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Complex population genetic structure in the endemic Canary Island pine revealed using chloroplast microsatellite markers

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Abstract The Canary archipelago, located on the northwestern Atlantic coast of Africa, is comprised of seven islands aligned from east to west, plus seven minor islets. All the islands were formed by volcanic eruptions and their geological history is well documented providing a historical framework to study colonization events. The Canary Island pine (*Pinus canariensis* C. Sm.), nowadays restricted to the westernmost Canary Islands (Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro), is considered an old (Lower Cretaceous) relic from an ancient Mediterranean evolutionary centre. Twenty seven chloroplast haplotypes were found in Canary Island pine but only one of them was common to all populations. The distribution of haplotypic variation in *P. canariensis* suggested the colonization of western Canary Islands from a single continental source located close to the Mediterranean Basin. Present-day populations of Canary Island pine retain levels of genetic diversity equivalent to those found in Mediterranean continental pine species, *Pinus pinaster* and *Pinus halepensis*. A hierarchical analysis of variance (AMOVA) showed high differentiation among populations within islands (approximately 19%) but no differentiation among islands. Simple differentiation models such as isolation by distance or stepping-stone colonization from older to younger islands were rejected based on product-moment correlations between pairwise genetic distances and both geographic distances and population-age divergences. However, the distribution of cpSSR diversity within the islands of Tenerife and Gran Canaria pointed towards the importance of the role played by regional Pliocene and Quaternary volcanic activity and long-distance gene flow in shaping the population genetic structure of the Canary Island pine. Therefore, conservation strategies at the population level are strongly recommended for this species.

Keywords Colonization · Gene diversity · Genetic structure · *Pinus canariensis* · Oceanic islands

Introduction

Oceanic islands are relatively simple systems which provide an ideal scenario for the study of patterns of gene flow and population subdivision (Francisco-Ortega et al. 1996; Hewitt 2001). Differentiation among islands has played a central role in the development of evolutionary theories from the 19th century onwards (see for example Grant 1986 or a recent review in Emerson 2002). To study colonization processes, adaptive radiation and inter-island vicariance, DNA data are better suited than morphology (Kim et al. 1996; Brown and Pestano 1998). In particular, organelle genomes (chloroplast and mitochondria) have recently received much attention in evolutionary biology. The relatively slow rate of sequence evolution, the small genome size of organelle DNA and the absence of sexual recombination make markers based on chloroplast and mitochondria DNA ideal for molecular phylogeographic studies (Avise 2000). Increasing use of this kind of markers for phylogeographic surveys has focused inter-island studies towards reconstructing the historical dispersal and evolutionary events that have led to present-

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day distributions of closely related groups of species (Brown and Pestano 1998).

The Canary archipelago, located on the northwestern Atlantic coast of Africa, is comprised of seven islands aligned from east to west: Lanzarote, Fuerteventura, Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro, plus seven minor islets. All the islands were formed by volcanic eruptions and their geological history is well documented (Ancochea et al. 1990; Coello et al. 1992). Isotopic datings of volcanic rocks reveal a general decrease in the age of the islands from east to west: Fuerteventura and Lanzarote formed 15–20 million (Ma) BP, Gran Canaria 13.5–14 Ma BP, La Gomera 11–12 Ma BP, Tenerife 5–8.5 Ma BP, La Palma 2 Ma BP and El Hierro less than 1 Ma BP. None of the Canary Islands seem to have been connected to the African continent, although distances among islands and between islands and the continent could have been smaller during ice ages due to a lower sea level. Given the prevailing trade winds and sea currents (from NE to SW), the Canaries have two possible sources of colonizers: northwestern Africa and the Iberian Peninsula (Juan et al. 2000). Rapid divergent evolution following colonization can be expected in oceanic islands. In the case of Canary Islands, between 25% and 45% of the native flora is endemic (Bramwell 1976, 1990), including several species which have developed insular woody habits from an herbaceous continental ancestor (see for instance Böhle et al. 1996 or a recent review in Baldwin et al. 1998).

Recently, the Canary Islands have become a focus for studies on colonization and diversification of different organisms (see review in Juan et al. 2000). Because of the general reduction of island age from east to west, a simple stepping-stone colonization model from older to younger islands has been proposed for some organisms (*Gallotia galloti*, Thorpe et al. 1994; genus *Pimelia*, Juan et al. 1995; genus *Hegeter*, Juan et al. 1996; *Drosophila subobscura*, Pinto et al. 1997; *Olea europaea* ssp. *cerasiformis*, Hess et al. 2000; *Apis mellifera*, De la Rúa et al. 2001). Conversely, other organisms show a genetic structure that was not in agreement with a directional stepping-stone pattern. In a phylogeography study of skinks (genus *Chalcides*), Brown and Pestano (1998) found that local extinctions and recolonizations during periods of intense volcanic activity were the main factor explaining the distribution of gene diversity in Canary Islands. In addition, multiple independent colonization events from the continent is a better model to explain the phylogenetic patterns found in several species (genus *Tarentola*, Nogales et al. 1998; *Dactylis glomerata*, Sahuquillo and Lumaret 1999; genus *Calathus*, Emerson et al. 1999, 2000) than a simple stepping-stone model based on a single colonization event.

Tree species typically show high within-population diversity and low among-population differentiation, even in geographical regions where recent colonizations have taken place (Hamrick et al. 1992; Austerlitz et al. 2000). The genetic population structure of forest trees has been extensively studied in several tropical and temperate species (see reviews in Hamrick et al. 1992; Loveless 1992; Ledig 1998 for *Pinus*), but island forest species studies are scarce.

The Canary Island pine (*Pinus canariensis* C. Sm.) is an endemic conifer from the western Canary Islands. Fossil records of *P. canariensis* have been found in Tertiary deposits from Mediterranean and Central Europe suggesting the differentiation of the species in an ancient Mediterranean evolutionary centre (Klaus 1989). Recent analyses of nuclear ribosomal and plastid DNA agreed about the close relation between *P. canariensis* and other Mediterranean diploxyl pines (Liston et al. 1999; Wang et al. 1999; López et al. 2002). In fact, Wang et al. (1999) found that Mediterranean pines formed one strongly supported clade within the subgenus *Pinus*, and within that clade *Pinus pinea, P. canariensis*, and *P. pinaster*, formed one group, albeit with weak (<50%) bootstrap support. The present-day native distribution of *P. canariensis* is restricted to the five westernmost Canary Islands: Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro. The origin of many plantations of *P. canariensis* in La Gomera is uncertain, but small relict pine forests (El Garabato and Roques de Imada and Agando) are present (Climent et al. 1996). No clear geographic structure at the population level was found in a wide-range study of morphological variation of *P. canariensis* cones (Gil et al. 2002). Moreover, allozymic studies showed high gene flow among islands and sharp genetic differences among populations within islands (Korol et al. 1999), which suggests both isolation at the population level and a weak island effect (Schiller et al. 1999).

The conservation of *P. canariensis* genetic resources in the Canary Islands is ecologically and economically rewarding. The Canary Island pine exhibits a remarkable adaptation to forest fires resulting from numerous traits, namely wide bark, presence of serotinous cones and sprouting ability. This adaptation, in combination with its ability to colonize poor soils in a dry environment, highlights the ecological importance of this species in the Canary Islands, where no other forest tree grows. In addition, *P. canariensis* forests play a crucial role in the water cycle of the islands through high water mist capture (Aboal et al. 2000). Beyond its natural range, plantations for wood production cover wide areas in Morocco and South Africa, and it is an object of great interest in reforestation programs in Israel and Australia. The Canary Island pine is also widely used for ornamental purposes in regions with a Mediterranean climate (primarily in the western Mediterranean Basin and California). The study of the distribution of genetic diversity, population structure and evolutionary features in the native range of the species is relevant in order to maintain the species' long-term variability and fitness and to direct genetic conservation programs. More realistic conservation strategies are possible if the distribution of genetic diversity between islands and populations within islands is better known.

The main aim of this paper is to elucidate the processes that have shaped the distribution of gene diversity in *P. canariensis*. Populations representative from the range-wide distribution of the species were analysed using chloroplast microsatellite markers (cpSSRs). The study of Canary Island pine has three main advantages compared to molecular studies in other Mediterranean diploxyl pines: (1) the restricted range of Canary Island pine makes it possible to sample the whole distribution of the species, (2) the well-established geological history of the islands provides a sound historical framework to study colonization events, and (3) the comparison between the endemic *P. canariensis* and closely related pine species from the continent (principally *P. pinaster* and *P. pinea*) can provide valuable information about general colonization and evolutionary processes in Mediterranean pines.

Materials and methods

Plant material

Needles were collected from trees in a provenance trial, established in 1999 in the Canary Islands. Eight populations covering the native range of the species were selected (Table 1), and 24 trees were randomly sampled from each of them. The island of La Gomera was excluded at this stage due to the small size of its native populations. All sampled populations belong to identified seed sources of putative native origin (Climent et al. 1996). Needles were stored at 4 °C until DNA isolation took place. Total DNA was extracted using a modified Doyle and Doyle (1990) protocol.

Molecular markers

Six pairs of chloroplast microsatellite primers were used: PT15169, PT30204, PT71936, PT87268, PT26081 and PT36480 (coded as in Vendramin et al. 1996). Primers were selected in order to obtain polymorphic regions for the species based on preliminary screening. The DNA amplifications by polymerase chain reaction (PCR) were carried out in a Perkin Elmer 9600 thermocycler, with the following PCR profile: 5 min denaturing at 95 °C, followed by 25 cycles of 1 min denaturing at 94 °C, 1 min annealing at 55 °C and 1 min extension at 72 °C, with a final extension of 8 min at 72 °C. The volume of the reaction mixture was 25 μl and contained: four dNTPs (each 0.2 mM), 2.5 mM of $MgCl_2$, 0.2 μM of each primer, 1× reaction buffer (Ecogen), 2.5 ng of template DNA and 0.5 U of *Taq*-polymerase (Ecogen). An aliquot of the amplification was denatured by adding an equal amount of formamide buffer and heating for 3 min at 94 °C. Fragments were loaded (0.65 μl) on 6% denaturing acrylamide/bisacrylamide 19:1 gels containing 7 M urea and 1× TBE buffer, and run (45 W constant power) together with external standards in a Li-Cor 4200 automatic sequencer. Fragments sizes were calculated by comparing them with the standards.

Data analysis

Genetic diversity estimates

Since the chloroplast genome does not undergo recombination, it can be considered as a single locus, and the size scores for the six polymorphic fragments analyzed were combined in order to derive the chloroplast haplotype of each individual. Nevertheless, we will use the term *locus* to refer to a cpSSR region, and *allele* to refer to a size variant at a given cpSSR region. The following population genetic parameters were computed for each population: haplotype frequency (p_i), number of haplotypes (n_h), number of private haplotypes (n_{hp}), number of polymorphic loci (n_p), effective number of haplotypes (n_e), and Nei's unbiased haplotypic diversity (H_e; Nei 1987). Within-population genetic distance between haplotypes, D^2, as defined by Goldstein et al. (1995) was also computed. This distance is based on the differences among the number of repeat units at the microsatellite regions (stepwise mutation model, SMM) considering the chloroplast DNA as a single locus.

Population genetic structure

The population genetic structure of Canary Island pine was investigated using an analysis of variance framework (AMOVA, see Excoffier et al. 1992 for details) based on the sum of the squared number of repeat differences between two haplotypes (Slatkin 1995). A hierarchical analysis of variance was used to partition the total variance into covariance components due to within-population variation, variation among populations within islands and variation among islands. The significance of covariance components was tested using permutation tests (1,000 permutations) at different levels (haplotypes among populations among islands, haplotypes among populations within islands and populations among islands). Only *P*-values lower than 0.05 were considered significant. The AMOVA analysis and significance tests were performed using Arlequin ver. 2000 (Schneider et al. 2000).

Specific hypotheses about spatial patterns in the distribution of chloroplast gene diversity at the population level were tested using Mantel tests (Mantel 1967). Comparisons were made computing the product-moment correlation between a matrix of pairwise genetic distances based on allele size differences (Goldstein et al. 1995) and (1) a matrix of Euclidean geographical distances, and (2) a matrix of divergence ages between pairs of islands estimated to be the difference between the maximum geological age of each individual island (see Juan et al. 1996 and Pinto et al. 1997 for similar spatial analyses). A correlation between genetic distances and divergence ages is expected if, indeed, a simple stepping-stone colonization model from older to younger islands has occurred for this species. In both cases, a null hypothesis of no correlation of the matrices was constructed using a permutation procedure. Cells of one of the matrices were randomly permutated, whereas cells of the other were not. For each permutation the product-moment correlation between the two distance matrices was computed. The procedure was repeated 1,000 times and the actual value was compared to the distribution of the null hypothesis. Finally, we also tested if gene dispersal across water was lower than gene dispersal

Table 1 Location of Canary Island pine populations. The provenance region refers to breeding units as described in Climent et al. (1996)

Code	Population	Provenance region	Latitude	Longitude	Altitude
TF1a	La Guancha	1.a Tenerife	28°22′	16°40′	700
TF1b	Vilaflor	1.b Tenerife	28°11′	16°38′	1900
TF1c	La Esperanza	1.c Tenerife	28°25′	16°23′	1100
LP2a	Garafía	2.a La Palma	28°47′	17°55′	1500
LP2b	Fuencaliente	2.b La Palma	28°31′	17°50′	1050
EH3	San Salvador	3 Hierro	27°42′	18°00′	1000
GC4a	Tamadaba	4.a Gran Canaria	28°03′	15°41′	1100
GC4b	Mogán	4.b Gran Canaria	27°56′	15°44′	900

across land by computing haplotypic genetic distances from each population to its closest neighbouring population along a sea and a land pathway. Differences among means were then tested using a signed rank test for pairs of data.

Results

Distribution of haplotypes and genetic diversity estimates

The combination of 20 chloroplast microsatellite alleles found in six polymorphic loci resulted in a total number of 27 haplotypes (Table 2). Eight haplotypes from the 27 detected were shared among populations and only four of them were present in more than two islands. The frequencies of shared and population-private haplotypes for each population are shown in Fig. 1. Only one haplotype is common to all populations (H23). The frequency of this haplotype is high in all but two populations. The exceptions were TF1a (La Guancha) and GC4b (Mogán), where less than 25% of the individuals had this haplotype. Three other haplotypes (H19, H20, H21) were common to most populations. Populations from eastern Tenerife (TF1b and TF1c) lacked two of these haplotypes (H20 and H21) and shared one specific haplotype (H10). Population GC4b from southern Gran Canaria showed the highest percentage of haplotype H20 (54%) and shared a specific haplotype (H27) with GC4a (Tamadaba) in northern Gran Canaria. H21 was distributed mainly throughout the western Canary Islands (western Tenerife-TF1a, La Palma and El Hierro) but also in northern Gran Canaria. As H21 is only one mutational step from the widespread H23 it is not surprising to find a disjoint distribution for this haplotype as it could have been independently produced several times over. The number of population-private haplotypes was very high (from 12.5% in LP2a to 41.7% in TF1a) in all populations except TF1b (Vilaflor) and GC4a (Tamadaba). These populations could have been created by migration from populations in the same islands (TF1c and GC4b, respectively), which show very similar haplotypic compositions. It is also noteworthy that in Tenerife, the western population TF1a had only three haplotypes (out of the 15 present in this island) in common with the other two populations from Tenerife, TF1b and TF1c, while these last populations shared all haplotypes.

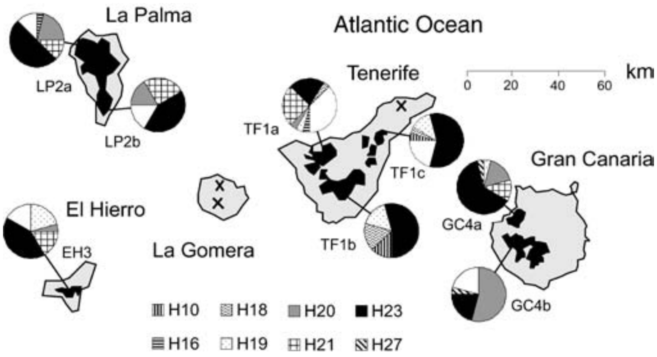

Fig. 1 Distribution of shared and population-private haplotypes in present-day populations of Canary Island pine. *White sectors in circular graphs* indicate the percentage of population-private haplotypes. The *shadowed area* represents the main range of the species; relic populations have been indicated with a *cross*

Table 2 Haplotype definition resulting from the combination of 20 chloroplast microsatellite alleles found in six polymorphic loci

Code	PT15169	PT30204	PT71936	PT87268	PT26081	PT36480
H1	118	142	149	162	110	138
H2	118	143	149	163	110	138
H3	118	141	148	165	111	138
H4	118	143	150	162	111	138
H5	118	143	149	164	111	137
H6	118	143	148	165	111	137
H7	118	142	149	165	110	138
H8	118	141	150	165	110	138
H9	118	143	150	163	111	138
H10	118	143	149	164	111	138
H11	118	142	150	164	111	138
H12	118	143	148	165	111	138
H13	118	142	149	165	111	138
H14	118	143	150	164	110	138
H15	118	143	149	165	110	138
H16	118	142	150	165	110	138
H17	118	143	150	165	109	138
H18	118	143	150	164	111	138
H19	118	143	149	165	111	138
H20	118	142	150	165	111	138
H21	118	143	150	165	110	138
H22	119	142	150	165	111	138
H23	118	143	150	165	111	138
H24	118	143	150	166	110	138
H25	118	143	150	167	110	138
H26	118	143	153	165	110	138
H27	118	143	153	165	111	138

Table 3 Genetic diversity parameters in Canary Island pine populations; sample size (n), number of haplotypes (n_h), number of private haplotypes (n_{hp}), number of polymorphic loci (n_p), effective number of haplotypes (n_e), Nei's unbiased haplotypic diversity (H_e), and within-population genetic distance between haplotypes (D^2)

Code	n	n_h	n_{hp}	n_p	n_e	H_e	D^2
TF1a	24	10	5	4	6	0.86	1.06
TF1b	24	4	0	2	3	0.66	0.22
TF1c	24	8	4	3	3	0.66	0.58
LP2a	24	6	3	3	3	0.71	0.46
LP2b	24	6	2	4	4	0.76	0.51
EH3	24	8	4	4	4	0.78	0.39
GC4a	24	5	0	3	2	0.59	0.34
GC4b	24	5	2	4	3	0.66	0.35
Average	24	6.5	2.5	3.4	3.3	0.73	0.49

Genetic diversity parameters based on haplotypes are shown in Table 3. The number of haplotypes in each population ranged from ten in TF1a (La Guancha) to four in TF1b (Vilaflor). Considering all populations together, mean values of the effective number of haplotypes, the percentage of polymorphic loci and Nei's genetic diversity were 3.3, 56% and 0.73, respectively. A wide range of genetic diversity was found at the population level. The effective number of haplotypes (n_e) and genetic diversity (H_e) had the highest values in population TF1a (6 and 0.86) whereas the lowest values occurred in population GC4a (2 and 0.59). At island level, mean Nei's genetic diversity values for Tenerife, La Palma and El Hierro were similar (0.73, 0.74 and 0.78, respectively) and higher than in Gran Canaria (0.63). The stepwise mutation model D^2 showed a similar trend to H_e except in one notable case, TF1b (Vilaflor), which had a low D^2 value even when H_e was relatively high (note that D^2 in TF1b was less than half that of TF1c but H_e was 0.66 in both populations). The distribution of pairwise cpSSR repeat-length differences among individuals within TF1b showed that most pairs of haplotypes were differentiated by only one or two mutational steps (data not shown). This fact, together with the lack of population-private haplotypes, could indicate a relatively recent origin of the TF1b population.

Population genetic structure

The hierarchical analysis of variance (AMOVA) showed high differentiation among populations within islands (approximately 19%, $P \leq 0.00$) but no differentiation among islands. Only when populations from the westernmost islands (La Palma and El Hierro) were grouped, some significant differentiation among islands was detected (approximately 3%, $P \leq 0.03$). The computation of pairwise SMM genetic distances (Table 4) provided further evidence of within islands differentiation, in Tenerife the average distance between TF1c and TF1a/TF1b was 0.074, and genetic similarity occured among populations from La Palma and El Hierro (distances not significantly different from zero). It is also noticeable the relatively low pairwise genetic distances between populations from Gran Canaria and those located at La Palma and El Hierro (primarily pairs LP2a-GC4a, LP2b-GC4a and LP2a-GC4b). No correlation was found between pairwise SMM genetic distances and either Euclidean geographical distances (–0.016 n.s.) or divergence ages (–0.115 n.s.). Therefore, simple differentiation models as isolation by distance or stepping-stone colonization from older to younger islands can be rejected. The complex physiography of the territory or differences in dispersal across the sea (i.e. among islands) and across land (i.e. among populations within islands) could confound simple isolation by distance models. In the present case, no significant differences between gene dispersal across water (mean genetic distance: 0.076, SD: 0.037) and gene dispersal across land (mean genetic distance: 0.057, SD: 0.021) were observed.

Discussion

Levels of chloroplast gene diversity in Canary Island pine were within the range of those found in mainland populations of Mediterranean continental pines. In fact, chloroplast genetic variation in *P. canariensis* was halfway between that found in *P. pinaster* and *Pinus halepensis* from the Iberian Peninsula (mean values per population are: n_h = 11.2, n_e = 6.9 and H_e = 0.85, and n_h = 5, n_e = 2.52 and H_e = 0.56 for *P. pinaster* and *P. halepensis*, respectively; G.G. Vendramin personal communication; own unpublished results). On the other hand, *P. canariensis* showed a higher number of private haplotypes per population than both *P. pinaster* and *P. halepensis* (2.5 in *P. canariensis* versus less than 1 in *P. pinaster* and *P. halepensis*). Allozyme analysis had previously revealed similar levels of genetic variation in *P. canariensis* compared with other Mediterranean hard pine species (Schiller et al. 1999 and references therein).

Table 4 Matrix of pairwise genetic distances between populations based on the stepwise mutation model statistic of Goldstein et al. (1995); *ns*: values not significantly different from zero ($P \geq 0.05$) as shown by a permutation test (1,000 permutations of haplotypes between populations)

Pop.	TF1a	TF1b	TF1c	LP2a	LP2b	EH3	GC4a
TF1b	0.079						
TF1c	0.070	*ns*					
LP2a	0.067	0.032	0.045				
LP2b	0.098	0.088	0.113	0.030			
EH3	0.047	0.033	0.045	*ns*	*ns*		
GC4a	0.101	0.036	0.053	*ns*	*ns*	0.021	
GC4b	0.141	0.113	0.124	0.050	0.092	0.099	0.055

The fact that Canary Island pine, with fragmented distribution, retained levels of genetic variation equivalent to those found in continuously distributed continental pines, might mean that effective population sizes in this species have remained large enough over time to prevent the erosion of genetic diversity by drift.

Twenty seven haplotypes were detected and only one was common to all populations (H23). Three other haplotypes, which are differentiated from H23 by only one mutational step, were common to most islands although not to all populations and they could represent different chloroplast lineages derived from H23. The higher frequencies and the wider geographical distribution of these haplotypes may reflect their ancient origin (Crandall and Templeton 1996). The Canary Island pine is considered an old (Lower Cretaceous) relic from an ancient Mediterranean evolutionary centre (Klaus 1989). Fossil cones of *P. canariensis* have been found in Austrian Miocene basins, southern France (Oligocene and Miocene) and southern Spain (Pliocene). In addition, three-needled bundles found along the Mediterranean Basin are frequently described as fossils of this species. Only one fossil record from Canary Islands (La Palma) has been reported dating back as far as the Pliocene (Gregor 1980). Colonization from the Mediterranean basin into Canary Islands have been described for several plant species. Unique colonization events in the late Miocene or early Pliocene following rapid radiation have been suggested for plants with an insular woody habit (genera *Sonchus*, Asteraceae and *Echium*, Boraginaceae) based on molecular evidence (Böhle et al. 1996; Kim et al. 1996). Moreover, the monophyly of *Argyranthemum* in Macaronesia suggested a unique continental origin of this genus (Francisco-Ortega et al. 1996). In contrast, Sahuquillo and Lumaret (1999) showed that cpDNA introgression from the Mediterranean has occurred more than once in Canarian *Dactylis glomerata*. With respect to the Canary Island pine, the existence of only one widespread haplotype (H23), from which different geographically structured chloroplast lineages were probably derived, might indicate a common origin of present-day populations. This agrees with the preliminary construction of phylogenetic trees based on haplotypes that were consistently monophyletic when haplotypes from other closely related pine species as *P. pinea* were included in the analysis (data not shown). *P. canariensis* is nowadays absent from the oldest Canary Islands (Fuerteventura 20 Ma BP and Lanzarote 15.5 Ma BP, in the east) but there is certain evidence (toponyms, pollen records and charcoals) that indicates its historical presence (Climent et al. 1996; Machado-Yanes 1996). These eastern islands are situated upstream of the prevailing winds and sea currents. Thus, Fuerteventura and Lanzarote could have acted as migration pathways from a single continental source located close to the Mediterranean Basin to the rest of the Canary Islands.

Population genetic structure is complex in the Canary Island pine and both simple isolation by distance and stepping-stone models (as those suggested for the Canarian olive tree; Hess et al. 2000) can be rejected. The distribution of cpSSR diversity and the pairwise SMM genetic distance matrix for *P. canariensis* within the islands of Tenerife and Gran Canaria pointed towards some degree of differentiation between northern and southern populations (Gran Canaria) and eastern and western populations (Tenerife). This molecular divergence may result from geographical or ecological isolation. In the case of Tenerife, a repeated phylogeographic pattern associated with the Anaga (northeastern Tenerife) and Teno (western Tenerife) massifs has been found in several diverse organisms (see review in Juan et al. 2000). These old, formerly isolated pre-Tenerife islands were joined by relatively recent volcanic activity (<2 Ma BP), explaining present-day within-Tenerife distribution as well as the phylogeography of lizards, skinks, beetles, mites, spiders and cockroaches. A similar pattern of genetic structure is clearly shown by Canary Island pine, where eastern populations are strongly differentiated from western populations, the latter being more closely related to western Canary Islands (La Palma and El Hierro) than to eastern Tenerife populations. Present-day geographical isolation among populations within the island could result from the younger volcanic sequences formed at the central composite volcano of Las Cañadas and along a SW-NE ridge linking Las Cañadas and Anaga (named Cordillera Dorsal).

A different explanation is required to account for differences found in Gran Canaria as this island is believed to have risen in a single short and intense shield-building phase, and since its origin has always been formed by a single unit (Nogales et al. 1998). Local extinctions and recolonizations from diverse sources during periods of intense volcanic activity (3.4–4.5 Ma BP; Juan et al. 2000; Rees et al. 2001) could explain differentiation between northern and southern populations in Gran Canaria. In fact, the GC4a population in northern Gran Canaria (Tamadaba) showed no private haplotypes and a mixed haplotypic composition that may have originated from relatively recent gene flow from eastern Tenerife and southern Gran Canaria (the GC4b population). This coincides with the late pattern (Quaternary) of volcanic activity in Gran Canaria which showed a concentration of volcanism in the northern part of the island (Funck et al. 1996). Figure 2 shows a correlation graph between pairwise genetic distances and divergence ages between pairs of islands taking into account the eastern and western Tenerife populations as originated in the Anaga (5.8 Ma BP) and Teno (7.4 Ma BP) massifs, respectively, as well as a relatively recent origin of the GC4a population (4 Ma BP). In this particular case, the correlation is significant (0.527 $P \leq 0.030$). Only pairs including the population from southern Gran Canaria (GC4b) fail to follow the general trend, resulting from a late colonization of the island or some gene flow between southern Gran Canaria and the La Palma and El Hierro islands in the westernmost range of the species.

The high amount of private haplotypes found in most *P. canariensis* populations (12.5% to 41.7%) indicates a

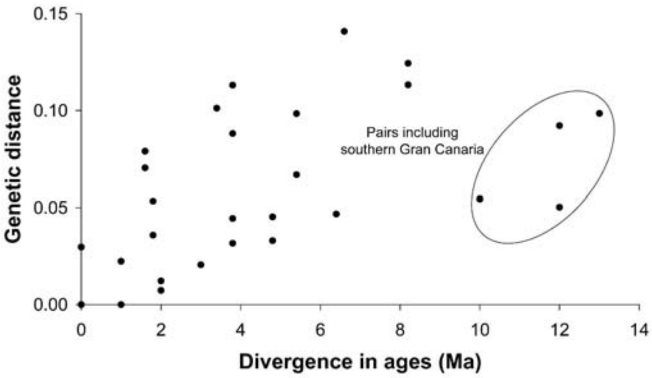

Fig. 2 Pairwise correlation between Goldstein et al. (1995) genetic distance and the time of inter-population divergence estimated from geological data. Population age was considered equal to the island maximum geological age for populations located in southern Gran Canaria, La Palma and El Hierro. Population ages of present-day Tenerife populations were considered equal to the age of pre-Tenerife Anaga (5.8 Ma BP) and Teno (7.4 Ma BP) massifs for eastern (TF1b and TF1c) and western (TF1a) Tenerife populations, respectively. GC4a population (northern Gran Canaria) was considered to be a recent colonization (4 Ma BP) after extinction caused by volcanic activity

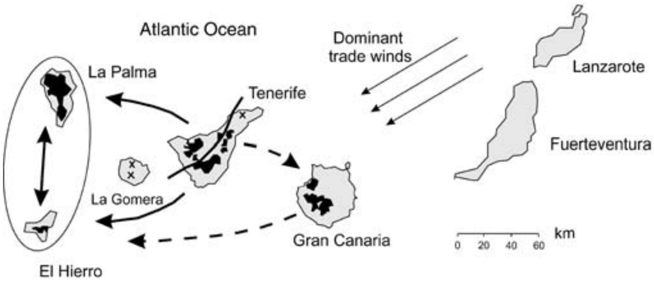

Fig. 3 Groups of populations and reconstruction of colonization events in Canary Island pine based on cpSSR markers. The *shadowed area* represents the main range of the species; relic populations have been indicated with a *cross*

certain degree of isolation. However, historical gene interchange among islands seems frequent (Fig. 3). Juan et al. (2000) noted that extensive inter-island dispersal between similar ecological zones has been a significant factor in the evolution of the Canarian flora. The populations of Canary Island pine located in the younger islands (La Palma and El Hierro) show similar haplotypic compositions, and pairwise SMM genetic distances were not significantly different from zero, in agreement with gene-flow vectors detected by allozymes in this species (Korol et al. 1999) and phylogeographic studies in other organisms (Coleoptera: Tenebrionidae, Juan et al. 1996; Rees et al. 2001; *Drosophila subobscura*, Pinto et al. 1997). *Pinus canariensis* seems to have dispersed from Tenerife to the west following two independent pathways: one from northwestern Tenerife to La Palma and the other from southeastern Tenerife to El Hierro, the latter probably through La Gomera where some relic populations of *P. canariensis* can still be found. Curiously, a similar geographical pattern has been found for the endemic lizard of the western Canary Islands *G. galloti* (Thorpe et al. 1994). The distribution of haplotypes, along with the genetic distances among populations, also indicates the probability of some gene interchange between Gran Canaria and the westernmost islands. Long-distance dispersal between the western and eastern Canary Islands has been proposed in order to explain the complex phylogenetic pattern of the Canarian endemic genus *Argyranthemum* (Francisco-Ortega et al. 1996). Sahuquillo and Lumaret (1999) found two chlorotypes (based on cpDNA RFLP patterns) in *D. glomerata* from the Canary Islands. One of them was distributed throughout Lanzarote, La Palma and Gomera, whereas the other was restricted to Tenerife and La Palma. Chloroplast gene interchange in Canary Island pine among islands could be facilitated by extensive current pollen flow and/or long-distance seed gene flow. Pollen flow is expected to be considerable in pine species both in terms of dispersal distances and quantity. Long-distance seed gene flow in *P. canariensis* could, in turn, be facilitated by its seed traits. *P. canariensis* possesses seeds with undetachable wings (adnate seed wings), a rare attribute shared with other island pines as *Pinus caribaea*, a tropical conifer from the Caribbean region. When such seeds are released, they will not loose their wings thus increasing their dispersal ability. *P. caribaea*, similarly to the Canary Island pine, retains levels of genetic variation equivalent to those found in continental pine species and a complex population genetic structure (Zheng and Ennos 1999). Klaus (1989) suggested that pines with adnate seed wings have the capacity to 'island hop', which could explain their Tertiary spread throughout the Tethys and present-day long-distance colonization.

The distribution of genetic diversity detected by cpSSR markers in *P. canariensis* and the putative monophyly of the species in Canary Islands suggest a single continental origin for the Canary Island pine which could have found refuge in the Canary Islands after the climatological changes occurring in the Mediterranean region in the late Tertiary. Long-distance dispersal, Pliocene and Quaternary volcanic activity and ecological constraints (e.g. forest fires and drought) can be considered major factors in shaping the present-day range of *P. canariensis*. Population genetic structure is complex in Canary Island pine and differentiation among populations within each island is high. Thus, conservation strategies for this species should be developed at the population level. The existence of ancient populations with several population-specific haplotypes along with recent populations with a remarkable lack of genetic diversity makes the study of population haplotypic variation a necessary prerequisite before the establishment of genetic reserves for conservation purposes. In particular, genetic analysis of candidate pine forest reserves in islands with a complex geological history, as is the case in Gran Canaria and Tenerife, should play an integral part in any conservation policy decision-making.

On-going research in Canary Island pine includes phylogenetic studies based on a wide range of markers (namely allozymes, cpSSRs, mtDNA RFLP fragments and DNA sequence data) and the analysis of relict populations that are typically formed by only a few old individuals (La Gomera and Roques de Anaga in Tenerife). The new insights to be provided by these on-going studies will allow us to describe in more detail the patterns of population genetic structure in Canary Island pine and to design specific conservation strategies for this ecologically and economically important endemic pine species.

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