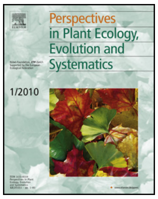
Perspectives in Plant Ecology, Evolution and Systematics 16 (2014) 190–202

Contents lists available at ScienceDirect

Perspectives in Plant Ecology, Evolution and Systematics

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



Research article

Narrow endemics to Mediterranean islands: Moderate genetic diversity but narrow climatic niche of the ancient, critically endangered *Naufraga* (Apiaceae)

Mario Fernández-Mazuecos[a,*], Pedro Jiménez-Mejías[a,1], Xavier Rotllan-Puig[b], Pablo Vargas[a]

[a] *Real Jardín Botánico (RJB-CSIC), Plaza de Murillo 2, 28014 Madrid, Spain*
[b] *Institut Mediterrani d'Estudis Avançats (CSIC-UIB), Miquel Marqués 21, 07190 Esporles, Mallorca, Balearic Islands, Spain*

ARTICLE INFO

Article history:
Received 18 November 2013
Received in revised form 18 March 2014
Accepted 4 May 2014
Available online 16 May 2014

Keywords:
Palaeoendemism
Amplified fragment length polymorphism
Plastid DNA haplotypes
Species distribution modelling
Balearic Islands
Apiaceae

ABSTRACT

Narrow endemics constitute the cornerstone of Mediterranean plant diversity. *Naufraga balearica* (Apiaceae) is a critically endangered, extremely narrow endemic plant from the western Mediterranean island of Majorca. Because the species belongs to a monotypic genus, *N. balearica* was hypothesized to be a palaeoendemism. Here we conducted phylogenetic dating, population genetic and climatic niche analyses in order to understand the evolutionary history and conservation perspectives of this flagship species. Phylogenetic dating analysis of nuclear and plastid DNA sequences revealed a late Miocene to early Pliocene divergence between *Naufraga* and its sister genus *Apium*, supporting the palaeoendemic status of the former. Amplified fragment length polymorphism (AFLP) markers and plastid DNA sequences of the five *Naufraga* populations revealed moderate genetic diversity. This diversity is in line with that of other palaeoendemisms from western Mediterranean islands, as revealed by a comparison with 22 other narrow endemic species from this region. Despite the fact that all *Naufraga* populations are located at a maximum distance of 10 km in a straight line, a strikingly strong population differentiation was found for AFLP markers, which is explained by long-term isolation likely related to short-range pollination and dispersal strategies of the species. While the species is not genetically impoverished, species distribution modelling and microclimatic monitoring revealed that narrow ecological requirements underlie the current extreme rarity of *Naufraga* and may jeopardize its long-term survival. Our results indicate that a multidisciplinary approach provides powerful tools to develop conservation strategies for evolutionarily singular lineages.

Introduction

The Mediterranean Basin, with c. 25,000 plant species (Quézel, 1985), constitutes one of the world's major biodiversity hotspots (Myers et al., 2000). Around 60% of plant species endemic to the Mediterranean region are narrow endemics, i.e. species whose distribution is restricted to a single, small area (Thompson, 2005). Hence, narrow endemics (both palaeo- and neoendemics) are considered to constitute the cornerstone of Mediterranean plant diversity (Thompson, 2005). Some of these species, termed 'extremely narrow endemics' (ENEs), are known from one or very few populations (≤5) and display very small census sizes (≤500 individuals) (López-Pujol et al., 2013). ENEs are of particular conservation concern because of the high extinction risk associated to rarity (O'Grady et al., 2004). In addition, ENEs usually display low levels of genetic diversity (Gitzendanner and Soltis, 2000; López-Pujol et al., 2013), which may limit their evolutionary viability. This genetic impoverishment is variously viewed as either a cause or consequence of rarity (Gitzendanner and Soltis, 2000). Indeed, the causes of species rarity have long been discussed, and a combination of ecological, historical and genetic factors is generally invoked to account for it (Kruckeberg and Rabinowitz, 1985).

Naufraga balearica (Fig. 1A) is an extremely narrow endemic plant from the northern coast of the western Mediterranean island

---

[*] Corresponding author at: Current address: Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK.
Tel.: +44(0)1223333934.
E-mail address: mfmazuecos@rjb.csic.es (M. Fernández-Mazuecos).

[1] Current address: School of Biological Sciences, Washington State University, Pullman 99164 WA, USA.

http://dx.doi.org/10.1016/j.ppees.2014.05.003
1433-8319/

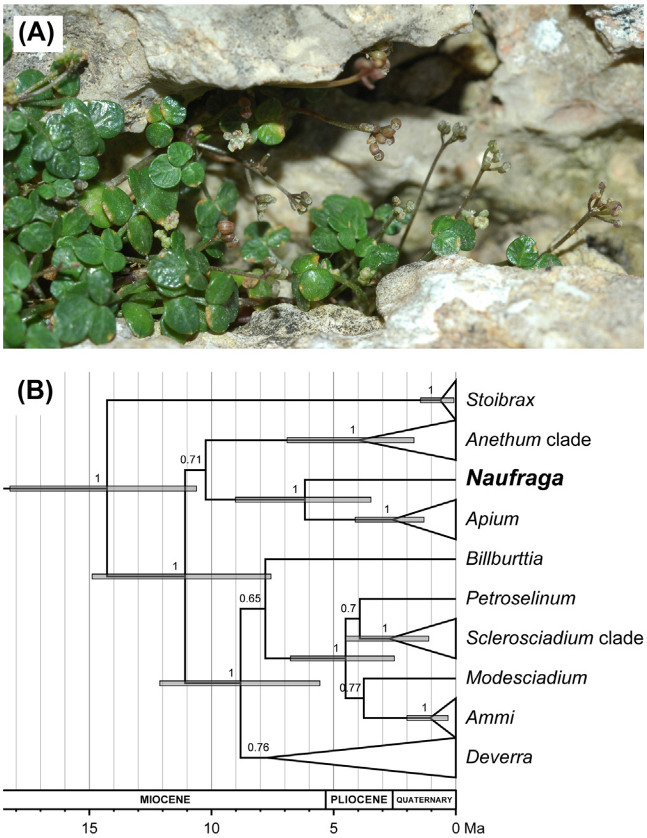


Fig. 1. (A) Specimens of *Naufraga balearica* with flowers and fruits, Cap de Catalunya population (photograph by P. Vargas). (B) Phylogenetic dating analysis of the tribe Apieae based on combined nuclear (ITS) and plastid (*rps*16) DNA sequences. The maximum clade credibility tree produced by a relaxed molecular-clock analysis in BEAST is shown. Outgroup taxa have been pruned for clarity. Values above branches indicate Bayesian posterior probabilities (PP). Node bars represent the 95% highest posterior density intervals for the divergence time estimates of clades with PP = 1.

of Majorca (Balearic Islands, Spain) (Rosselló, 2010). It is listed as critically endangered (CR) in the IUCN Red List (Moreno, 2011) and the Red List of Spanish vascular flora (Moreno, 2008). The species was first described from a single locality (Coves Blanques) discovered in 1962 (Constance and Cannon, 1967; Duvigneaud, 1970). Additional populations were found decades later in the Formentor Peninsula, not far from the *locus classicus* (Bibiloni and Soler, 2002). One locality (Finucchiaghia) was also reported in Corsica in 1981, but became extinct shortly after (Gamisans et al., 1996; Fridlender, 2001). Genetic analysis of cultivated Corsican plants indicated a very close relationship with the Coves Blanques population (but not with other populations from Majorca), which casted doubt on the spontaneity of *N. balearica* in Corsica (Fridlender and Boisselier-Dubayle, 2000).

It has long been hypothesized that *N. balearica* is a palaeoendemism (Duvigneaud, 1970). Palaeoendemics have historically been defined as relics of earlier floras which have survived in a limited portion of their past territory (Wulff, 1943; Favarger and Contandriopoulos, 1961). They are systematically isolated as a result of their early divergence, and they did not necessarily originate in the area they currently occupy. On the contrary, neoendemics originated recently in a given region and have not yet spread beyond it. They are closely related to other species, frequently in the same region. Phylogenetic analyses based on nuclear internal transcribed spacer (ITS) sequences have revealed that *Naufraga* is sister to the genus *Apium* (c. 20 spp.), and divergence between the two genera has been dated back to >4.8 Ma (Spalik et al., 2010; Banasiak et al., 2013). These results are congruent with a palaeoendemic status of *Naufraga*. However, additional evidence (from phylogenetics, population genetics and ecology) is required before firm conclusions can be drawn.

A previous population genetic analysis based on random amplified polymorphic DNA (RAPD) markers found strong genetic differentiation between populations of *N. balearica* (Fridlender and Boisselier-Dubayle, 2000). However, the population sampling in that study was unsatisfactory (number of localities included) and the reliability of RAPD markers has been questioned because of their low reproducibility (Newton et al., 1999). More reliable markers, together with a deeper sampling of individuals and populations, are therefore needed for a reliable genetic characterization of *N. balearica* populations.

Here, phylogenetic and population genetic analyses (based on nuclear and plastid DNA markers) and species distribution modelling were conducted to achieve the following objectives: (1) to estimate the divergence time of *N. balearica*; (2) to disclose the genetic diversity and spatial genetic structure of extant populations; and (3) to characterize the climatic niche of the species both at the macro- and microclimatic scales. Our results were further integrated with previous ecological and life history data in order to understand the evolutionary history and conservation perspectives of the species. Our working hypothesis in this study was that the current rarity of *N. balearica* can be explained by its narrow ecological requirements, together with its short-range pollination and dispersal strategies.

Materials and methods

Study species

Naufraga balearica Constance & Cannon (Apiaceae, Apioideae, Apieae) (hereafter *Naufraga*) is a perennial herb (Fig. 1A) inhabiting shady, humid sites on calcareous coastal cliffs, 25–250 m above sea level (Fridlender, 2001; Bibiloni and Soler, 2002). Its distribution range encompasses a short (c. 15 km) stretch of the northern Majorcan coast (Fig. 2A). It is a xenogamous, ant-pollinated species (Cursach and Rita, 2012). Flowering starts in April and ends in August, and fruiting occurs from June to September (Rosselló, 2010). Barochory seems to be its only mode of seed dispersal (Fridlender, 2001; Moragues, 2005). Vegetative reproduction by stolons is frequent (Moragues, 2005). A diploid chromosome number of $2n = 20$, with 0–2 accessory chromosomes, has been reported (Castro and Rossello, 2005). Demographic analyses have shown that populations are declining (Cursach et al., 2013; Cursach and Rita, 2013) and that seedling survival is low (Cursach and Rita, 2012). Major threats include interspecific competition, changing climatic conditions, soil erosion, umbel predation, collection and fires (Rosselló, 2010; Cursach and Rita, 2012; Cursach et al., 2013; Cursach and Rita, 2013). A certain degree of herbivore pressure is thought to benefit *Naufraga* populations by reducing interspecific competition (Cursach et al., 2013).

Phylogenetic dating

Spalik et al. (2010) and Banasiak et al. (2013) obtained the first estimates for the divergence time of *Naufraga* based on broad-scale analyses of ITS sequences of subfamily Apioideae. In order to obtain a more precise estimate, a deeper sampling of closely related genera and additional DNA markers are required. To this end, we conducted a dating analysis using sequence matrices from a separate phylogenetic study of the tribe Apieae (Jiménez-Mejías and Vargas, under review). Forty-four nuclear ITS sequences and 44

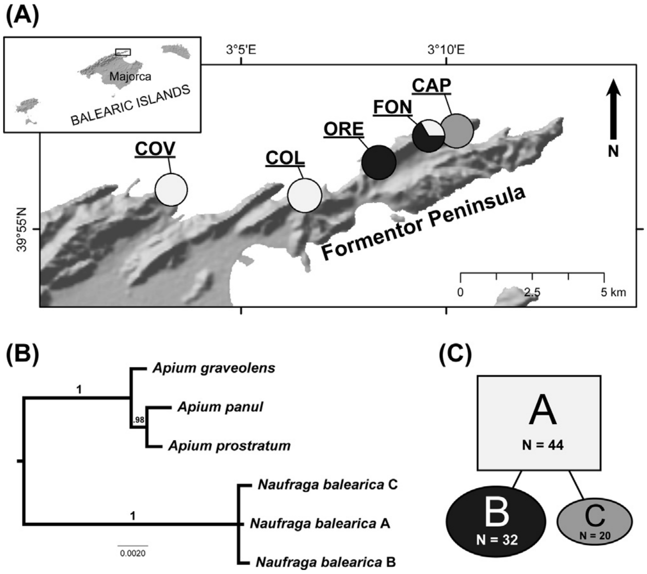


Fig. 2. Analysis of ptDNA (*rpl*32-*trn*L^{UAG}/*ycf*6-*psb*M) haplotypes of *Naufraga balearica*. The three haplotypes are represented as different shades of grey in both A and C. (A) Geographical distribution of haplotypes across the five sampled populations. Pie charts represent haplotype frequencies, obtained by sequencing 19–20 individuals per population. (B) Fifty per cent majority-rule consensus tree of the Bayesian phylogenetic analysis of ptDNA haplotypes; numbers above branches are Bayesian posterior probabilities. (C) Statistical parsimony network of ptDNA haplotypes; lines represent single nucleotide substitutions. The rectangle (haplotype A) indicates the haplotype suggested as ancestral by the TCS software. Oval and rectangle sizes are proportional to the number of sequences (N) obtained for each haplotype.

plastid DNA (ptDNA) *rps*16 sequences from 33 Apieae and 11 outgroup species (one individual per species; Table S1) were analyzed using the relaxed molecular clock approach implemented in BEAST 1.7.5 (Drummond et al., 2006; Drummond and Rambaut, 2007). Since no fossils of Apieae appropriate for calibration are known to date (Martínez-Millán, 2010; Banasiak et al., 2013), we implemented a secondary calibration based on the result of Banasiak et al.'s (2013) dating analysis of Apioideae. A lognormal distribution was fitted to the posterior distribution of ages (in million years) for the crown age of Apieae obtained by Banasiak et al. (2013) using the R package MASS (Ł. Banasiak, pers. comm.). The obtained distribution, with log(mean) = 2.724 and log(stdev) = 0.138, was then used as a prior to calibrate the crown age of Apieae in our dating analysis. Models of nucleotide substitution were selected for each DNA region under the Akaike information criterion (AIC) in jModelTest 0.1 (Guindon and Gascuel, 2003; Posada, 2008). A birth-death process (Gernhard, 2008) was employed as tree prior. The substitution rate variation was modelled using an uncorrelated lognormal distribution. Based on previous estimates for herbaceous plants, uniform prior distributions were set for the substitution rates, with ranges 5×10^{-4}–5×10^{-2} substitutions per site per Myr for ITS, and 1×10^{-4}–1×10^{-2} substitutions per site per Myr for ptDNA (see Blanco-Pastor et al., 2012 for details). For each dataset, four MCMC analyses with 10 million generations each and a sample frequency of 1000 were run through the CIPRES Science Gateway (Miller et al., 2010). Parameter analysis in Tracer 1.5 (Rambaut and Drummond, 2007) showed adequate chain length, with effective sample size (ESS) values above 300. Chains were combined using LogCombiner 1.7.5, after discarding the first 10% of sampled generations as burn-in. Trees were summarized in a maximum clade credibility (MCC) tree obtained in TreeAnotator 1.7.5 and visualized in FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Population genetics

Sampling strategy and DNA isolation

Plant materials were sampled from 100 individuals in the five known populations of *Naufraga* covering its entire known distribution (Bibiloni and Soler, 2002) (Fig. 2A; Table 1). As a result, twenty individuals were sampled per population. Given the stoloniferous character of the species, care was taken to sample distinct individuals. All plant material was collected in the field and dried in silica gel. Based on previous phylogenetic results (Spalik et al., 2010), three individuals of different species of *Apium* (*A. graveolens*, *A. prostratum* and *A. panul*) were additionally sampled to be used as the outgroup in phylogenetic and phylogeographic analyses (see below). Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen Inc., California) following the manufacturer's recommended protocols.

ptDNA sequence variation

Procedures used for ptDNA amplification and sequencing followed Fernández-Mazuecos and Vargas (2011). First, a pilot study using five individuals (one per population) of *Naufraga* was performed to find consistently amplified and variable ptDNA regions. We tested eight regions previously used in phylogeographic analyses: *rpl*32-*trn*L^{UAG}, *trn*Q-*rps*16, *trn*S-*trn*G, *trn*H-*psb*A, *pet*L-*psb*E, *psb*J-*pet*A, *ycf*6-*psb*M and *atp*I-*atp*H (Hamilton, 1999; Shaw et al., 2005, 2007; Hollingsworth et al., 2009). All regions were consistently amplified and sequenced. The two regions (*rpl*32-*trn*L^{UAG} and *ycf*6-*psb*M) that yielded nucleotide variation in the pilot study were then sequenced for all sampled individuals of *Naufraga* and outgroup. In all cases, the same standard primers were employed for amplification and sequencing. Sequences were assembled in Geneious Pro (Drummond et al., 2010). Sequences of each DNA region were separately aligned using MAFFT 6 (Katoh et al., 2002) with default parameters, and further adjustments were made by visual inspection. The three ptDNA regions were concatenated in a single matrix. All new sequences were deposited in the GenBank database (see Table S2 for accession numbers).

The concatenated dataset was analyzed using the statistical parsimony algorithm (Templeton et al., 1992), as implemented in TCS 1.21 (Clement et al., 2000), in order to infer genealogical relationships among haplotypes and phylogeographic patterns. The maximum number of differences resulting from single substitutions among haplotypes was calculated with 95% confidence limits, treating gaps as missing data.

Relationships among haplotypes were additionally assessed using Bayesian inference. Models of nucleotide substitution

Table 1
Geographic location, average AFLP gene diversity ± standard deviation, DW index values and BAPS clustering of *Naufraga balearica* populations.

Population	Label	Coordinates	Gene diversity	DW	BAPS clustering
Coves Blanques	COV	39.933° N 3.055° E	0.1995 ± 0.2016	45.15	I
Coll de la Creueta	COL	39.930° N 3.109° E	0.1149 ± 0.1763	30.73	II
Orelles de l'Ase	ORE	39.944° N 3.139° E	0.0531 ± 0.1300	19.98	III
Les Fonts Salades	FON	39.955° N 3.160° E	0.2233 ± 0.2002	48.57	IV
Cap de Catalunya	CAP	39.957° N 3.171° E	0.1970 ± 0.1879	40.57	IV
Total			0.2686 ± 0.1621		

(GTR for both DNA regions) were selected under the AIC in jModelTest 0.1. The Bayesian analysis was performed in MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) using two searches with 10 million generations each and a sample frequency of 1000. The two regions were partitioned, and substitution models were unlinked across partitions. A fifty-percent majority rule consensus tree with Bayesian posterior probabilities (PP) of clades was calculated after removing the first 10% generations as burn-in.

Amplified fragment length polymorphism

The genetic diversity and spatial genetic structure of *Naufraga* populations were analyzed using AFLPs (amplified fragment length polymophisms; Vos et al., 1995). Laboratory procedures followed Gaudeul et al. (2000). First, we performed a pilot study in which 32 primer combinations were screened in six samples (one individual per population, plus one replicate). We chose the four primer combinations that yielded the highest numbers of reproducible fragments and informative characters: *Eco*RI ACT (FAM) – *Mse*I CTG, *Eco*RI AGG (VIC) – *Mse*I CTA, *Eco*RI AGA (FAM) – *Mse*I CTA, and *Eco*RI AGG (VIC) – *Mse*I CTA. The 100 sampled individuals were then analyzed using the four chosen primer combinations. Selective PCR products were run on a capillary sequencer (ABI PRISM 3700; Applied Biosystems, Foster City, CA, USA) with the internal size standard GeneScan 500 LIZ (Applied Biosystems). Data collection and fragment sizing were performed using the software GeneMapper v3.7 (Applied Biosystems). Fragments in the range 50–500 bp were automatically scored and manually revised. The results were exported as a presence/absence (1/0) matrix. Reproducibility was estimated based on nine replicated samples (9% of the sampling) as the average proportion of correctly replicated bands (Bonin et al., 2004). Markers with low reproducibility were excluded, resulting in a final error of 1.9%. Linked alleles were removed from the matrix.

Phenotype diversity was evaluated using the R script AFLPdat ver. 2008 (Ehrich, 2006; updated in 24th June 2010). Clones were identified as those phenotypes that differed in a proportion of bands below the error rate (2%, see above). We used Arlequin v3.5 (Excoffier and Lischer, 2010) to evaluate gene diversity of populations according to Nei's formula (Nei, 1987). The 'frequency-down-weighted marker' (DW) value, a rarity measure (Schönswetter and Tribsch, 2005), which accounts for differences in sample size (Ehrich et al., 2008), was also calculated using AFLPdat. Several methods were employed to evaluate the genetic structure of populations. An unrooted neighbour-joining (NJ) tree of AFLP genotypes was obtained in PAUP* v4.0b10 (Swofford, 2002) using Nei-Li distances (Nei and Li, 1979). Branch support was evaluated with 10,000 bootstrap replicates. A principal coordinate analysis (PCoA) was performed in the package GenAlEx v6.5 (Peakall and Smouse, 2006) using Euclidean distances among samples (Huff et al., 1993). The Bayesian clustering software BAPS v6.0 (Corander et al., 2004) was also used to estimate population structure. A genetic mixture analysis was conducted at the individual level, with an upper bound to the number of populations $K = 20$. An admixture analysis was then conducted with default parameters. Analysis of molecular variance (AMOVA; Excoffier et al., 1992) was conducted in Arlequin to assess genetic differentiation among populations and BAPS groups. Finally, a Mantel test (Smouse et al., 1986) was performed in GenAlEx to evaluate isolation-by-distance, i.e. correlation between genetic and geographic distances.

Climatic niche

Species distribution modelling

Species distribution modelling (SDM) was performed to evaluate the potential distribution of *Naufraga* under present macroclimatic conditions. We employed the maximum entropy algorithm, as implemented in Maxent v3.3 (Phillips et al., 2006), because it is appropriate for low numbers of presence-only data and its good predictive performance has been demonstrated (Elith et al., 2006; Pearson et al., 2007). We retrieved a set of 19 bioclimatic variables under current conditions from the WorldClim website (www.worldclim.org; Hijmans et al., 2005). Following Fernández-Mazuecos and Vargas (2013), we then selected a set of seven variables that are uncorrelated in the western Mediterranean (including the Balearic Islands): bio3 (isothermality), bio4 (temperature seasonality), bio5 (maximum temperature of warmest month), bio6 (minimum temperature of coldest month), bio13 (precipitation of wettest month), bio14 (precipitation of driest month) and bio15 (precipitation seasonality). The seven variables were used as predictors to calibrate the distribution model in Maxent. Analyses were conducted at two different geographic scales: western Mediterranean region (latitude 32–47° N; longitude 11° W to 19° E), and Balearic Islands (latitude 38.5–40.3° N; longitude 1–4.5° E). In the occurrence dataset, we included precise coordinates of the five Balearic populations sampled for genetic analyses. Due to the doubtful spontaneity of the extinct Corsican population (Fridlender and Boisselier-Dubayle, 2000), analyses at the western Mediterranean scale were conducted both excluding and including this locality. The predictive power of Maxent with as few as five localities was demonstrated by Pearson et al. (2007). Given the low number of presence records, we did not split localities into training and test data (Pearson et al., 2007). Jackknife analyses were employed to evaluate variable contributions to the models. To convert continuous suitability values to discrete presence/absence (1/0) values, we chose the 'minimum training presence' threshold, which provides a conservative estimate of suitable areas (Pearson et al., 2007).

Microclimatic niche

To complement the modelling results, we characterized the climatic niche of *Naufraga* at a smaller spatial and temporal scale. Temperature and relative humidity in the five sampled localities were monitored in the course of one year, from September 2012 to August 2013. We used ten HOBO U23 Pro v2 data loggers (Onset Computer Corporation, Bourne, MA, USA), which recorded the two variables at 30-min intervals. Two loggers were placed at each locality, one in a shady slope facing north, with presence of *Naufraga*, and the other in a sunny slope facing south, in which *Naufraga* was absent. Distance between loggers in the same locality ranged between 75 and 310 m, while altitude differences ranged between 2 and 130 m. Resulting data were processed using the HOBOware software.

Results

Phylogenetic dating

The phylogenetic dating analysis of nuclear and plastid sequences of Apieae (Fig. 1B) recovered a sister relationship between *Naufraga* and *Apium* (PP = 1), and estimated a divergence time between the two genera in the late Miocene to early Pliocene (95% highest posterior density interval 3.5–9.0 Ma).

Population genetics

ptDNA sequence variation

Two ptDNA regions, *rpl*32-*trn*L$^{\text{UAG}}$ and *ycf*6-*psb*M, yielded nucleotide diversity within *Naufraga* in the pilot study (one nucleotide substitution each). After extensive sequencing of the two regions (Fig. 2A–C), the combined matrix consisted of 1896 bp from 96 individuals of *Naufraga* and three of *Apium*. In the TCS

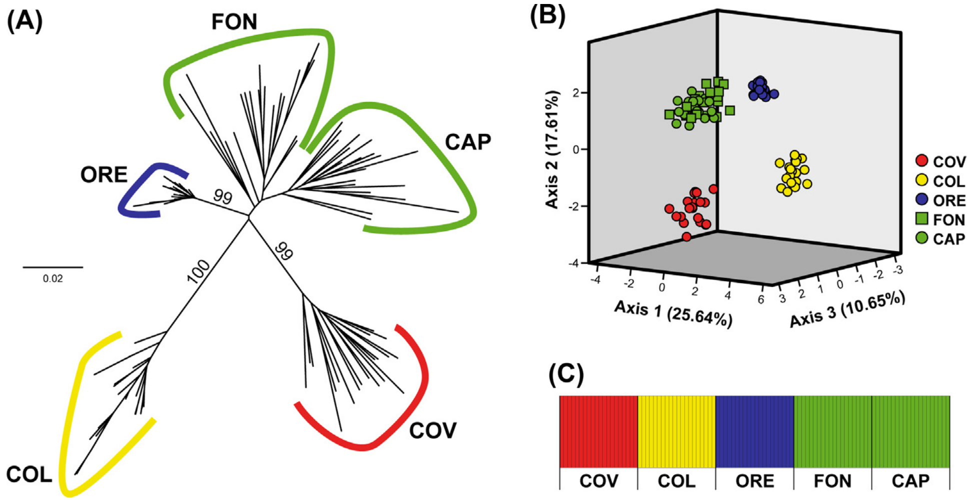


Fig. 3. Analysis of the population genetic structure of *Naufraga balearica* based on AFLP markers. Colours represent genetic clusters obtained in BAPS: red, cluster I; yellow, cluster II; blue, cluster III; green, cluster IV. Populations are labelled as in Table 1. (A) Neighbour-joining tree of AFLP genotypes. Numbers above branches are bootstrap values (in percentage). (B) Principal coordinate analysis (PCoA). Values for the first three axes are plotted, and percentage of variation explained by each axis is shown in brackets. (C) Genetic clustering of individuals based on Bayesian analysis in BAPS. The result of the admixture analysis is shown. Each vertical bar represents a single individual, with colours representing the genetic contributions of the four genetic clusters detected in the mixture analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

analysis, two unconnected networks were obtained: one formed by three haplotypes of *Naufraga*, and the other formed by three *Apium* haplotypes (one per species; results not shown). The three *Naufraga* haplotypes (A–C) formed a network with no loops and no missing haplotypes (Fig. 2C). The three haplotypes constituted a monophyletic group (PP = 1) in the Bayesian phylogenetic analysis, but relationships between them were unresolved (Fig. 2B). The central haplotype A was the most widely distributed (Fig. 2A): it was the only haplotype found in western populations (COV, COL), and it was also found in one eastern population (FON). Haplotype B was found in central-eastern populations (ORE, FON). Haplotype C was exclusively found in the easternmost population (CAP). Remarkably, all haplotypic variation was represented in the two easternmost populations (FON, CAP), separated by c. 1 km but displaying no shared haplotypes between them (Fig. 2A).

Amplified fragment length polymorphism

The final AFLP matrix had 185 characters. Gene diversity for the whole species was 0.27. Only one sample was identified as a putative clone considering an error rate of 2%. The highest population diversity and rarity (DW) values were found in population FON, while ORE displayed the lowest values (Table 1). The NJ tree (Fig. 3A), the PCoA (Fig. 3B) and the BAPS analysis (Fig. 3C) recovered congruent results. All three analyses revealed four distinct genetic clusters, each constituted by individuals from one or two populations. All individuals from each of the populations belonged to one of the clusters detected by BAPS (Fig. 3C). Clusters I, II and III were formed by individuals from populations COV, COL and ORE respectively, while cluster IV included individuals from the adjacent populations FON and CAP. No admixture between genetic clusters was detected by BAPS (Fig. 3C). In the population-based AMOVA (Table 2), similar percentages of variation were found within (53.42%) and among (46.58%) populations. When analysing BAPS groups, higher variation was found among groups (36.55%) than among populations within group IV (11.99%) (Table 2). The result of the Mantel test was not significant ($R^2 = 0.0598$; $P = 0.350$).

Climatic niche

Species distribution modelling

According to the distribution model at the scale of the western Mediterranean region excluding the Corsican locality (Fig. 4A), suitable areas for *Naufraga* include northern Majorca (where the species actually occurs), and four other small areas dispersed across the region: the adjacent island of Minorca, the north-western coast of Portugal, the coast of Kabylie in northern Algeria, and three islands of the Italian offshore Tuscan Archipelago. No suitability was inferred in the Corsican locality. According to the jackknife test (results not shown), the minimum temperature of coldest month (bio6) was the most important variable for the model, followed by the temperature seasonality (bio4). When including the Corsican locality in the model (Fig. S1), the potential distribution was still restricted in the context of the western Mediterranean, but an unrealistically broad potential distribution was obtained both in the Balearic Islands and Corsica.

Table 2
AMOVA analyses of AFLP genotypes in *Naufraga balearica* populations.

Grouping compared and source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Populations				
Among populations	4	1075.120	12.71029	46.58%
Within populations	95	1384.550	14.57421	53.42%
BAPS groups				
Among groups	3	992.645	10.35028	36.55%
Among populations within groups	1	82.475	3.39504	11.99%
Within populations	95	1384.550	14.57421	51.46%

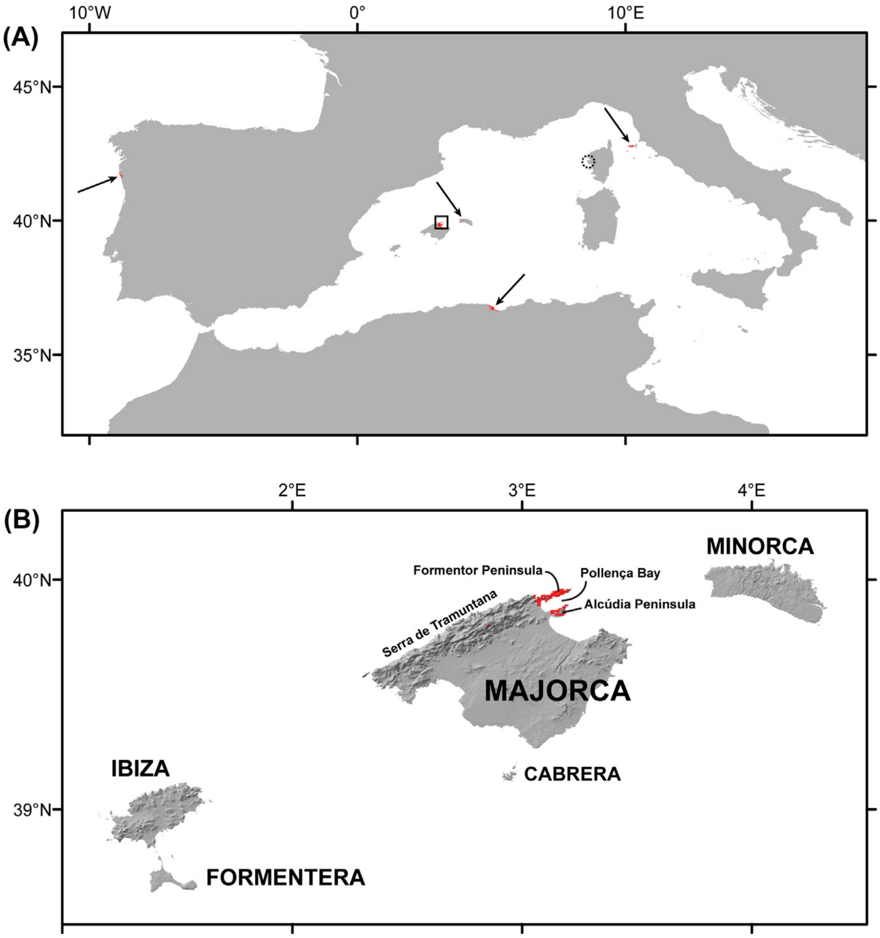


Fig. 4. Maximum entropy distribution models of *Naufraga balearica* fitted to current climatic conditions. The 'minimum training presence' threshold was applied to the logistic output of Maxent in order to obtain presence (red)/absence data. (A) Distribution model at the scale of the western Mediterranean region. Location of known populations (northern Majorca) is indicated by a small box. Other suitable areas are marked with arrows. The dotted circle marks the location of the previously reported (but now extinct) Corsican population (Gamisans et al., 1996), which was excluded from the analysis due to its doubtful spontaneity. Notice the lack of habitat suitability for this station. (B) Distribution model at the scale of the Balearic Islands. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In the distribution model at the scale of the Balearic Islands (Fig. 4B), the inferred potential distribution was restricted to the island of Majorca. No climatic suitability was inferred for the remaining islands of the Balearic archipelago. The main suitable area was located around the Pollença Bay in the north of Majorca, including the Formentor and Alcúdia peninsulas. In addition, a very small suitable area was inferred in one the highest peaks of the island, in the Serra de Tramuntana mountain range (Puig de Massanella, 1364 m). According to the jackknife test (results not shown), the precipitation of the wettest month (bio13) was the most important variable for the model.

Microclimatic niche

The one-year monitoring of temperature and relative humidity in sunny and shady sites of the five localities is summarized in Fig. 5 and Table 3. Sunny sites displayed much wider diurnal ranges of temperature (particularly in spring-summer), and much higher maximum temperatures than shady sites. Temperatures never fell below 0 °C at shady sites, and only occasionally at sunny sites. Differences in the diurnal range of relative humidity were also found, particularly in spring-summer, when lower minimum values were clearly recorded in sunny sites. More similar patterns of temperature and humidity variation were found across localities at shady than at sunny sites.

Discussion

Naufraga balearica, a pre-Mediterranean endemism

Continental islands in the central-western Mediterranean (Balearic Islands, Corsica, Sardinia, Sicily) constitute one of the regional hotspots and glacial refugia of plant diversity within the large Mediterranean hotspot (Médail and Quézel, 1997; Médail and Diadema, 2009). They represent both a reservoir of genetic diversity for widespread Mediterranean plants (Fineschi et al., 2005; López de Heredia et al., 2005) and a centre of palaeo- and neoendemism (Cardona and Contandriopoulos, 1977). Palaeoendemisms seem to be particularly frequent in the Balearic Islands (Cardona and Contandriopoulos, 1977; Vilà and Muñoz, 1999). A palaeoendemic status of *Naufraga* has long been suggested (Duvigneaud, 1970; Cardona and Contandriopoulos, 1977). Based on available knowledge, including our results, *Naufraga* fulfils several predictions of the palaeoendemic hypothesis, as classically formulated (Braun-Blanquet, 1923; Wulff, 1943; Favarger and Contandriopoulos, 1961): (1) divergence between the monotypic *Naufraga* and the

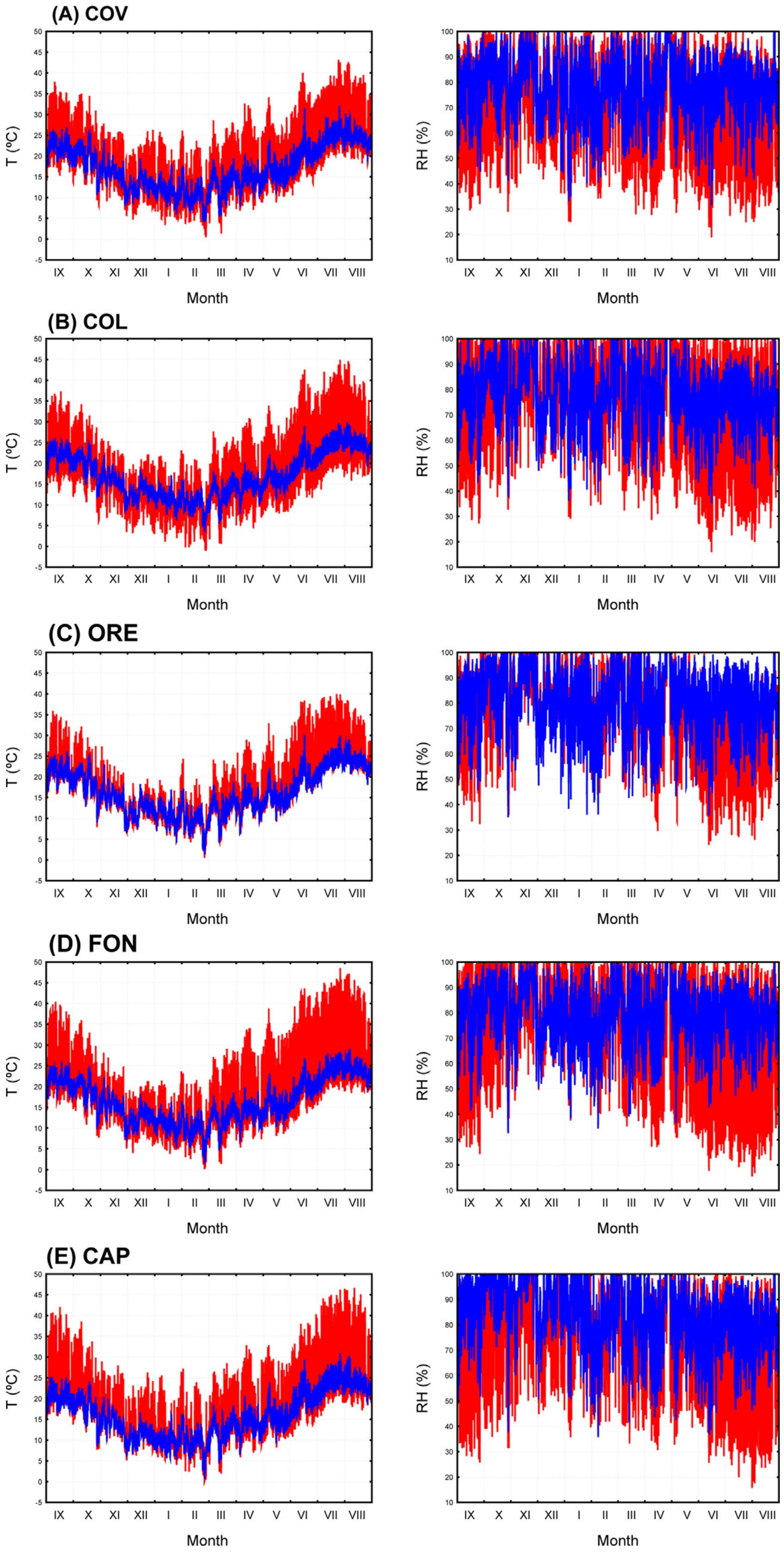


Fig. 5. One-year monitoring of temperature (left) and relative humidity (right) in five localities (A–E) of *Naufraga balearica*. For each locality, values in a north-oriented shady site (with presence of *Naufraga*) are shown in blue, and values in a south-oriented sunny site (in which *Naufraga* is absent) are shown in red. Values from September 2012 to August 2013 are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3

Descriptive statistics of temperature and relative humidity in the five localities of *Naufraga balearica* monitored during one year (September 2012–August 2013). For each locality, one north-oriented shady site (with presence of *Naufraga*) and one south-oriented sunny site (in which *Naufraga* is absent) were studied. Annual mean, minimum and maximum values for each variable are shown, as well as mean, minimum and maximum diurnal ranges (DR).

Locality	Site	Coordinates	Altitude (m)	Temperature (°C)						Relative humidity (%)					
				Mean	Min.	Max.	Mean DR	Min. DR	Max. DR	Mean	Min.	Max.	Mean DR	Min. DR	Max. DR
COV	Shady	39.93265° N, 3.05501° E	69	17.0	4.1	32.1	4.3	0.7	10.8	78	32	100	26	0	58
	Sunny	39.93062° N, 3.05459° E	98	18.5	0.5	43.2	13.8	2.3	23.3	72	19	100	46	3	73
COL	Shady	39.93034° N, 3.10927° E	64	17.1	3.5	29.9	3.7	1.0	8.6	78	35	100	25	0	59
	Sunny	39.92784° N, 3.10936° E	194	17.1	−1.1	44.9	14.7	2.3	27.2	78	16	100	47	0	79
ORE	Shady	39.94378° N, 3.13934° E	239	16.1	1.3	30.1	3.8	0.8	11.1	81	35	100	27	0	64
	Sunny	39.94349° N, 3.14013° E	217	16.8	0.5	40.0	9.7	1.5	19.9	79	24	100	35	0	67
FON	Shady	39.95458° N, 3.15956° E	140	16.3	1.5	29.1	3.9	1.2	10.2	80	33	100	25	0	61
	Sunny	39.95180° N, 3.16021° E	135	18.0	0.1	48.5	15.6	2.1	27.0	77	15	100	48	0	84
CAP	Shady	39.95659° N, 3.17083° E	170	15.8	0.7	30.9	4.4	0.9	9.2	84	36	100	24	0	62
	Sunny	39.95591° N, 3.17075° E	172	17.4	0.1	46.7	14.4	1.5	27.5	78	16	100	44	0	81

relatively species-rich sister genus *Apium* (3.5–9.0 Ma; Fig. 1B; see also Spalik et al., 2010; Banasiak et al., 2013) older than the establishment of current environmental conditions (onset of the Mediterranean climate c. 3.2 Ma; Suc, 1984); (2) systematic isolation, as shown by its phylogenetic and morphological distinctness (Ronse et al., 2010; Jiménez-Mejías and Vargas, under review); (3) marked ecological specialization that leads to a restricted potential distribution under current environmental conditions (Figs. 4 and 5); and (4) limited capacity for dispersal and range expansion (see below).

Some authors have recently proposed a different concept of island palaeo- and neoendemics based on the relative timing of speciation and island formation (Mansion et al., 2008, 2009; Salvo et al., 2010). According to these authors, an island palaeoendemic would be an island endemic whose speciation predated the formation of the island. Under this concept, *Naufraga* would not be considered a palaeoendemism, as the Balearic Islands were separated from continental Europe in the Oligocene (30–28 Ma; Rosenbaum et al., 2002), well before the split between *Naufraga* and *Apium* in the late Miocene-early Pliocene (3.5–9.0 Ma). Therefore, the presence of *Naufraga* in Majorca may be the result of long-distance dispersal. Regardless, here we followed the classical concept of palaeoendemics, as it is applicable not only to island endemics, but to any endemic to the Mediterranean basin.

In the absence of fossils, the biogeographic history of *Naufraga* cannot be easily unravelled. Such an old lineage may have been subject to considerable extinction and unrecorded biogeographic events, and is thus poorly informative in biogeographic terms (Crisp et al., 2011). Nevertheless, the fact that the divergence of *Naufraga* appears to be older than the onset of the Mediterranean climate (c. 3.2 Ma; Suc, 1984), together with its strict ecological requirements (Figs. 4 and 5; see below), suggest that *Naufraga* may be a relict from the more humid climate that prevailed in the western Mediterranean in the late Tertiary (Postigo Mijarra et al., 2009; Jiménez-Moreno et al., 2010). Assuming that *Naufraga* has since conserved a similar climatic niche, fail to adapt to increasing aridification, to the establishment of summer drought and to subsequent Quaternary glacial cycles would have hindered diversification and led the species to take refuge in a restricted area with favourable conditions. A similar hypothesis has been proposed for a Sicilian palaeoendemism belonging to the same family (Apiaceae), *Petagnaea gussonei*, whose divergence time has been estimated to be >15 Ma (Kadereit et al., 2008; De Castro et al., 2009).

Patterns of genetic diversity in narrow endemics to Mediterranean islands

Narrow endemic species of angiosperms from central-western Mediterranean islands show a wide variation in genetic diversity, depending on population sizes, distribution range, as well as historical and ecological factors (Table 4). A better comprehension of these factors is important to understand not only the evolutionary history of species, but also their conservation perspectives (Frankham, 1995). The palaeo- vs. neoendemic status of Mediterranean island species may be one of the historical factors influencing the genetic diversity of current populations. Thus, the moderate genetic diversity of *Naufraga* contrasts with the extremely low diversity found in another extremely narrow endemic species of Apiaceae from Majorca, *Coristospermum huteri* (López-Pujol et al., 2013). *C. huteri* is probably a neoendemism, and its low diversity has been explained by a founder effect resulting from hypothetical recent dispersal from the continent (López-Pujol et al., 2013). Another island narrow endemic with low genetic diversity, *Delphinium requienii*, seems to be also a neoendemism (Orellana et al., 2009; Jabbour and Renner, 2012). Genetic

Table 4
Population genetic studies of narrow endemic species of angiosperms from Central-Western Mediterranean continental islands.

Taxon	Distribution	Markers	Main genetic features	Source
Anchusa crispa (Boraginaceae)	Corsica, Sardinia	Allozymes	No variation within populations or within geographic regions; low differentiation among regions.	Quilichini et al. (2004)
Corso-Sardinian endemic *Anchusa* spp. (Boraginaceae)	Corsica, Sardinia	AFLP	Relatively high diversity; very low divergence between populations and minor role of isolation-by-distance.	Coppi et al. (2008)
Sardinian endemic *Aquilegia* spp. (Ranunculaceae)	C and E Sardinian mountains	AFLP	Low intra-population diversity; high spatial structure due to extreme isolation and genetic drift.	Garrido et al. (2012)
Centaurea horrida (Asteraceae)	N coast of Sardinia and associated islands	SSR	Considerable genetic variation; medium-high differentiation among populations.	Mameli et al. (2008)
Coristospermum huteri (Apiaceae)[b]	Tramuntana Range, Majorca (Balearic Islands)	Allozymes	Extreme low diversity, probably due to founder effect.	López-Pujol et al. (2013)
Crepis triasii (Asteraceae)	Majorca, Minorca, Cabrera (Balearic Islands)	Allozymes, cpSSR	High diversity and high structure both at the regional and at the landscape level.	Mayol et al. (2012)
Cytisus aeolicus (Fabaceae)	Aeolian Archipelago (N Sicily)	Allozymes	Low diversity probably due to founder effect; low differentiation among populations.	Conte et al. (1998)
Daphne rodriguezii (Thymelaeaceae)	Minorca (Balearic Islands)	AFLP, ptDNA sequences	Low diversity within populations; low population differentiation for AFLP, clear differentiation for ptDNA.	Calviño-Cancela et al. (2012)
Delphinium requienii (Ranunculaceae)	Hyères Archipelago (S France)	Allozymes	Low diversity and evidence of inbreeding.	Orellana et al. (2009)
Digitalis minor (Plantaginaceae)	Majorca, Minorca, Cabrera (Balearic Islands)	RAPD	Moderate diversity; low population differentiation.	Sales et al. (2001)
Femeniasia balearica (Asteraceae)[a]	N coast of Minorca (Balearic Islands)	AFLP	Relatively high diversity; high differentiation between geographical groups of populations.	Vilatersana et al. (2007)
Hippocrepis balearica, *H. grosii* (Fabaceae)	Balearic Islands	RAPD	Moderate diversity; highly structured geographic pattern.	Rosselló et al. (2002)
Lamyropsis microcephala (Asteraceae)	C Sardinian mountains	ISSR	Diversity ranging from low to moderate; differentiation between populations detected.	Bacchetta et al. (2013)
Leopoldia gussonei (Asparagaceae)	S Sicily	AFLP	Low diversity; significant differentiation among populations.	Vandepitte et al. (2013)
Linaria capraria (Plantaginaceae)	Tuscan Archipelago	ISSR	Medium-high diversity; generally high differentiation among islands.	Coppi et al. (2013)
Lysimachia minoricensis (Myrsinaceae)[c]	Minorca (Balearic Islands)	Allozymes, RAPD	No detectable genetic variation in plants preserved in botanical gardens.	Ibáñez et al. (1999), Calero et al. (1999)
Medicago citrina (Fabaceae)	Ibiza, Cabrera (Balearic Islands), Columbretes Islands	AFLP	Overall high diversity; high differentiation among populations, variable differentiation among islands.	Juan et al. (2004)
Naufraga balearica (Apiaceae)[a,b]	N Majorca (Balearic Islands)	RAPD, AFLP, ptDNA sequences	Moderate diversity; high population differentiation for AFLP and RAPD, lower differentiation for ptDNA sequences.	Fridlender and Boisselier-Dubayle (2000), this study
Petagnaea gussonei (Apiaceae)[a]	NE Sicilian mountains	cpSSR, AFLP	Relatively high diversity; low differentiation among populations.	De Castro et al. (2013)
Rhamnus ludovici-salvatoris (Rhamnaceae)	Majorca, Cabrera (Balearic Islands)	RAPD	Lower diversity and stronger structure than the closely related, widespread *R. alaternus*.	Ferriol et al. (2009)
Rhamnus persicifolia (Rhamnaceae)	C and E Sardinian mountains	ISSR	High overall diversity but depauperation of peripheral populations; moderate differentiation correlated with distance.	Bacchetta et al. (2011)
Senecio rodriguezii (Asteraceae)	Majorca, Minorca (Balearic Islands)	ptDNA sequences	High levels of genetic diversity; significant geographical structuring.	Molins et al. (2009)
Zelkova sicula (Ulmaceae)	S Sicily	ISSR, AFLP	Extremely reduced diversity, monomorphic patterns found with both markers	Fineschi et al. (2004)

[a] Species belonging to monotypic genera.
[b] Species confirmed as extremely narrow endemics (ENEs, ≤5 populations, ≤500 individuals).
[c] Species extinct in the wild.

impoverishment may make these species more prone to extinction as a result of inbreeding depression (Frankham, 1998, 2005; but see Bouzat, 2010). On the contrary, higher genetic diversity levels, similar to those of *Naufraga*, are found in other monotypic genera and putative palaeoendemics. In particular, the above mentioned palaeoendemic Apiaceae *P. gussonei*, from Sicily, displays relatively high diversity based on both AFLP and cpSSR markers (De Castro et al., 2013). Similarly, *Femeniasia balearica*, an endemic Asteraceae from the northern coast of Minorca (Balearic Islands), does not show genetic depauperation based on AFLP markers (Vilatersana et al., 2007). Interestingly, this species seems to have diverged around the same time as *Naufraga* (Barres et al., 2013). Even if these palaeoendemic species are genetically healthy, a deep knowledge of their population genetic structure may help in designing strategies to ensure their long-term survival (see below for *Naufraga*). Indeed, maintaining genetic diversity, and thus evolutionary potential, can reduce the probability of extinction (Frankham, 2005), and it can play a role in the local adaptation of populations (Leimu and Fischer, 2008).

Some species do not follow the patterns described above, such as the neoendemic but genetically diverse *Linaria capraria* (Coppi et al., 2013; Fernández-Mazuecos et al., 2013) and the putative palaeoendemic but depauperate *Cytisus aeolicus* (Conte et al., 1998; Troia, 2012). Therefore, other factors are at work, and additional phylogenetic dating and population genetic studies are needed to determine the correlates of genetic diversity in Mediterranean island endemics and their consequences for conservation.

Long term isolation of Naufraga populations

The geographic structure of genetic diversity displayed by *Naufraga* is striking, particularly for an outcrossing species (Hamrick and Godt, 1996). AFLP markers revealed very strong differentiation and no evidence of recent gene flow between four clusters located at a maximum distance of 10 km in a straight line (Fig. 3). This is congruent with previous results based on RAPD markers (Fridlender and Boisselier-Dubayle, 2000). This remarkable structure would be the result of genetic drift acting on small isolated populations (Ellstrand and Elam, 1993). The absence of gene flow between nearby populations can be explained by life history traits, particularly the short-range pollination and dispersal strategies of the species. *Naufraga* is exclusively pollinated by ants (myrmecophily), mostly *Plagiolepis pygmaea* and *Lasius grandis* (Cursach and Rita, 2012). Myrmecophilous pollination is generally thought to result in short-distance gene flow (Wyatt and Stoneburner, 1981; Svensson, 1985; Peakall and Beattie, 1991), which may lead to the genetic structuring of populations, even at very small scales (Peterson et al., 2002). Likewise, *Naufraga* seeds lack any obvious adaptation for long-distance dispersal. Barochory is the only reported dispersal mechanism (Fridlender, 2001; Moragues, 2005), which probably contributes to the lack of gene flow and strong differentiation between populations. Indeed, populations FON and CAP, separated by c. 1 km, display differentiation as based on ptDNA sequences (no shared haplotypes; Fig. 2), but are undifferentiated according to nuclear AFLP markers (Fig. 3). Given that plastids seem to be maternally inherited in Apiaceae (Corriveau and Coleman, 1988), and therefore dispersed by seeds, the ptDNA differentiation pattern between FON and CAP suggests that gene flow by seeds is highly restricted in *Naufraga*. *F. balearica* also shows geographically structured genetic diversity over short distances (18 km), which has been explained by its poor dispersal capacity (Vilatersana et al., 2007). On the contrary, *P. gussonei* populations show low genetic differentiation as a result of efficient pollen and seed flow (De Castro et al., 2013).

Narrow ecological requirements jeopardize the survival of Naufraga

The ecological requirements of *Naufraga*, resulting from the historical factors discussed above, probably constitute one of the main determinants of its current rarity. According to the distribution model at the scale of the western Mediterranean region (excluding the Corsican locality; Fig. 4A), macroclimatic conditions appropriate for *Naufraga* are extremely rare in the western Mediterranean. The presumed non-native status of *Naufraga* on Corsica (Fridlender and Boisselier-Dubayle, 2000) is supported by the unrealistically broad potential distribution in the Balearic Islands and Corsica obtained when including the Corsican locality in the model, together with the fact that no suitability was recovered in Corsica when excluding that locality.

A similar pattern of habitat rarity was found when the potential distribution was modelled at the scale of the Balearic Islands (Fig. 4B). Habitat suitability was obtained for Minorca in the Mediterranean-scale model, but not in the Balearic Island-scale model, which may have been a result of the different background used in the two models. Despite the overall habitat rarity obtained under the two models, they both suggested that most of the Formentor Peninsula and adjacent areas in northern Majorca are climatically suitable for *Naufraga*. Yet, populations are restricted to a few isolated localities in the north-facing coastal slopes of Formentor. Our monitoring of temperature and humidity in these localities (Fig. 5) revealed that microclimatic conditions are crucial for the survival of *Naufraga*. Remarkable differences in diurnal ranges of both temperature and humidity, as well as in maximum temperatures (particularly during the flowering and fruiting periods), were encountered when comparing shady sites inhabited by *Naufraga* with sunny sites where the plant is absent. This suggests that the potential distribution of *Naufraga* is even narrower than that recovered by the distribution model.

In addition to climate, other ecological factors not analyzed here, including substrate preferences (calcareous cliffs) and interspecific competition (ruderalization), may restrict the distribution of *Naufraga* in Majorca (Duvigneaud, 1970; Rosselló, 2010; Cursach and Rita, 2013) and probably contribute to the genetic isolation of populations. These factors are known to determine the distribution of other Balearic endemics (Galmes et al., 2009). In particular, a morphologically similar (but phylogenetically unrelated) species of Apiaceae, *Helosciadium bermejoi*, is distributed in coastal environments of the adjacent island of Minorca, and seems to be influenced by somehow similar ecological restrictions (Ronse et al., 2010; Banasiak et al., 2013; Rita and Cursach, 2013).

Given its ecological requirements, range expansion of *Naufraga* would depend on long-distance dispersal to other distant suitable areas in the western Mediterranean (Fig. 4A). Recurrent long-distance dispersal events have been reported in Mediterranean plant species with unspecialized dispersal mechanisms, but with larger distributions as a result of their ecological requirements broader than those of *Naufraga* (Guzmán and Vargas, 2009; Fernández-Mazuecos and Vargas, 2010, 2011). Indeed, the genetic differentiation detected among *Naufraga* populations suggests that the recent role of long-distance dispersal has merely been incidental (if any). In addition, interspecific competition (Cursach et al., 2013) may have contributed to hinder the establishment of new populations. Therefore, a combination of narrow ecological requirements, unassisted dispersal and interspecific competition seems to have contributed to the current extreme rarity of our study species.

Consequences for conservation biology

The conservation biology of *Naufraga* has been extensively investigated by previous authors using approaches from reproductive biology, ecology, genetics and demography (Fridlender and Boisselier-Dubayle, 2000; Cursach and Rita, 2012, 2013; Cursach et al., 2013). The marked population decline of the species (Cursach and Rita, 2013) makes it crucial to undertake scientifically sound measures to guarantee the long-term survival of this unique palaeoendemism. Indeed, our results provide new genetic and ecological insights for the conservation and management of the species. A moderate genetic diversity was detected both at the species and population levels, which is evenly distributed within and among populations (Tables 1 and 2). This seems to rule out genetic impoverishment and inbreeding depression as immediate causes of threat. We have identified four evolutionary significant units (ESUs), i.e. units within species that are sufficiently differentiated to require separate management (Frankham, 2010). These ESUs are the strongly differentiated genetic clusters identified using AFLP markers, corresponding to the COV, COL, ORE and FON-CAP populations. The FON-CAP unit preserves the highest genetic diversity (considering both AFLP and ptDNA sequence markers; Figs. 2 and 3; Table 1), but all units display a high number of rare AFLP markers (see DW index values in Table 1). Therefore, a broad genetic sample from the four ESUs should be preserved by ex situ conservation programmes (seed banks, botanic gardens). This will potentially allow future reintroductions or population reinforcements (e.g. Rita and Cursach, 2013), whose success will heavily depend upon the genetic quality of the available ex situ sample (see Calero et al., 1999; Ibáñez et al., 1999 for the possible genetic causes behind the failed reintroduction attemps of *Lysimachia minoricensis* in Minorca). Given the strong differentiation and apparently old divergence of the four ESUs, gene flow among them should, in principle, be avoided, and any future population reinforcement should be conducted using samples from the same population in order to avoid outbreeding depression and to preserve potentially adaptive lineages (see Frankham et al., 2011). Finally, our macro- and microclimatic results should also be considered when selecting appropriate sites for future reintroductions.

Conclusions

Our results reveal that a combination of species distribution modelling and microclimatic monitoring provides a powerful tool to analyze the climatic niches of narrow endemics. The future of Mediterranean narrow endemics with strict climatic requirements is uncertain given the expected climate change (e.g. Blanco-Pastor et al., 2013). Under this scenario, a thorough knowledge of genetic features, including population diversity and geographic structure, is needed in order to develop conservation strategies for palaeoendemic species such as *Naufraga*.

Acknowledgements

The authors thank Emilio Cano and Alberto Herrero for technical support; S.R. Downie for DNA samples of *Apium*; K. Spalik and Ł. Banasiak for advice on the dating analysis. This research was funded by Banco Santander, the Spanish National Research Council (CSIC) and Fundación General CSIC (FGCSIC) through a *Proyecto Cero* on endangered species 2010 (“Do all endangered species hold the same value?: origin and conservation of living fossils of flowering plants endemic to Spain”).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ppees.2014.05.003.

References

Bacchetta, G., Fenu, G., Gentili, R., Mattana, E., Sgorbati, S., 2013. Preliminary assessment of the genetic diversity in *Lamyropsis microcephala* (Asteraceae). Plant Biosyst. 147, 500–507.

Bacchetta, G., Fenu, G., Mattana, E., Zecca, G., Grassi, F., Casazza, G., Minuto, L., 2011. Genetic variability of the narrow endemic *Rhamnus persicifolia* Moris (Rhamnaceae) and its implications for conservation. Biochem. Syst. Ecol. 39, 477–484.

Banasiak, Ł., Piwczyński, M., Uliński, T., Downie, S.R., Watson, M.F., Shakya, B., Spalik, K., 2013. Dispersal patterns in space and time: a case study of Apiaceae subfamily Apioideae. J. Biogeogr. 40, 1324–1335.

Barres, L., Sanmartín, I., Anderson, C.L., Susanna, A., Buerki, S., Galbany-Casals, M., Vilatersana, R., 2013. Reconstructing the evolution and biogeographic history of tribe Cardueae (Compositae). Am. J. Bot. 100, 867–882.

Bibiloni, G., Soler, J., 2002. Notes florístiques de les Illes Balears (XIV). Aportació al coneixement de la flora de Mallorca. Boll. Soc. Hist. Nat. Balears 45, 51–57.

Blanco-Pastor, J.L., Fernández-Mazuecos, M., Vargas, P., 2013. Past and future demographic dynamics of alpine species: limited genetic consequences despite dramatic range contraction in a plant from Sierra Nevada (Spain). Mol. Ecol. 22, 4177–4195.

Blanco-Pastor, J.L., Vargas, P., Pfeil, B.E., 2012. Coalescent simulations reveal hybridization and incomplete lineage sorting in Mediterranean *Linaria*. PLoS ONE 7, e39089.

Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F., Brochmann, C., Taberlet, P., 2004. How to track and assess genotyping errors in population genetics studies. Mol. Ecol. 13, 3261–3273.

Bouzat, J.L., 2010. Conservation genetics of population bottlenecks: the role of chance, selection, and history. Conserv. Genet. 11, 463–478.

Braun-Blanquet, J., 1923. L'origine et le développement des flores dans le massif central de France. L. Lhomme, Paris.

Calero, C., Ibáñez, O., Mayol, M., Rosselló, J.A., 1999. Random amplified polymorphic DNA (RAPD) markers detect a single phenotype in *Lysimachia minoricensis* J.J. Rodr. (Primulaceae), a wild extinct plant. Mol. Ecol. 8, 2133–2136.

Calviño-Cancela, M., Escudero, M., Rodríguez-Pérez, J., Cano, E., Vargas, P., Velo-Antón, G., Traveset, A., 2012. The role of seed dispersal, pollination and historical effects on genetic patterns of an insular plant that has lost its only seed disperser. J. Biogeogr. 39, 1996–2006.

Cardona, M.A., Contandriopoulos, J., 1977. L'endemisme dans les flores insulaires méditerranéennes. Mediterránea 2, 49–77.

Castro, M., Rossello, J.A., 2005. Chromosome numbers in plant taxa endemic to the Balearic Islands. Bot. J. Linn. Soc. 148, 219–228.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1659.

Constance, L., Cannon, J.F.M., 1967. *Naufraga* – a new genus of Umbelliferae from Mallorca. Feddes Repert 74, 1–4.

Conte, L., Troia, A., Cristofolini, G., 1998. Genetic diversity in *Cytisus aeolicus* Guss. (Leguminosae), a rare endemite of the Italian flora. Plant Biosyst. 132, 239–249.

Coppi, A., Guidi, T., Viciani, D., Foggi, B., 2013. Genetic structure of *Linaria capraria* Mill. (Plantaginaceae) and endemic species of the Tuscan Archipelago (central Mediterranean). Plant Biosyst. 148, 249–258.

Coppi, A., Mengoni, A., Selvi, F., 2008. AFLP fingerprinting of *Anchusa* (Boraginaceae) in the Corso-Sardinian system: genetic diversity, population differentiation and conservation priorities in an insular endemic group threatened with extinction. Biol. Conserv. 141, 2000–2011.

Corander, J., Waldmann, P., Marttinen, P., Sillanpää, M.J., 2004. BAPS 2: enhanced possibilities for the analysis of genetic population structure. Bioinformatics 20, 2363–2369.

Corriveau, J.L., Coleman, A.W., 1988. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Am. J. Bot. 75, 1443–1458.

Crisp, M.D., Trewick, S.A., Cook, L.G., 2011. Hypothesis testing in biogeography. Trends Ecol. Evol. 26, 66–72.

Cursach, J., Moragues, E., Rita, J., 2013. Herbivory and plant competition effects on the population dynamics of *Naufraga balearica*, a threatened narrow endemic species of the Balearic Islands. Plant Biosyst. 147, 508–517.

Cursach, J., Rita, J., 2012. Implications of the reproductive biology of the narrow endemic *Naufraga balearica* (Apiaceae) for its conservation status. Plant Syst. Evol. 298, 581–596.

Cursach, J., Rita, J., 2013. *Naufraga balearica*, a threatened narrow endemism of the Balearic Islands (western Mediterranean basin): assessing the population dynamics of two subpopulations. Plant Spec. Biol. 29, 192–201.

De Castro, O., Cennamo, P., De Luca, P., 2009. Analysis of the genus *Petagnaea* Caruel (Apiaceae), using new molecular and literature data. Plant Syst. Evol. 278, 239–249.

De Castro, O., Sepe, F., Di Maio, A., Cennamo, P., De Luca, P., Gianguzzi, L., Menale, B., 2013. Genetic structure in the paleoendemic and endangered *Petagnaea gussonei* (Spreng.) Rauschert (Saniculoideae, Apiaceae) and implications for its conservation. Plant Syst. Evol. 299, 209–223.

Drummond, A.J., Ashton, B., Cheung, M., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T., Wilson, A., 2010. Geneious version 5.0. http://www.geneious.com

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, 699–710.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Duvigneaud, J., 1970. Écologie de *Naufraga balearica* Constance et Cannon (Umbelliferae). Bull. Soc. Roy. Bot. Belgique 103, 31–38.

Ehrich, D., 2006. AFLPdat: a collection of R functions for convenient handling of AFLP data. Mol. Ecol. Notes 6, 603–604.

Ehrich, D., Alsos, I.G., Brochmann, C., 2008. Where did the northern peatland species survive the dry glacials: cloudberry (*Rubus chamaemorus*) as an example. J. Biogeogr. 35, 801–814.

Elith, J., Graham, C.H., Anderson, R.P., Dudík, M., Ferrier, S., Guisan, A., Hijmans, R.J., Huettmann, F., Leathwick, J.R., Lehmann, A., 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography 29, 129–151.

Ellstrand, N.C., Elam, D.R., 1993. Population genetic consequences of small population size: implications for plant conservation. Annu. Rev. Ecol. Systemat. 24, 217–242.

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567.

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

Favarger, C., Contandriopoulos, J., 1961. Essai sur l'endémisme. B. Soc. Bot. Suisse 71, 384–408.

Fernández-Mazuecos, M., Blanco-Pastor, J.L., Vargas, P., 2013. A phylogeny of toadflaxes (*Linaria* Mill.) based on nuclear internal transcribed spacer sequences: systematic and evolutionary consequences. Int. J. Plant Sci. 174, 234–249.

Fernández-Mazuecos, M., Vargas, P., 2010. Ecological rather than geographical isolation dominates Quaternary formation of Mediterranean *Cistus* species. Mol. Ecol. 19, 1381–1395.

Fernández-Mazuecos, M., Vargas, P., 2011. Historical isolation *versus* recent long-distance connections between Europe and Africa in bifid toadflaxes (*Linaria* sect. *Versicolores*). PLoS ONE 6, e22234.

Fernández-Mazuecos, M., Vargas, P., 2013. Congruence between distribution modelling and phylogeographical analyses reveals Quaternary survival of a toadflax species (*Linaria elegans*) in oceanic climate areas of a mountain ring range. New Phytol. 198, 1274–1289.

Ferriol, M., Llorens, L., Gil, L., Boira, H., 2009. Influence of phenological barriers and habitat differentiation on the population genetic structure of the balearic endemic *Rhamnus ludovici-salvatoris* Chodat and *R. alaternus* L. Plant Syst. Evol. 277, 105–116.

Fineschi, S., Cozzolino, S., Migliaccio, M., Musacchio, A., Innocenti, M., Vendramin, G.G., 2005. Sicily represents the Italian reservoir of chloroplast DNA diversity of *Quercus ilex* L. (Fagaceae). Ann. For. Sci. 62, 79–84.

Fineschi, S., Cozzolino, S., Migliaccio, M., Vendramin, G.G., 2004. Genetic variation of relic tree species: the case of Mediterranean *Zelkova abelicea* (Lam.) Boisser and *Z. sicula* Di Pasquale, Garfıànd Quézel (Ulmaceae). Forest. Ecol. Manag. 197, 273–278.

Frankham, R., 1995. Conservation genetics. Annu. Rev. Genet. 29, 305–327.

Frankham, R., 1998. Inbreeding and extinction: island populations. Conserv. Biol. 12, 665–675.

Frankham, R., 2005. Genetics and extinction. Biol. Conserv. 126, 131–140.

Frankham, R., 2010. Challenges and opportunities of genetic approaches to biological conservation. Biol. Conserv. 143, 1919–1927.

Frankham, R., Ballou, J.D., Eldridge, M.D.B., Lacy, R.C., Ralls, K., Dudash, M.R., Fenster, C.B., 2011. Predicting the probability of outbreeding depression. Conserv. Biol. 25, 465–475.

Fridlender, A., 2001. *Naufraga balearica* Constance et Cannon (Apiaceae); description, écologie, répartition et projets de conservation en Corse. J. Bot. Soc. Bot. France, 7–24.

Fridlender, A., Boisselier-Dubayle, M.C., 2000. Comparaison de la diversité génétique (RAPD) de collections ex situ et de populations naturelles de *Naufraga balearica* Constance & Cannon. C.R. Acad. Sci. III-Vie 323, 399–406.

Galmes, J., Conesa, M.A., Cifre, J., Gulías, J., Medrano, H., Ribas-Carbó, M., Flexas, J., 2009. Ecofisiología de las plantas endémicas de las Islas Baleares en el contexto Mediterráneo. Ecosistemas 19, 10–23.

Gamisans, J., Moret, J., Fridlender, A., Deschâtres, R., Dutartre, G., 1996. Le *Naufraga balearica* est-il éteint en Corse? Étude du site originel, recherche de stations comparables, possibilités de réintroduction. Candollea 51, 552–557.

Garrido, J.L., Fenu, G., Mattana, E., Bacchetta, G., 2012. Spatial genetic structure of *Aquilegia* taxa endemic to the island of Sardinia. Ann. Bot. 109, 953–964.

Gaudeul, M., Taberlet, P., Till-Bottraud, I., 2000. Genetic diversity in an endangered alpine plant, *Eryngium alpinum* L. (Apiaceae), inferred from amplified fragment length polymorphism markers. Mol. Ecol. 9, 1625–1637.

Gernhard, T., 2008. The conditioned reconstructed process. J. Theor. Biol. 253, 769–778.

Gitzendanner, M.A., Soltis, P.S., 2000. Patterns of genetic variation in rare and widespread plant congeners. Am. J. Bot. 87, 783–792.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Guzmán, B., Vargas, P., 2009. Long-distance colonization of the Western Mediterranean by *Cistus ladanifer* (Cistaceae) despite the absence of special dispersal mechanisms. J. Biogeogr. 36, 954–968.

Hamilton, M.B., 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Mol. Ecol. 8, 521–523.

Hamrick, J.L., Godt, M.J.W., 1996. Effects of life history traits on genetic diversity in plant species. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 351, 1291–1298.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25, 1965–1978.

Hollingsworth, P.M., Forrest, L.L., Spouge, J.L., Hajibabaei, M., Ratnasingham, S., van der Bank, M., Chase, M.W., Cowan, R.S., Erickson, D.L., Fazekas, A.J., Graham, S.W., James, K.E., Kim, K.-J., Kress, W.J., Schneider, H., van AlphenStahl, J., Barrett, S.C.H., van den Berg, C., Bogarin, D., Burgess, K.S., Cameron, K.M., Carine, M., Chacón, J., Clark, A., Clarkson, J.J., Conrad, F., Devey, D.S., Ford, C.S., Hedderson, T.A.J., Hollingsworth, M.L., Husband, B.C., Kelly, L.J., Kesanakurti, P.R., Kim, J.S., Kim, Y.-D., Lahaye, R., Lee, H.-L., Long, D.G., Madriñán, S., Maurin, O., Meusnier, I., Newmaster, S.G., Park, C.-W., Percy, D.M., Petersen, G., Richardson, J.E., Salazar, G.A., Savolainen, V., Seberg, O., Wilkinson, M.J., Yi, D.-K., Little, D.P., 2009. A DNA barcode for land plants. Proc. Natl. Acad. Sci. U.S.A. 106, 12794–12797.

Huff, D.R., Peakall, R., Smouse, P.E., 1993. RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.]. Theor. Appl. Genet. 86, 927–934.

Ibáñez, O., Calero, C., Mayol, M., Rosselló, J.A., 1999. Isozyme uniformity in a wild extinct insular plant, *Lysimachia minoricensis* J.J. Rodr. (Primulaceae). Mol. Ecol. 8, 813–817.

Jabbour, F., Renner, S.S., 2012. A phylogeny of Delphinieae (Ranunculaceae) shows that *Aconitum* is nested within *Delphinium* and that Late Miocene transitions to long life cycles in the Himalayas and Southwest China coincide with bursts in diversification. Mol. Phylogenet. Evol. 62, 928–942.

Jiménez-Moreno, G., Fauquette, S., Suc, J.P., 2010. Miocene to Pliocene vegetation reconstruction and climate estimates in the Iberian Peninsula from pollen data. Rev. Palaeobot. Palynol. 162, 403–415.

Juan, A., Crespo, M.B., Cowan, R.S., Lexer, C., Fay, M.F., 2004. Patterns of variability and gene flow in *Medicago citrina*, an endangered endemic of islands in the western Mediterranean, as revealed by amplified fragment length polymorphism (AFLP). Mol. Ecol. 13, 2679–2690.

Kadereit, J.W., Repplinger, M., Schmalz, N., Uhink, C.H., Wörz, A., 2008. The phylogeny and biogeography of Apiaceae subf. Saniculoideae tribe Saniculeae: from south to north and south again. Taxon 57, 365–382.

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066.

Kruckeberg, A.R., Rabinowitz, D., 1985. Biological aspects of endemism in higher plants. Annu. Rev. Ecol. Systemat. 16, 447–479.

Leimu, R., Fischer, M., 2008. A meta-analysis of local adaptation in plants. PLoS ONE 3, e4010.

López-Pujol, J., Martinell, M.C., Massó, S., Blanché, C., Sáez, L., 2013. The 'paradigm of extremes': extremely low genetic diversity in an extremely narrow endemic species, *Coristospermum huteri* (Umbelliferae). Plant Syst. Evol. 299, 439–446.

López de Heredia, U., Jiménez, P., Díaz-Fernández, P., Gil, L., 2005. The Balearic Islands: a reservoir of cpDNA genetic variation for evergreen oaks. J. Biogeogr. 32, 939–949.

Mameli, G., Filigheddu, R., Binelli, G., Meloni, M., 2008. The genetic structure of the remnant populations of *Centaurea horrida* in Sardinia and associated islands. Ann. Bot. 101, 633–640.

Mansion, G., Rosenbaum, G., Schoenenberger, N., Bacchetta, G., Rosselló, J.A., Conti, E., 2008. Phylogenetic analysis informed by geological history supports multiple, sequential invasions of the Mediterranean Basin by the angiosperm family Araceae. Syst. Biol. 57, 269–285.

Mansion, G., Selvi, F., Guggisberg, A., Conti, E., 2009. Origin of Mediterranean insular endemics in the Boraginales: integrative evidence from molecular dating and ancestral area reconstruction. J. Biogeogr. 36, 1282–1296.

Martínez-Millán, M., 2010. Fossil record and age of the Asteridae. Bot. Rev. 76, 83–135.

Mayol, M., Palau, C., Rosselló, J.A., González-Martínez, S.C., Molins, A., Riba, M., 2012. Patterns of genetic variability and habitat occupancy in *Crepis triasii* (Asteraceae) at different spatial scales: insights on evolutionary processes leading to diversification in continental islands. Ann. Bot. 109, 429–441.

Médail, F., Diadema, K., 2009. Glacial refugia influence plant diversity patterns in the Mediterranean Basin. J. Biogeogr. 36, 1333–1345.

Médail, F., Quézel, P., 1997. Hot-spots analysis for conservation of plant biodiversity in the Mediterranean basin. Ann. Missouri Bot. Gard. 84, 112–127.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), pp. 1–8.

Molins, A., Mayol, M., Rosselló, J.A., 2009. Phylogeographical structure in the coastal species *Senecio rodriguezii* (Asteraceae), a narrowly distributed endemic Mediterranean plant. J. Biogeogr. 36, 1372–1383.

Moragues, E., (Master thesis) 2005. *Naufraga balearica* (Apiaceae) especie endémica y vulnerable de las Islas Baleares. Biología reproductiva y efecto de la herbivoría. Universitat des Illes Balears, Palma de Mallorca.

Moreno, J.C., 2011. *Naufraga balearica*. IUCN Red List of Threatened Species. Version 2012.2. IUCN, www.iucnredlist.org (accessed 19.06.13).

Moreno, J.C., 2008. Lista Roja 2008 de la flora vascular española. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino) y Sociedad Española de Biología de la Conservación de Plantas , Madrid.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Nei, M., Li, W.H., 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. U.S.A. 76, 5269–5273.

Newton, A.C., Allnutt, T.R., Gillies, A.C.M., Lowe, A.J., Ennos, R.A., 1999. Molecular phylogeography, intraspecific variation and the conservation of tree species. Trends Ecol. Evol. 14, 140–145.

O'Grady, J.J., Reed, D.H., Brook, B.W., Frankham, R., 2004. What are the best correlates of predicted extinction risk? Biol. Conserv. 118, 513–520.

Orellana, M.R., Blanché, C., Simon, J., Bosch, M., 2009. Genetic diversity within and among disjunct populations of the Mediterranean Island endemic *Delphinium pictum* and *D. requienii* (Ranunculaceae). Folia Geobot. 44, 47–63.

Peakall, R., Beattie, A.J., 1991. The genetic consequences of worker ant pollination in a self-compatible, clonal orchid. Evolution 45, 1837–1848.

Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6, 288–295.

Pearson, R.G., Raxworthy, C.J., Nakamura, M., Townsend Peterson, A., 2007. Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. J. Biogeogr. 34, 102–117.

Peterson, A., Bartish, I.V., Peterson, J., 2002. Genetic structure detected in a small population of the endangered plant *Anthericum liliago* (Anthericaceae) by RAPD analysis. Ecography 25, 677–684.

Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190, 231–259.

Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.

Postigo Mijarra, J.M., Barrón, E., Gómez Manzaneque, F., Morla, C., 2009. Floristic changes in the Iberian Peninsula and Balearic Islands (south-west Europe) during the Cenozoic. J. Biogeogr. 36, 2025–2043.

Quézel, P., 1985. Definition of the Mediterranean region and the origin of its flora. In: Gómez-Campo, C. (Ed.), Plant conservation in the Mediterranean area. W. Junk, Dordrecht, pp. 9–24.

Quilichini, A., Debussche, M., Thompson, J.D., 2004. Geographic differentiation of morphological traits and isozymes in the Mediterranean island endemic *Anchusa crispa*: implications for the conservation of a protected species. Biol. Conserv. 118, 651–660.

Rambaut, A., Drummond, A.J., 2007. Tracer version 1.4. http://beast.bio.ed.ac.uk/Tracer

Rita, J., Cursach, J., 2013. Creating new populations of *Apium bermejoi* (Apiaceae), a critically endangered endemic plant on Menorca (Balearic Islands). Anales Jard. Bot. Madrid 70, 27–38.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Ronse, A.C., Popper, Z.A., Preston, J.C., Watson, M.F., 2010. Taxonomic revision of European *Apium* L. s.l.: *Helosciadium* W.D.J.Koch restored. Plant Syst. Evol. 287, 1–17.

Rosenbaum, G., Lister, G.S., Duboz, C., 2002. Reconstruction of the tectonic evolution of the western Mediterranean since the Oligocene. J. Virtual Explorer 8, 107–130.

Rosselló, J.A., 2010. *Naufraga balearica* Constance & Cannon. In: Bañares, A., Blanca, G., Güemes, J., Moreno, J.C., Ortiz, S. (Eds.), Atlas y libro rojo de la flora vascular amenazada de España. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino) y Sociedad Española de Biología de la Conservación de Plantas, Madrid, pp. 46–47.

Rosselló, J.A., Cebrián, M.C., Mayol, M., 2002. Testing taxonomic and biogeographical relationships in a narrow Mediterranean endemic complex (*Hippocrepis balearica*) using RAPD markers. Ann. Bot. 89, 321–327.

Sales, E., Nebauer, S.G., Mus, M., Segura, J., 2001. Population genetic study in the Balearic endemic plant species *Digitalis minor* (Scrophulariaceae) using RAPD markers. Am. J. Bot. 88, 1750–1759.

Salvo, G., Ho, S.Y.W., Rosenbaum, G., Ree, R., Conti, E., 2010. Tracing the temporal and spatial origins of island endemics in the Mediterranean region: a case study from the citrus family (*Ruta* L., Rutaceae). Syst. Biol. 59, 705–722.

Schönswetter, P., Tribsch, A., 2005. Vicariance and dispersal in the alpine perennial *Bupleurum stellatum* L. (Apiaceae). Taxon 54, 725–732.

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E., Small, R.L., 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Am. J. Bot. 92, 142–166.

Shaw, J., Lickey, E.B., Schilling, E.E., Small, R.L., 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am. J. Bot. 94, 275–288.

Smouse, P.E., Long, J.C., Sokal, R.R., 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst. Zool. 35, 627–632.

Spalik, K., Piwczyński, M., Danderson, C.A., Kurzyna-Młynik, R., Bone, T.S., Downie, S.R., 2010. Amphitropic amphiantarctic disjunctions in Apiaceae subfamily Apioideae. J. Biogeogr. 37, 1977–1994.

Suc, J.P., 1984. Origin and evolution of the Mediterranean vegetation and climate in Europe. Nature 307, 429–432.

Svensson, L., 1985. An estimate of pollen carryover by ants in a natural population of *Scleranthus perennis* L. (Caryophyllaceae). Oecologia 66, 373–377.

Swofford, D., 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer, Sunderland, MA.

Templeton, A.R., Crandall, K.A., Sing, C.F., 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132, 619–633.

Thompson, J.D., 2005. Plant Evolution in the Mediterranean. Oxford University Press, New York.

Troia, A., 2012. Insular endemism in the Mediterranean vascular flora: the case of the Aeolian Islands (Sicily, Italy). Biodivers. J. 3, 369–374.

Vandepitte, K., Gristina, A.S., De Raedt, R., Roldán-Ruiz, I., Marcenò, C., Sciandrello, S., Honnay, O., 2013. Conservation genetics of an endemic from the Mediterranean Basin: high genetic differentiation but no genetic diversity loss from the last populations of the Sicilian Grape Hyacinth *Leopoldia gussonei*. Conserv. Genet. 14, 963–972.

Vilà, M., Muñoz, I., 1999. Patterns and correlates of exotic and endemic plant taxa in the Balearic islands. Ecol. Mediterr. 25, 153–161.

Vilatersana, R., Susanna, A., Brochmann, C., 2007. Genetic variation in *Femeniasia* (Compositae, Cardueae), an endemic and endangered monotypic genus from the Balearic Islands (Spain). Bot. J. Linn. Soc. 153, 97–107.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van De Lee, T., Hornes, M., Friters, A., Pot, J., Paleman, J., Kuiper, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23, 4407–4414.

Wulff, E.V., 1943. An Introduction to Historical Plant Geography. Chronica Botanica, Waltham, MA.

Wyatt, R., Stoneburner, A., 1981. Patterns of ant-mediated pollen dispersal in *Diamorpha smallii* (Crassulaceae). Syst. Bot. 6, 1–7.