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► **To cite this version:**

Zaida Lorenzo, Concetta Burgarella, Unai López de Heredia, Roselyne Lumaret, Remy R. Petit, et al.. Relevance of genetics for conservation policies: the case of Minorcan cork oaks. *Annals of Botany*, 2009, 104 (6), pp.1069-1076. 10.1093/aob/mcp200 . hal-02655478

HAL Id: hal-02655478

<https://hal.inrae.fr/hal-02655478v1>

Submitted on 31 Oct 2023

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Relevance of genetics for conservation policies: the case of Minorcan cork oaks

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Received: 5 May 2009 Returned for revision: 26 June 2009 Accepted: 10 July 2009 Published electronically: 11 August 2009

- **Background and Aims** Marginal populations of widely distributed species can be of high conservation interest when they hold a significant or unique portion of the genetic diversity of the species. However, such genetic information is frequently lacking. Here the relevance of genetic surveys to develop efficient conservation strategies for such populations is illustrated using cork oak (*Quercus suber*) from Minorca (Balearic Islands, Spain) as a case study. Cork oak is highly endangered on the island, where no more than 67 individuals live in small, isolated stands in siliceous sites. As a consequence, it was recently granted protected status.
- **Methods** Two Bayesian clustering approaches were used to analyse the genetic structure of the Minorcan population, on the basis of nuclear microsatellite data. The different groups within the island were also compared with additional island and continental populations surrounding Minorca.
- **Key Results** Very high genetic diversity was found, with values comparable with those observed in continental parts of the species' range. Furthermore, the Minorcan oak stands were highly differentiated from one another and were genetically related to different continental populations of France and Spain.
- **Conclusions** The high levels of genetic diversity and inter-stands differentiation make Minorcan cork oak eligible for specific conservation efforts. The relationship of Minorcan stands to different continental populations in France and Spain probably reflects multiple colonization events. However, discrepancy between chloroplast DNA- and nuclear DNA-based groups does not support a simple scenario of recent introduction. Gene exchanges between neighbouring cork oak stands and with holm oak have created specific and exceptional genetic combinations. They also constitute a wide range of potential genetic resources for research on adaptation to new environmental conditions. Conservation guidelines that take into account these findings are provided.

Key words: Nuclear microsatellites, cluster analysis, marginal populations, conservation guidelines, *Quercus suber* (cork oak), *Q. ilex* (holm oak), western Mediterranean, Minorca, Balearic Islands.

INTRODUCTION

Conservation of intraspecific variation in marginal areas of species distribution is considered to be important for long-term survival, allowing the species to adapt to changing environmental conditions (Channell and Lomolino, 2000; Petit *et al.*, 2005). Marginal populations might be genetically depauperate as a result of genetic drift or, in contrast, might harbour a significant share of the diversity of the species, for instance if they have been introduced from different non-native sources. Furthermore, they may also show specific genetic combinations due to adaptation to extreme environmental conditions (Hunter and Hutchinson, 1994; Lesica and Allendorf, 1995). Unfortunately, conservation measures very often have to be taken in marginal populations before clarifying their situation in this sense. Genetic surveys should help in assessing conservation priorities and developing effective conservation strategies (Newton *et al.*, 1999). This applies for cork oak (*Quercus suber*) in Minorca (Balearic Islands), in the western Mediterranean.

The Mediterranean Basin comprises almost 5000 islands and islets (Delanoë, 1996). Marine influence sheltered the area from glaciations, which led to severe species extinctions in continental regions. It is considered as one of the world's 25 zones with the highest conservation priority and 4.3 % of its vascular plants are endemics (Myers *et al.*, 2000). Médail and Quézel (1997) identified ten hotspots within the Mediterranean Basin by performing a global survey of plant richness and endemism, and they highlighted the western islands (i.e. Balearic Islands, Corsica, Sardinia and Sicily) as the most important areas. This diversity is currently under threat due to human impacts. Since the Neolithic era, human pressure has increasingly affected Mediterranean ecosystems, and its effects are even more prominent on small island ecosystems (Williams, 2000; Bover and Alcover, 2008; Panitsa *et al.*, 2008).

The emblematic sclerophyllous tree, cork oak (*Q. suber*), represents an ideal system to investigate the effect of historical processes on genetic diversity (Hampe and Petit, 2007; Magri *et al.*, 2007). The species has a rather discontinuous distribution that ranges from the Atlantic coasts of North Africa and the Iberian Peninsula to the south-eastern regions of Italy, including the Mediterranean islands, Provence

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(France), French and Spanish Catalonia, and the coastal belts of eastern Spain, Algeria and Tunisia. The discontinuity of the distribution is related to the fact that cork oak is a strictly siliceous species, unable to live in calcareous or dolomitic soils, unless these are decarbonated. Thus, cork oak forms extensive woodlands in the siliceous south-western part of the Iberian Peninsula, while in the calcareous eastern part and in the Balearic Islands, it appears exclusively in small, scattered sites. This is the case in Minorca, where small groups of trees appear in gullies and stream beds in calcareous sites. Hence, although located in the middle of the species range, Minorca can be considered an ecologically marginal area for cork oak. To our knowledge, only 67 cork oak trees grow on this island. Cork oak woodlands are protected by the European Union (Habitat Directive 92/43 EEC), and the species has been recently designated as 'sensitive to habitat destruction species' by the Balearic Government (BOIB, 2005). This protection status provides an impetus for the development of management plans. The present work describes the genetic diversity of cork oak in Minorca as a guide for subsequent conservation programmes.

By combining molecular and palaeoecological data, recent studies indicate that cork oak genetic diversity is strongly structured across its range and that chloroplast DNA (cpDNA) geographical patterns may date back to the Tertiary (Lumaret *et al.*, 2005; López de Heredia *et al.*, 2007a; Magri *et al.*, 2007). Evergreen oaks show four different cpDNA lineages; one of them, known as the *suber* lineage, appears exclusively in cork oak, whereas the other three are typical of holm and kermes oaks (*Q. ilex* and *Q. coccifera*), and are therefore named *ilex-coccifera* lineages I, II and III (López de Heredia *et al.*, 2007a). While most cork oak populations show cpDNA haplotypes belonging to the *suber* lineage, haplotypes of lineage *ilex-coccifera* I characterize the populations of French Catalonia, Eastern Spain and the Balearic Islands. Hybridization and introgression with holm oak, largely indifferent to soil nature, has been proposed as a plausible explanation for their presence in this predominantly calcareous environment (López de Heredia *et al.*, 2007b). López de Heredia *et al.* (2005a) found a complex phylogeographic pattern for Balearic evergreen oaks, especially for cork oak, with a mixture of *ilex-coccifera* I and *suber* cpDNA lineages. In particular, in the western part of Minorca, cork oak shows the so-called haplotype 66, belonging to the *ilex-coccifera* I lineage, which is distributed in eastern Iberia, while the easternmost stands of the island are characterized by haplotypes 1 and 2, also observed in western Iberia and in Sardinia, respectively, and belonging to the *suber* lineage. These results suggest that Minorca is a melting pot for the genetic diversity of the species.

Although the species has experienced an important expansion over much of its range during the 19th and the first decades of the 20th century, as a result of cork exploitation in some areas, historical human activities have favoured holm oak to the detriment of cork oak, due to the better acorn and firewood production of holm oak (Martín-Vicente and Fernández-Alés, 2006). In Minorca, the strong human pressure exerted on cork oak is evident in some specific areas. Cork oak management along the island has focused on firewood extraction and charcoal production, a widespread practice in sclerophyllous oak species

around the Mediterranean Basin (Thirgood, 1981; Blondel, 1995; Pardo and Gil, 2005).

The critical situation of cork oak populations in Minorca (López de Heredia *et al.*, 2005b) urges further analyses of its genetic structure as a support for the definition of a conservation plan. The study of nuclear DNA variation and its comparison with cpDNA variation previously analysed in the same plant material can bring to light additional valuable information about the native or introduced origin of the species in Minorca, the current state of its genetic diversity and its conservation status.

This work exemplifies the applicability of genetic surveys to develop conservation strategies in marginal populations, using the Minorcan cork oak population as a case study. We have analysed the complete population (67 adult trees) constituting nine small groups of individuals (stands) in order to: (a) describe patterns of genetic variation using nuclear DNA markers, and check its correspondence with that identified previously on the basis of cpDNA variation; (b) assess the genetic similarity among stands within Minorca and compare the genetic variation identified in the island with that observed in populations from continental and island areas surrounding the archipelago; and (c) provide information for the development of future conservation plans.

MATERIALS AND METHODS

Study site and sampling

Minorca is a small island with an area of 702 km². It is geologically the oldest of the Balearic Islands, dating back to the Carboniferous (325 Myears BP; Traveset, 2002). The southern area of the island is characterized by calcareous terrains of the upper Miocene (11–5 Myears BP), while the northern area consists of Palaeozoic hills (sandstones, schist, pelites and turbidites), quite rough, and Mesozoic plateaus (dolomites, sandstones, limestone and gypsum) limited by rocky cliffs. Its flora corresponds to a typical dry Mediterranean climate (Peinado and Rivas-Martínez, 1987), with monthly average temperatures ranging from 10 to 26 °C, average precipitation of 600 mm per year, and 4 or 5 months of drought.

The complete known population of Minorcan cork oaks *Q. suber* L. (67 individuals) distributed in nine small clumps or stands was sampled. The stands are found on different substrates and constitute either monospecific stands or mixed stands with holm oak, in which cork oak occupies a dominated status (Fig. 1, Supplementary Table S1, available online).

Eight other cork oak populations analysed in Burgarella *et al.* (2009) from both continental and island regions surrounding Minorca, and characterized by the different cpDNA lineages identified in Minorca, were used as reference populations (details in Table 1).

DNA analysis

Approximately 1 g of leaf material was collected from each individual and stored in liquid nitrogen. Extractions were performed according to Doyle and Doyle (1990) with modifications by Dumolin *et al.* (1995). Nine nuclear microsatellites were used, MSQ4 and MSQ13, first described in *Q. macrocarpa* (Dow *et al.*, 1995), *QpZAG9*, *QpZAG15*, *QpZAG36* and

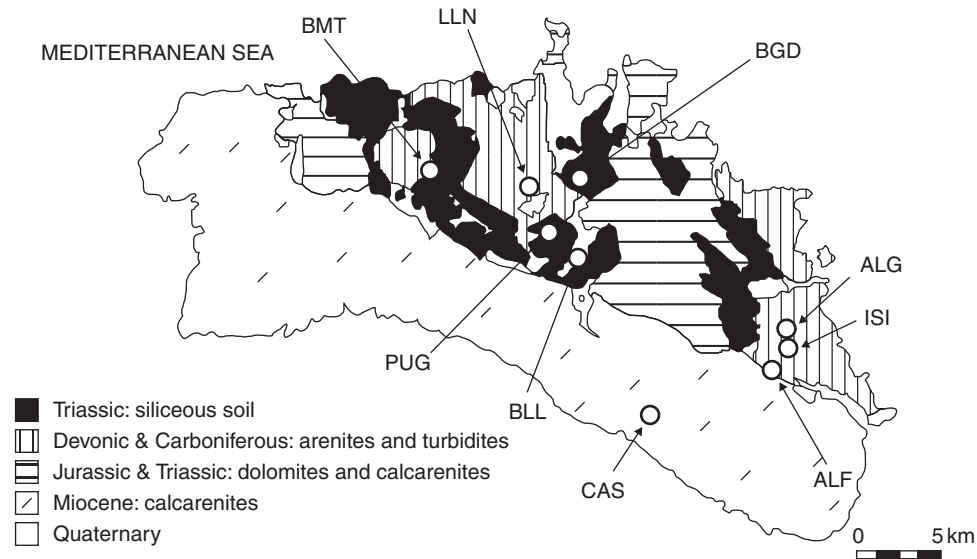


FIG. 1. Sampling localities of cork oak in Minorca and soil types identified throughout the island. The siliceous soils are indicated in black.

TABLE 1. Details of *Q. suber* populations used in this study

| Population | Location, country | Longitude | Latitude | Sample size | cpDNA lineage* |
|---------------------------------|--------------------|------------|------------|-------------|----------------|
| Alfavaret (ALF) | Minorca, Spain | 04°14'12"E | 39°54'06"N | 1 | s |
| Algarrovet (ALG) | Minorca, Spain | 04°15'02"E | 39°55'19"N | 13 | s |
| Binigurdo (BGD) | Minorca, Spain | 04°06'25"E | 40°00'10"N | 1 | i-c I |
| Binillubet (BLL) | Minorca, Spain | 04°06'18"E | 39°57'42"N | 3 | i-c I |
| Binimoti (BMT) | Minorca, Spain | 04°00'07"E | 40°00'25"N | 2 | i-c I |
| Es Puig Mal (PUG) | Minorca, Spain | 04°05'04"E | 39°58'31"N | 19 | i-c I |
| Llinarix Nou (LLN) | Minorca, Spain | 04°04'06"E | 39°59'57"N | 18 | i-c I |
| Sant Isidre (ISI) | Minorca, Spain | 04°14'47"E | 39°54'35"N | 5 | s |
| So Na Casana (CAS) | Minorca, Spain | 04°09'01"E | 39°52'48"N | 5 | i-c I |
| North Castilla La Mancha (NCM) | Toledo, Spain | 05°17'21"W | 39°54'51"N | 83 | s |
| South Castilla La Mancha (SCM) | Ciudad Real, Spain | 04°04'48"W | 38°30'00"N | 50 | i-c I |
| East Comunidad Valenciana (ECV) | Valencia, Spain | 00°17'00"W | 38°59'00"N | 69 | i-c I |
| Spanish Catalonia (SCT) | Girona, Spain | 02°49'48"E | 41°24'36"N | 73 | i-c I |
| French Catalonia (FCT) | Le Boulou, France | 02°50'34"E | 42°30'00"N | 100 | i-c I |
| Landes (LAN) | Seignosse, France | 01°21'47"W | 43°41'38"N | 32 | s |
| Var (PRO) | Les Maures, France | 06°16'46"E | 43°19'23"N | 50 | s |
| Sicily (SIC) | Madonie, Italy | 13°55'48"E | 37°54'36"N | 63 | s |

* Abbreviations: s, *suber*; i-c I, *ilex-coccifera* I (López de Heredia *et al.*, 2007a).

QpZAG46, developed in *Q. petraea* (Steinkellner *et al.*, 1997), and *QrZAG7*, *QrZAG11* and *QrZAG20*, developed in *Q. robur* (Kampfer *et al.*, 1998). The PCR amplification of microsatellite loci was done as previously described in Soto *et al.* (2003, 2007).

Data analysis

Standard genetic diversity parameters were calculated using the software package GENEPOP 3-4 (Raymond and Rousset, 1995). The G'_{ST} differentiation parameter (Hedrick, 2005) was calculated using SMOGD software (Crawford, 2009). The inbreeding coefficient, F_{IS} , was calculated for the largest stands (Es Puig Mal, Llinarix Nou and Algarrovet), and its statistical significance was estimated by the Markov chain method (Guo, 1992), with 100 batches of 10 000 iterations

per batch. Parent–offspring relationships within Llinarix Nou were tested using Famoz software (Gerber *et al.*, 2003), as the inbreeding coefficient in this stand was significantly positive.

Identification of genetic structuring

Within Minorca, cork oak appears to be scattered throughout the island, in small clumps. This may be the result of fragmentation of an ancestral widespread population or, alternatively, there may have been stable small stands since the colonization of the island from one or more sources. Clustering methods provide the best way to assess the genetic structure in cases of unknown genetic origin (Pritchard *et al.*, 2000; Dawson and Belkhir, 2001; Corander *et al.*, 2003; Falush *et al.*, 2003). Moreover, they allow testing for panmixia

(Manel *et al.*, 2005) and the establishment of the putative origin of each individual from different reference populations.

The genetic structure of Minorcan cork oaks was analysed using two Bayesian approaches. As a first step, we used STRUCTURE version 2 (Pritchard *et al.*, 2000; Pritchard and Wen, 2003), without prior information on the locality of origin, allowing the allele frequencies to be correlated among them. This configuration has been considered optimal in the case of limited population structure (Falush *et al.*, 2003). The number of populations (K) was set from a minimum of one to a maximum of nine (maximum number of known stands within the island), and ten simulations were run for each K -value with a burn-in of 100 000 and with 100 000 iterations each. The mean value of the posterior probability was calculated from the ten simulations for each K , and the most likely number of clusters was selected following the methodology proposed by Evanno *et al.* (2005).

A second Bayesian cluster analysis was performed implemented in the software BAPS 4.14 (Corander *et al.*, 2006) to detect similarities between the clusters detected in Minorca with STRUCTURE and the eight reference populations from the surrounding areas of the Mediterranean Basin. In contrast to the individual-based algorithm applied in STRUCTURE, the group-level option implemented in BAPS was used, so that the clusters detected within the island were considered as different populations, and this information was included in the mixture analysis. The most likely number of clusters (K) was obtained after ten independent simulations for values of K ranging from one to 11 (maximum number of known populations).

RESULTS

Genetic diversity and differentiation

Standard genetic diversity parameters for the 67 individuals of Minorca considered as a whole and for reference populations are shown in Table 2. It is noteworthy that the highest

TABLE 2. Measures of genetic diversity for the Minorcan and the reference populations

| Population | n | H_e | H_o | A | No. of total alleles |
|---------------------------------|-----|-------------|-------------|-----------|----------------------|
| French Catalonia (FCT) | 100 | 0.50 (0.08) | 0.44 (0.02) | 5.1 (2.1) | 46 |
| Minorca (MIN) | 67 | 0.55 (0.08) | 0.52 (0.02) | 6.4 (3.1) | 58 |
| Sicily (SIC) | 63 | 0.60 (0.06) | 0.58 (0.02) | 6.7 (2.5) | 60 |
| North Castilla-La Mancha (NCM) | 83 | 0.51 (0.08) | 0.54 (0.02) | 5.1 (2.5) | 46 |
| Spanish Catalonia (SCT) | 73 | 0.52 (0.08) | 0.53 (0.02) | 5.8 (2.5) | 52 |
| Provence (PRO) | 50 | 0.44 (0.09) | 0.42 (0.02) | 4.6 (2.3) | 41 |
| Landes (LAN) | 32 | 0.53 (0.08) | 0.55 (0.03) | 3.8 (1.1) | 34 |
| East Comunidad Valenciana (ECV) | 69 | 0.53 (0.06) | 0.52 (0.02) | 4.6 (1.7) | 41 |
| South Castilla-La Mancha (SCM) | 50 | 0.50 (0.08) | 0.48 (0.02) | 4.1 (1.2) | 38 |

n , sample size; H_e , expected heterozygosity; H_o , observed heterozygosity; A, average number of alleles per locus. No. of total alleles, number of alleles found in each population. The standard deviation is given in parentheses.

numbers of alleles per locus are observed in the two island populations, Sicily and Minorca. Moreover, Minorca shows the highest number and proportion of private alleles (not found in other populations): 13.8% (eight out of 58), whereas continental populations (e.g. Landes, East Comunidad Valenciana or South Castilla La Mancha) have <5% of private alleles (data not shown). The Minorcan population is the least divergent one, as shown by the minimum average pairwise F_{ST} value (0.032) and G'_{ST} value (0.068), compared with, for example, $F_{ST} = 0.096$ and $G'_{ST} = 0.164$ for the Var (Provence) population (Table 3).

Diversity parameters for each of the Minorcan stands are shown in Table 4. A total of 58 alleles were recorded over nine microsatellite loci in the 67 mature trees. The three largest stands (Algarrovet, Llinarix Nou and Es Puig Mal) show contrasting inbreeding coefficients (Table 4), suggesting contrasted demographic histories. The inbreeding coefficient for Algarrovet is not significantly different from zero, while the positive and significant inbreeding coefficient detected in Llinarix Nou ($F_{IS} = 0.134$) may be due to high biparental inbreeding (i.e. inbreeding among genetically related trees) within this small population. A parentage analysis done in Llinarix Nou pointed out seven parent-offspring relationships among adult trees, involving five parent trees, which might be responsible for this deviation (data not shown). In contrast, inbreeding coefficients for Es Puig Mal are significantly negative ($F_{IS} = -0.14$); this result could be due to different causes, such as isolate breaking (fusion of formerly isolated populations) or adaptive advantage of heterozygote individuals.

Differentiation among Minorcan stands is comparatively high. The F_{ST} value among stands with ≥ 5 individuals reaches 0.104 ($P < 0.001$), while G'_{ST} reaches 0.214. F_{ST} and G'_{ST} values among the groups established with STRUCTURE and BAPS (see below) reach 0.065 and 0.184, respectively (pairwise F_{ST} and G'_{ST} values among these clusters are provided in Supplementary Table S2, available online). In comparison, the global F_{ST} and G'_{ST} values for all reference populations plus Minorca are only 0.049 and 0.119, respectively. Many private alleles have been found within Minorca, mostly in peripheral stands of the island: eight alleles in Algarrovet, seven in Binillubet, four in Sant Isidre and Llinarix Nou, and one in So Na Casana.

Identification of genetic structuring

Using the program STRUCTURE, the highest posterior probability value was obtained for $K = 2$. For this number of genetic pools, the Es Puig Mal stand is singled out, with 16 out of its 19 individuals showing a q -value (the probability of belonging to one of the pools) > 0.90 ; on the other hand, Algarrovet and Sant Isidre stands cluster together, with q -values for the other pool > 0.85 (9/13 and 5/5 individuals, respectively). Following the instructions of the authors of the software, these stands were removed for a second STRUCTURE analysis, in an attempt to distinguish further subtle structuring among the remaining localities. However, no further clustering of individuals was found ($K = 1$).

BAPS analysis in group mode generated seven different clusters (Fig. 2), three of them involving Minorcan groups: (I) the Algarrovet and Sant Isidre group with South Castilla

TABLE 3. Pairwise genetic differentiation [upper section, G'_{ST} values, according to Hedrick (2005); lower section, F_{ST} values] of Minorca's population and the reference populations

| | MIN | ECV | FCT | LAN | NCM | PRO | SIC | SCM | SCT | Average pairwise G'_{ST} |
|---------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------------------------|
| MIN | – | 0.048 | 0.044 | 0.091 | 0.047 | 0.156 | 0.079 | 0.048 | 0.034 | 0.068 |
| ECV | 0.019 | – | 0.067 | 0.103 | 0.046 | 0.147 | 0.076 | 0.028 | 0.064 | 0.072 |
| FCT | 0.019 | 0.036 | – | 0.154 | 0.086 | 0.178 | 0.114 | 0.087 | 0.039 | 0.096 |
| LAN | 0.041 | 0.053 | 0.091 | – | 0.100 | 0.181 | 0.130 | 0.116 | 0.114 | 0.124 |
| NCM | 0.020 | 0.023 | 0.044 | 0.052 | – | 0.188 | 0.134 | 0.034 | 0.111 | 0.093 |
| PRO | 0.096 | 0.084 | 0.113 | 0.105 | 0.105 | – | 0.109 | 0.166 | 0.184 | 0.164 |
| SIC | 0.026 | 0.035 | 0.058 | 0.062 | 0.063 | 0.060 | – | 0.097 | 0.091 | 0.104 |
| SCM | 0.026 | 0.015 | 0.052 | 0.062 | 0.021 | 0.097 | 0.044 | – | 0.094 | 0.084 |
| SCT | 0.010 | 0.035 | 0.019 | 0.061 | 0.056 | 0.105 | 0.041 | 0.052 | – | 0.091 |
| Average pairwise F_{ST} | 0.032 | 0.037 | 0.054 | 0.066 | 0.048 | 0.101 | 0.049 | 0.046 | 0.047 | – |

TABLE 4. Measures of genetic diversity for each population of Minorcan cork oak (*Q. suber*), based on nine nuclear microsatellites

| Population | <i>n</i> | H_e | H_o | A | F_{IS} |
|--------------------|----------|-------------|-------------|-----------|----------|
| Alfavaret (ALF) | 1 | 0.75 (0.16) | 0.75 (0.15) | 1.7 (0.5) | – |
| Algarrovet (ALG) | 13 | 0.54 (0.09) | 0.55 (0.05) | 3.8 (1.9) | –0.03 |
| Binigurdo (BGD) | 1 | 0.67 (0.17) | 0.67 (0.16) | 1.7 (0.5) | – |
| Binillubet (BLL) | 3 | 0.66 (0.10) | 0.57 (0.10) | 3.0 (1.3) | – |
| Binimoti (BMT) | 2 | 0.43 (0.11) | 0.50 (0.12) | 1.8 (0.7) | – |
| Es Puig Mal (PUG) | 19 | 0.43 (0.09) | 0.49 (0.04) | 3.0 (1.4) | –0.14* |
| Llinarix Nou (LLN) | 18 | 0.56 (0.08) | 0.47 (0.04) | 3.8 (1.9) | 0.13* |
| Sant Isidre (ISI) | 5 | 0.50 (0.10) | 0.49 (0.08) | 2.6 (1.3) | – |
| So Na Casana (CAS) | 5 | 0.54 (0.08) | 0.68 (0.08) | 2.7 (1.1) | – |

Sample size (*n*), observed heterozygosity (H_o), expected heterozygosity (H_e), mean number of alleles (A) and inbreeding coefficient per population F_{IS} (* $P < 0.001$) are given for populations with five or more individuals. The standard deviation is given in parentheses.

La Mancha and East Comunidad Valenciana; (II) Binimoti, together with Llinarix Nou, So Na Casana, Binillubet, Binigurdó and Alfavaret, cluster with Spanish and French Catalonia; and (III) Es Puig Mal clusters independently. The last four populations cluster independently: Var (Provence, cluster IV), North Castilla La Mancha (cluster V), Landes (VI) and Sicily (VII). The same seven clusters were obtained whether the geographical location of populations was included as prior information in the analysis or not. It is noteworthy that clustering based on nuclear markers does not match the geographic distribution of the chloroplast lineages (e.g. Algarrovet and Sant Isidre, with cpDNA of the *suber* lineage, cluster with East Comunidad Valenciana and South Castilla La Mancha, cpDNA *ilex-coccifera* I; Es Puig Mal, cpDNA *ilex-coccifera* I, is very close to Var, cpDNA *suber*).

DISCUSSION

Singularity of the Minorcan cork oak population

Together with Sicily (the other island population used as reference in this study), Minorca shows the highest average number of alleles per locus and the highest number of private alleles. A comparatively high level of genetic differentiation has been detected within Minorca. The F_{ST} value among the Minorcan groups (see below) is approx. 33 % higher than the

F_{ST} value among populations across the species range (0.065 vs. 0.049). Moreover, the Minorcan population is the least differentiated of all populations analysed, as judged by average pairwise F_{ST} and G'_{ST} values (Table 3).

An introduced origin of at least some of the cork oak stands in Minorca cannot be ruled out. The relatively high level of genetic diversity for both chloroplast and nuclear markers, and the high differentiation among stands within the island with low divergence with other populations across the species range suggest that at least some of the stands are not native. The easternmost stands (Sant Isidre and Algarrovet), with a cpDNA haplotype typical of the western Iberian Peninsula (López de Heredia *et al.*, 2005a), are the most serious candidates for an introduced status. However, no documented evidence of human introductions is known, and the species has never been exploited industrially on the island, which could have justified an introduction from western Iberia. In addition, discrepancies between clustering of Minorcan and reference populations, based on nuclear markers, on the one hand, and the distribution of chloroplast lineages, on the other hand, do not fully fit with a simple scenario of recent introduction from these different places.

Due to the extremely patchy distribution of suitable soils for cork oak, large populations of this species are not expected on the island, in contrast to western Iberia populations. Carrión (2002) proposed that, without human intervention, cork oak would occur in mixed populations, together with maritime pine and other oaks, rather than in large monospecific stands. Most of the private alleles found in peripheral populations of the island are restricted to holm oak (data not shown) and are probably the product of hybridization and subsequent backcrossing (i.e. introgression). Consistently, a high level of introgression with holm oak has been detected in Minorca, more than in any other studied population (Burgarella *et al.*, 2009). These results also suggest that Minorcan stands have maintained small sizes, at least for the last generations, thereby facilitating inter-species hybridization and further introgression (see, for example, Currat *et al.*, 2008; Lepais *et al.*, 2009).

Population structure within and between stands

Three different groups can be distinguished among Minorcan cork oak stands, according to clustering analysis and similarities with surrounding populations. These pools correspond to (a) two

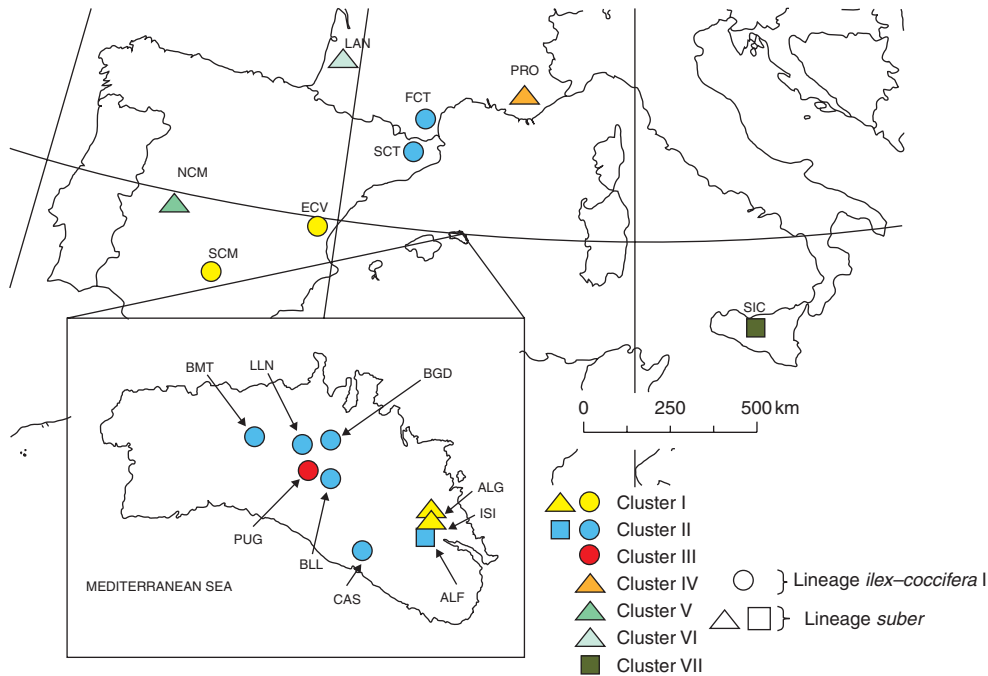


FIG. 2. Genetic structuring of Minorcan cork oak stands, and surrounding reference populations. Stands and populations are represented either with circles if they have the chloroplast lineage from Eastern Iberian *ilex-coccifera* I, with a triangle if they show the *suber* lineage from western Iberia, or with a square if they show Tyrrhenian haplotypes of the *suber* lineage. The colours correspond to clusters resulting from the BAPS analysis.

nearby clumps, on the easternmost part of the island, Algarrovet and Sant Isidre; (b) Es Puig Mal, in the centre of the island; and (c) the rest of the individuals. The recent origin of some stands, introduced from different sources, or the existence of effective long-term barriers to gene flow could account for this relative genetic isolation among groups.

Minorca lacks complex orography (Mount Toro, 354 m a.s.l., is the only peak in the island), favouring the homogenizing effect of gene flow through seed and especially pollen. However, edaphic discontinuities could constitute physical barriers to seed flow, given the specific soil requirements for cork oak. Genetic homogenization of Minorcan cork oak populations via pollen flow is probably hampered by the canopy of taller and bigger surrounding trees of other species, acting as a barrier. Phenological asynchronies in flowering (Levin, 1978), or pollen competition from other species (Waser, 1978) such as the predominant holm oak, may also account for the genetic isolation.

Implications for conservation

Cork oak is alarmingly endangered in Minorca. Its present population size is extremely low and no seedling was found during this study in any stand, probably due to the strong competition with other more heliophilous and frugal species (i.e. *Q. ilex*, *Pistacia lentiscus*, *Phillyrea* sp.). The high levels of diversity and differentiation reported here, together with the occurrence of unique genetic combinations observed in the island, make the Minorcan cork oak eligible for special conservation efforts (Eckert *et al.*, 2008). More detailed demogenetic monitoring of the Minorcan population could prove very valuable for our understanding of the role of hybridization and introgression in the evolutionary history of the species; for instance, some Minorcan cork oaks could have acquired

tolerance to non-siliceous soils by means of introgression of genes from holm oaks (indifferent to soil nature).

The protected status recently granted to the species in Minorca requires the adoption of active policies, although the native or exotic status of the species remains controversial. Conservation activities must take into account the three genetically differentiated groups identified in the present study. Conservation strategies should be focused on the viability of each group, favouring natural regeneration (i.e. reduction of overgrazing and competition with other species, soil improvement, etc.), rather than on generic policies for the species as a whole. The clusters based on nuclear genetic diversity will help to choose the plant stock provenance when reinforcement with seedlings from other populations is needed (e.g. in the smallest populations). The Alfavaret specimen, the only one on the island carrying the Tyrrhenian haplotype 2 of the *suber* chloroplast lineage (López de Heredia *et al.*, 2005a), deserves special protection. Fortunately, this individual has already been propagated using *in vitro* culture techniques (Hernández *et al.*, 2003, 2005), and the 50 ramets currently available from this individual will be useful for future conservation activities.

This work illustrates the importance of genetic information for the decision-making process on the allocation of efforts for the conservation of marginal populations. The special features reported here support the active conservation of the Minorcan population, and the factors discussed must be taken into account to generate effective conservation strategies.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxford-journals.org and consist of the following. Table S1: sampled localities of Minorcan cork oak trees, details of edaphology

and surrounding vegetation. Table S2: pairwise genetic differentiation of Minorca's clustered stands (G_{ST}' values and F_{ST} values).

ACKNOWLEDGEMENTS

We thank A. Hampe and M. Valbuena-Carabaña for their helpful comments. We also thank the editor and two anonymous referees for their constructive revision of a previous version. P. Hodgkinson revised the English language. We would also like to thank the Government of the Balearic Islands for their support. This work was partially funded by EU Project CREOAK (QLRT-2001-01594) and by the Spanish Ministry of Environment (DGB) through the covenant 'Evaluación y conservación de los recursos genéticos de los *Quercus* esclerófilos mediterráneos en España'.

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