Geographical distance and barriers explain population genetic patterns in an endangered island perennial

Elisabete F. Dias1, M. Moura1, H. Schaefer2 and Luís Silva1

1 CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Pólo dos Açores, Universidade dos Açores, Rua da Mãe de Deus, Apartado 1422, Ponta Delgada, 9501-801 Açores, Portugal
2 Plant Biodiversity Research, Technische Universität München, 85354 Freising, Germany

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Abstract. Island plants are frequently used as model systems in evolutionary biology to understand factors that might explain genetic diversity and population differentiation levels. Theory suggests that island plants should have lower levels of genetic diversity than their continental relatives, but this hypothesis has been rejected in several recent studies. In the Azores, the population level genetic diversity is generally low. However, like in most island systems, there are high levels of genetic differentiation between different islands. The Azores lettuce, Lactuca watsoniana, is an endangered Asteraceae with small population sizes. Therefore, we expect to find a lower level of genetic diversity than in the other more common endemic Asteraceae. The intra- and interpopulation genetic structure and diversity of L. watsoniana was assessed using eight newly developed microsatellite markers. We included 135 individuals, from all 13 known populations in the study. Because our microsatellite results suggested that the species is tetraploid, we analysed the microsatellite data (i) in codominant format using PolySat (Principal Coordinate Analysis, PCoA) and SPAgedi (genetic diversity indexes) and (ii) in dominant format using Arlequin (AMOVA) and STRUCTURE (Bayesian genetic cluster analysis). A total of 129 alleles were found for all L. watsoniana populations. In contrast to our expectations, we found a high level of intrapopulation genetic diversity (total heterozygosity = 0.85; total multilocus average proportion of private alleles per population = 26.5 %, \( F_{IS} = -0.19 \)). Our results show the existence of five well-defined genetic groups, one for each of the three islands São Miguel, Terceira and Faial, plus two groups for the East and West side of Pico Island (\( F_{ST} = 0.45 \)). The study revealed the existence of high levels of genetic diversity, which should be interpreted taking into consideration the ploidy level of this rare taxon.

Keywords: Azores; conservation; endemic plants; isolation by distance; microsatellites; population genetics; tetraploid.

Introduction

Oceanic islands have been important study systems for ecologists, evolutionary and conservation biologists and are widely recognized as natural laboratories, for studying evolution due to their discrete geographical nature and diversity of species and habitats (Emerson 2002). Oceanic islands generally have lower overall species numbers per unit area (Whittaker and Fernández-Palacios 2007) but show higher percentages of endemism than mainland areas (Kier et al. 2009).
Geological, geographical and ecological conditions, such as island age and area, geographical isolation by oceanic barriers, climatic stability, environmental heterogeneity, high habitat diversity and the absence of competitors, have been pointed out as key determinants of the speciation patterns observed within volcanic archipelagos (Gillespie and Baldwin 2009; García-Verdugo et al. 2014; Moiral et al. 2015).

Biodiversity of remote islands has arisen through evolution and adaptation of the few initial colonists (Gillespie 2007), with high levels of endemism displaying unique genetic patterns in comparison with continental relatives (Crawford et al. 1987; Stuessy and Ono 1998; Emerson 2002; Whittaker and Fernandez-Palacios 2007, Bramwell and Caujapé-Castells 2011; Silva et al. 2015; Takayama et al. 2015). Differentiation of island populations is further enhanced by intrinsic traits, such as reduced dispersal capabilities, evolutionary innovations, size and reproductive changes (Price and Wagner 2004; Kaiser-Bunbury et al. 2010; Hutsemékers et al. 2011) which, in addition to ecological opportunity and chance, play a key role in successful island colonization, and often result in adaptive radiation (Gillespie 2007).

Several classical studies have been directed to estimate the genetic diversity of island endemic plants, including the Hawaiian silversword alliance (Baldwin 2009), the Juan Fernandez Islands groups (Crawford et al. 2001) and several Canary Island taxa (Francisco-Ortega et al. 2000; Caujapé-Castells et al. 2010). Recent studies from Macaronesia (Moura et al. 2013; García-Verdugo et al. 2015) rejected the hypothesis that island populations always have a low genetic diversity due to bottleneck effects, small population size and adaptation to specific ecological conditions (Bouzet 2010; López-Pujol et al. 2013; Meloni et al. 2015). Similar results had already been obtained in other parts of the world (e.g., Abies nebrodensis—Conte et al. 2004; Nothofagus australis—Torres-Díaz et al. 2007; Cedrus brevifolia—EIaides et al. 2011). These high levels of genetic diversity may have been the result of genetic drift (Dias et al. 2014) or might have resulted from changes in ploidy levels (Crawford et al. 2009, 2015).

In the Azores, the endemic plant species studied so far showed a considerable range of population genetic patterns: (i) high genetic diversity but low population differentiation—Picconia azorica (Martins et al. 2013); (ii) relatively low genetic diversity and low population differentiation—Prunus azorica (Moreira et al. 2013); and (iii) high genetic diversity (with the exception of very small populations) and high level of differentiation—Juniperus brevifolia (Silva et al. 2011). Some of this supports the hypothesis of a possible Linnean shortfall in the Azorean flora (Schaefer et al. 2011; Moura et al. 2015a), with previously overlooked diversity in several taxa (Leontodon, Moura et al. 2015b; Platanthera, Bateman et al. 2013; Viburnum, Moura et al. 2013).

Among the endemic Asteraceae, the genera Leontodon and Tolpis have already been studied and showed high population genetic diversity and a complex genetic structure, with clear geographical-linked patterns (Dias et al. 2014; Moura et al. 2015b; Silva et al. 2016).

The Azores lettuce (alfacinha), L. watsoniana, is a perennial herb, endemic to the Azores, today restricted to four of the nine islands of the archipelago (Faial, Pico, São Miguel and Terceira). It is probably extinct in São Jorge Island, from where two specimens exist in the Lisbon University herbarium (LISU), collected between the valleys of ‘Ribeira do Salto’ and ‘Ribeira de S. João’. Lactuca watsoniana is today restricted to the steep slopes of craters, ravines, and temperate juniper rain forest, between 600 and 800 m above sea level (Schaefer 2005; Silva et al. 2009; ED, pers. obs.).

Estimates for its total population size range from 500 to 2000 individuals (Schaefer 2005; Silva et al. 2009, 2011), but recent field observations indicate lower numbers (probably fewer than 500 individuals). Like many other endemic plants from oceanic islands (Francisco-Ortega et al. 2000; Caujapé-Castells et al. 2010) it is considered a priority species for conservation and was listed as endangered [EN B2ab(i,ii,iii); C2a(ii)] on the IUCN Red List 2013. Furthermore, it was included in Habitats Directive as a Priority species (Annex B-II) and also in Bern Convention (Appendix 1, Annex 1). The species is threatened by habitat loss and degradation resulting from changes in land use, namely expansion of pastureland, invasion by exotic species, introduced herbivores and disturbance of sensitive areas by tourists and locals (Silva et al. 2009). Lactuca watsoniana propagules are dispersed by wind and water (Schaefer 2003; Silva et al. 2009) but it is unknown, how efficient these strategies are under the specific conditions in the Azores archipelago with predominantly westerly winds and individual islands separated by distances of 6 km (Faial to Pico) to 600 km (Santa Maria to Corvo) of open ocean.

As consequence, the remaining often small and geographically isolated populations on the different islands are likely to have low genetic diversity and could suffer from inbreeding depression (Lowe et al. 2005).

Besides the scientific interest related to the study of evolutionary processes in islands and particularly in the Azores, where many scientific gaps still exist (Carine and Schaefer 2010; Schaefer et al. 2011; Moura et al. 2015b, c), practical issues are also involved when it comes to the conservation of endangered endemic plants. Recently,
Silva et al. (2015) proposed that population genetic studies should be included in more holistic approaches to research devoted to rare island plants, since different views exist about crucial aspects such as propagule provenance (Edmands 2007; Silva et al. 2011; Weeks et al. 2011; Dias et al. 2014; Silva et al. 2016).

In the context of a holistic view, and considering that a previous germination study was already conducted for *L. watsoniana*, we additionally performed a comprehensive population genetic study of *L. watsoniana* populations using newly developed microsatellite (Simple Sequence Repeats, SSR) markers. Based on the relative depauperation of the extant populations, we expected to find relatively low levels of genetic diversity.

Our objectives were (1) to determine the population genetic structure of *L. watsoniana*; (2) to estimate intra and inter-population genetic variation patterns; (3) to identify populations with low genetic variability and in-breeding; and (4) to verify the occurrence of other putative operational taxonomic units.

Beyond the conservation of *L. watsoniana*, we also aim to use the results of the present study to better understand the evolution of island plants in the Azores as well as in other relatively remote and geologically young island systems.

**Methods**

**Study site**

The Azores Archipelago includes nine volcanic islands, located in the NE of the Atlantic Ocean, between 36° and 43° N and 25° and 31° W, extending for more than 600 km and lying in a northwest–southeast direction. The closest mainland is the Iberian Peninsula, approximately 1363 km East, Newfoundland is 2272 km Northwest, Bermuda 3455 km southwest and Madeira 340 km southeast. There are nine major Azorean islands in three main groups: Flores and Corvo, to the west; Graciosa, Terceira, São Jorge, Pico and Faial in the centre; and São Miguel, Santa Maria and the Formigas Reef to the east. The archipelago surface is c. 2334 km². However, the islands reveal very uneven dimensions: the larger, São Miguel (745.8 km²), Pico (448.4 km²) and Terceira (403.4 km²), represent 70% of the total surface; São Jorge (245.9 km²), Faial (173.8 km²) and Flores (141.6 km²) have an intermediate size; Santa Maria (97.1 km²), Graciosa (61.2 km²) and Corvo (17.2 km²) are the smallest of the archipelago. Santa Maria is the oldest island of the archipelago (6.3 M years) and Pico being the youngest island of the Azorean Archipelago (0.27 M years; Avila et al. 2016). The Azorean climate is temperate oceanic with a mean annual temperature of 17 °C at sea level, low thermal amplitude, high mean relative humidity, persistent wind and rainfall ranging from 800 to 3000 mm/m², increasing with altitude (Azevedo 1996).

The vascular plant flora is currently thought to comprise c. 1110 taxa, including 73 endemic taxa (Silva et al. 2010). However, these numbers likely underestimate the true diversity, since recent molecular studies have repeatedly revealed new endemic taxa (Schaefer and Schönfelder 2009; Bateman et al. 2013; Moura et al. 2015b, c; Schaefer 2015). Many species introductions and land use changes led to the replacement of natural plant communities, with more than 60% of the surface today covered by pasture land (Schaefer 2003; Lourenço et al. 2011; Costa et al. 2012; Marcelino et al. 2013).

**Plant material and sampling**

A total of 13 different populations and 135 individuals of *L. watsoniana* were sampled, along with a subsample of five individuals of *Lactuca palmensis*, one or two individuals from selected North American species and one individual of *L. sativa* (Table 1). All the places from which *L. watsoniana* populations have been mentioned in historic or recent records, or which were known to local botanists or to the Environmental Services were visited, but only 13 could be confirmed. We searched without success for the only population known from São Jorge and for one population in São Miguel (Lagoa do Fogo, see Table 1).

In 2012 and 2013, trips to Terceira and Pico islands were carried out in order to complement the samples of *L. watsoniana* already available at the DNA bank collection of the AZB herbarium (Biology Department, Azores University). Samples from every single individual were taken in populations with up to 30 plants and in larger populations, 20 samples were collected in populations. *Lactuca palmensis* from La Palma Island (Canary Islands) and North American species—*L. biennis*, *L. canadensis*, *L. floridana*, *L. graminifolia*, *L. hirsuta*, *L. ludovician* and *L. tenerrima*—were obtained from herbarium specimens of the United States National Herbarium, Smithsonian Institute; *L. tenerrima* samples were also obtained from specimens at the BM herbarium of The Natural History Museum, London; and a *L. sativa* sample was obtained in the local market. Depending on leaf size, we collected one or two leaves per individual and immediately stored them in a plastic bag with silica gel. After drying, the leaves were vacuum sealed in plastic bags and stored in folders. The locations of all populations were geo-referenced and mapped using ArcMap® 10.2.2 (Fig. 1).
DNA extraction and microsatellites development

DNA was extracted from dry leaves using a modified Doyle and Dickson CTAB protocol (Doyle and Doyle 1987). Due to the difficulties encountered in obtaining high quality DNA, modifications proposed by Borges et al. (2009). DNA quality and quantity were measured using a Nanodrop 2000 (Thermo Fisher Scientific) spectrophotometer. Samples were conserved at –20 °C until use.

Total DNA from fresh leaves of two individuals of *L. watsoniana* was sent to the Savannah River Ecology Laboratory (University of Georgia) where an Illumina paired-end (IPE) shotgun library was prepared for microsatellite sequencing (Castoe et al. 2012). The resulting sequences were analysed to identify microsatellite repeats for which primers were designed with PAL_FINDER_v0.02.03.

Out of the 48 sequences of primer pairs provided by the Savannah River Ecology Lab we selected 24 primer pairs with expected PCR products ranging between 100 and 400 bp to allow multiloding of PCR products (Oetting et al. 1995). All the primer pairs (with the tag sequence included) were selected on criteria of non-complementarities within and between primers, low secondary structures and 3'-end instability (Rychlik 1995).

One primer from each pair was extended on the 5'-end with an engineered sequence (M13R tag 5'-GGAAACAGCTATGACCAT-3') to enable the use of a third primer identical to the M13R, which allows for an inexpensive fluorescent labelling of the PCR product.

### Table 1. Geographical distribution Lactuca watsoniana populations sampled in the Azores archipelago, and different herbarium specimens included in this study (lactuca palmensis and several Lactuca species from North America). A total of 150 individuals were sampled; (N), total number of individuals per island/location, (n) number of individuals per population. * Population excluded from the analyses (less than 6 individuals).

| Species                | Location (N) | Populations            | Codes  | n    |
|------------------------|--------------|------------------------|--------|------|
| *L. watsoniana*        | Faial (11)   | Caldeira               | FACA   | 11   |
|                        | Pico (65)    | Painha                 | PIAP   | 13   |
|                        |              | Caveiro                | PICA   | 17   |
|                        |              | Gruta dos Montanheiros | PIGM   | 13   |
|                        |              | Subida Montanha        | PISM   | 21   |
|                        |              | Caldeira Ribeirinha*   | PIRB   | 1    |
| Terceira (33)          |              | Grota das Alfacinhas*  | TEGA   | 2    |
|                        |              | Ribeira dos Gatos*     | TENR   | 2    |
|                        |              | Rocha do Chambre       | TERC   | 13   |
|                        |              | Serra do Galhardo      | TESG   | 16   |
| São Miguel (26)        |              | Caminho Criação*       | SMCC   | 1    |
|                        |              | Lagoa do Canário       | SMLC   | 19   |
|                        |              | Miradouro Canário      | SMMC   | 6    |
| *L. biennis*           | N.A (1)      | Cultivated BGBM*       | LB     | 1    |
| *L. sativa*            | São Miguel (1) | Mercado Graça*        | LSMG   | 1    |
| *L. palmensis*         | Canary Islands (5) | Las palma*            | LPLP   | 5    |
| *L. canadensis*        | Dominican Republic (1) | Provincia Santiago*   | LC     | 1    |
|                        | Minnesota(1) | Hubbard County*        |        | 1    |
| *L. floridana*         | Florida (1)  | Lake Okalumpka*        | LFL    | 1    |
| *L. graminifolia*      | South Carolina (1) | Brunswick County*      | LG     | 1    |
| *L. tenerrima*         | Malacitona (1) | BM herbarium*         | LT     | 1    |
| *L. hirsuta*           | Louissiana (1) | New Orleans*         | LH     | 1    |
| *L. ludoviciana*       | Nebraska (1)  | Hitchcock County*      | LL     | 1    |
|                        | South Dakota (1) | Deuel County*      |        | 1    |
obtained (Schuelke 2000). A GTTT ‘pigtail’ was added to the 5′-end of the untagged primer to facilitate accurate genotyping (Brownstein et al. 1996).

Microsatellite selection

In the first phase of the test, all 24 primer pairs were tested on four samples of *L. watsoniana* using a final volume of 25 µl, including 25 ng of DNA, 1× NH₄ Buffer, 1.5 mM MgCl₂, 0.4 µM of FAM-M13R, 0.08 µM of tag primer, 0.36 µM of unlabelled tag primer, 200 µM of dNTPs, 1U of polymerase (Immolase, Bioline) and using a Biometra TGradient thermocycler. Touchdown thermal cycling programmes (Don et al. 1991) encompassing a 10 °C span of annealing temperatures, ranging between 64 and 54 °C, were used for all loci. The PCR program consisted of the following steps: 95 °C for 7 min (hot start); 95 °C for 3 min; 20 cycles at 95 °C for 30 s, the highest annealing temperature of 65 °C (decreased by 0.5 °C per cycle) for 30 s, and 72 °C for 30 s; 20 cycles of 95 °C for 30 s, 55 °C for 30 s and 70 °C for 30 s; and finally, 72 °C for 10 min for the final extension of the PCR products. Five microliters of PCR products were run on a 3.5% agarose gel, stained with SafeView™ Nucleic Acid Stain (ab) and visualized under UV light to check for amplification, polymorphism and scorability of the bands.

Eleven primer pairs exhibited scorable amplified products of the expected length range and with at least two alleles. In the second phase, the variability of the 11 polymorphic loci was assessed in 13 samples, one from each population. After analysis of the quality of the PCR products obtained with the universal primer M13R, eight primer pairs with acceptable to high scorability were selected to run the complete study (Table 2).

Full-scale genotyping

After optimization, all samples were amplified following Dias et al. (2014). The M13R was labelled either with PET, FAM, NED or VIC. The Taq polymerase used with all the markers was Immolase (Bioline). Amplification products were diluted, multiloaded, run on an ABI-3130xl Genetic Analyser and sized with LIZ500 size standard. The genotypes obtained were scored using the software Geneious R8 (http://www.geneious.com; Kearse et al. 2012). The population matrix is available at DEMIURGE (http://www.demiurge-project.org/) with digest code D-CPMIC-101.
Data analyses
SSR preliminary analyses showed a pattern compatible with tetraploidy since all individuals displayed four alleles per locus. Cytological analysis of seedling root tip material later confirmed the tetraploid nature of *L. watsoniana* (manuscript in prep). This information was used to define the statistical analysis methods, since polyploid organisms raise analytical limitations, namely, problems associated with inferring allele frequencies and assumptions regarding inheritance (Obbard et al. 2006; Dufresne et al. 2014).

Genetic diversity. Considering the tetraploid nature of *L. watsoniana*, we used specific software to generate the genetic dataset. Therefore, genetic diversity for each population was calculated using a codified co-dominant matrix with SPAGeDi (Hardy and Vekemans 2002), as a multilocus average for the eight loci, including the number of alleles (NA), the allelic richness (AR), Nei’s gene diversity (1978) (He), global inbreeding coefficient (*F*), computed for all loci and individual inbreeding coefficient (*F*), computed as kinship coefficient between homologous gene copies within individuals. The total genetic diversity (*H*), was determined using the unbiased estimate method of Nei (1987). The percentage of private alleles was calculated using Microsoft Excel based on SPAGeDi allelic frequency data.

Population structure. Taking into consideration the ploidy level, we first analysed the population structure with a Principal Coordinate Analysis (PCoA), based on the matrix of Bruvo distances between individuals, using the R package PolySat (Clark and Jasieniuk 2011). Then we used a Bayesian approach estimate the number of genetic clusters excluding populations with fewer than six plants (PIRB, SMCC, TEGA and TENR) which resulted in a final number of 129 individuals in the analyses. The data were exported from PolySat to a dominant matrix (presence/absence of alleles), which was run with the program STRUCTURE version 2.3.4 (Pritchard et al. 2000; Kumar et al. 2009). We followed the admixture model along with the assumption of correlated allele frequencies between groups (Falush et al. 2003), with 500 000 MCMC repetitions, exclusion of a burn-in period of 50 000 and 10 replicates for each *K* value ranging from 1 to 10. Estimation of best *K* value was conducted with STRUCTURE Harvester (Earl and von Holdt 2012) following the approach of Evanno et al. (2005). The optimal *K* repetitions were permuted in Clumpp version 1.1.2 (Jakobsson and Rosenberg 2007) using the Greedy algorithm and results were graphically represented using Distruct version 1.1 (Rosenberg 2004). For Analysis of Molecular Variance (AMOVA), we used the dominant matrix and Arlequin 3.5 (Excoffier et al. 1992; Excoffier and
Lischer 2010) to (1) calculate the partition of genetic variability within populations, among populations within islands, and among islands and (2) estimate gene flow among populations and the global Wright index, \( F_{ST} \), under the null hypothesis of Hardy–Weinberg (HW) equilibrium.

**Geographical barriers and isolation by distance.** To determine the order of barriers to gene flow possibly occurring within the archipelago, the Nei’s genetic distance matrix corresponding to the Azorean populations was analysed with the Monmonier algorithm, using BARRIER version 2.2 (Manni et al. 2004) and allowing a maximum number of nine barriers (the total number of populations analysed after excluding four populations with fewer than six individuals). Nei’s genetic distance (Nei 1972, 1978) was calculated using a dominant matrix with GenAlEx 6.5 (Peakall and Smouse 2012).

We followed the approach of Meirmans (2012) to discern between a Hierarchical Island model and a Stepping Stone model using standard, stratified and partial Mantel tests using the package VEGAN (Oksanen et al. 2009) in R (http://www.r-project.org/). We also modelled Nei’s genetic distance as a function of geographical distance and of the presence of geographical barriers, using beta regression with the BETAREG package in R (Cribari-Neto and Zeileis 2010). We compared the model based on geographical distance alone with models including an increasing number of geographical barriers. We defined the geographical barriers in two ways: (i) considering the existence of barriers between the different clusters identified with STRUCTURE; and (ii) considering the existence of barriers between populations as determined with BARRIER. Model fit was compared using AIC and pseudo-\( R^2 \).

**Results**

**Genetic diversity**

The eight developed primers showed acceptable to high scorability results, with exception for the \( L.\ sativa \) sample (all loci) and \( L.\ biennis \) (loci Lw-01.08037 and Lw-01.08435). A total of 129 alleles were found for \( L.\ watsoniana \), with a total genetic diversity \( (H_T) = 0.85 \), an overall excess of heterozygotes (Multilocus \( F_{Ts} = -0.1912, P < 0.001 \); Average Multilocus \( F_{T} \) values \( = -0.074, P < 0.001 \)), and with a total multilocus average proportion of private alleles of 26.5 %.

The number of alleles per locus varied from 10 to 31 (Table 2).

The ‘Lagoa do Canário’ population of São Miguel Island displayed the highest percentage of private alleles (7.0 %) and the highest multilocus number of alleles (9), while the ‘Miradouro do Canário’ population in São Miguel Island and ‘Subida da Montanha’ in Pico Island did not show any private alleles (Table 3). The mean allelic richness ranged from 4.58 alleles in the ‘Subida da Montanha’ population up to 6.18 alleles in the ‘Lagoa do Canário’ population. The populations showed similar values for gene diversity (0.7 and 0.8, Table 3).

**Population genetic structure**

The initial analysis of 148 samples (all except \( L.\ sativa \) e \( L.\ biennis \) samples) from 13 populations, based on eight microsatellite loci, revealed the existence of a close relationship between the North American species and \( L.\ watsoniana \) populations and a clear separation from \( L.\ palimensis \), as shown by PCoA (Fig. 2). \( L.\ watsoniana \) individuals were also separated according to their geographical location, but with one cluster combining the populations of the neighbouring islands Faial and Pico (Fig. 2). A Bayesian analyses, showed a peak of \( \Delta K \) for \( K = 5 \). Taking into consideration, the variation across runs, and the partitioning of individuals, we assumed \( K = 5 \) as the most likely value. The results obtained with STRUCTURE (Fig. 3) show a non-admixture pattern with the definition of five genetic clusters corresponding to each of the islands of origin (Faial, Terceira and São Miguel), and to the separation of the four Pico Island populations into two genetic clusters (PIAP and PICA, more to the west versus PIGM and PISM, more to the east). This result is in accordance with those obtained with PolySat (Fig. 2) and GenAlEx 6.5 (data not shown).

**Table 3.** Genetic diversity parameters based on eight SSR loci within nine populations of \( L.\ watsoniana \), with multilocus average. Abbreviations: number of samples (\( N \)), number of alleles (NA), allelic richness (AR), gene diversity (He) and inbreeding coefficient (\( F_I \)), significance levels (\( P_V \): * \( P < 0.01 \); ** \( P < 0.05 \)), and average percentage of private alleles (Pa).

| Island          | Populations | \( N \) | NA | AR | He | \( F_I \) | \( P_V \) | Pa |
|-----------------|-------------|--------|----|----|----|--------|--------|----|
| Faial           | Caldeira    | 11     | 7  | 5.66 | 0.79 | -0.27 * | 1.69   |
| Pico            | Prainha     | 13     | 8  | 6.07 | 0.80 | -0.14 * | 4.55   |
|                 | Caveiro     | 17     | 7  | 5.46 | 0.77 | -0.17 * | 1.72   |
|                 | Gruta dos    | 13     | 6  | 4.97 | 0.74 | -0.18 * | 5.88   |
|                 | Montanheiros |        |    |     |     |         |        |
|                 | Subida Montanha | 21   | 6  | 4.58 | 0.71 | -0.15 * | 0.00   |
| Terceira        | Rocha do Chambre | 13 | 6  | 4.95 | 0.74 | -0.35 * | 1.96   |
|                 | Serra do Galhando | 16 | 7  | 4.86 | 0.76 | -0.26 * | 3.64   |
| São Miguel      | Lagoa do Canário | 19 | 9  | 6.18 | 0.82 | -0.16 * | 7.04   |
|                 | Miradouro Canário | 6  | 6  | 5.52 | 0.80 | -0.10 **| 0.00   |
| **Total**       |             | 129    | 15.88 | 7.38 | 0.85 | -0.07 * | 26.49  |

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Regarding isolation by distance (IBD) models, *L. watsoniana* seems to correspond to an intermediate case between hierarchical island and stepping stone models, with no clear discrimination based on the results obtained from standard, stratified and partial Mantel tests (Table 6). Globally, the Mantel test indicated that there was a significant correlation between genetic distance and geographical distance ($r = 0.77$, $R^2 = 0.66$, Fig. 5). However, model fit increased from the model including geographical distance alone ($AIC = -140.40$, pseudo-$R^2 = 0.58$) to the models including an increasing number of geographical barriers: (i) considering the existence of barriers between different populations clusters as determined with STRUCTURE (best model including all the barriers, $AIC = -168.5$, pseudo-$R^2 = 0.90$); (ii) considering the existence of barriers between populations as determined with BARRIER (best model including the first to the third order barriers, $AIC = -161.5$, pseudo-$R^2 = 0.84$). The graphical representation of the best fitting model shows how the inclusion of barriers increases the fit of the geographical model (Fig. 5). Also, on average, Pico populations seem to be genetically closer to all the other populations (Fig. 5).

**Discussion**

**Implications of tetraploidy**

The SSR profile obtained in this study supported that *L. watsoniana* is tetraploid. To the best of our knowledge, only two of the nine endemic Azorean Asteraceae are polyploid, namely, *Pericallis malviflora* (Jeffrey 1992; Jones et al. 2014) and *L. watsoniana* while seven are diploids: *Bellis azorica*, *Leontodon filii*, *L. hochstetteri*, *L. rigens*, *Solidago azorica*, *Tolpis azorica* and *T. suculenta* (Lack 1981; Fiz et al. 2002). In the genus *Lactuca*, Feráková (1977) distinguished three main groups based on chromosome number: the first group with $n = 8$ includes perennial species from Europe and the Himalayas; the second group with $n = 9$ comprises the majority of the taxa from Europe, Mediterranean, Middle East, Africa and India; and the third group includes North American species with $n = 17$, considered to be of amphidiploid origin (Doležalová et al. 2002). The ploidy level of *L. watsoniana* was previously unknown (Feráková 1977; Lack 1981). Our results suggested by the SSR pattern observed, point to a closer phylogenetic relationship with the North American taxa.

In the Canary Islands, species-rich lineages like the Asteraceae originated from diploid colonizers (Suda et al. 2003), while in other archipelagos (Galapagos Islands, Hawaiian Islands, Robinson Crusoe Islands and St. Helena) the endemic diversity apparently originated

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**Figure 2.** Principal Coordinate Analysis (Bravo distance) based on the data set of nine microsatellite loci for all 148 *Lactuca* individuals included in this study. Inertia percentage of axes 1 (13.72 %) and 2 (10.41 %). Each individual is represented by a symbol according to its geographical distribution (sampling site labels as in Table 1). Red ellipse limits the individuals from São Miguel, individuals from Terceira are limited with a blue ellipse and Pico populations seem to be genetically closer to all the other populations (Fig. 4).
from polyploid colonists (Harbaugh 2008; Crawford et al. 2009). In the Azores Islands, ploidy levels for most endemic colonizers have not been studied yet, although many taxa are now diploid (Bateman et al. 2013; Dias et al. 2014). However, tetraploids have been recently found for *Prunus lusitanica* ssp. *azorica* (Moreira et al. 2013). It was hypothesized that diploid colonizers might be more common in archipelagos closer to continental source areas while successful polyploid colonizers could be more frequent at more remote islands (Carine et al. 2004, Crawford et al. 2009). However, at this stage, the number of detected polyploids in the Azorean flora does not support such a claim.

Two possible ancestral colonization events may have occurred in the case of *L. watsoniana*: (a) an allotetraploid colonizer from North America or (b) an ancestral diploid population leading to an autotetraploid form, with later extinction of the ancestral diploid. Considering the absence of other endemic or native *Lactuca* species in the Azores, and the existence of allotetraploid North American *Lactuca* species (Doležalová et al. 2002) closely related with *L. watsoniana* (Fig. 2), an early colonization from North America seems a more plausible hypothesis. This is a very rare colonization pathway for vascular plants in the Azores, but was confirmed for another endemic Asteraceae, the Azores goldenrod *Solidago azorica* (Schaefer 2015). Therefore, *L. watsoniana* is not likely to be a neopolyploid, although formation of new cytotypes and their demographic establishment have been recorded for ocean islands (Crawford et al. 2009; Stuessy et al. 2014). More comprehensive morphological and molecular phylogenetic analyses are currently underway to verify this hypothesis.

**Genetic diversity**

The genetic variability found in the *L. watsoniana* populations was comparably high to that found for other Azorean herbaceous endemic taxa (Dias et al. 2014; Silva et al. 2015), and we found no evidence for inbreeding, despite the population decline that most likely took place in the past five centuries. It might also be a direct expression of polyploidy which causes an increase in gene dosage at the genome. Polyploid taxa often contain higher levels of genetic variation to their diploid relatives (Osbald et al. 2006; Garcia-Verdugo et al. 2015). This high diversity of tetraploid colonizers together with self-compatibility would have been an advantage in the establishment of a sexually reproducing population (Crawford and Stuessy 1997, Crawford et al. 2009), providing favourable conditions for establishment in one island with subsequent dispersal to other parts of the

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**Table 4.** Analysis of molecular variance (AMOVA) conducted on SSR profile of nine populations of *Lactuca watsoniana* from São Miguel, Terceira, Faial and Pico. The clusters were identified with STRUCTURE, *P* < 0.001 for all components.

| Populations | FACA | PIAP | PIGM | PISM | TESG | TERC | SMMC | SMLC |
|-------------|------|------|------|------|------|------|------|------|
| FACA        | 0.54 | 0.35 | 0.65 | 0.85 | 1.00 | 1.54 | 1.23 | 0.96 |
| PIAP        | 0.46 | 0.22 | 0.34 | 0.37 | 0.95 | 0.99 | 0.84 | 0.64 |
| PIGM        | 0.47 | 1.11 | 0.38 | 0.44 | 0.78 | 0.72 | 0.63 | 0.54 |
| PISM        | 0.39 | 0.74 | 0.66 | 0.35 | 1.07 | 1.13 | 1.09 | 0.81 |
| TESG        | 0.29 | 0.67 | 0.57 | 0.70 | 1.25 | 1.14 | 1.16 | 0.81 |
| TERC        | 0.25 | 0.26 | 0.32 | 0.23 | 0.20 | 0.70 | 1.43 | 1.08 |
| SMMC        | 0.16 | 0.25 | 0.34 | 0.22 | 0.22 | 0.36 | 1.28 | 0.93 |
| SMLC        | 0.20 | 0.30 | 0.40 | 0.23 | 0.22 | 0.17 | 0.20 | 0.13 |

**Figure 3.** Results of genetic admixture analysis performed with STRUCTURE (*K* = 5, genetic clusters indicated in different colours) based on the SSR loci scored for *L. watsoniana*. Each vertical bar corresponds to an individual, populations are indicated below the graphic and islands, above.
Archipelago. However, studies addressing Azorean endemic plant reproductive strategies are very limited (see Crawford et al. 2015 for Tolpis spp.). Meanwhile, field and laboratory observations indicate that isolated L. watsoniana individuals flower regularly but mostly produce infertile seeds whereas in relatively large populations, such as those found in Pico Island, a very high number of viable seeds are produced, annually. In this sense, according to Ferrer and Good-Avila (2007), the basal members of the tribe Cichorieae are dominated by self-compatibility or partial self-incompatibility exemplified by members of the genera Cichorium and Lactuca. Further studies should be undertaken to enlighten this matter, namely experiments of forced self-pollination and cross-pollination (Zhang et al. 2015).

Population genetic structure

We found five genetic groups in L. watsoniana, supported by AMOVA, PCA and Bayesian model-based clustering. This genetic structure coincides spatially with the studied islands, with the exception of Pico Island, which is home to two genetic groups. The average $F_{st}$ value (0.45) was higher than those found for many other plant species (Bottin et al. 2005; Lucio 2011; Chaves 2013). We also observed that genetic variation was mainly found within populations (55.0 %), which is in accordance with the results obtained for Leontodon filii and L. rigens (Dias et al. 2014) and in contrast with Canary Islands endemic species, where most genetic variation was found between populations ($G_{st} = 77.3 \%$) (Francisco-Ortega et al. 2000). However, a relatively high genetic variation was found among populations within islands (17.7 %), only surpassed in the Azores by the dioecious Juniperus brevifolia (Silva et al. 2011). An even larger proportion of variation was found among islands (27.3 %), an extreme case in the Azores so far.

Estimated gene flow values were always below 1, contrary to the much higher values obtained for Leontodon (Dias et al. 2014), but similar to the values obtained for Viburnum treleasei (Moura et al. 2013), although the latter study was conducted with dominant markers. The highest gene flow value (1.94) between two São Miguel populations (Table 5), and between two Pico populations (1.11), likely reflect their geographic proximity and the

**Table 5.** Genetic differentiation between pairs of Azorean Lactuca watsoniana populations. $F_{st}$ values (above diagonal) and gene flow values calculated using the Slatkin formula (below diagonal).

| Source of variation            | $df$ | Sum of squares | Variance component | Percentage of variation (%) |
|-------------------------------|------|---------------|--------------------|-----------------------------|
| Among clusters                | 4    | 562.36        | 3.82               | 27.30                       |
| Among populations within clusters | 4    | 165.12        | 2.47               | 17.68                       |
| Within populations            | 120  | 924.34        | 7.70               | 55.02                       |
| Total                         | 128  | 1651.81       | 14.00              |                             |

**Figure 4.** Barriers to gene flow possibly present between nine populations of L. watsoniana from São Miguel, Terceira, Faial and Pico (Azores archipelago), calculated by the Monmonier’s algorithm (1973) with the program BARRIER. Barriers are labelled from a to h, alphabetically ordered by relevance.
Lactuca watsoniana diaspores disperse by anemochory and hydrochory (Schaefer 2003; Silva et al. 2009), which are more efficient in small distance than in long distance dispersal. In this case, plants in spatially closer populations will be more similar genetically than those from more distant populations (Meirmans 2012). A limitation in dispersal ability is expected to be a key factor in geographical isolation (Kisel and Barraclough 2010), with a significant impact on relatedness within plant taxa (Papadopoulos et al. 2011).

**Disturbance from volcanism**

After birth and growth of volcanic islands, destructive events such as secondary eruptions, landslides and thick volcanic deposits on the islands surface, might reshape the islands flora due to a massive population extinction by soil sterilization (Mairal et al. 2015; Borges Silva et al. 2016), and lead to habitat fragmentation and genetic isolation of populations, which in due course could lead to differentiation and speciation within or among-islands (Gillespie and Roderick 2014; Mairal et al. 2015).

Considering our results, differentiation within Pico populations, grouped into two clusters, might be a consequence of the existence of Pico Mountain, acting as a natural geological barrier.

On the other hand, besides geographical distance and the existence of barriers, population genetic structure might be the result of the populations sampled being relics, resulting from geological events, such as volcanic eruptions, as hypothesized for Juniperus brevifolia and Tolpis azorica (Silva et al. 2011, Borges Silva et al. 2016) as an explanation of the population genetic patterns found in Terceira Island. ‘Sete Cidades’ and ‘Lagoa do Fogo’ have been among the most active volcanic systems in São Miguel Island during the past 5000 years (Booth et al. 1978; Gaspar et al. 2015), where the most recent explosive eruption occurred just before human colonization in the 15th century (Ferreira et al. 2015). In Pico, Macedo (1981a, b) and Machado (1962) documented four major volcanic eruptions in the 16th and 18th centuries, in ‘Prainha’ (1562), ‘Santa Luzia, São João’ (1718) and ‘Silveira’ (1720). These episodes, with deposits of thick pumice, might have exterminated nearby populations of L. watsoniana, contributing to the unique observed patterns of genetic variation. Implications of island age on the observed genetic patterns are difficult to determine at this stage, due to the possibility of the later occurrence of volcanic disturbance, as illustrated above, and to a considerable effect of human disturbance (see below). Moreover, during a preliminary analysis, we included island age and differences in island age between populations, in our models to

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**Figure 5.** Correlation between pairwise genetic distance (Nei) and geographical distance (m) in the studied Azorean Islands. The dashed line corresponds to the geographical model only (AICc = −140.40, pseudo-R² = 0.58). ‘Predicted’ correspond to the genetic distances predicted by the best model, including geographical and barriers (AICc = −168.5, pseudo-R² = 0.92), as defined by the five STRUCTURE genetic clusters. The symbols represent comparisons between populations within the same genetic cluster (same cluster), or within different genetic clusters (e.g. ‘F x P1’). Abbreviations of STRUCTURE genetic clusters correspond to: F = Faial (1 population); P1 = Pico (2 populations); P2 = Pico (2 populations); T = Terceira (2 populations); S = São Miguel (2 populations).

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**Geographical distance and barriers**

Lactuca watsoniana show a strong geographic structure. Isolation by distance (IBD) and the existence of effective barriers to gene flow, the Atlantic Ocean and Pico Mountain, were found to be related with genetic distance. Therefore, L. watsoniana seems to be an intermediate case between hierarchical island and stepping stone models (Table 6) in what could be seen as an approximation to a two or three dimensions Kimura and Weiss (1964) stepping stone model, complicated by inexistente stones or by insurmountable barriers, i.e. the sea. This may be due to the existence of regional structuring (Morand et al. 2002), since oceanic barriers are, in general, more effective than topographic barriers at promoting isolation in insular systems (Gillespie and Clague 2009).
explain genetic distance, but without significant improvement of the modelling results.

**Anthropogenic disturbance**

Human colonization has had a strong impact on oceanic islands endemic plants since pristine ecosystems have evolved or persisted in the absence of novel or exotic disturbances (Connor et al. 2012). Human population settlement on previously pristine ecosystems, with large scale changes in land use, originated habitat loss and fragmentation both of which contribute to a decline in biological diversity (Wilcox and Murphy 1985; Kuussaari et al. 2009; Krauss et al. 2010; Triantis et al. 2010). In the Azores, widespread and persistent vegetation and soil use changes in the last 500 years, together with the introduction of alien plants (Silva and Smith 2004; Connor et al. 2012; Costa et al. 2012), and herbivores (Moura et al. 2013; Dias et al. 2014; Silva et al. 2015), led to a decline in native vegetative cover and in the number of endemic plant populations throughout the archipelago. Our field work allowed us to directly observe the results of herbivore consumption on L. watsoniana individuals, from almost complete consumption of above ground plant material (goats in Sâo Miguel Island) to the removal of the developing synflorescence (cattle in Terceira Island). Damage caused by human intervention in the native vegetation is the most probable explanation for the possible extinction of L. watsoniana in Sâo Jorge Island, where the native vegetation has been replaced by invasive plants in the site of the historical collection. Pico Island holds the highest number of individuals including many young plants, what could be a direct consequence of being one of the less altered islands in the archipelago. One justification would be that Pico represents a sample of a formerly wider genetic pool that was eroded in the remaining islands, which through possible genetic bottlenecks only kept part of that wider pool, similarly to what might have happened to Tolpis azorica populations in the central sub-archipelago of the Azores (Borges Silva et al. 2016). Whether this actually happened and whether it was related with volcanic or human disturbance remains to be ascertained. Bottleneck analyses are possible for diploid species, but there are no analysis tools presently available to test them in tetraploids (Espinosa and Noor 2002). Therefore, although the impact of human disturbance was observed in most populations, at this stage L. watsoniana populations managed to maintain a high degree of genetic diversity and low levels of inbreeding. However, the continuation of this level of disturbance might still lead to more pronounced population decline with unpredictable consequences on future diversity levels.

**Implications in conservation**

Our results, of a high genetic diversity, with substantial population differentiation among islands, strongly advocate that the concept of propagule provenance should be taken into consideration when developing augmentation or reintroduction strategies (Silva et al. 2011; Weeks et al. 2011; Dias et al. 2014; Borges Silva et al. 2016). For example, the heterogeneity between the western and eastern populations in Pico means that seeds or individuals from the eastern part of this island should not be used in reintroduction programs on the western part of the island or even on the other islands. Since the species shows a considerable degree of genetic diversity, high seed production and high germination rate (Dias et al. 2015), some of the conservation actions that should be implemented or established are: (i) running a seed bank and a plant nursery (Attorre et al. 2011), where plants from the different islands could be produced following specific protocols for L. watsoniana (Dias et al. 2015); (ii) reinserion of the plantlets in the original natural populations, increasing the number of effectives and preserving the genetic diversity. Inter-island and inter-population transfers of seedlings and plantlets should be avoided at all cost, since differentiation levels are high but
inbreeding was not found (Moura et al. 2013; Dias et al. 2014; Borges Silva et al. 2016).

The uniqueness of some of the studied populations and the existence of populations with extremely low number of individuals, indicate that the populations of Pico (PIRB, PIAP, PICA, PIGM and PISM), ‘Caminho da Criação’ on São Miguel (SMCC) and Terceira (TEGA and TENR), should be considered priority for conservation.

The awareness of the threats by habitat loss and degradation resulting from changes in land use indicates that extreme care should be taken regarding the implementation of augmentation conservation strategies. Some measures have been implemented to locally control invasive species (Costa et al. 2013). However, L. watsoniana populations in São Miguel island are severely threatened by herbivores (feral goats and rabbits), which in Terceira have already alarmingly obliterated most of the Serra do Galhardo population. These animals should be controlled or eradicated from these protected areas.

Conclusion

Our research underscores the importance of considering a broad array of intrinsic traits and external factors when studying the evolution and management of plants in oceanic islands.

Regarding intrinsic traits, our results support previous claims suggesting that high levels of genetic variation are often associated with polyploid taxa, therefore highlighting the potential importance of this evolutionary mechanism in island systems (Oubbard et al. 2006; Garcia-Verdugo et al. 2015). Indeed, while showing signs of population reduction, fragmentation, and local extinction, we found an example of a rare species that still maintains high levels of genetic diversity. External factors such as geographical distance and geographical barriers improved our ability to model genetic distance. Therefore, the classical models should be tested with newer and more integrated versions, including not only geographical distances but also relevant geographical barriers. While addressing oceanic island floras, discrepancies between the hierarchical and stepping stone classical models could be unravelled (Meirmans 2012), contributing to the eventual development of a more unified modelling approach for genetic distance, including also other dimensions such as temporal aspects (Anderson et al. 2010).

Finally, this study revealed that L. watsoniana populations show considerable genetic diversity, also representing another case in the Azores of pronounced genetic differentiation among populations in different islands. This information on the remaining Lactuca populations in the archipelago should be taken into consideration in the design of a recovery plan.

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Contributions by the Authors

E.F.D., participated in sampling, performed genetic analyses, and wrote the first draft; E.F.D and L.S. conceived the idea for the paper; L. S. was involved in the statistical analysis; M.M. was involved in the genetic analysis; H.S. participated in field work. All authors helped revise the manuscript.

Conflict of Interest Statement

None declared.

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Literature Cited

Anderson CD, Epperson BK, Fortin M-J, Holderegger R, James PMA, Rosenberg MS, Scribner KT, Spear S. 2010. Considering spatial and temporal scale in landscape-genetic studies of gene flow. Molecular Ecology 19:3565–3575.

Attorre F, Taleb N, De Sanctis M, Farcomeni A, Guillet A, Vitale M. 2011. Developing Conservation strategies for endemic tree species when faced with time and data constraints: Boswellia ssp. on Socotra (Yemen). Biodiversity and Conservation 20:1483. DOI 10.1007/s10531-011-0039-7.

Ávila SP, Melo C, Berning B, Cordeiro R, Landau B, da Silva CM. 2016. Persististrombus coronatus (Mollusca: Strombidae) in the early Pliocene of Santa Maria Island (Azores: NE Atlantic): paleoecology, paleoecology, paleoecology, paleoecology, paleoecology, paleoecology. Palaeogeography, Palaeoclimatology, Palaeoecology 441: 912–923.
Azevedo EB. (1996) Modelação do clima insular à escola local. Modelo CIELO aplicado à ilha Terceira, PhD Dissertation, University of Azores, Portugal. 247 p.

Bateman RM, Rudall PJ, Moura M. 2013. Systematic revision of Platanthera in the Azorean archipelago: not one but three species, including arguably Europe’s rarest orchid. Peer Journal 1: e218. DOI 10.7717/peerj.218.

Baldwin BG. 2009. Silverswords. In: Gillespie, RG Clague, DA, eds., Booth B, Croasdale R, Walker GPL. 1978. A quantitative study of five

Bateman RM, Rudall PJ, Moura M. 2013. Systematic revision of Platanthera in the Azorean archipelago: not one but three species, including arguably Europe’s rarest orchid. Peer Journal 1: e218. DOI 10.7717/peerj.218.

Baldwin BG. 2009. Silverswords. In: Gillespie, RG Clague, DA, eds., Booth B, Croasdale R, Walker GPL. 1978. A quantitative study of five

Borges A, Rosa MS, Recchia GH, Queiroz-Silva JR, Bressan EA, Veasey EA. 2009. CTAB Methods for DNA Extraction of sweetpotato for microsatellite analysis. Scientia Agricola 66:529–534.

Borges Silva L, Sardos J, Menezes de Sequeira M, Silva L, Crawford D, Moura M. 2016. Understanding intra and inter-archipelago population genetic patterns within a recently evolved insular endemic lineage. Plant Systematics and Evolution DOI 10.1007/s00606-015-1267-1.

Booth B, Crossdale R, Walker GPL. 1978. A quantitative study of five thousand years of volcanism on São Miguel, Azores. Philosophical Transactions Royal Society, London 288:271–319.

Bouzat JL. 2010. Conservation genetics of population bottlenecks: the role of chance, selection, and history. Conservation Genetics 11:463–478.

Bottin L, Vaillant A, Sire P, Cordi C, Bouvet JM. 2005. Isolation and characterization of microsatellite loci in Santulium austrocaledonicum, Santalaceae. Molecular Ecology Notes 5:800–802.

Bramwell D, Caujape- Castells J. 2011. The biology of island floras. Cambridge: Cambridge University Press.

Brownstein MJ, Carpten JD, Smith JR. 1996. Modulation of non-

Brownstein MJ, Carpten JD, Smith JR. 1996. Modulation of non-

Bramwell D, Caujape- Castells J. 2011. The biology of island floras. Cambridge: Cambridge University Press.

Brownstein MJ, Carpten JD, Smith JR. 1996. Modulation of non-

Brownstein MJ, Carpten JD, Smith JR. 1996. Modulation of non-

Carine MA, Russel SJ, Santos-Guerra A, Francisco-Ortega J. 2004. Relationships of the Macaronesian and Mediterranean floras: molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in Convolvulus (Convolulaceae). American Journal of Botany 91:1070–1085.

Carine MA, Schaefer H. 2010. The Azorean diversity enigma: Why are there so few Azorean endemic flowering plants and why are they so widespread? Journal of Biogeography 37:77–89.

Castoe TA, Poole AW, de Koning APJ, Jones KL, Tomback DF, et al. 2012. Rapid microsatellite identification from illumina paired-end genomic sequencing in two birds and a snake. PLoS One 7: e30953.

Caujape- Castells J, Tye A, Crawford DJ, Santos-Guerra A, Sakai A, Beaver K, Lobin W, Mable BK. 2014. Recent progress and future genomic sequencing in two birds and a snake. Nucleic Acids Research 42:e29.

Chaves CL. (2013) Estrutura genética de Hypochaeris catharinensis Cabrera (Asteraceae) utilizando marcadores moleculares. 67 pp. Dissertação Mestrado em Agronomia. Universidade Estadual de Londrina, Londrina.

Clark LV, Jasieniuk M. 2011. POLYSAT: an R package for polyploid microsatellite analysis. Molecular Ecology Resources 11: 562–566.

Connor SE, Van Leeuwen JFN, Rittenour TM, Van Der Knaap WO, Briggsa Ammann B, Björck S. 2012. The ecological impact of oceanic island colonization—a palaeoecological perspective from the Azores. Journal of Biogeography 39:1007–1023.

Conte L, Cotti C, Schirchi R, Raimondo FM, Christofolini G. 2004. Detection of ephemeral genetic sub-structure in the narrow endemic Abies nebrodensis (Lojac.) Mattei (Pinaceae) using RAPD markers. Plant Biosyst 138:279–289.

Costa H, Arando SC, Lourenço P, Medeiros V, Brito de Azevedo E, Silva L. 2012. Predicting successful replacement of forest invaders by native species using species distribution models: the case of Pittosporum undulatum and Morella faya in the Azores. Forest Ecology and Management 279:90–96.

Dias EF, Medeiros V, Azevedo EB, Silva L. 2013. Evaluating ecological-niche factor analysis as a modelling tool for environmental weed management in island systems. Weed Research 53:221–230.

Crawford DJ, Witkus R, Stuessy TF. (1987). Plant evolution and speciation on oceanic islands. In: Urbanska KM, ed. Differentiation and diversification of (and plants). Tokyo: Springer Verlag, 183–199.

Crawford DJ, Stuessy TF. (1997). Plant speciation on oceanic islands. In: Iwatsuki K, Raven PH, eds. Evolution and diversification in land plants. Tokyo: Springer, 249–267.

Crawford DJ, Ruiz E, Stuessy TF, Tepe E, Aqeveque P, Gonzalez F, et al. 2001. Allozyme diversity in endemic flowering plant species of the Juan Fernandez Archipelago, Chile: ecological and historical factors with implications for conservation. American Journal of Botany 88:2195–2203.

Crawford DJ, Lowrey TK, Anderson GJ, Bernardello G, Santos-Guerra A, Stuessy TF. 2009. Genetic diversity in the colonizing ancestors of Asteraceae endemic to oceanic islands: Baker’s law and polyploidy. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ, eds. Systematics, evolution and biogeography of the compositae. Vienna: International Organization of Plant Taxonomy, 139–151.

Crawford DJ, Anderson GJ, Silva LB, Sequeira MM, Moura M, Santos-Guerra A, Kelly JK, Mort ME. 2015. Breeding systems in Tolpis (Asteraceae) in the Macaronesian islands: the Azores, Madeira and the Canaries. Plant Systematics and Evolution 301:1981–1993.

Cribari-Neto F, Zeileis A. 2010. Beta regression in R. Journal of Statistical Software 34:1–24.

Dias EF, Sardos J, Silva L, Maciel MGB, Moura M. 2014. Microsatellite markers unravel the population genetic structure of the Azorean Leontodon: implications in conservation. Plant Systematics and Evolution 300:987–1001.

Dias EF, Moura M, Schaefer H, Silva L. 2015. Interactions between temperature, light and chemical promoters trigger seed germination of the rare Azorean lettuce, Lactuca watsoniana (Asteraceae). Seed Science and Technology 43:133–144.

Doleželová I, Kristová E, Lebeda A, Vinter V. 2002. Description of morphological characters of wild Lactuca spp., genetic resources (English-Czech version). Horticultural Sciences (Prague) 29:56–83.

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS. 1991. Touchdown’ PCR to circumvent spurious priming during gene amplification. Nucleic Acids Research 19:4008.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry Bulletin 19:11–15.

Dufresne F, Stift M, Vergilino R, Mable BK. 2014. Recent progress and challenges in population genetics of polyploid organisms: an overview of current state-of-the-art molecular and statistical tools. Molecular Ecology 23: 40–69.
Earl DA, vonHoldt BM. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4: 359–361.

Edmands S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology 16:463–475.

Eliades N-GH, Gailing O, Leinemann L, Fady B, Finkeldey R. 2011. High genetic diversity and significant population structure in Cedrus brevifolia Henry, a narrow endemic Mediterranean tree from Cyprus. Plant Systematics and Evolution (2011) 294: 185–198.

Emerson BC. 2002. Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. Molecular Ecology 11:951–966.

Evanno G, Regnault S, Goudet J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14:2611–2620.

Espinosa NR, Noor MAF. 2002. Population genetics of a polyploid: is there hybridization between lineages of Hylia versicolor? The Journal of Heredity 93:81–85.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Feráková V. (1977) The genus Lactuca L. in Europe. Bratislava, CSR: Univerzita Komenského.

Ferreira T, Gomes A, Gaspar JL, Guest J. 2015. Distribution and significance of basaltic eruptive centers: São Miguel, Azores. In: Gaspar JL, Guest JE, Duncan AM, Barriga F-JAS, Chester DK, eds. Volcanic geology of São Miguel Island (Azores archipelago), Vol. 44. London, Memoirs: Geological Society, 135–146.

Ferrer MM, Good-Avila SV. 2007. Macrophylogenetic analyses of the gain and loss of self-incompatibility in the Asteraceae. New Phytologist 173:401–414.

Fiz O, Valcárcel V, Vargas P. 2002. Phylogenetic position of Mediterranean Asteraceae and character evolution of daisies (Bellis, Asteraceae) inferred from nrDNA ITS sequences. Molecular Phylogenetics and Evolution 25:157–171.

Francisco-Ortega J, Santos-Guerra A, Kim S-C, Crawford DJ. 2000. Plant genetic diversity in the Canary Islands: a conservation perspective. American Journal of Botany 87:909–919.

García-Verdugo C, Forrest AD, Fay MF, Vargas P. 2010. The relevance of gene flow in metapopulation dynamics of an oceanic island endemic, Olea europaea subsp. guanchica. Evolution 64: 3525–3536.

García-Verdugo C, Calleja JA, Vargas P, Silva L, Moreira O, Pulido F. 2013. Polyploidy and microsatellite variation in the relict tree Prunus lusitana L.: how effective are refugia in preserving genotypic diversity of clonal taxa? Molecular Ecology 22: 1546–1557.

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

García-Verdugo C, Sajeva M, La Mantia T, Harrouni C, Msanda F, Caujapé-Castells J. 2015. Do island plant populations really have lower genetic variation than mainland populations? Effects of selection and distribution range on genetic diversity estimates. Molecular Ecology 24:726–741.

Gaspar JL, Guest J, Queiroz G, Pacheco J, Pimentel A, Gomes A, Marques R, Felpeto A, Pacheco T, Wallenstein N. 2015. Eruptive frequency and volcanic hazards zonation in São Miguel Island, Azores. In: Gaspar JL, Guest JE, Duncan AM, Barriga F-JAS, Chester DK, eds. Volcanic geology of São Miguel Island (Azores archipelago), Vol. 44. London, Memoirs: Geological Society, 155–166.

Gillespie RG. 2007. Oceanic islands: models of diversity. In: Levin SA, ed. Encyclopedia of biodiversity Oxford: Elsevier Ltd, pp.1–13.

Gillespie RG, Clague DA. 2009. Encyclopedia of islands. Berkeley, California: University of California Press.

Gillespie RG, Baldwin BG. 2009. Island biogeography of remote archipelagos: interplay between ecological and evolutionary processes. In: Losos JB, Ricklefs RE, eds. The theory of island biogeography (Revisited). Princeton, NJ: Princeton University Press, 358–387.

Gillespie RG, Roderick GK. 2014. Evolution: Ecology and climate drive diversification. Nature 509:297–298.

Harbaugh D. 2008. Polyploid and hybrid origins of Pacific island sand-dwolds (Santalum, Santalaceae) inferred from low-copy nuclear and flow cytometry data. International Journal of Plant Sciences 169:677–685.

Hardy OJ, Vekemans X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2:618–620.

Hutsemékers V, Szövényi P, Shaw AJ, González-Mancebo J-M, Munoz J, Vanderpoorten A. 2011. Oceanic islands are not sinks of biodiversity in spore-producing plants. Proceedings of the National Academy of Sciences of the United States of America 108:18989–18994.

Jakobsson M, Rosenberg NA. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multi-modality in analysis of population structure. Bioinformatics 23: 1801–1806.

Jeffrey C. 1992. The tribe Senecioneae (Compositae) in the Mascarene Islands with an annotated world check-list of the genera of the tribe. Notes on Compositae VI. Kew Bulletin 47: 49–109.

Jones KE, Reyes-Betancort JA, Hiscock SJ, Carine MA. 2014. Allopatric diversification, multiple habitat shifts, and hybridization in the evolution of Pericallis (Asteraceae), a Macaronesian endemic genus. American Journal of Botany 101:1–15. 2014.

Kaiser-Bunbury CN, Travest A, Hansen DM. 2010. Conservation and restoration of plant–animal mutualisms on oceanic islands. Perspectives in Plant Ecology, Evolution and Systematics 12: 131–143.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Kelso A, Li S, PyACEMENTA, Godfree R, Dimmer E, Aslett L, Findlay L, Graf V, Hamelryck T, Lunt C, McAnally L, Makin J, Mitchell K, O’Dowd M, Peñalva OJ, Saunders M, Turner C, Xu Y, Heald P, stuffing C. 2012. Geneious Basic: an integrated application to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2:618–620.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Kelso A, Li S, PyACEMENTA, Godfree R, Dimmer E, Aslett L, Findlay L, Graf V, Hamelryck T, Lunt C, McAnally L, Makin J, Mitchell K, O’Dowd M, Peñalva OJ, Saunders M, Turner C, Xu Y, Heald P, stuffing C. 2012. Geneious Basic: an integrated application to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2:618–620.

Kier G, Kreft H, Lee TM, Jetz W, Ibisch PL, Nowicki C, Mutke JM Barthlott W. 2009. A global assessment of endemism and
species richness across island and mainland regions. *Proceedings of the National Academy of Sciences of the United States of America* **106**:9322–9327.

Kimura M, Weiss GH. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics **49**:561–576.

Kisel Y, Barraclough TG. 2010. Speciation has a spatial scale that depends on levels of gene flow. *The American Naturalist* **175**:316–334.

Krauss J, Bommarco R, Guardiola M, Heikkinen RK, Lindborg R, Oechtering J, Bommarco R, Heikkinen RK, Helm A, Krauss J, Lindborg R, Oechtering J, Pöntinen M, Steffan-Dewenter I. 2009. Extinction debt: a challenge for biodiversity conservation. *Trends in Ecology and Evolution* **24**:564–571.

Lack HW. 1981. Die Lactuceae (Asteraceae) der Azorischen Inseln. [The Lactuceae (Asteraceae) of the Azores Archipelago]. *Wildenowia* **11**:211–247.

López-Pujol J, Martínez MC, Masso S, Blanche C, Sáez L. 2013. The ‘paradigm of extremes’: extremely low genetic diversity in an extremely narrow endemic species, *Coristospermum huteri* (Umbelliferae). *Plant Systematics and Evolution* **299**:439–446.

Loureno P, Medeiros V, Gil A, Silva L. 2011. Distribution, habitat and biomass of *Pittosporum undulatum*, the most important woody plant invader in the Azores Archipelago. *Forest Ecology and Management* **262**:178–187.

Lowe AJ, Bossier D, Ward M, Bacles CFE, Navarro C. 2005. Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity* **95**:255–273.

Lucio KCF. 2011. Estudios genéticos en populações da espécie *Hypochaeris ciliennis* (Asteraceae) utilizando marcadores microsatelitares. Dissertação de Mestrado em Genética e Biologia Molecular, 101 pp. Londrina: Universidade Estadual de Londrina.

Macedo ALS. 1981a. História das Quatro Ilhas que Formam o Distrito do Horta. Direcção Regional dos Assuntos Culturais, S.R.E.C. (Ed.), Angra do Heroísmo, Reimpressão fac-símilada da edição de 1871, Gráfico Maiadouro, Vol. I. 571 p.

Macedo ALS. 1981b. História das Quatro Ilhas que Formam o Distrito do Horta. Direcção Regional dos Assuntos Culturais, S.R.E.C. (Ed.), Angra do Heroísmo, Reimpressão fac-símilada da edição de 1871, Gráfico Maiadouro, Vol. III. 296 p.

Machado F. 1962. Erupções históricas do sistema vulcânico Faial-Pico-S.Jorge. Atlântida 6:84–91.

Mani F, Guérard EF, Heyer E. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by “Monmonier’s algorithm”. *Human Biology* **76**:173–190.

Marcelino JA, Silva L, Garcia PV, Weber E, Soares AO. 2013. Using species spectra to evaluate plant community conservation value along a gradient of anthropogenic disturbance. *Environment Monitoring Assessment* **185**:6221–6233.

Mairal M, San-Martín I, Aldosorio JJ, Cilshav V, Manolopoulou I, Alarcón M. 2015. Palaeo-islands as refugia and sources of genetic diversity within volcanic archipelagos: the case of the widespread endemic *Canaria canariensis* (Campanulaceae). *Molecular Ecology* **24**:3944–3963.

Martins JM, Moreira OCB, Sardos J, Maciel MG, Silva L, Moura M. 2013. Population genetics and conservation of the Azorean tree *Picconia azorica*. *Biochemical Systematics and Ecology* **49**:135–143.

Meirmans PG. 2007. The trouble with isolation by distance. *Molecular Ecology* **21**:2839–2846.

Meloni M, Reid A, Caujape-Castells J, Soto M, Fernández-Palacios JM, Conti E. 2015. High genetic diversity and population structure in the endangered Canarian endemic Ruta oreojasme (Rutaceae). *Genetica* **143**:571–580.

Morand M-E, Brachet S, Rossignol P, Dufour J, Frascaria-Lacoste N. 2002. A generalized heterozygote deficiency assessed with microsatellites in French common ash populations. *Molecular Ecology* **11**:377–385.

Moreira O, Martins JM, Moreira OCB, Sardos J, Maciel MG, Silva L, Moura M. 2013. Population genetic structure and conservation of the Azorean tree *Prunus azarica* (Rosaceae). *Plant Systematics and Evolution* **299**:1737–1748.

Moura M, Silva L, Caujapé-Castells J. 2013. Population genetics in the conservation of the Azorean shrub *Viburnum treleasei* Gand. *Plant Systematics and Evolution* **299**:1809–1817.

Moura M, Carine MA, Dias EF, Menezes de Sequeira M, Schoafer H, Silva L, Melo C, Hipólito A, Ávila SP, Silva L. 2015a. Decrypting plant evolution in the Azores archipelago. *Annual Meeting of the Society for Molecular Biology and Evolution (SMBE)*, 12–16 July 2015, Vienna, Austria.

Moura M, Silva L, Dias EF, Schoafer H, Carine MA. 2015b. A revision of the genus *Leontodon* (Asteraceae) in the Azores based on morphological and molecular evidence. *Phytotaxa* **210**:024–046.

Moura M, Carine MA, Menezes de Sequeira M. 2015c. *Aichryson santamariensis* (Crassulaceae): a new species endemic to Santa Maria in the Azores. *Phytotaxa* **234**:037–050.

Nei M. 1972. Genetic distance between populations. *American Naturalist* **106**:283–392.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**:583–590.

Nei M. (1987) *Molecular evolutionary genetics*. New York: Columbia University Press.

Obbard DJ, Harris SA, Pannell JR. 2006. Simple allelic-phenotype di-
Island. Proceedings of the National Academy of Sciences of the United States of America 108:188–193.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539.

Price JP, Wagner WL. 2004. Speciation in Hawaiian angiosperm lineages: cause, consequence, and mode. Evolution 58:2185–2200.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of 685 population structure using multilocus genotype data. Genetics 155:945–959.

Rosenberg N. 2004. Distruct: a program for the graphical display of population structure. Molecular Ecology Notes 4:137–138.

Rychlik W. 1995. Selection of primers for polymerase chain reactions. Molecular Biotechnology 3:129–134.

Scheafer H. 2003. Chorology and diversity of the Azorean flora. Dissertationes Botanicae 374:1–130.

Scheafer H. (2005) Flora of the Azores. A field guide, 2nd edn. Germany: Margraf Publishers.

Scheafer H, Schönfelder P. 2009. Smilax azorica, S. canariensis (Smilacaceae) and the genus Smilax in Europe. Monografías Del Instituto De Estudios Canarios 78:297–307.

Scheafer H, Moura M, Maciel MGB, Silva L, Rumsey F, Carine MA. 2011. The Linnean shortfall in oceanic island biogeography: a case study in the Azores. Journal of Biogeography 38:1345–1355.

Scheafer H. 2015. On the origin and systematic position of the Azorean goldenrod, Solidago azorica (Asteraceae). Phytotaxa 210:47–59.

Schuelke M. 2000. An economic method for the fluorescent labeling of PCR fragments: a poor man's approach to genotyping for research and high-throuput diagnostics. Nature Biotechnology 18:233–234.

Sibrant ALR, Hildrenbrant A, Marques FO, Weiss B, Boulesteix T, Hübscher C, Lüdemann T, Costa ACG, Cislaru JC. 2015. Morpho-structural evolution of a volcanic island developed inside an active oceanic rift: S. Miguel Island (Terceira Rift, Azores). Journal of Volcanology and Geothermal Research 301:90–106.

Silva L, Martins M, Maciel G, Moura M. (2009) Azorean vascular flora. Priorities in conservation. Ponta Delgada: Amigos dos Açores and CCPA, 116 p.

Silva L, Moura M, Schaefer H, Rumsey F, Dias EF. (2010) List of vascular plants (Tracheobionta). In: Borges PAV, Costa A, Cunha R, Gabriel R, Gonçalves V, Martins AF, Melo I, Parente M, Raposeiro P, Rodrigues P, Santos RS, Silva L, Vieira P, Vieira V, eds. A list of the terrestrial and marine biota from the Azores. Cascais:Principia, 9–33.

Silva L, Elias RB, Moura M, Meimberg H, Dias E. 2011. Genetic variability and differentiation among populations of the Azorean endemic gymnosperm Juniperus brevifolia: baseline information for a conservation and restoration perspective. Biochemical Genetics 49:715–734.

Silva L, Dias EF, Sardas J, Azevedo EB, Schaefer H, Moura M. 2015. Towards a more holistic research approach to plantconservation: the case of rare plants on oceanic islands. AoB Plants 7:plv066.

Silva L, Smith W. 2004. A characterization of the non-indigenous flora of the Azores Archipelago. Biological Invasions 6:193–204.

Stuessy TF, Oo M. 1998. Evolution and speciation of island plants. Cambridge: Cambridge University Press, 358 p.

Stuessy TF, Takayama K, Lopez-Sepulveda P, Crawford DJ. 2014. Interpretation of patterns of genetic variation in endemic plant species of oceanic islands. Botanical Journal of the Linnean Society 174:276–288.

Suda J, Kyncl T, Freiová R. 2003. Nuclear DNA amounts in Macaronesian angiosperms. Annals of Botany 92:153–164.

Takayama K, Lopez-Sepulveda P, Greimler J, Crawford DJ, Penalillo P, Baeza M, Ruiz E, Kohl G, Tremetsberger K, Gatica A, Letelier L, Novoa P, Novak J, Stuessy TF. 2015. Genetic consequences of cladogenetic vs. anagenetic speciation in endemic plants of oceanic islands. AoB PLANTS 7:plv102.

Torres-Díaz C, Ruiz E, González F, Fuentes G, Cavieres L. 2007. Genetic diversity in Nothofagus alessandrii (Fagaceae), an endangered endemic tree species of the coastal Maulino forest of Central Chile. Ann Bot 100:75–82.

Trantis KA, Borges PAV, Ladle RJ, Hortol J, Cardoso P, Gaspor C, Dinis F, Mendonça E, Silveira LMA, Gabriel R, Melo C, Santos AMC, Amorim IR, Ribeiro SP, Serrano ARM, Quartoat JAcute;, Whittaker R. 2010. Extinction debt on oceanic islands. Ecography 33:285–294.

Weeks AR, Sgro CM, Young AG, Frankham R, Mitchell NJ, Miller KA, Byrne M, Coates DJ, Eldridge MD, Sunnucks P, Breed MF, James EA, Hoffmann AA. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evolutionary Applications 4:709–725.

Wilcox BA, Murphy DD. 1985. Conservation strategy: the effects of fragmentation on extinction. The American Naturalist 125:879–887.

Whittaker R, Fernandez-Palacios JM. (2007) Island biogeography: ecology, evolution, and conservation, 2nd edn. New York: Oxford University Press.

Zhang W, Wei X, Meng H-L, Ma C-H, Jiang N-H, Zhang G-H, Yang S-C. 2015. Transcriptomic comparison of the self-pollinated and cross-pollinated flowers of Erigeron breviscapus to analyze candidate self-incompatibility-associated genes. BMC Plant Biology 15:248.