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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

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• *Background and Aims* Archipelagos are unique systems for studying evolutionary processes promoting diversification and speciation. The islands of the Mediterranean basin are major areas of plant richness, including a high proportion of narrow endemics. Many endemic plants are currently found in rocky habitats, showing varying patterns of habitat occupancy at different spatial scales throughout their range. The aim of the present study was to understand the impact of varying patterns of population distribution on genetic diversity and structure to shed light on demographic and evolutionary processes leading to population diversification in *Crepis triasii*, an endemic plant from the eastern Balearic Islands.
• *Methods* Using allozyme and chloroplast markers, we related patterns of genetic structure and diversity to those of habitat occupancy at a regional (between islands and among populations within islands) and landscape (population size and connectivity) scale.
• *Key Results* Genetic diversity was highly structured both at the regional and at the landscape level, and was positively correlated with population connectivity in the landscape. Populations located in small isolated mountains and coastal areas, with restricted patterns of regional occupancy, were genetically less diverse and much more differentiated. In addition, more isolated populations had stronger fine-scale genetic structure than well-connected ones. Changes in habitat availability and quality arising from marine transgressions during the Quaternary, as well as progressive fragmentation associated with the aridification of the climate since the last glaciation, are the most plausible factors leading to the observed patterns of genetic diversity and structure.
• *Conclusions* Our results emphasize the importance of gene flow in preventing genetic erosion and maintaining the evolutionary potential of populations. They also agree with recent studies highlighting the importance of restricted gene flow and genetic drift as drivers of plant evolution in Mediterranean continental islands.

Key words: Allozymes, Balearic flora, chloroplast microsatellites, continental islands, *Crepis triasii*, fragmentation, genetic diversity, genetic drift, Quaternary, spatially structured populations, SGS.

INTRODUCTION

Insular environments constitute unique ecosystems often comprising many endemic species and high biological diversity (Losos and Ricklefs, 2009). Several factors have been suggested to explain the high diversification of island biotas (e.g. Stuessy *et al.*, 2006; Rundell and Price, 2009). For instance, remote oceanic islands arising *de novo* have often provided multiple ecological opportunities for a few number of colonizers that have been able to radiate and develop a number of new species. Well-known examples of adaptive radiations include the Hawaiian silverswords (Carlquist *et al.*, 2003) or Darwin's finches from the Galapagos archipelago (Grant and Grant, 2008). Similarly, adaptive radiation has been invoked to account for diversification of Bromeliaceae in 'terrestrial habitat islands' (Barbará *et al.*, 2008; Givnish *et al.*, 2011). By contrast, it has been hypothesized that diversification in continental islands might have a major non-adaptive component, with a prevalent role of drift in population divergence and speciation. Some examples are land snails of the genus *Albinaria* from Crete (Gittenberger, 1991) as well as particular plant groups in the Aegean Archipelago (*Nigella* complex: Comes *et al.*, 2008).

Islands constitute one of the major hotspots of diversity of the Mediterranean region, characterized by high endemicity rates (10–12 % in the larger islands, i.e. Balearic Islands, Crete, Corsica, Cyprus, Sardinia and Sicily) and species diversity (Médail, 2008; Médail and Diadema, 2009). Most islands in the Mediterranean are of the continental type, and have become progressively isolated from each other and the mainland by a complex combination of tectonic and glacio-eustatic processes. Such events, operating at different temporal and spatial scales, have promoted geographical and genetic isolation among plant populations, and thus have favoured

allopatric speciation via selection and/or genetic drift (Thompson, 2005). For instance, schizoendemic taxa restricted to the Tyrrhenian islands (e.g. *Erodium corsicum* from Corsica–Sardinia and *E. reichardii* from Majorca–Minorca) have probably evolved from an ancestral species present in the Protoligurian massif, a west Mediterranean Hercynian formation that was fragmented during the Oligocene (30–28 Mya), leading to the current position of Corsica, Sardinia and the Balearic Islands, among other territories (Alvarez, 1972; Alvarez *et al.*, 1974; Rosenbaum *et al.*, 2002). Similarly, the separation into western and eastern Balearic Islands since the opening of the Gibraltar strait at the end of the Messinian salinity crisis (5·33 Mya, Duggen *et al.*, 2003) has probably led to the evolution of independent lineages within several endemic taxa, such as the sister plant species *Hippocrepis balearica–H. grosii* (Rosselló *et al.*, 2002) or the lizards *Podarcis lilfordi–P. pityusensis* (Terrasa *et al.*, 2004).

In addition to major geographical barriers caused by tectonic movements, climate changes affecting the Mediterranean region since the end of the Plio-Pleistocene have probably been decisive in shaping distributions of Mediterranean insular plants through eustatic sea-level changes linked to glacial/interglacial cycles. Oscillations in the level of the sea have modified the shape, size and connections of emerged lands, promoting or restricting intraspecific gene flow, fragmenting populations and enhancing their divergence (e.g. *Senecio rodriguezii*; Molins *et al.*, 2009), and ultimately providing new opportunities for allopatric speciation (e.g. *Nigella arvensis* complex, Bittkau and Comes, 2009). Thus, continental islands of the Mediterranean are one of the best natural laboratories to improve our understanding of the effects of geographical isolation and long-term fragmentation on population divergence and speciation.

The long-term survival of populations in Mediterranean islands probably has depended on the existence of ecologically stable areas buffering the impact of Quaternary climatic extremes and, more recently, human pressures. Therefore, it is perhaps no coincidence that most endemic species in the Mediterranean flora occur on rocky habitats, whose intrinsic properties (e.g. relatively low incidence of disturbances with limited above-ground competition) make them optimal for long-term persistence of many plant populations (Thompson *et al.*, 2005). The occurrence of endemic species in rocky habitats is frequent in several Mediterranean archipelagos as, for example, the Balearic Islands (Alomar *et al.*, 1997) or the Aegean region (Médail and Quézel, 1997). The spatial configuration (size and isolation) of these habitats might have further contributed to between-population divergence through restricted gene flow, increased drift and local selection pressures, which in turn may have eventually led to the formation of new species. Hence, endemic species from Mediterranean islands usually show varying geographical patterns of occupancy at different spatial scales, and provide ideal model systems (1) to link the study of population differentiation with that of speciation (Thompson, 1999), and (2) to explore the long-term genetic consequences of habitat fragmentation as a result of progressive changes in environmental quality.

There is a general agreement that a comprehensive understanding of plant differentiation and speciation requires consideration of genetic patterns at a range of spatio-temporal scales (Comes, 2004). Assessment of spatial genetic structure at different spatial scales can provide insights into the importance and scale of genetic drift, gene flow and natural selection as drivers of evolutionary divergence between populations. However, few studies analysing genetic diversity in this region provide information for the same species at multiple scales (but see Lambertini *et al.*, 2008), and no one is centred on island endemics. Moreover, population genetic processes are linked to the spatial structure of the landscape, so the explicit understanding of evolution requires taking into account the effects of geography and habitat features on genetic variation. In the context of fragmented habitats, the degree of population isolation or the quality of habitats surrounding the fragments may be major determinants of population genetic structure (Gibbs, 2001).

In the present study we used allozymes and chloroplast microsatellite markers to analyse the spatial organization of genetic diversity in *Crepis triasii* (Asteraceae), an iteroparous, outcrossing endemic plant species found on limestone cliffs in the eastern Balearic Islands (Majorca, Minorca and Cabrera). We focused on nine localities across the species range and determined the spatial genetic structure both at the regional and at the local scale. As population size and degree of isolation are fundamental factors in explaining spatial genetic structure, we also estimated population sizes and the degree of isolation in each locality. The following specific questions were addressed: (1) Does genetic structure match current patterns of occupancy and isolation at a broad geographical scale, i.e. between islands and among populations within islands? (2) Do within-population genetic diversity and structure relate to population size and isolation at the landscape level? The results obtained at both spatial scales are combined to infer the most influential historical and contemporary demographic processes promoting diversification in this species.

MATERIAL AND METHODS

Study species

Crepis triasii (Cambess.) Fries is a long-lived perennial endemic to the eastern Balearic Islands (Majorca, Minorca and Cabrera), in the western Mediterranean Basin (Alomar *et al.*, 1997). Populations are mainly found in mesic slopes of cliffs and rocky open habitats, from sea level to about 1400 m a.s.l. In the north-western (Serra de Tramuntana) and eastern (Serres de Llevant) mountain ranges of Majorca, the species has a relatively continuous distribution, while its occurrence is much more discontinuous and restricted in the remaining distribution area (Fig. 1). The largest populations in Minorca are located at El Toro, in the central part of the island (Fig. 1). Plants are iteraparous and self-incompatible (Palau, 2004). From May to June they produce 1–132 capitula per plant (mean = 9·3), with 17–106 (mean = 57·6) hermaphroditic flowers which are visited mainly by generalist insect pollinators (M. Riba, CREAF-UAB, Barcelona, unpubl. res.). Fruits are cypselas showing a conspicuous pappus, and mature fruits are dispersed by wind in July.

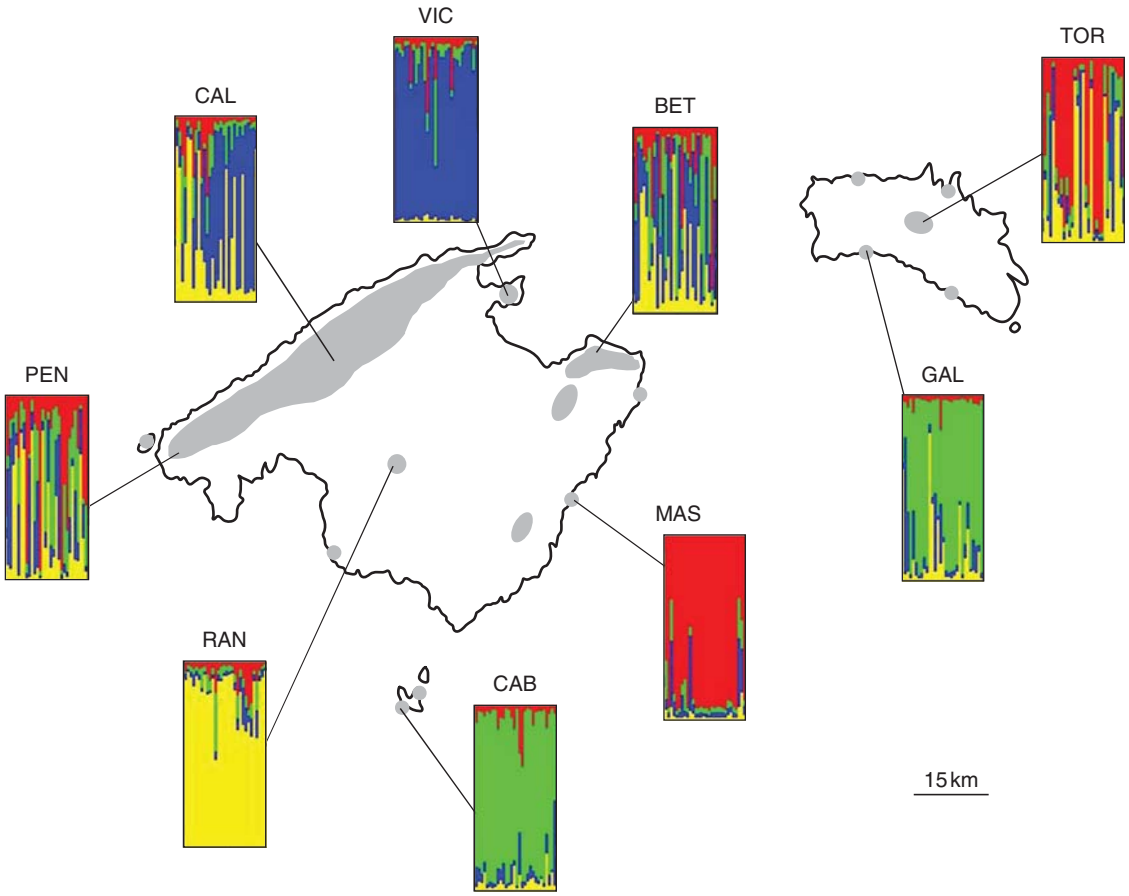

FIG. 1. Sampled populations and summary of the clustering results obtained with the Bayesian approach of Pritchard *et al.* (2000) for the *Crepis triasii* allozyme data assuming four populations ($K = 4$). Graphs indicate the proportional assignment of individuals to the four genetic clusters (1, red; 2, green; 3, blue; 4, yellow) within each population. Site codes are defined in Table 1. The grey area indicates the current distribution range of the species in the eastern Balearic Islands (Majorca, Minorca and Cabrera) based on Alomar *et al.* (1997).

Population selection and sample collection

Previous information about the geographical distribution of *C. triasii* (Alomar *et al.*, 1997) was used to select nine locations (hereafter called 'landscapes') covering the entire species range: six from Majorca, two from Minorca and one from Cabrera (Table 1, Fig. 1). Landscapes were selected to cover a wide range of sizes and degrees of isolation as well as to represent the full geographical range of the species. In 2001, and within each landscape, we selected a 'focal population', defined as a patch or group of patches clearly delimited by physiographic elements (e.g. a cliff, an isolated hill or a valley slope). In each focal population, leaves from 31 adult individuals were collected and stored in liquid nitrogen until extraction (Table 1). Plants were sampled as regularly spaced as possible taking into account both density and population extent. All samples were analysed for allozyme markers while a randomly selected subset of 21–24 samples per population was analysed for chloroplast microsatellites (Table 1). Sampled individuals were marked and georeferenced for the analysis of fine-scale spatial genetic variability.

Allozyme electrophoresis

For allozyme electrophoresis, leaves were crushed in a chilled mortar using the following extraction buffer, slightly modified from Soltis *et al.* (1983): 0·1 M Tris–HCl, pH 8·5, 0·04 g mL^{-1} PVP-40, 0·2 mg mL^{-1} EDTA, 4 mg mL^{-1} $MgCl_2$, 40 μL mL^{-1} glycerol, 1 μL mL^{-1} β-mercaptoethanol and 1 mg mL^{-1} bovine serum albumin. Protein extracts were run in vertical discontinuous polyacrylamide gels (stacking gel at 4 % in 0·125 M Tris-HCl, pH 6·8; separating gel at 10 % in 0·375 M Tris-HCl, pH 8·8). Of 26 enzyme systems tested, ten showed a good and repeatable activity: alkaline phosphatase (ALP, EC 3·1·3·1), aspartate aminotransferase (AAT, EC 2·6·1·1), formaldehyde dehydrogenase (FHOH, EC 1·1·1·284), formate dehydrogenase (FDH, EC 1·2·1·2), glucose 6-phosphate isomerase (PGI, EC 5·3·1·9), β-glucosidase (β-GLU, EC 3·2·1·21), glutamate dehydrogenase (GDH, EC 1·4·1·2), isocitrate dehydrogenase (IDH, EC 1·1·1·42), phosphoglucomutase (PGM, EC 5·4·2·2) and triose-phosphate isomerase (TPI, EC 5·3·1·1). All buffer and stain recipes followed Wendel and Weeden (1989). Genotypes were inferred from the resulting banding patterns based on previous knowledge concerning the quaternary structure and the number of subcellular isoenzymes for each enzyme system (Weeden and Wendel, 1989).

DNA extraction and chloroplast microsatellite markers (cpSSR)

Total genomic DNA was extracted following the CTAB method of Doyle and Doyle (1987), scaled down to perform

TABLE 1. *Location, description and codes of the nine* Crepis triasii *populations/landscapes included in this study*

Island	Population	Code	Lat./Long.	Altitude (m)	Pop. size	D (m)	CPop	PO (%)	N_n/N_{cp}
Majorca	Ermita de Betlem	BET	39·73°N, 3·32°E	310	832	50	7	30·81	31/21
	Castell d'Alaró	CAL	39·73°N, 2·79°E	770	450	31	4	7·51	31/24
	S'Estany d'En Mas	MAS	39·51°N, 3·30°E	5	69	15 000	0	0·42	31/23
	Penyal d'Enric	PEN	39·57°N, 2·36°E	300	217	25	3	3·31	31/23
	Puig de Randa	RAN	39·52°N, 2·92°E	520	607	35	1	5·60	31/22
	La Victòria	VIC	39·87°N, 3·17°E	295	258	40	3	17·06	31/24
	Total								186/137
Cabrera	N'Ensiola	CAB	39·13°N, 2·92°E	80	364	225	2	4·84	31/23
	Total								31/23
Minorca	Cala Galdana	GAL	39·93°N, 3·96°E	15	68	1500	0	0·76	31/21
	El Toro	TOR	39·98°N, 4·11°E	320	230	30	2	1·78	31/22
	Total								62/43
Overall total									279/203

Population sizes were estimated as the harmonic mean number of flowering plants per population between 2001 and 2005. D, distance to the nearest contributing population; CPop, number of contributing populations in a radius of 500 m; PO, proportion of landscape area occupied by the species in a 1-km-diameter circle; *N*, number of sampled individuals (N_n, allozyme markers; N_{cp}, chloroplast markers). For further explanation about population sizes and connectivity, see text.

the process in 1·5-mL microfuge tubes. To detect polymorphic markers, ten pairs of chloroplast microsatellite primers were initially tested using two individuals per island: ccmp2, ccmp3, ccmp4, ccmp5, ccmp6, ccmp7 and ccmp10 (Weising and Gardner, 1999) and NTCP13, NTCP37 and NTCP39 (Bryan *et al.*, 1999). Seven primer pairs that amplified well and were polymorphic were selected for the final study: ccmp2, ccmp3, ccmp5, ccmp7, NTCP13, NTCP37 and NTCP39. Amplifications were carried out in a Primus 96 Thermal Cycler (MWG Biotech, Ebersberg, Germany), with the following profile: an initial denaturation step of 5 min at 94 °C, followed by 40 cycles of 1 min at 94 °C, 50 s at 50 °C and 1 min at 72 °C, and a final extension step at 72 °C for 10 min. Reactions were performed in 10-μL total volume, containing 5 ng of template DNA, 1× reaction buffer (Ecogen, Barcelona, Spain), 2 mM $MgCl_2$, 0·2 mM of each dNTP, 0·2 μM of each primer and 0·15 U of *Taq* DNA polymerase (Ecogen). The forward primers were labelled with fluorescent dyes (TAMRA™, HEX™ or FAM™; Applied Biosystems, Foster City, CA, USA). PCR amplifications were performed separately for each locus, and amplification products were divided into two multiplexes according to fragment size and fluorescent dye (mix-1: ccmp2, ccmp3, NTCP13, NTCP37; mix-2: ccmp5, ccmp7, NTCP39). Amplified fragments were separated by capillary electrophoresis on an ABI 310 automated sequencer (Applied Biosystems), using GeneScan™ 350 ROX™ (Applied Biosystems) as the internal size standard. Fragment sizes were determined using GENESCAN® and GENOTYPER version 3·5 software (Applied Biosystems). Some ccmp2 PCR products were sequenced as a 5-bp indel was apparently present between one of the alleles and the rest (see Results). Sequencing was carried out with purified PCR products using the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems) and standard protocols.

Genetic diversity and structure at the regional scale

The following population genetic statistics were calculated for allozyme data using GENETIX version 4·04 (Belkhir *et al.*, 2001): the mean number of alleles per locus (*A*), the percentage of polymorphic loci (*p*), the observed heterozygosity (H_O) and Nei's unbiased expected heterozygosity (H_E; Nei, 1978). Inbreeding coefficients (F_{IS}) were estimated according to Weir and Cockerham (1984) for each population, and departure from Hardy–Weinberg equilibrium was assessed by a permutation test using 10 000 replicates. Linkage disequilibria between allozyme loci was tested using GENEPOP version 4·0·7 (Rousset, 2008). Genetic diversity statistics for chloroplast markers were calculated using ARLEQUIN version 2·000 (Schneider *et al.*, 2000). Chloroplast haplotypes were defined as the combination of the different fragments obtained for each cpSSR locus, and the following parameters were calculated: number of haplotypes (N_h), number of private haplotypes (N_{ph}), and Nei's unbiased haplotypic diversity (*H*; Nei, 1987). To visualize the phylogenetic relationship among chloroplast haplotypes, a median-joining network (Bandelt *et al.*, 1999) was constructed using the software NETWORK version 4·2·0·1 (available at www.fluxus-engineering.com).

The geographical structure of genetic diversity was analysed using different approaches and molecular markers. First, genetic differentiation among populations ($F_{ST} = \theta$) was estimated according to Weir and Cockerham (1984) using GENETIX version 4·04 (Belkhir *et al.*, 2001) and a test for population differentiation was conducted by permutation using 10 000 replicates. Then, we tested for isolation by distance (IBD) according to Rousset (1997): the correlation between the matrix of genetic $[F_{ST}/(1 - F_{ST})]$ and geographical distances (logarithmic scale) among pairs of populations was analysed with a Mantel test (10 000 permutations) using MANTEL version 2·0 (Liedloff, 1999). Second, an analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was used to partition the total variance into covariance components, at different hierarchical levels: (1) among and within populations, without regional grouping; (2) among islands, among populations within islands and among individuals within populations. The significance levels of the covariance components were obtained by a non-parametric permutation using 10 000 replicates. The AMOVA and significance tests

were performed using ARLEQUIN version 2·000 (Schneider *et al.*, 2000).

For allozyme markers only, we used a Bayesian model-based clustering method to identify clusters of genetically similar individuals without prior knowledge of their population of origin (program STRUCTURE version 2·2; Pritchard *et al.*, 2000). The program was run with the admixture ancestral model, ignoring the population affiliation when assigning individuals, and assuming correlated allele frequencies among populations. To estimate the number of potential source populations (K) we conducted ten independent runs of 100 000 Markov chain Monte Carlo (MCMC) iterations after a burn-in period of 10 000 for each K (K from 2 to 9). As our values of $\ln P(D)$ increased gradually with increasing values of K, we calculated ΔK, as proposed by Evanno *et al.* (2005). This statistic is based on the rate of change in the log probability of data between K values, and takes into account the variance among estimates in the multiple runs. The optimum number of clusters, K, was determined following guidelines from the authors (Pritchard and Wen, 2004) and the simulation study of Evanno *et al.* (2005).

Finally, we also estimated the pollen/seed gene flow ratio (P/S) following Ennos (1994), using the formula: $[A(1 + F_{IS}) - 2C]/C$, where $A = (1/F_{ST} - 1)$ for biparentally inherited markers (allozymes), and $C = (1/F_{ST} - 1)$ for maternally inherited markers (cpSSR).

Fine-scale spatial genetic structure (SGS)

Fine-scale genetic structure was investigated for nuclear data (allozymes) by analysing the relationship between pairwise relatedness coefficients and the spatial distance between individuals. Genetic co-ancestry was computed using the kinship coefficient (F_{ij}) developed by J. Nason (described in Loiselle *et al.*, 1995). For each population, F_{ij} values were averaged over a set of ten distance classes, automatically defined to contain equal numbers of pairwise comparisons within each distance interval, and regressed on the spatial distance between individuals, d_{ij}, and its natural logarithm, $\ln d_{ij}$, to provide the regression slopes b_{lin} and b_{log}, respectively. Regressions with distance are adequate for lineal (one-dimension) habitats whereas those with the logarithm of distance are used for bidimensional space (Rousset, 2004). The statistical significance of values obtained for both slopes was tested using a randomization procedure where individuals of each population were permuted 10 000 times among locations. Standard errors of b_{lin} and b_{log} were calculated by jackknifing over loci. Finally, the statistic Sp developed by Vekemans and Hardy (2004) was used to compare the extent of SGS among populations. Sp was calculated as $-b/(1 - F_1)$, where F_1 is the average kinship coefficient at the first distance class. All calculations were performed using SPAGeDi version 1·2 software (Hardy and Vekemans, 2002).

Effect of population size and landscape features

To study the influence of habitat occupancy on genetic diversity and structure, in each landscape we identified and mapped all other *C. triasii* patches within 500 m radius around the focal population (hereafter called 'contributing populations'). The area of each population and the distances between focal and contributing populations were measured from 1 : 10 000 scale maps using the UTHSCSA ImageTool program (developed at the University of Texas Health Science Center at San Antonio, Texas, and available from http://ddsdx.uthscsa.edu/dig/). These data were used to calculate different measures of landscape occupancy related to the isolation and connectivity of the focal populations: the proportion of landscape area occupied by the species (PO), the number of contributing populations within the landscape (CPop) and the distance from the focal population to its nearest contributing population (D). For landscapes with no observed contributing populations within a 500-m-radius circle, we used the distance to the nearest known population. Finally, to assess the potential effects of population size on genetic diversity, in each focal population we counted the number of flowering individuals during the springs of 2001, 2002, 2003, 2004 and 2005. For later analyses, we considered the population size as the harmonic mean of the number of flowering plants in these five years.

The relationship between genetic diversity (A, H_O, H_E) and fine-scale structure parameters (Sp_{lin}, Sp_{log}) with population size and landscape structure (PO, CPop, D) was assessed using Spearman's rank correlation tests. As *C. triasii* is distributed along a marked latitudinal and longitudinal gradient of both rainfall and temperature, the potential relationship between latitude, longitude and altitude with genetic diversity, population size and isolation was also assessed. All correlation analyses were performed using SPSS version 15·0 (SPSS Inc.).

RESULTS

Allozyme diversity and departure from random mating

Ten polymorphic (AAT-1, FDH-1, FHODH-1, GDH-1, IDH-1, IDH-2, PGI-1, PGM-2, TPI-1, TPI-2) and two monomorphic (ALP-1, GLU-1) loci were adequately resolved and scored. At the species level, 83·3 % of the loci were found to be polymorphic (p), with an average number of 2·92 alleles per locus (A) and overall genetic diversity of 0·281 (H_E, Table 2). At the population level, values of genetic diversity varied widely: population MAS had the lowest values of diversity ($p = 50$ %, $A = 1{\cdot}58$, $H_E = 0{\cdot}114$), whereas CAL was the most diverse ($p = 83{\cdot}3$ %, $A = 2{\cdot}33$, $H_E = 0{\cdot}301$; Table 2). No significant linkage disequilibrium was observed between any pair of loci after sequential Bonferroni correction ($P < 0{\cdot}05$).

In contrast to the high values of expected heterozygosity obtained, observed heterozygosity was low, with a mean of 0·169 ($\pm$ 0·036 s.d.) over all sampled populations (Table 2). We found significant deviations from Hardy–Weinberg equilibrium in all but two populations (CAB and VIC, Table 2), and the mean fixation index (F_{IS}) across all loci and populations was 0·222 $\pm$ 0·129.

Chloroplast diversity and relationships among haplotypes

Two loci (ccmp7 and NTCP13) amplified more than one PCR product in some samples, and were excluded from further analysis. We found 15 different variants for the remaining five chloroplast loci, with values ranging from two at locus

TABLE 2. *Summary of genetic diversity estimates obtained for* Crepis triasii

Island	Population	Allozyme markers						Chloroplast markers		
		A	*p*	H_E	H_O	F_{IS}	F_{ST}	*H*	N_h	N_{ph}
Majorca	BET	2·17	83·3	0·252	0·179	0·292***	0·170	0·467	2	1
	CAL	2·33	83·3	0·301	0·199	0·344***	0·134	0·790	7	3
	MAS	1·58	50·0	0·114	0·089	0·219***	0·306	0	1	0
	PEN	2·25	66·7	0·287	0·202	0·299***	0·173	0·490	3	0
	RAN	2·08	66·7	0·261	0·194	0·256***	0·279	0·541	3	1
	VIC	1·67	58·3	0·154	0·159	−0·035 n.s.	0·309	0·659	4	4
Cabrera	CAB	2·00	66·7	0·184	0·176	0·044 n.s.	0·228	0·403	2	2
Minorca	GAL	1·75	58·3	0·192	0·137	0·289***	0·238	0·467	2	1
	TOR	2·08	83·3	0·259	0·185	0·291***	0·187	0·454	2	1
Species level		2·92	83·3	0·281	0·169	0·222	0·226	0·903	19	13

A, mean number of alleles per locus; *p*, percentage of polymorphic loci; H_E, Nei's unbiased expected heterozygosity (Nei, 1978); H_O, observed heteroygosity; F_{IS}, inbreeding coefficient (calculated according to Weir and Cockerham, 1984); F_{ST}, mean pair-wise differentiation obtained for each population; *H*, Nei's unbiased haplotypic diversity (Nei, 1987); N_h, number of haplotypes; N_{ph}, number of private haplotypes. ***$P<0·001$; n.s., not significant.

NTCP37 and NTCP39, to five at locus ccmp5. All individuals from CAB and one individual from PEN showed variants differing by 5 bp from the others at locus ccmp2. Once sequenced, we found that the presence of this variant was due to a 5-bp deletion in the microsatellite flanking region rather than a decrease of the microsatellite length itself. Thus, the fragment sizes for these individuals at locus ccmp2 were assigned according to the repeat motif of the microsatellite region (i.e. without considering the indel). The combination of the 15 variants resulted in a total number of 19 haplotypes (Figs 2 and 3; Supplementary Data Table S1, available online). Most of the haplotypes (13) were exclusively from one locality, and only six of them were present in more than one population (Figs 2 and 3, Table 2). The most frequent haplotype (haplotype 8, 21·7 %) was found in three populations (BET, GAL and MAS) from two islands (Majorca and Minorca). Haplotype 16 was also shared by two populations from different islands (RAN and TOR), but its frequency was lower (9·8 %). Finally, four haplotypes were shared among populations from Majorca: haplotypes 1, 5 and 6 were present in populations CAL and PEN, and haplotype 4 was shared by CAL and RAN (Figs 2 and 3; Supplementary Data Table S1).

Considering all populations together, haplotypic diversity (*H*) was 0·903, but there was a wide range of variation in the populations studied (Table 2). Population MAS was the only one showing no variation at chloroplast loci ($H = 0$), while seven different haplotypes were found in population CAL ($H = 0·790$).

The haplotype network showed several loops (ambiguities), suggesting the existence of homoplasy (Fig. 3). Nevertheless, the network could be divided into two main groups connected by intermediate missing haplotypes (Fig. 3). The eight haplotypes (8–14 and 19) present in the populations from eastern Majorca, southern Minorca and Cabrera (BET, CAB, GAL, MAS, VIC) were grouped together, whereas a second group contained the remaining haplotypes found in the populations from western Majorca and central Minorca (CAL, PEN, RAN, TOR), and in population BET (haplotypes 1–7 and 15–18; Fig. 3). With the exception of BET, none of the populations had haplotypes belonging to the two groups (Fig. 3).

Genetic structure at the regional scale

Genetic differentiation at nuclear loci was high (overall $F_{ST} = 0·226$, $P < 0·05$). All pair-wise F_{ST} values were significant, and ranged from 0·027 for the pair GAL–CAB to 0·445 for the pair MAS–VIC. There was no evidence for isolation by distance at this geographical scale, as the pair-wise correlation between genetic and geographical distances was not significant (Mantel test: $r = -0·296$, $P = 0·103$). Very distant populations located in different islands (e.g. CAB–GAL) were genetically more similar than others geographically close. In agreement with these results, AMOVA showed that 22·64 % of the variation occurred among populations, but there was no significant variation due to differences between islands (Table 3). For chloroplast markers, the proportion of variation explained by differences among populations was greater than for nuclear loci (70·06 %), but differences among islands were not significant (Table 3).

The model-based clustering method implemented in STRUCTURE (applied to allozyme data) suggested that the model with $K = 4$ was better than alternative models. Log-likelihood values for *K* showed a pattern of gradual increase with increasing *K*, but the maximum modal value of ΔK occurred at $K = 4$ (Fig. 1). Some populations were assigned mainly to one gene pool each (MAS, RAN, VIC) while CAB and GAL shared the same gene pool. BET, CAL, PEN and TOR included several gene pools and admixed individuals. Interestingly, these latter populations, located in both Majorca and Minorca, did not fall in a contact zone of different clusters. Populations comprising various gene pools had significantly lower values of pair-wise differentiation and significantly higher genetic diversity than those assigned to one specific pool (mean $F_{ST} = 0·166$ vs. 0·272; mean $A = 2·208$ vs. 1·816; mean $H_E = 0·275$ vs. 0·181, for admixed and non-admixed populations, respectively; Mann–Whitney's *U*-test: $P < 0·05$; Table 2). In the correlated allele frequencies model used for STRUCTURE runs, it is assumed that the four populations represented in our sample have each undergone independent drift away from the allele frequencies present in a hypothetical 'ancestral' population,

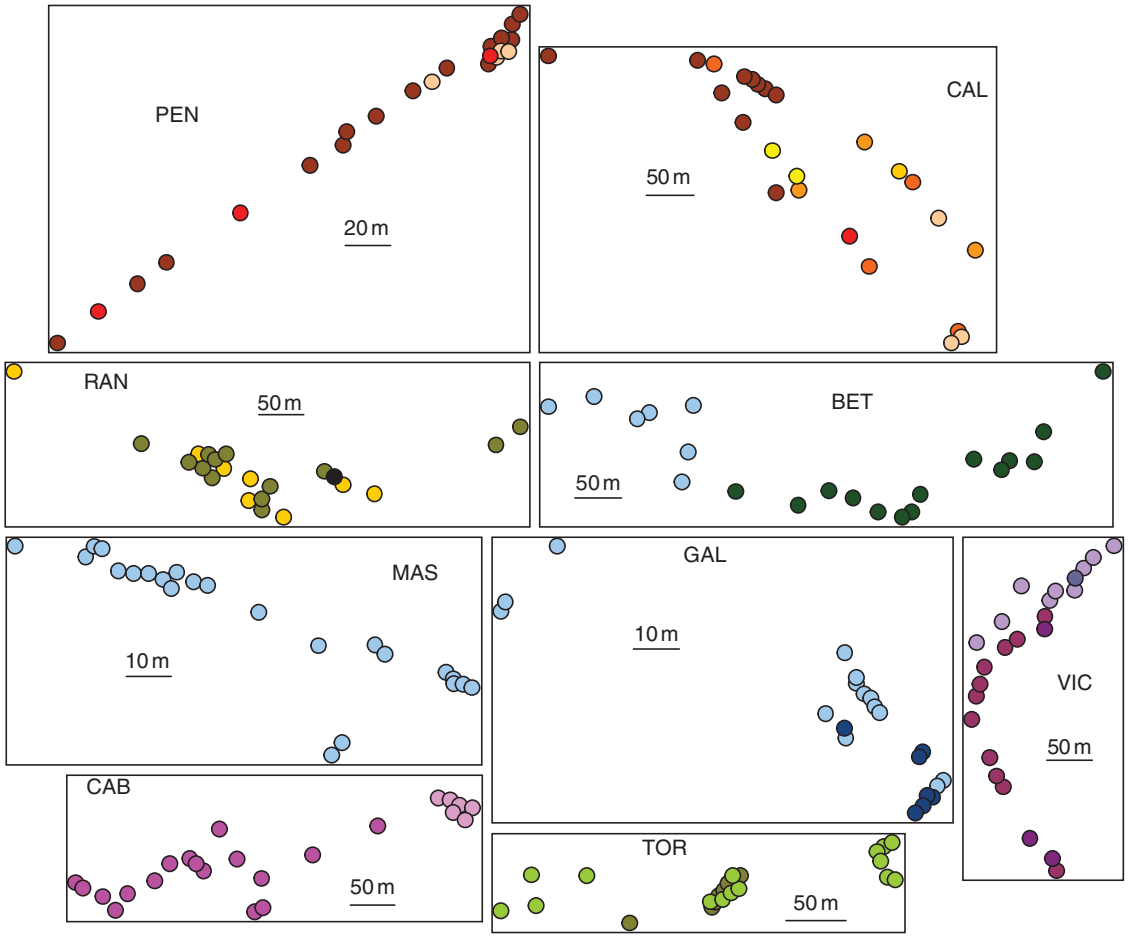

FIG. 2. Within-population spatial distribution of chloroplast haplotypes in each of the nine populations of *Crepis triasii* analysed. Haplotype colours correspond to those provided in Fig. 3. Site codes are defined in Table 1.

TABLE 3. *Analysis of molecular variance (AMOVA) based on allozyme and chloroplast microsatellite data for* Crepis triasii

	Allozyme markers			Chloroplast markers		
Source of variation	d.f.	Sum of squares	Percentage of variation	d.f.	Sum of squares	Percentage of variation
(a)						
Among populations	8	157·56	22·64***	8	181·81	70·06***
Within populations	549	564·76	77·36	194	82·01	29·94
(b)						
Among islands	2	39·28	–0·04 n.s.	2	49·57	0 n.s.
Among populations within islands	6	118·28	22·67***	6	215·73	69·11***
Within populations	549	564·76	77·37***	194	135·25	30·89***

(a) Assuming no regional differentiation. (b) Populations grouped into three islands (Majorca, Minorca and Cabrera). ***$P < 0{\cdot}001$ (significance test after 10000 permutations); n.s., not significant.

at a rate that is parameterized by the mean F_{ST} values obtained for each cluster (Falush *et al.*, 2003). The F_{ST} values obtained were 0·454, 0·390, 0·412 and 0·091, for the 'red', 'green', 'blue' and 'yellow' clusters, respectively (Fig. 1). Thus, the populations assigned to 'red', 'green' and 'blue' clusters (MAS, CAB–GAL and VIC, respectively) showed higher divergence from the hypothetical ancestral population than those from the 'yellow' one (RAN).

The ratio of pollen flow to seed flow was calculated as 7·77, which suggests a greater contribution of pollen to gene migration.

Fine-scale SGS

Significant SGS was detected with allozyme markers when all individuals were included in the analyses (Table 4 and

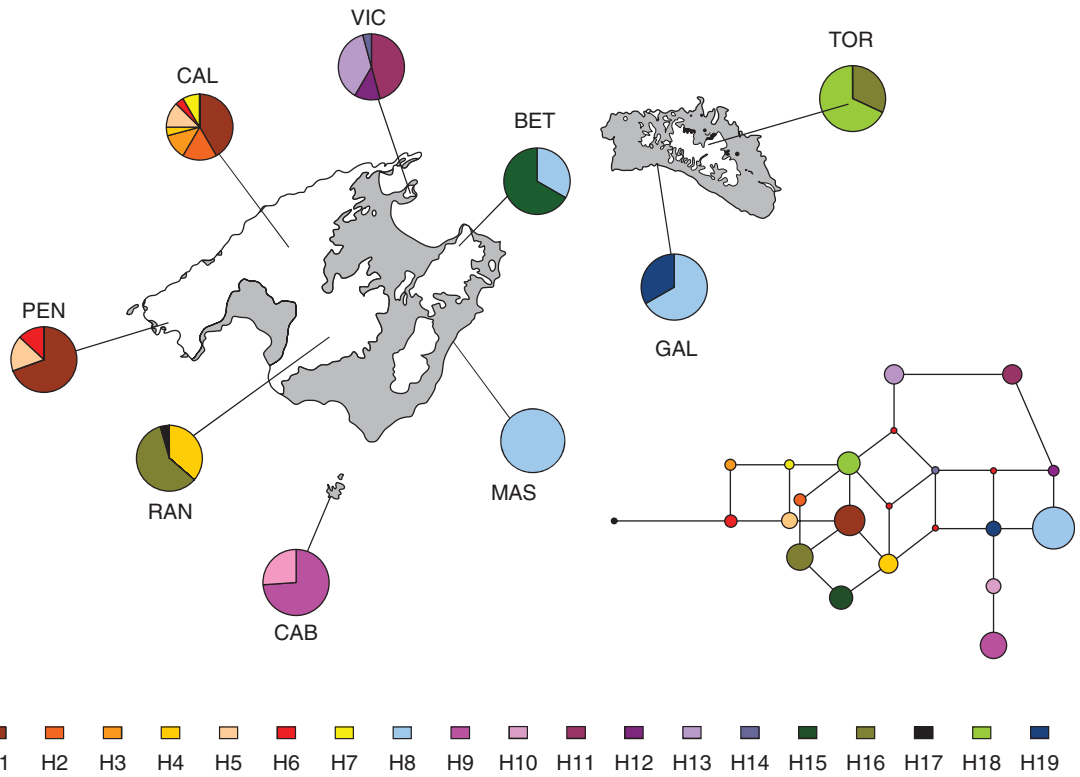

FIG. 3. Geographical distribution of 19 cpSSR haplotypes found in nine populations of *Crepis triasii* in the eastern Balearic Islands (Majorca, Minorca and Cabrera). For population codes, see Table 1. On the right is the median-joining network among the 19 cpSSR haplotypes detected in this study. The size of the circle is proportional to the frequency of each haplotype in the total sample. Median vectors are shown in red. The map shows the current distribution of emerged lands of the Balearic Islands (grey area), and the approximate geographical configurations of these islands during the maximum transgression of the Pleistocene (white area). Modified from Gràcia *et al.* (2001).

TABLE 4. *Spatial genetic structure parameters for* Crepis triasii *using nuclear markers*

Island	Population	F_1	b_{lin}	Sp_{lin} (s.e.)	b_{log}	Sp_{log} (s.e.)
Majorca	BET	−0·0043 n.s.	−0·00011 n.s.	0·00011 (0·00011)	−0·01112 n.s.	0·01108 (0·01872)
	CAL	0·0297 n.s.	−0·00009 n.s.	0·00009 (0·00009)	−0·01499 n.s.	0·01546 (0·01525)
	MAS	0·1584***	−0·00146*	0·00174 (0·00067)	−0·03083***	0·03664 (0·01452)
	PEN	−0·0153 n.s.	0·00000 n.s.	0·00000 (0·00013)	0·00374 n.s.	−0·00368 (0·00667)
	RAN	0·0345 n.s.	−0·00024***	0·00025 (0·00005)	−0·03031***	0·03139 (0·00784)
	VIC	0·0348 n.s.	−0·00008 n.s.	0·00009 (0·00006)	−0·00989 n.s.	0·01024 (0·00709)
Cabrera	CAB	0·1066**	−0·00048***	0·00054 (0·00016)	−0·04749**	0·05316 (0·01434)
Minorca	GAL	0·0213 n.s.	−0·00078*	0·00079 (0·00039)	−0·00682 n.s.	0·00697 (0·00580)
	TOR	0·0328 n.s.	−0·00030*	0·00031 (0·00016)	−0·00872 n.s.	0·00902 (0·00789)
Total		0·0235**	−0·00021***	0·00022 (0·00004)	−0·01703***	0·01743 (0·00498)

F_1, average kinship coefficient between individuals at the first distance class; b_{lin} and b_{log}, regression slope of kinship coefficient values (F_{ij}) as a function of the spatial distance (d_{ij}) between individuals and its logarithm ($\ln d_{ij}$), respectively; *Sp* was calculated as $-b/(1 - F_1)$. Following Hardy *et al.* (2006), we present the standard error (s.e.) of *b* (calculated by jackknifing over loci) as an estimate of the variability of *Sp*. For locality abbreviations see Table 1.
***$P < 0{\cdot}001$; **$P < 0{\cdot}01$; *$P < 0{\cdot}05$; n.s., not significant.

Supplementary Data Fig. S1). When analyses were conducted in each population separately, different spatial structure patterns were obtained depending on the population considered (Table 4, Supplementary Data Fig. S1). In populations CAB, MAS and RAN, b_{lin} and b_{log} were both significant (Table 4). For GAL and TOR, kinship coefficients decreased significantly with the geographical distance but not with its logarithm (Table 4). However, it must be noted that all populations but PEN had negative values of b_{lin} and b_{log}, indicating that individuals spatially close to each other were, on average, more likely to be genetically related than individuals separated by larger distances (Table 4). Kinship coefficients between neighbours at the first distance class (F_1) were also different between sites, with a maximum value of $F_1 = 0{\cdot}1584$ in population MAS (Table 4). Comparison of *Sp* statistics revealed that populations composed of different gene pools (BET, CAL, PEN, TOR) tended to have lower values than those assigned to only one pool (CAB, GAL, MAS, RAN, VIC; mean

$Sp_{lin} = 0{\cdot}00013$ vs. 0·00068, mean $Sp_{log} = 0{\cdot}00797$ vs. 0·02768 for each group of populations, respectively), indicating a more pronounced fine-scale genetic structure in the latter (Table 4).

Strong genetic structure at the local scale was also found for chloroplast markers, as most populations showed a non-random distribution of haplotypes, independent of the number of genetic pools detected in each population for nuclear markers (Fig. 2).

Effect of population size and landscape features

Although genetic variation tended to be reduced in small compared with large populations, no significant correlations between genetic diversity (A, H_O, H_E) and population size were found (Table 5). In contrast, we found a strong relationship between landscape structure and both population diversity and within-population genetic structure (Table 5 and Supplementary Data Fig. S2). We found significant negative relationships between log-transformed distance to the nearest contributing population and the number of alleles per locus, the observed heterozygosity and the expected heterozygosity. The number of alleles per locus was also positively correlated with the number of contributing populations within the landscape. Genetic structure within populations (Sp_{lin}) was positively correlated with the logarithmic distance to the nearest contributing population, and negatively correlated with the number of contributing populations and the proportion of the landscape occupied by the species.

Genetic diversity was not related to either latitude or longitude. By contrast, genetic diversity significantly increased with altitude (A: Spearman's $\rho = 0{\cdot}795$, $P < 0{\cdot}01$; H_O: $\rho = 0{\cdot}817$, $P < 0{\cdot}01$; H_E: $\rho = 0{\cdot}833$, $P < 0{\cdot}01$). This was because populations located at higher altitudes were significantly larger ($\rho = 0{\cdot}683$, $P < 0{\cdot}05$) and less isolated ($\rho = -0{\cdot}750$, $P < 0{\cdot}05$) than those located at lower elevations.

DISCUSSION

Genetic diversity and structure at the regional scale

The island endemic *C. triasii* was found to be highly variable for both nuclear and cytoplasmic markers (Table 2). At the species level, values of p and H_E for allozymes were much higher than those found by Hamrick and Godt (1996) for long-lived perennial, animal-outcrossed plants with wind-dispersed seeds, and were also higher than those provided for endemic and narrow-ranged species in their review. The scarcity of population genetic studies based on chloroplast microsatellite data for plants other than trees or shrubs precludes a direct comparison of diversity statistics with those of the present study for this marker. However, the number of cpSSR haplotypes found in *C. triasii* falls between those observed in some more widely distributed angiosperms, such as *Alyssum bertolonii* (35 haplotypes; Mengoni *et al.*, 2003), *Capsella bursa-pastoris* (eight haplotypes; Ceplitis *et al.*, 2005) or *Silene paradoxa* (27 haplotypes; Mengoni *et al.*, 2001), although comparisons among different genera must be taken with caution. Our results are in accordance with recent studies providing evidence that the Balearic Islands constitute a reservoir of genetic diversity, both for widespread Mediterranean taxa (evergreen oaks; López de Heredia *et al.*, 2005) and for narrowly distributed endemics (*Senecio rodriguezii*: Molins *et al.*, 2009; *Hippocrepis balearica*: our unpubl. res.). Some factors that may have contributed to maintain these high levels of genetic diversity are the outcrossing mating system of the species, the long life span of adult plants and their high resprouting ability (M. Mayol and M. Riba, pers. obs.).

The AMOVA results showed that this high genetic diversity was highly structured, with significant population differentiation for both nuclear and chloroplastic markers (Table 3). The patterns of genetic differentiation did not match the current island pattern of isolation, as no significant differentiation was found among islands. The distribution of chloroplastic haplotypes within the archipelago suggested the existence of two basic geographical groups (see Fig. 3). With the exception of BET, all populations belonged to one of these two groups, the first one grouping populations extending along the coast of the archipelago (CAB, GAL, MAS and VIC), and the second one grouping populations from the westernmost area of Majorca (CAL, PEN and RAN) and central Minorca (TOR). Very similar results are obtained when analysing cpDNA sequences on a much wider sampling (25 populations; M. Mayol *et al.*, unpubl. res.). Populations belonging to the first cluster, occurring mainly at low elevations, tended to have lower allozymic diversity than those belonging to the second group (Mann–Whitney's U-test: $P < 0{\cdot}05$ for A, H_O and H_E), all of them located at higher elevations (above 300 m). These data point to the existence of two main lineages evolving under different demographic scenarios in different regions across the Balearic archipelago.

The existence of varying demographic processes associated with patterns of regional occupancy is highlighted by the results of the Bayesian clustering analyses performed on allozyme data. In populations CAB, GAL, MAS, RAN and VIC, all of them located in small, isolated mountains or coastal areas (see Fig. 1), more than 70 % of individuals in the sample were assigned to one specific gene pool, and these populations were, on average, highly divergent from all others (Table 2). In contrast, populations located on the Tramuntana (CAL, PEN) and Llevant Mountains (BET), as well as in the central part of Minorca (TOR), where the

TABLE 5. *Spearman rank correlations between genetic diversity and landscape structure parameters*

	Pop. size	PO	CPop	D
A	0·502 n.s.	0·427 n.s.	0·682*	−0·736*
H_O	0·433 n.s.	0·350 n.s.	0·589 n.s.	−0·900***
H_E	0·350 n.s.	0·250 n.s.	0·439 n.s.	−0·800**
Sp_{lin}	−0·383 n.s.	−0·667*	−0·785*	0·750*
Sp_{log}	0·417 n.s.	0·100 n.s.	−0·160 n.s.	0·483 n.s.

A, mean number of alleles per locus; H_E, Nei's unbiased expected heterozygosity (Nei, 1978); H_O, observed heterozygosity; Sp, within-population genetic structure; PO, proportion of landscape area occupied by the species in a 1-km-diameter circle; CPop, number of contributing populations in a radius of 500 m; D, distance to the nearest contributing population. ***$P < 0{\cdot}001$; **$P < 0{\cdot}01$; *$P < 0{\cdot}05$; n.s., not significant.

species has a more continuous regional distribution, had lower levels of genetic differentiation and many individuals were estimated to have a mixed ancestry (Table 2, Fig. 1). Therefore, these data suggest that gene flow among populations is much more reduced within regions where the extent of habitat occupancy is also more limited (see also next section below).

Two historical factors may underlie the observed pattern of genetic structure at this regional scale. First, changes in sea level associated with northern hemisphere glaciations have recurrently modified the shape and size of emerged Balearic Islands, and are likely to have modified the genetic diversity and structure of many plant populations. The marine transgressions that occurred during warmer interglacial periods in the Early Pleistocene (1·6–0·7 Mya) caused sea levels to rise up to 100 m above the current coastline (Cuerda, 1975; Gràcia *et al.*, 2001). During these episodes, the island of Majorca was divided into two major islands, the size of Minorca was considerably reduced and most of the Cabrera archipelago was submerged (Fig. 3). These processes have probably produced the long-term isolation of several territories, limiting gene flow and promoting divergence among populations currently located on the same island. For example, in the past, population VIC was completely isolated from all others due to rising sea levels, which could explain its high among-population divergence for both nuclear and chloroplast markers. Populations currently occurring along the modern coastline (e.g. GAL and MAS) have probably been subjected to repeated founding events associated with changes in sea level, a fact that might have decreased their genetic variability. In contrast, populations located on the higher Balearic mountains in the western coast of Majorca could have maintained an elevated connectivity even during the more severe marine transgressions. Indeed, all haplotypes found in population PEN were also present in population CAL (Fig. 3), suggesting that gene flow has been widespread within this region, at least in the long past. The lowest F_{ST} value obtained for the 'yellow' cluster from a hypothetical 'ancestral' population, together with the fact that populations CAL and RAN also shared one haplotype, further suggests that the isolation of population RAN has been relatively recent.

A second factor that could have played a major role in shaping the regional genetic patterns observed is the vegetation change that occurred in the Balearic Islands during the middle Holocene (5000–4000 yr BP) in response to the progressive aridification of the climate, showing the replacement of mesophyllous communities with *Fagus*, *Corylus*, *Alnus*, deciduous *Quercus* and *Buxus* by xeric-type formations with a dominance of *Olea* (Yll *et al.*, 1997; Ninyerola *et al.*, 2007). The progressive warming and drying of the climate has probably led to the extinction of some species and to the fragmentation of formerly large populations into small and isolated fragments as a consequence of climate-driven habitat deterioration. In fact, our long-term demographic data on some populations (to be published elsewhere) shows that both recruitment rate and population growth rate are positively correlated with summer rainfall. These changes in environmental conditions probably had a lesser impact on mountain areas, where large evergreen oak forests and relicts of mesic woods can still be found, allowing gene flow to have occurred due to a higher presence of neighbouring populations in these areas. At lower elevations, however, deteriorating environmental conditions could have accelerated the loss of favourable habitat for the species, increasing isolation and decreasing levels of gene flow between populations. In agreement with this scenario, detailed analysis of the patterns of habitat occupancy at a landscape level (see below) do show that populations located at higher altitudes are significantly larger and less isolated.

Population size, isolation and genetic variation at the landscape scale

Our data on population sizes and patterns of landscape occupancy provided further insights into the role of migration in explaining population genetic variability and structure in *C. triasii*, as well as additional evidence on the impact of population isolation and habitat degradation, i.e. long-term fragmentation. Thus, for instance, differences among populations in genetic variation (A, H_O, H_E) and within-population structure (Sp_{lin}, Sp_{log}) were not related to population size, but significantly correlated with measures of landscape occupancy (Table 5). In particular, population genetic diversity decreased exponentially with isolation. Many empirical studies have assessed the effects of reduced population size on the maintenance of genetic variation (recently reviewed by Leimu *et al.*, 2006; Honnay and Jacquemyn, 2007), but the consequences of different levels of isolation for the preservation of genetic diversity have received considerably less attention. Some studies have reported that small-sized populations located on well-connected landscapes, i.e. close to large populations, do not show significant reductions on genetic diversity as compared with more isolated remnants of similar size (e.g. Prober and Brown, 1994; Cruzan, 2001). These results point to the existence of 'isolation thresholds' that are critical for the long-term maintenance of genetic diversity and evolutionary potential, both at the regional and at the local level, and beyond which long-distance dispersal may not occur at a high enough rate to offset the effects of genetic drift (Cruzan, 2001). Recent studies on *Mercurialis annua* clearly show the evolutionary consequences of isolation thresholds, represented in this case by geographical barriers (Pujol *et al.*, 2010). In this species, isolated populations after range expansion show reduced ability to respond to selection (Pujol and Pannell, 2008) and reduced inbreeding depression (Pujol *et al.*, 2009), a key factor favouring the evolution of self-fertilization. In these cases, gene flow among interconnected populations plays a more important role than population size itself in the maintenance of genetic diversity through a 'rescue effect', as genetic erosion is prevented by the immigration of individuals (Brown and Kodric-Brown, 1977) or genes (Richards, 2000) from nearby populations. Such a 'rescue effect' through gene flow, providing increased genetic diversity and heritable adaptive potential, has been reported by Lavergne and Molofsky (2007) for North American invasive populations of *Phalaris arundinacea*.

In *Crepis triasii*, however, gene flow is not only limited at large spatial scales due to long-term fragmentation. The significant fine-scale genetic structure detected for nuclear markers within some of the populations analysed (Table 4), together with the clumped distribution of chloroplast haplotypes

(Fig. 2), indicates that gene movement by pollen and seeds is highly restricted within populations. In agreement with recent reviews, levels of pollen flow were found to be larger than those of seeds (Petit *et al.*, 2005), which suggests that restricted gene flow through seeds is quantitatively more important in shaping the current genetic architecture across populations. However, the significant levels of inbreeding detected in almost all populations, despite this being an outcrossing species, also indicate limitations to pollen movement within populations. In agreement with the results obtained for genetic diversity, the observed variability in the levels of within-population structure was not related to population size but rather to measures of landscape occupancy, Sp_{lin} decreasing with the number of contributing populations or the proportion of landscape area occupied by the species. This suggests that the same factors limiting effective dispersal at a regional scale might also be acting on a much finer spatial scale, at least partly, and that gene flow is not limited only by geographical distance. Far beyond the effects of isolation and population size per se, environmental processes leading to progressive habitat fragmentation might also alter habitat quality within remnant populations (Young *et al.*, 1996). Habitat quality might determine the spatial distribution of individuals as a result of the combined effects of the availability of favourable sites for establishment and limited seed dispersal around maternal individuals, promoting the spatial aggregation of related genotypes (e.g. Cruse-Sanders and Hamrick, 2004). Habitat quality might have other indirect effects on patterns of gene flow through changes in plant density, demographic behaviour or individual plant traits. For instance, degradation of habitat quality might cause a low local density (e.g. Lienert and Fischer, 2003) and, in turn, reduce among-plant pollinator movements (Karron *et al.*, 1995). Furthermore, and in the case of insect-pollinated plants, the amount of individual floral display can reduce pollen transfer among plants (Harder and Barrett, 1995) and hence gene flow through pollen dispersal. In the case of *C. triasii*, the more isolated populations (e.g. GAL, MAS and RAN) show limited recruitment and are usually composed by old-aged individuals with large floral displays at low densities (C. Palau *et al.*, unpubl. res.). These changes in plant and demographic structure, as well as a lower level of external gene flow (see above), could have contributed to enhance within-population genetic structure in *C. triasii*, and might help to explain the stronger fine-scale SGS detected in more isolated populations of the species. Therefore, our findings stress the importance of examining relationships between genetic patterns and landscape structure, and provide empirical evidence to support that long-term habitat fragmentation and prolonged isolation may alter genetic diversity and species evolutionary potential at different spatial scales.

Concluding remarks

The patterns of genetic structure found in this study support a complex evolutionary history of *C. triasii* across the Balearic archipelago, and suggest that contrasting patterns of regional occupancy have been important in determining the structure observed. Changes in habitat availability linked to marine transgressions and an increase of aridity taking during the Quaternary are the most likely reasons underlying these observations at the range-wide scale.

The significant pair-wise genetic differentiation among populations, the lack of a pattern of isolation by distance and the high variance in divergence estimates (F_{ST}) across all geographical distances suggest that random genetic drift has been an important force structuring genetic variation in *C. triasii* (Hutchison and Templeton, 1999). This is in accordance with some recent studies indicating that genetic drift has acted historically as a major evolutionary force driving plant evolution in other Mediterranean insular systems, such as the Aegean archipelago (Edh *et al.*, 2007; Comes *et al.*, 2008). In *C. triasii*, however, the high genetic diversity detected in more connected populations, as well as the fact that several populations from different islands share chloroplast haplotypes and nuclear gene pools, suggest that genetic drift must be a relatively recent (and probably still ongoing) process that has not completely eliminated shared ancestral variation. Such a scenario should have important evolutionary consequences, as genetic variation is a prerequisite for selection to act upon. The progressive and slow reduction of diversity in response to habitat changes, and subsequent population divergence and gene flow restriction, provides conditions for initial drift-induced differences to be further amplified by selection to the local environment (given enough levels of remaining genetic variation, as seems the case for most populations). This mode of evolution has been proposed, for example, to explain speciation of *Melanoplus* grasshoppers on separate mountain blocks ('sky islands') in the Rocky Mountains during the Pleistocene (Knowles and Richards, 2005), and may be a plausible model to understand diversification of some plant lineages in Mediterranean continental islands. This may be especially true for species occurring along altitudinal or climatic gradients, conditions potentially promoting diversifying selection and local adaptation and, eventually, speciation. Hence, endemic species in continental islands, such as *Crepis triasii*, may be particularly well-suited models to elucidate the role of natural selection in the initial stages of species formation, a question largely neglected so far in studies addressing evolution on Mediterranean islands.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: Microsatellite chloroplast haplotypes identified on nine populations of *Crepis triasii*. Figure S1: Average kinship coefficient values plotted against the geographical distance between individuals for each population and the overall sample. Figure S2: Relationship between landscape features and estimates of genetic variation for nine populations of *Crepis triasii*.

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LITERATURE CITED

Alomar G, Mus M, Rosselló JA. 1997. *Flora Endèmica de les Balears*. Palma de Mallorca, Spain: Consell Insular de Mallorca.

Alvarez W. 1972. Rotation of Corsica–Sardinia microplate. *Nature Physical Science* **235**: 103–105.

Alvarez W, Cocozza T, Wezel FC. 1974. Fragmentation of alpine orogenic belt by microplate dispersal. *Nature* **248**: 309–314.

Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**: 37–48.

Barbará T, Lexer C, Martinelli G, Mayo S, Fay MF, Heuertz M. 2008. Within-population spatial genetic structure in four naturally fragmented species of a neotropical inselberg radiation, *Alcantarea imperialis*, *A. geniculata*, *A. glaziouana* and *A. regina* (Bromeliaceae). *Heredity* **101**: 285–296.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. 2001. *GENETIX version 4·04, logiciel sous Windows*^{TM} *pour la génétique des populations*. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, France.

Bittkau C, Comes HP. 2009. Molecular inference of a Late Pleistocene diversification shift in *Nigella* s. lat. (Ranunculaceae) resulting from increased speciation in the Aegean archipelago. *Journal of Biogeography* **36**: 1346–1360.

Brown JH, Kodric-Brown A. 1977. Turnover rates in insular biogeography: effect of immigration on extinction. *Ecology* **58**: 445–449.

Bryan GJ, McNicoll J, Ramsay G, Meyer RC, De Jong WS. 1999. Polymorphic simple sequence repeat markers in chloroplast genomes of Solanaceous plants. *Theoretical and Applied Genetics* **99**: 859–867.

Carlquist S, **Baldwin BG**, **Carr GD.** (eds) **2003.** *Tarweeds and silverswords: evolution of the Madiinae (Asteraceae)*. St Louis: Missouri Botanical Garden Press.

Ceplitis A, Su Y, Lascoux M. 2005. Bayesian inference of evolutionary history from chloroplast microsatellites in the cosmopolitan weed *Capsella bursa-pastoris* (Brassicaceae). *Molecular Ecology* **14**: 4221–4233.

Comes HP. 2004. The Mediterranean region – a hotspot for plant biogeographic research. *New Phytologist* **164**: 11–14.

Comes HP, Tribsch A, Bittkau C. 2008. Plant speciation in continental island floras as exemplified by *Nigella* in the Aegean Archipelago. *Philosophical Transactions of the Royal Society of London, series B* **363**: 3083–3096.

Cruse-Sanders JM, Hamrick JL. 2004. Spatial and genetic structure within populations of wild american ginseng (*Panax quinquefolius* L., Araliaceae). *Journal of Heredity* **95**: 309–321.

Cuerda J. 1975. *Los tiempos cuaternarios en Baleares*. Palma de Mallorca, Spain: Instituto de Estudios Baleáricos.

Cruzan MB. 2001. Population size and fragmentation thresholds for the maintenance of genetic diversity in the herbaceous endemic *Scutellaria montana* (Lamiaceae). *Evolution* **55**: 1569–1580.

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

Duggen S, Hoernle K, van den Bogaard P, Rüpke L, Morgan JP. 2003. Deep roots of the Messinian salinity crisis. *Nature* **422**: 602–606.

Edh K, Widén B, Ceplitis A. 2007. Nuclear and chloroplast microsatellites reveal extreme population differentiation and limited gene flow in the Aegean endemic *Brassica cretica* (Brassicaceae). *Molecular Ecology* **16**: 4972–4983.

Ennos RA. 1994. Estimating the relative rates of pollen and seed migration among plant populations. *Heredity* **72**: 250–259.

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

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.

Gibbs JP. 2001. Demography versus habitat fragmentation as determinants of genetic variation in wild populations. *Biological Conservation* **100**: 15–20.

Gittenberger E. 1991. What about non-adaptive radiation? *Biological Journal of the Linnean Society* **43**: 263–272.

Givnish TJ, Barfuss MHJ, Van Ee B, *et al*. 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: insights from an eight-locus plastid phylogeny. *American Journal of Botany* **98**: 872–895.

Gràcia F, Clamor B, Landreth R, Vicens D, Watkinson P. 2001. Evidències geomorfològiques dels canvis del nivell marí. *Monografíes de la Societat d'Història Natural de les Balears* **9**: 91–119.

Grant PR, Grant BR. 2008. *How and why species multiply: the radiation of Darwin's finches*. Princeton, NJ: Princeton University Press.

Hamrick JL, Godt JW. 1996. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London Series B* **351**: 1291–1298.

Harder LD, Barrett SCH. 1995. Mating cost of large floral displays in hermaphrodite plants. *Nature* **373**: 512–515.

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.

Hardy OJ, Maggia L, Bandou E, *et al*. 2006. Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. *Molecular Ecology* **15**: 559–571.

Honnay O, Jacquemyn H. 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology* **21**: 823–831.

Hutchison DW, Templeton AR. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53**: 1898–1914.

Karron JD, Thumser NN, Tucker R, Hessenauer AJ. 1995. The influence of population-density on outcrossing rates in *Mimulus ringens*. *Heredity* **75**: 175–180.

Knowles LL, Richards CL. 2005. Importance of genetic drift during Pleistocene divergence as revealed by analyses of genomic variation. *Molecular Ecology* **14**: 4023–4032.

Lambertini C, Gustafsson MHG, Frydenberg J, Speranza M, Brix H. 2008. Genetic diversity patterns in *Phragmites australis* at the population, regional and continental scales. *Aquatic Botany* **88**: 160–170.

Lavergne S, Molofsky J. 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences USA* **104**: 3883–3888.

Leimu R, Mutikainen P, Koricheva J, Fischer M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology* **94**: 942–952.

Liedloff A. 1999. *MANTEL version 2·0: Mantel Nonparametric Test Calculator*. Brisbane: Queensland University of Technology.

Lienert J, Fischer M. 2003. Habitat fragmentation affects the common wetland specialist *Primula farinosa* in north-east Switzerland. *Journal of Ecology* **91**: 587–599.

Loiselle BA, Sork VL, Nason J, Graham C. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* **82**: 1420–1425.

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

Losos JB, Ricklefs RE. 2009. Adaptation and diversification on islands. *Nature* **457**: 830–836.

Médail F. 2008. A natural history of the islands' unique flora. In: Arnold C. ed. *Mediterranean islands*. London: Mediterranean Islands c/o Survival Books, 26–33.

Médail F, Diadema K. 2009. Glacial refugia influence plant diversity patterns in the Mediterranean Basin. *Journal of Biogeography* **36**: 1333–1345.

Médail F, Quézel P. 1997. Hot-spots analysis for conservation of plant biodiversity in the Mediterranean Basin. *Annals of the Missouri Botanical Garden* **84**: 112–127.

Mengoni A, Barabesi C, Gonnelli C, Galardi F, Gabbrielli R, Bazzicalupo M. 2001. Genetic diversity of heavy metal-tolerant populations in *Silene*

paradoxa L. (Caryophyllaceae): a chloroplast microsatellite analysis. *Molecular Ecology* **10**: 1909–1916.

Mengoni A, Gonnelli C, Brocchini E, *et al*. 2003. Chloroplast genetic diversity and biogeography in the serpentine endemic Ni-hyperaccumulator *Alyssum bertolonii*. *New Phytologist* **157**: 349–356.

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

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.

Ninyerola M, Sáez L, Pérez-Obiol R. 2007. Relating postglacial relict plants and Holocene vegetation dynamics in the Balearic Islands through field surveys, pollen analysis and GIS modeling. *Plant Biosystems* **141**: 292–304.

Palau C. 2004. *Estructura i dinàmica demogràfica bàsiques de l'endemisme Crepis triasii (Cambess.) Nyman en relació a la fragmentació de l'hàbitat.* Barcelona: DEA Ecology, Autonomous University of Barcelona.

Petit RJ, Duminil J, Fineschi S, Hampe A, Salvini D, Vendramin GG. 2005. Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Molecular Ecology* **14**: 689–701.

Pritchard JK, Wen W. 2004. *Documentation for STRUCTURE software version 2·2*. Chicago: Department of Human Genetics, University of Chicago (available at http://pritch.bsd.uchicago.edu).

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

Prober SM, Brown AHD. 1994. Conservation of the grassy white box woodlands: population genetics and fragmentation of *Eucalyptus albens*. *Conservation Biology* **8**: 1003–1013.

Pujol B, Pannell JR. 2008. Reduced responses to selection after species range expansion. *Science* **321**: 96.

Pujol B, Zhou S-R, Sanchez-Vilas J, Pannell JR. 2009. Reduced inbreeding depression after species range expansion. *Proceedings of the National Academy of Sciences USA* **106**: 15379–15383.

Pujol B, Obbard DJ, Pannell JR. 2010. Symptoms of population range expansion: lessons from phenotypic and genetic differentiation in hexaploid *Mercurialis annua*. *Plant Ecology & Diversity* **3**: 103–108.

Richards CM. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. *American Naturalist* **155**: 383–394.

Rosenbaum G, Lister GS, Duboz C. 2002. Reconstruction of the tectonic evolution of the western Mediterranean since the Oligocene. *Journal of the Virtual Explorer* **8**: 107–126.

Rosselló JA, Cebrián C, Mayol M. 2002. Testing taxonomic and biogeographical relationships in a narrow Mediterranean endemic complex (*Hippocrepis balearica*) using RAPD markers. *Annals of Botany* **89**: 321–327.

Rousset F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* **145**: 1219–1228.

Rousset F. 2004. *Genetic structure and selection in subdivided populations*. Princeton, NJ: Princeton University Press.

Rousset F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* **8**: 103–106.

Rundell RJ, Price TD. 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology and Evolution* **24**: 394–399.

Soltis DE, Haufler CH, Darrow DC, Gastony GJ. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American Fern Journal* **73**: 9–27.

Schneider S, Roessli D, Excoffier L. 2000. *ARLEQUIN version 2·000: a software for population genetics data analysis*. Geneva: Genetics and Biometry Laboratory, University of Geneva.

Stuessy TF, Jakubowsky G, Salguero Gómez R, *et al*. 2006. Anagenetic evolution in island plants. *Journal of Biogeography* **33**: 1259–1265.

Terrasa B, Picornell A, Castro JA, Ramón MM. 2004. Genetic variation within endemic *Podarcis* lizards from the Balearic Islands inferred from partial Cytochrome b sequences. *Amphibia–Reptilia* **25**: 407–414.

Thompson JD. 1999. Population differentiation in Mediterranean plants: insights into colonization history and the evolution and conservation of endemic species. *Heredity* **82**: 229–236.

Thompson JD. 2005. *Plant evolution in the Mediterranean*. New York: Oxford University Press.

Thompson JD, Lavergne S, Affre L, Gaudeul M, Debussche M. 2005. Ecological differentiation of Mediterranean endemic plants. *Taxon* **54**: 967–976.

Vekemans X, Hardy OJ. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology* **13**: 921–935.

Weir BS, Cockerham CC. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.

Weising K, Gardner RC. 1999. A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. *Genome* **42**: 9–19.

Weeden NF, Wendel JF. 1989. Genetics of plant isozymes. In: Soltis DE, Soltis PS. eds. *Isozymes in plant biology*. Portland, OR: Dioscorides Press, 46–72.

Wendel JF, Weeden NF. 1989. Visualization and interpretation of plant isozymes. In: Soltis DE, Soltis PS. eds. *Isozymes in plant biology*. Portland, OR: Dioscorides Press, 5–45.

Yll E-I, Pérez-Obiol R, Pantaleon-Cano J, Roure JM. 1997. Palynological evidence for climatic change and human activity during the Holocene on Minorca (Balearic Islands). *Quaternary Research* **48**: 339–347.

Young A, Boyle T, Brown T. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* **11**: 413–418.