' (random) nested in 'species' (random) as predictors. Tukey HSD *post hoc* tests were performed to identify statistically homogeneous groups of species based on dispersability (V_T) values.

Plastid DNA data

Leaves from five individuals from a minimum of two populations per island were sampled for genetic analyses ([Supplementary Data Table S1](#)). In the case of *Periploca*, genetic data were obtained from [García-Verdugo et al. \(2017\)](#), but two populations that were not considered in the previous study were newly analysed. For *Kleinia*, leaves were transported to the laboratory, cut into small pieces and left on a bench for 5–7 d. When the leaf pieces were completely dry, ~300 mg of leaf material from each individual was subjected to the washing pretreatment described in [Sun et al. \(2009\)](#), followed by the DNA extraction protocol detailed in [Dellaporta et al. \(1983\)](#). DNA extracts were then purified with commercial kits (UltraClean PCR Clean-Up, MoBio, Carlsbad, CA, USA).

Following [Shaw et al. \(2007\)](#), sequences from three non-coding plastid regions for each species (*trnS*^(GCU)–*trnG*^(UCC), *psbJ*–*petA* and *ndhF*–*rpl32* for *Periploca* and *rpl32*–*trnL*^(UAG), *trnV*^(UAC)–*ndhC* and *rps16*–*trnK*^(UUU) for *Kleinia*) were compiled for the total sample size ($N = 95$, *Periploca*; $N = 91$, *Kleinia*). The selection of these regions was based on the levels of polymorphism detected in a previous screening of six plastid regions commonly used in population-level studies ([Shaw et al., 2007](#)). Data from the three plastid regions were concatenated using DnaSP v5 ([Librado and Rozas, 2009](#)). Newly generated sequences were deposited in GenBank (accession numbers MH660938 to MH661210).

Phylogeographical framework for analyses of trait variation

Genetic data were used to analyse how genetic structure and population age may impact the expression of phenotypic traits. To test for an effect of isolation by distance (IBD) across the archipelago, genetic and log-transformed geographical distances between pairs of populations were analysed for each species in a Mantel test using GenAlEx v.6 ([Peakall and Smouse, 2006](#)). Matrices of genetic distances were computed with DnaSP v5 as the average number of nucleotide substitutions per site between populations ([Nei, 1987](#)). The level of significance in Mantel tests was tested with 999 permutations.

In addition, we computed levels of population differentiation (G_{ST}) at the island level using DnaSP v5. For this analysis, we expected to observe a correlation between dispersability and population genetic structure. If dispersability varied significantly across or within islands, we would expect contrasting levels of genetic structure at the island level across the archipelago (i.e. diaspora dispersability accounts for population structure; Gibson, 2001). The association between mean dispersability values at the island level and G_{ST} estimates was tested by means of Pearson r correlations.

Lastly, to test for the effect of population age on trait variation, we generated a phylogeographical framework for each species. In this case, we would expect more recently established island populations to display significant phenotypic differences when compared with older populations either due to the predominant effect of random processes in young areas of colonization (Jordan and Snell, 2008) or selection against dispersability in older areas (Cody and McOverton, 1996). Because inference of divergence times at microevolutionary scales typically implies broad ranges of uncertainty, we adopted a conservative approach by focusing on two contrasting levels of inferred age classes: old (ancestral) populations and more recently established populations.

For *Periploca*, a broad-scale study on Macaronesian lineages identified the populations on the island of Gran Canaria as the oldest within the Canarian archipelago, whereas those on La Palma (at the western limit) and Lanzarote and Fuerteventura (at the eastern limit) were inferred as the youngest (García-Verdugo et al., 2017). A recent paper focusing on the population genetics of *Kleinia* island populations (Sun and Vargas-Mendoza, 2017) tested different scenarios of inter-island colonization applying a Bayesian approach with nuclear microsatellite data. Although the results varied substantially depending on the model selected, the authors chose as the most likely scenario a pattern of bidirectional colonization of westernmost and easternmost islands from Gran Canaria.

To provide an alternative phylogeographical approach in *Kleinia*, we used the information of uniparentally inherited markers (plastid DNA) generated in our study to obtain inferences on population age. Similarly, plastid sequence data in *Periploca* were reanalysed to focus on the phylogeographical patterns of the Canarian archipelago. We followed the same approach previously adopted across Macaronesian *Periploca* lineages (García-Verdugo et al., 2017). Briefly, we combined Bayesian dating analyses (Drummond et al., 2012) and inferences on phylogeographical patterns (Lemey et al., 2009) to obtain information on population ages and dispersal patterns within the archipelago (Supplementary Data Notes S2). For *Kleinia*, secondary age constraints were obtained from the most comprehensive phylogenetic reconstruction of the genus available to date (Pokorny et al., 2015).

Contrast analysis was used to test the statistical significance of predictions of trait variation based on phylogeographical inferences. Specifically, we used the phenotypic data matrix to investigate via ANOVA and subsequent contrast analyses if more recent island populations displayed significant differences compared with the oldest populations. The level of significance of each contrast was adjusted following a sequential Bonferroni correction (Rice, 1989).

RESULTS

Phenotypic variation between regions and across species

Populations of island taxa displayed mean leaf sizes approximately four (*Kleinia*) and three (*Periploca*) times larger than those of mainland species (Figs 1 and 2). Similarly, estimates of dispersability were higher in the island taxa than those obtained in their mainland congeners (i.e. smaller V_T values on islands), representing an ~15 % increase for both island species (Fig. 2). By contrast, seed size showed opposite patterns of variation between lineages: while island *Periploca* populations showed a 23 % mean increase in seed size with respect their mainland counterparts, this trait in *Kleinia* was 25 % smaller in island than in mainland populations (Fig. 2). Differences in trait values among populations within the Canary Islands were significant in all cases (Supplementary Data Table S3), which suggested further differentiation at the archipelago scale.

Although *Periploca* and *Kleinia* island populations showed higher estimates of dispersability than mainland congeners, both species displayed the smallest values when compared with other island species [nested ANOVA for factor ‘species’, $F_{5,39} = 10.27$, $P < 0.001$ (Supplementary Data Table S2)]. Thus, *Periploca* and *Kleinia* diaspora phenotypes represented the right tail of the pooled frequency distribution of V_T values observed across species, which was associated with larger diaspora sizes (i.e. low size of dispersal mechanism in relation to diaspora mass; Fig. 3, Supplementary Data Table S2).

Hierarchical levels of phenotypic variation in the island setting

Variance component analyses showed similar patterns of hierarchical variation between species at the archipelago scale. A substantial percentage of trait variation (~50–80 %) was found within populations, and the among-island component also accounted for a significant (10–45 %) proportion of phenotypic variance, particularly for dispersability and seed size in *Kleinia* (Fig. 2). The most remarkable difference was observed among traits for the factor ‘exposure within islands’, which was negligible for dispersability and seed size (<1 % in both *Kleinia* and *Periploca*) but explained ~25 % of leaf size variation in both species (Fig. 2).

Post hoc tests showed that ten out of 13 comparisons of leaf size variation between contrasting exposures of the same island were significant and followed the same pattern: populations at north exposures typically displayed larger sizes than those measured at southern island exposures (Fig. 4). The few cases of non-significant differences between exposures were restricted to peripheral islands: La Palma and Lanzarote in *Kleinia*, and El Hierro in *Periploca*, with leaf sizes deviating from the mean trait values of each species (Fig. 4).

Phylogeographical analyses and trait variation

The combination of polymorphism detected within three plastid regions revealed 19 haplotypes in *Kleinia*, whereas no new haplotypes ($N = 18$) were detected in *Periploca* after the analysis of two new populations (Supplementary Data

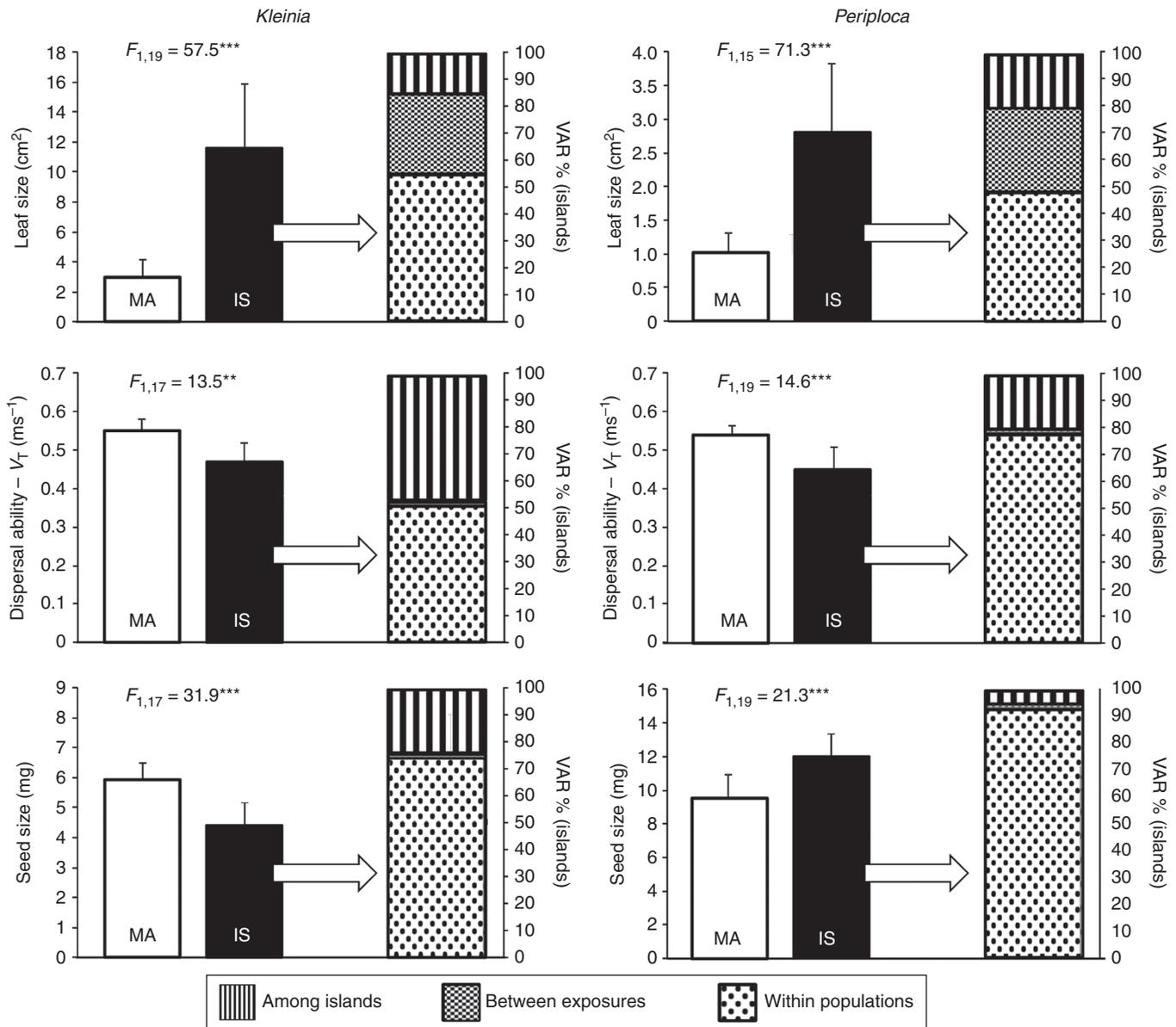


Fig. 2. Mean (+ s.d.) values of traits between mainland (MA) and island (IS) regions for the two study lineages, and partitioning of variance (VAR) in island traits across hierarchical scales (among islands, between exposures within islands and within populations). F -ratios and significances for island–mainland comparisons are shown above bars: ** $P < 0.01$; *** $P < 0.001$.

Tables S1 and S4). Haplotypes common to two or more neighbouring islands were rare, and the number of private haplotypes was higher on the central islands than on the other areas of the archipelago (Supplementary Data Fig. S4).

Mantel tests indicated a significant IBD pattern of differentiation across the archipelago, for both *Kleinia* ($r = 0.60$, $P = 0.001$) and *Periploca* ($r = 0.49$, $P = 0.01$) (Supplementary Data Fig. S5).

Dating analyses indicated that both *Kleinia* and *Periploca* island lineages diverged from mainland congeners within a similar time-frame, i.e. the Plio-Pleistocene [*Kleinia*, 3.26 Ma, 95 % highest posterior density (HPD) = 2.29–4.23; *Periploca*, 2.11 Ma, 95 % HPD = 0.10–3.37; Fig. 5, Supplementary Data Fig. S6]. In agreement with previous studies, the combination

of dating analyses and ancestral area reconstructions suggested similar patterns of inter-island dispersal for both species, with the two central islands identified as the oldest areas (Fig. 5); Gran Canaria for *Periploca* (divergence from sister island clades = 0.75 Ma, 95 % HPD = 0.03–1.71), and Gran Canaria and Tenerife for *Kleinia* (1.42 Ma, 95 % HPD = 0.91–1.92). In addition, the island populations inferred as the youngest occupied the peripheral areas of the island distribution of each species. At the western limit, the island of La Palma was inferred as the most recent colonization from El Hierro populations in *Periploca* (0.25 Ma, 95 % HPD = 0.003–0.61), whereas populations on El Hierro in *Kleinia* were in turn inferred as a relatively recent dispersal (0.20 Ma, 95 % HPD = 0.04–0.46) from the island of La Gomera (Fig. 5). For both species, easternmost islands were also inferred

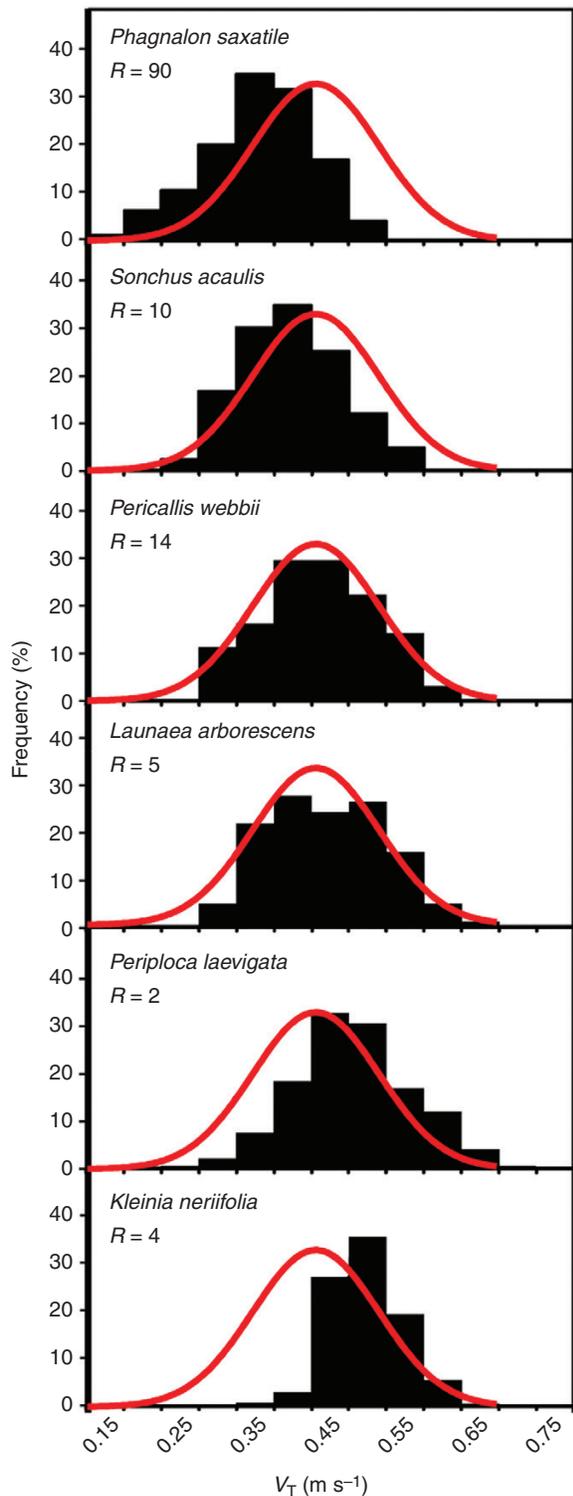


FIG. 3. Estimates of dispersability (inverse of V_T) in six island species displaying morphological traits related to anemochory. The red line represents the frequency distribution of dispersability for all species pooled. The ratio between size of the dispersal mechanism (pappus or coma) and diaspore mass (R , mm mg^{-1}) is indicated for each species.

as recently colonized areas despite their older geological ages, with Lanzarote and Fuerteventura being the result of at least one well-supported event of dispersal from Gran Canaria in *Kleinia*

(0.20 Ma, 95 % HPD = 0.05–0.48) and the only population of *Periploca* sampled on Fuerteventura resulting from a recent colonization from mainland populations (Fig. 5).

Thirteen out of 24 phenotypic contrasts between island age classes remained significant after the sequential Bonferroni correction (Table 1). In both species, leaf size tended to increase from eastern to western island populations rather than between age classes. Dispersability and seed size showed a more consistent pattern with regard to population age. Thus, in five out of eight tests, dispersability was higher in ancestral areas than the values observed across younger island populations (Table 1), with two remarkable cases of reduced dispersability compared with ancestral populations: El Hierro (–21 %) in *Kleinia* and Fuerteventura (–25 %) in *Periploca*. In turn, seed size showed no significant variation among *Periploca* populations, but in *Kleinia* Fuerteventura plants produced larger seeds than those of the central islands, while El Hierro populations showed significantly smaller seeds than the central islands (Table 1).

For both species, estimates of G_{ST} at the island level ranged from 0.14 (on large islands) to 0, indicating a very weak population structure within islands (Fig. 6). Correlations between mean dispersability and G_{ST} across islands were not significant in either case (*Kleinia*, $N = 7$, $r = 0.19$, $P = 0.69$; *Periploca*, $N = 6$, $r = 0.44$, $P = 0.38$).

DISCUSSION

Convergent patterns of trait variation between mainland–island species pairs

Our results suggest that two phylogenetically distant lineages follow similar patterns of phenotypic variation in two traits tightly related to dispersal and island colonization. Thus, we found that insular populations of *Kleinia* and *Periploca* displayed parallel increases in leaf size and diaspore dispersability when compared with their respective mainland sister species. Convergent phenotypic patterns between unrelated lineages support the idea of directional selection shaping these traits upon island colonization (Clegg *et al.*, 2002; Mahler *et al.*, 2013), and further suggest that the evolution of plant phenotypes under similar island conditions may be repeatable (Carlquist, 1974; Baldwin, 2007). However, our study also highlights that parallel variation between species in a complex trait (dispersability) can be associated with disparate patterns in some of its components (seed size) (see Talavera *et al.*, 2012, for similar results in other diaspore types).

The notion that leaf size increases under island conditions (Burns *et al.*, 2012; Cox and Burns, 2017) was reinforced by our data. In our study, island taxa displayed up to a 4-fold increase in leaf size with respect to mainland populations. Release from herbivore pressures, competitive advantages for light capture and climatic stability related to island habitats are some of the ecological factors that have been invoked to explain this recurrent pattern (Bowen and Van Buren, 1997; Burns *et al.*, 2012). Contrasting temperature regimes between Mediterranean-type habitats and neighbouring Macaronesian islands (e.g. García-Verdugo, 2014) probably represent a predominant factor driving phenotypic divergence in leaf size in our study system (Wright *et al.*, 2017). Nevertheless, the explanatory power of single ecological factors for this common observation is likely

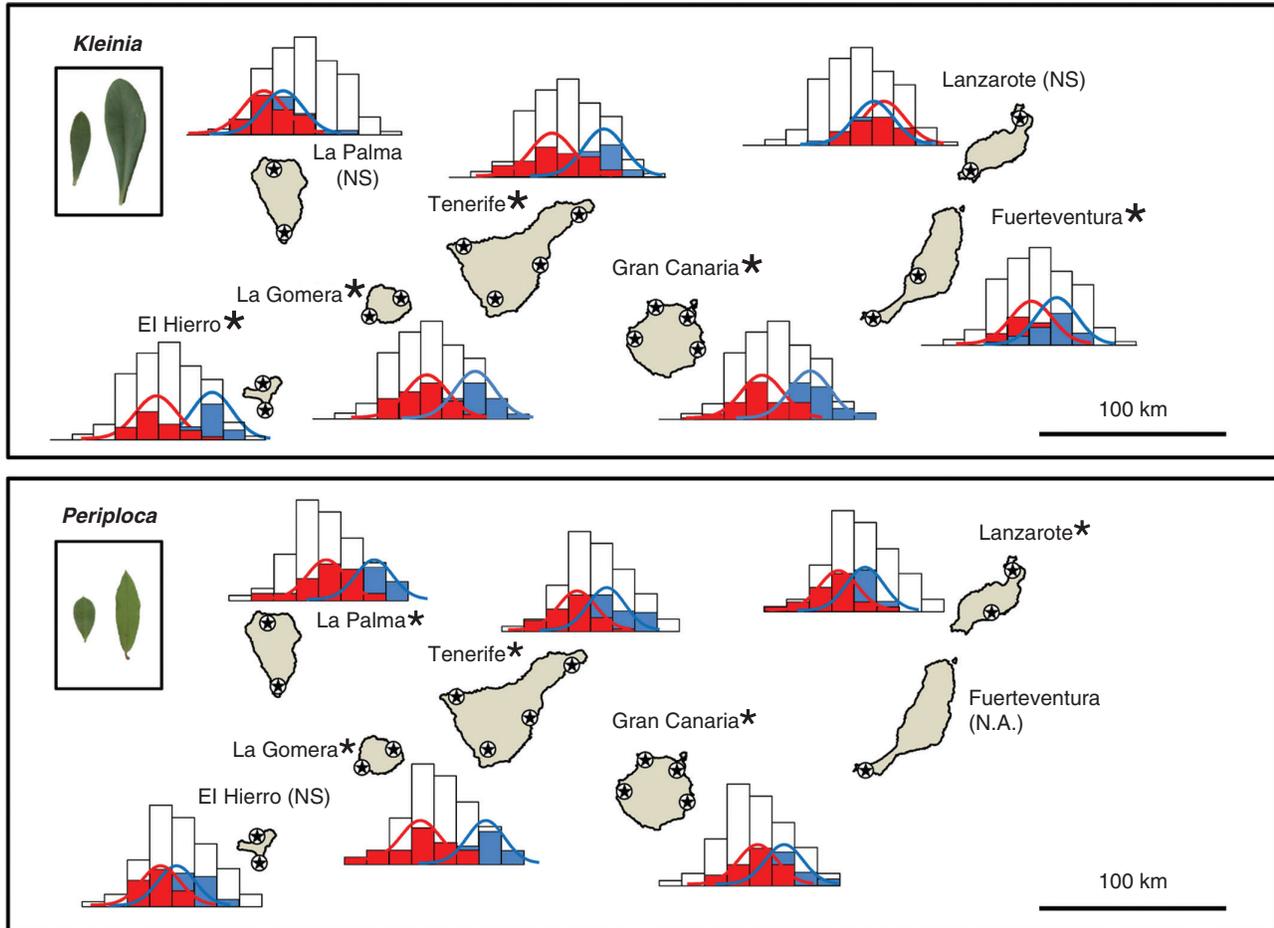


FIG. 4. Patterns of leaf size variation between populations having south exposure (red) and north exposure (blue) at the island level. Background histograms show log-transformed frequency values for the whole island dataset ($N = 1018$, *Kleinia*; $N = 1462$, *Periploca*), with larger leaf sizes represented towards the right tail. Stars indicate sampling populations, and asterisks next to island names identify significant differences between exposures according to *post hoc* tests. NS, non-significant difference.

idiosyncratic to each island system/species (Hochberg, 1980; Bowen and Van Buren, 1997; Burns *et al.*, 2012), given the peculiarities of island and source mainland habitats and the disparate phylogenetic histories of the insular floras worldwide.

Periploca populations also followed the general pattern of increased seed size documented on islands (Kavanagh and Burns, 2014), which is intrinsically linked to loss of dispersability in anemochorous diaspores (Burns, 2018). Contrary to this expectation, however, *Periploca* island diaspores exhibited a net increase in dispersability when compared with that of mainland populations, due to substantial investment in the dispersal mechanism (García-Verdugo *et al.*, 2017). In contrast, increased dispersability in island *Kleinia* populations was apparently explained by a smaller investment in seed size. There are, however, some limitations in the unambiguous interpretation of the geographical pattern of variation observed in *Kleinia* diaspore phenotypes. Unlike *Periploca*, the most common recent ancestor of the *Kleinia* sister species considered in our study remains unknown (Pelsner *et al.*, 2007). Hence, if the ancestral character state of the *Kleinia* species pair in e.g. seed size was similar to that observed under island conditions, island–mainland phenotypic divergence could be

also attributable to strong directional selection in the mainland region (e.g. larger seeds selected in more xeric environments; Hallett *et al.*, 2011) and stabilizing selection on the islands (Hansen and Martins, 1996). A broader phylogenetic approach is therefore needed to draw more accurate inferences on the selective regimes driving the evolution of *Kleinia* diaspore traits (García-Verdugo *et al.*, 2017).

Microevolutionary patterns among islands: linking phylogeography, plant traits and lineage differentiation

At the archipelago scale, our genetic analyses also revealed some similarities in dispersal patterns between species. Phylogeographical reconstructions highlighted the pivotal role of central islands, which served as sources of migrants to both westernmost (*Kleinia* and *Periploca*) and easternmost (*Kleinia*) islands. In addition, mainland areas were found to act as secondary areas of dispersal in *Periploca*, providing the easternmost islands with colonizers in recent times. These patterns exemplify the main scenarios of dynamic dispersal proposed for the Canarian archipelago (Sanmartín *et al.*, 2008; Caujapé-Castells

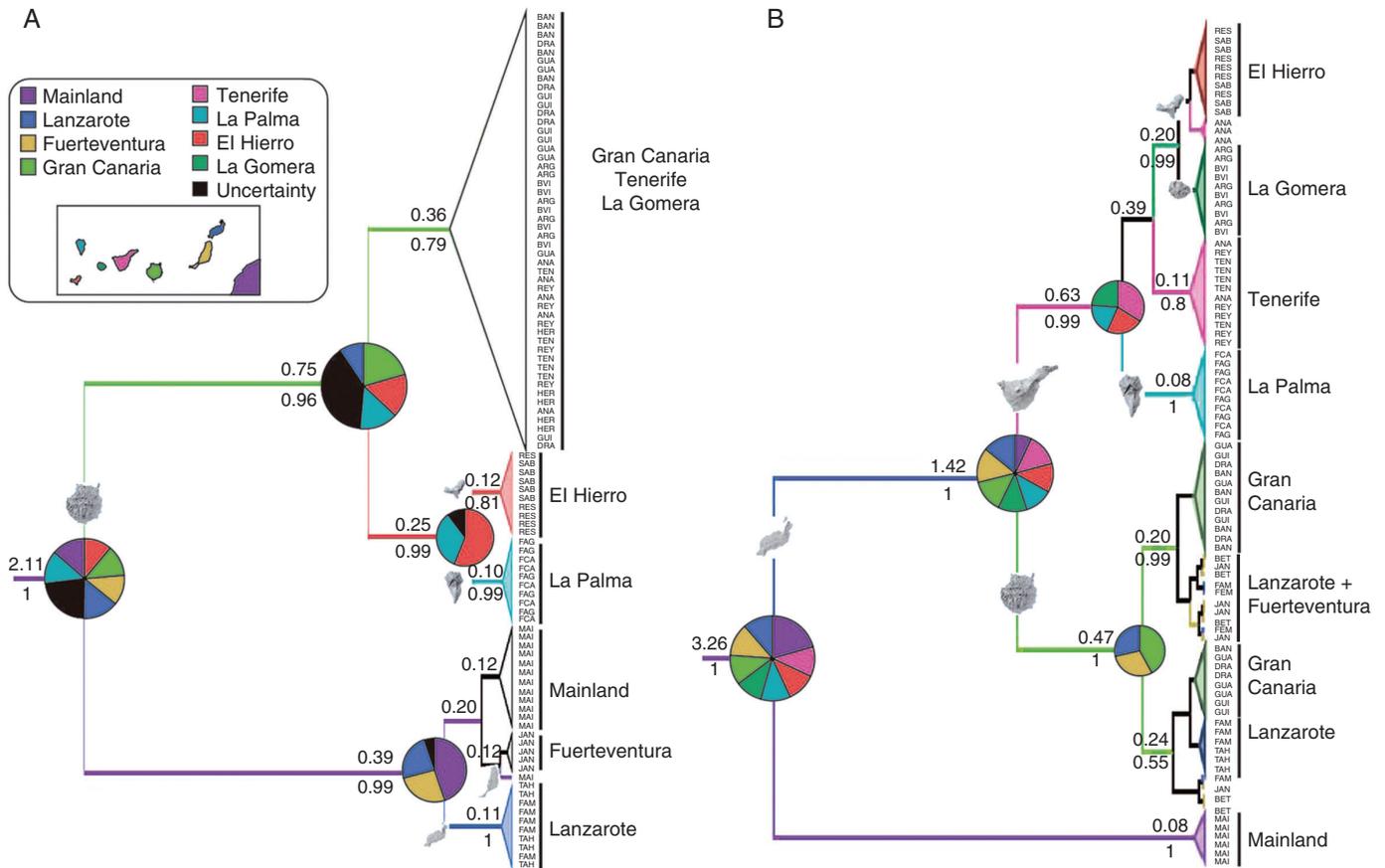


FIG. 5. Representation of Bayesian ancestral range reconstruction and divergence time analyses for *Periploca* (A) and *Kleinia* (B) lineages. Coloured branches represent the ancestral range with the highest posterior probability for each area (see key), whereas node pie charts show marginal probabilities for alternative ancestral ranges. Numbers above branches show mean ages and numbers below branches represent Bayesian posterior probabilities.

TABLE 1. Contrast analysis of phenotypic traits [leaf size, dispersability (V_T) and seed size] between islands inferred as ancestral (GC, Gran Canaria; TF, Tenerife) and more recently colonized (HI, El Hierro; LP, La Palma; LZ, Lanzarote; FT, Fuerteventura) following phylogeographical analyses. Mean island values are indicated in parentheses for each trait, and t-values and significance (* $P < 0.003$) following sequential Bonferroni correction are shown for each contrast

<i>Kleinia</i>				<i>Periploca</i>		
Leaf size (cm ²)	HI (13.5)	LZ (12.9)	FT (10.9)	Leaf size (cm ²)	LP (3.56)	FT (2.51)
GC (11.8)	2.57	2.13	-1.34	GC (3.05)	2.19	2.61*
TF (11.0)	4.32*	3.99*	-0.49			
V_T (m s ⁻¹)	HI (0.540)	LZ (0.467)	FT (0.492)	V_T (m s ⁻¹)	LP (0.431)	FT (0.529)
GC (0.447)	13.35*	3.38*	7.09*	GC (0.422)	2.25	7.16*
TF (0.491)	5.90*	-3.22*	0.01			
Seed size (mg)	HI (3.72)	LZ (4.33)	FT (5.13)	Seed size (mg)	LP (11.5)	FT (11.0)
GC (4.34)	4.29*	0.10	-5.80*	GC (11.6)	0.31	1.49
TF (4.49)	5.05*	1.10	-4.49*			

et al., 2017), and account for the underlying genetic model of differentiation observed across island populations.

A clear IBD pattern was detected across the widespread distribution of both study species but, as explained above, such a spatial pattern does not appear to be the consequence of an

east-to-west sequential colonization route (i.e. the ‘progression rule’; Shaw and Gillespie, 2016). Rather, it appears to be mainly the result of asymmetrical rates of migration from the oldest areas of distribution (i.e. central islands) to more recent ones (i.e. peripheral islands). Under an IBD model of differentiation,

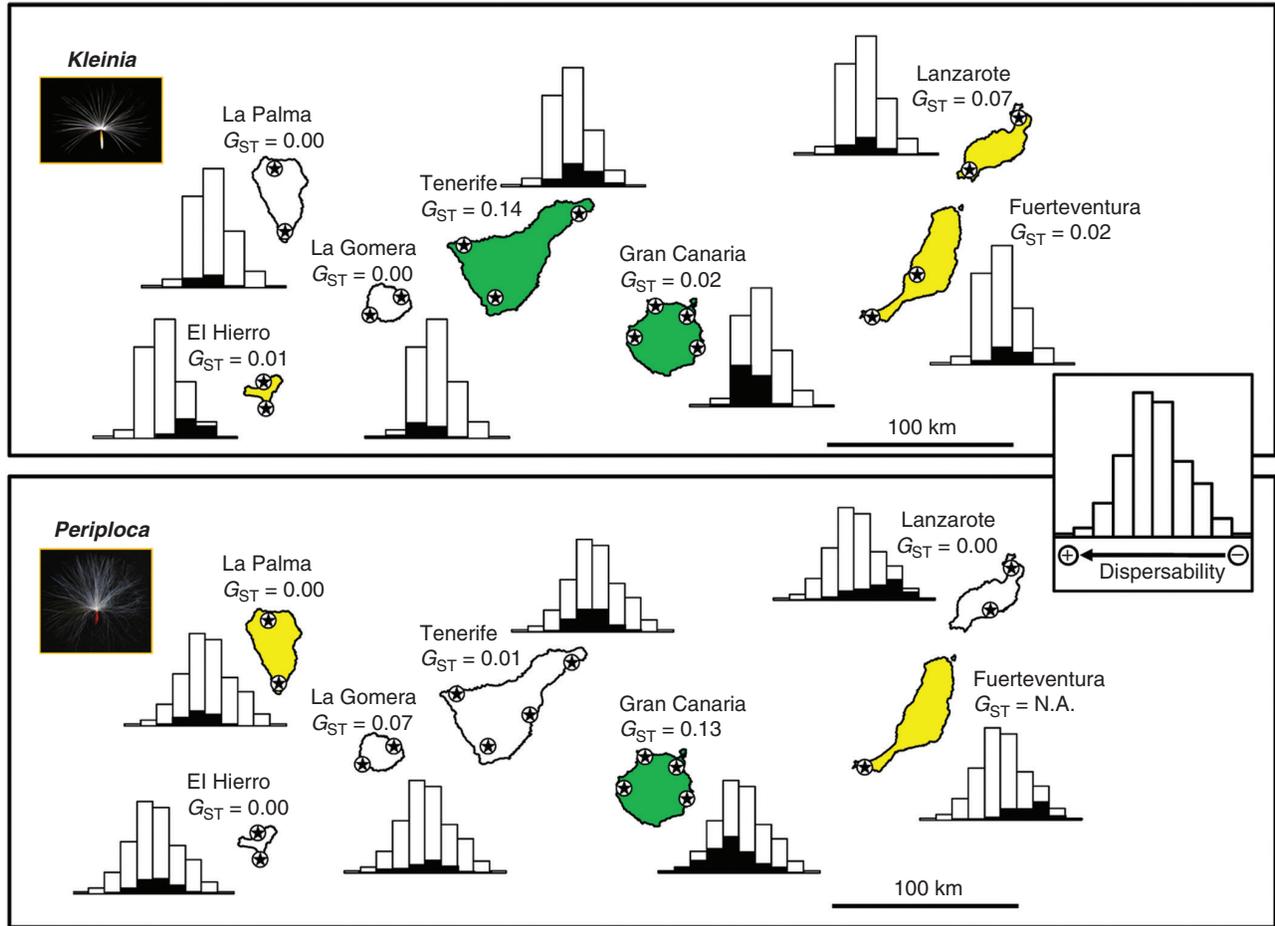


FIG. 6. Relationships between dispersability (frequency histograms), population structure (G_{ST}) and population age (represented by colours) at the island level. Following the results of phylogeographical reconstructions, ancestral areas are represented in green, whereas islands with more recently founded populations are coloured in yellow. Stars indicate sampling populations.

genetic drift is predicted to be pronounced in areas distant from source populations (Nistelberger *et al.*, 2015). In our study, the higher occurrence of fixed haplotypes in populations on peripheral islands, in addition to lower levels of heterozygosity than those reported for central islands (García-Verdugo *et al.*, 2015; Sun and Vargas-Mendoza, 2017), point towards genetic drift as a relevant evolutionary force on peripheral islands. Rare events of inter-island dispersal therefore allow successful colonization across the archipelago, but may also have set the stage for allopatric differentiation driven by random processes (Jordan and Snell, 2008).

Evidence of the contribution of random processes to lineage differentiation was mostly supported by the disparate patterns displayed between traits across co-occurring distributions. For instance, dispersability and seed size were shown to be affected by factors associated with inter-island colonization (the ‘among-island’ component, in Fig. 2) rather than by environmental variation related to contrasting island exposures. One remarkable example was provided by *Kleinia* populations on the peripheral island of El Hierro. Our analyses indicated an atypical case of loss of dispersability. This being the result of a relatively recent dispersal from a source area displaying

highly dispersive phenotypes (island of La Gomera; Fig. 6), we would expect the opposite pattern (i.e. high dispersability on a newly colonized island; Cody and McOverton, 1996). El Hierro is the smallest of the sampled islands, but limited habitat availability can hardly be conceived as a selective factor against dispersability, since the actual distribution of populations on this island extends over hundreds of square kilometres. Interestingly, loss of dispersal potential is not the consequence of selection for larger seeds, as appears to be the reason in the case of Fuerteventura (Table 1) (Burns, 2018). We hypothesize that factors related to stochastic colonization provide a more plausible explanation. For instance, as a likely consequence of recent island colonization by a limited number of colonists, *Kleinia* populations on El Hierro display significant levels of inbreeding (Sun and Vargas-Mendoza, 2017), a trait that has been linked to loss of dispersal potential in anemochorous plants (Mix *et al.*, 2006). Alternatively, lack or scarcity of efficient pollinators on this island may have selected for shifts in mating system (selfing) and reduced dispersal (Cheptou, 2012). While the ecological reasons behind loss of dispersability in these populations remain unclear, El Hierro offers an interesting framework for future research on this topic.

Variation in phenotypic traits related to colonization at the island scale

Contrary to the archipelago scale, estimates of genetic differentiation portray single islands as gene-flow-dominated systems. This finding strongly suggests that both diaspore dispersal and subsequent population establishment are frequent within islands, even across the bigger ones (i.e. Tenerife, Gran Canaria and Fuerteventura). Large diaspore sizes in our focal species (Fig. 3) probably contribute to high rates of colonization success (Moles and Westoby, 2006), but acclimation of leaf phenotypes to contrasting environments also reflects the high colonization abilities of these two species (Maron et al., 2004). Both *Kleinia* and *Periploca* showed consistent differences in leaf size between contrasting island exposures in a pattern that clearly parallels the adaptive scenario described in radiating lineages, where closely related taxa typically display leaf phenotypes that match contrasting island conditions (Humphries, 1976; Santiago and Kim, 2009; García-Verdugo et al., 2013b).

Another remarkable parallelism with radiating anemochorous lineages is the finding of limited inter-island dispersal and weak within-island genetic structure (Jones et al., 2014), even for species like *Pericallis*, for which we obtained high potential for wind dispersal (Fig. 3). This observation suggests that dispersability in anemochorous species does not promote genetic connectivity across wide geographical scales, and provides further support to the idea that stochastic events of inter-island dispersal could be mediated (or constrained) by factors other than wind, such as alternative dispersal vectors or habitat filtering (Carlquist, 1974; Stuessy et al., 2017).

Conclusions

The analysis of widespread island taxa within a comparative phylogeographical framework can provide valuable insights into the patterns and processes associated with early stages of lineage differentiation. While leaf size generally showed predictable patterns of variation across hierarchical geographical scales, spatial variation in dispersability followed a more stochastic pattern between species and islands. Our results question the effectiveness of wind dispersal in population connectivity at large (among-island) scales. The stochastic nature of dispersal, linked to other (i.e. establishment) island filters, probably represents the main factors enhancing population structure and, ultimately, triggering allopatric differentiation.

SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Figure S1: distribution maps of the island species. Figure S2: leaf size variation in *Kleinia* between contrasting island exposures. Figure S3: relationship between observed and predicted values of terminal velocity in *Kleinia*. Figure S4: distribution of haplotypes for both island species. Figure S5: graphical representation of Mantel tests. Figure S6: confidence intervals for divergence age estimates. Notes S1: expanded methods for estimation of dispersability. Notes S2: Expanded phylogeographical methods. Table S1: phenotypic and genetic data of each population.

Table S2: comparison of dispersal-related traits among anemochorous island species. Table S3: ANOVA results for the comparison of plant traits between island and mainland areas. Table S4: definition of haplotypes in *Kleinia*.

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