Heredity (2004) 93, 307–315

www.nature.com/hdy

Allozyme variation and structure of the Canarian endemic palm tree *Phoenix canariensis* (Arecaceae): implications for conservation

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Electrophoretic analysis of 18 allozyme loci was used to estimate the levels and structuring of genetic variation within and among natural populations of the protected endemic palm species from the Canary Islands (*Phoenix canariensis*) to evaluate its genetic relationship with the widespread congener *P. dactylifera*, and to assess comparatively the genetic variation in the populations where the two species coexist with morphologically intermediate plants (mixed populations). Our survey revealed that the within-population component explains roughly 75% of the genetic variation levels detected in *P. canariensis* ($A = 1.59$; $P = 41.8$; $He = 0.158$), which rank higher than those reported for other species of the Arecaceae. A Principal Component analysis (PCA) based on allele frequencies consistently separates populations of *P. canariensis* and *P. dactylifera*, and reveals a close genetic relationship between *P. canariensis* and the mixed populations. Reduced levels of genetic variation in *P. canariensis* with respect to *P. dactylifera*, the fact that the genetic makeup of the Canarian endemic (with no unique alleles) is a subset of that found in *P. dactylifera*, and the high genetic identity between both species strongly suggest that *P. canariensis* is recently derived from a common ancestor closely related to *P. dactylifera*.
Heredity (2004) **93,** 307–315. doi:10.1038/sj.hdy.6800507
Published online 9 June 2004

Keywords: allozymes; Canary Islands; conservation genetics; genetic differentiation; hybrids; *Phoenix canariensis*; *Phoenix dactylifera*

Introduction

Phoenix canariensis Hort. ex Chabaud and *P. dactylifera* L. are diploid ($n = 18$), long-lived, dioecious, arborescent monocots that are closely related (Barrow, 1998; Sosa *et al*, 1998) and exhibit strikingly different distribution ranges. While *P. canariensis* is endemic to the Canary Islands (Kunkel and Kunkel, 1974), *P. dactylifera* is distributed from western Asia to north-eastern Africa, including the Canarian archipelago. The need to conserve *P. canariensis* has been formalised by the Canarian Government through approving a decree that recognises its threatened status (BOC, 1991), and its communities are included in the European Habitat Directive (92/43/EEC) as priority. However, the implementation of sensible management strategies to warrant long-term survival of this Canarian endemic has been stalled by the lack of knowledge regarding the levels and distribution of its genetic variability. Although Sosa *et al* (1998) reported data on the levels of isozyme genetic variation within *P. canariensis*, only two populations from Gran Canaria were analysed in that study.

The population structure of the Canarian endemic *P. canariensis* presents several interlinked problems that interfere with the design of strategies to conserve its threatened variability. One of them stems from the difficulty of establishing the identity of individuals or populations for anthropogenic and biological reasons. For example, Canarian farmers have often introduced date palm (*P. dactylifera*) specimens in natural populations of the Canary palm (*P. canariensis*) for enhanced exploitation (Santana and Toledo, 1997). However, the lack of historical records and the *ad hoc* nature of these introductions make it difficult to determine where planting was performed and where populations grew naturally.

Closely related to the above, the distinction of *P. canariensis* from *P. dactylifera* is at present based solely on morphological traits, and it would be easily accomplished if the differences among individuals of both species were as obvious as described by different authors (Kunkel and Kunkel, 1974; Chabaud, 1882; Barrow, 1998), who agree in that *P. canariensis* has a bushier crown, darker green leaves and a thicker trunk with no offshoots, and smaller fruits than *P. dactylifera*. However, in nature, juvenile individuals of both species are impossible to distinguish using these indicators, and we frequently find adult specimens that share features of both species.

In addition, hybridisation between *P. dactylifera* and *P. canariensis* in nature has been hypothesised by different authors (Kunkel and Kunkel, 1974;

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Received 10 July 2003; accepted 1 April 2004; published online 9 June 2004

Hodel, 1995; Barrow, 1998; Morici, 1998; Sosa *et al*, 1998). The ability of *P. canariensis* to hybridise with *P. dactylifera* might pose the biggest problem for conservation, because it could foster the coexistence of both species and their supposed hybrids in stands where planting was carried out and because it would be difficult to detect and remove the hybrid individuals (Barrow, 1998).

Unwitting planting in the past, the inherent plasticity of morphological traits and the apparent hybridisation of *P. dactylifera* with *P. canariensis* hinder the characterisation of many palm populations in the Canaries.

Allozyme polymorphisms have provided valuable data to assess the genetic variability of endemic species (Allphin *et al*, 1998; Godt and Hamrick, 1998; Batista *et al*, 2001), and their population genetic structuring (Schnabel *et al*, 1991; Shapcott, 1995; Caujapé-Castells *et al*, 1999). They have also been used successfully to characterise and differentiate close by related species, ecological varieties or natural and artificial hybrids (Rieseberg *et al*, 1989; Booij *et al*, 1995; Bendiab *et al*, 1998; Elisiário *et al*, 1999). Therefore, they could provide valuable insights into the genetic problems posed by the Canarian endemic *P. canariensis*.

Our aims in this paper are to (1) explore the levels of genetic isozymic variation in *Phoenix* populations from the Canary Islands; (2) analyse the genetic structure of the natural populations of *P. canariensis*; (3) compare *P. dactylifera*, *P. canariensis* and their putative hybrids using allozyme variation and (4) use the resulting information to provide practical guidelines for the conservation genetics of *P. canariensis*.

Materials and methods

Plant material

We sampled 20 populations (Figure 1) that were classified into one of three classes according to morphological traits: (i) nine natural populations of phenotipically pure *P. canariensis* localised in La Palma and La Gomera (where *P. dactylifera* has not been recorded; Izquierdo *et al*, 2001), and in very isolated areas of Tenerife and Gran Canaria located several kilometres from the nearest *P. dactylifera* population; (ii) four populations of phenotipically pure *P. dactylifera*: three of them imported recently from the Mediterranean region and planted in landscape areas, and one from Elche (a locality in the mid east of Spain); and (iii) seven populations from Gran Canaria, Lanzarote and Fuerteventura that contained individuals of *P. canariensis*, *P. dactylifera* and a continuous range of morphologically intermediate plants, designated mixed populations. Hybrid zones rarely represent a single generation and hybrids from these zones are a mosaic of parental and intermediate characters rather than solely intermediate ones (Rieseberg and Ellstrand, 1993).

Finally, we also included one population of *P. theophrasti* (a Mediterranean species from the Island of Crete, Barrow, 1998) that was used as an external reference.

The leaves of the sampled individuals were kept in a portable cooler until subsequent manipulation in the laboratory, where they were rinsed with distilled water and dried. After this procedure, the samples were kept at −80°C until electrophoresed.

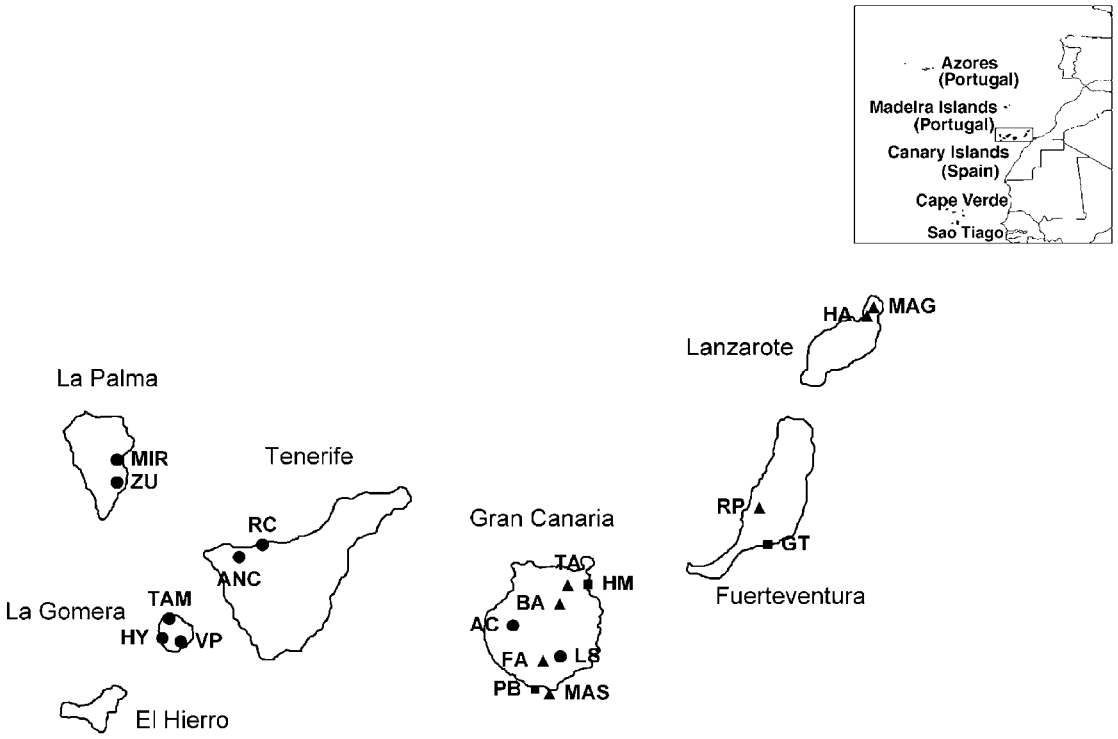

Figure 1 Populations sampled of *Phoenix canariensis* (●), *Phoenix dactylifera* (■) and mixed populations (▲) in the Canary Islands. Population codes are as in Table 1.

Electrophoretic analyses

Enzymes were extracted by crushing leaves with liquid nitrogen using a mortar and pestle until a fine-grained powder was obtained. Subsequently, an extraction buffer (Torres and Tisserat, 1980) was added to the cold, powdered leaf material to dissolve and stabilise the enzymes. Enzyme extracts were absorbed onto Whatman n°3 paper wicks and kept at −80°C until analysed electrophoretically.

Horizontal starch gel electrophoresis was carried out for 10 isozyme systems, namely aconitase (ACO, EC. 4.2.1.3), alcohol dehydrogenase (ADH, EC. 1.1.1), glucose-6-phosphate dehydrogenase (G6PDH, EC. 1.1.1.49), isocitrate dehydrogenase (IDH, EC. 1.1.1.42), malate dehydrogenase (MDH, EC. 1.1.1.37), malic enzyme (ME, EC. 1.1.1.40), phosphoglucose isomerase (PGI, EC. 5.3.1.9), phosphoglucose mutase (PGM, EC. 5.4.2.2), shikimate dehydrogenase (SDH, EC. 1.1.1.25) and 6-phosphogluconate dehydrogenase (6pgdh, EC. 1.1.1.44).

Electrophoresis was conducted in 12% starch gels in different electrode/gel buffer systems. For ADH, G6PDH, IDH, MDH, ME, SDH and 6PGDH, we used Morpholine-Citrate 6.1/Morpholine-Citrate 6.1 (Clayton and Tretiak, 1972). Systems ACO and PGI were resolved on Boric acid pH 8.7/Tris Citrate pH 7.9 (Torres and Tisserat, 1980). Finally, PGM was resolved on Tris-Citrate pH 7/Histidine-Citrate 5.7 (Stuber *et al*, 1977). All the staining recipes were based on Wendel and Weeden (1989), although, for some isozymes, some modifications related to substrate concentration and final pHs of the staining solutions were introduced to improve band resolution.

Data interpretation

For each locus, the alleles were labelled and ordered following the alphabetical sequence in order of their mobility towards the anode. In most cases, the number of bands in heterozygous individuals was consistent with the expected quaternary structures of the corresponding enzyme (Wendel and Weeden, 1989), although null alleles were inferred in *Mdh-1* and *Pgm-1* (González-Pérez, 2001).

Data analysis

Elementary genetic allozymic variability statistics, genetic diversity indices (Nei, 1973) and genetic distance values (Nei, 1972) were calculated from genotype data per population using BIOSYS-1 (Swofford and Selander, 1981) and GENSTAT-PC 3.31 (Lewis, 1993).

Exact Hardy–Weinberg tests to measure the significance of deviations from the null hypothesis of random union of gametes (Weir, 1990; Guo and Thompson, 1992) were carried out on natural populations of *P. canariensis* and on the mixed populations using GENEPOP 3.1 (Raymond and Rousset, 1997). Nei's (1973) statistics of intra- and interpopulation variation (G_{ST}, H_T, D_{ST}) were calculated with GENSTAT-PC 3.31 (Lewis, 1993).

A multivariate representation of the palm populations sampled was carried out by subjecting allele frequencies to a Principal Component Analysis (PCA) in SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). The genetic differentiation among individual populations and among the three classes of populations identified was studied using the genetic differentiation coefficient (F_{ST}) calculated through the genetic analysis software GENEPOP 3.1 (Raymond and Rousset, 1997).

Results

Allozyme diversity within and among populations

The 10 isozyme systems analysed allowed us to interpret 18 putative loci, four of which (*G6pdh*, *Mdh-2*, *Mdh-3* and *Pgi-1*) were monomorphic throughout, while all the remaining loci were polymorphic. Four of the total 43 alleles were exclusive to *P. dactylifera* populations (*Mdh1*-c, *Mdh1*-e, *Pgm2*-a and *Pgm2*-e). One exclusive locus (*Mdh-4*) was detected in the eastern Mediterranean species *P. theophrasti*. Furthermore, populations of *P. canariensis* and *P. dactylifera* had diverged conspicuously in terms of allele frequencies at several loci (eg *Idh2*-a, *Mdh1*-b and *Pgm2*-c). The frequencies of these alleles ranged between 0.013 and 0.319 in the mixed populations, while they reached values of 1.000 in *P. dactylifera*. There were no alleles shared exclusively by *P. canariensis* and the mixed populations.

On an average, *P. canariensis* showed lower values of genetic variability than *P. dactylifera*, and mixed populations (Table 1). Overall, the level of genetic variability varied widely among populations. Thus, the number of alleles per locus ranged from 1.25 in La Culata (ANC, *P. canariensis*) to 2.17 in Maspalomas (MAS, mixed populations). The lowest percentage of polymorphic loci was detected in *P. canariensis* ($P = 18.8$ in La Culata, ANC) and the highest in the mixed populations of Maspalomas (MAS) and Río Palma (RP), both with $P = 72.2$ (Table 1).

Genetic diversity statistics (Table 2) indicated that most of the genetic variability in *P. canariensis*, *P. dactylifera* and the mixed populations was contained within the populations, ($H_s = 75.12\%$, $H_s = 79.48\%$ and $H_s = 85.59\%$, respectively).

Population genetic structure

Fixation indices (F_{IS}) varied widely, and none of the populations surveyed was found to fit Hardy–Weinberg equilibrium proportions (Table 1). All *P. canariensis* populations (except for Acusa and La Sorrueda) showed heterozygosity excess, while all mixed populations (except for Barranco Angostura) exhibited a slight heterozygosity deficit (Table 1).

Genetic relationships within and among species

PCA, the first two eigenvectors of which accounted for 81.64% of the total variance (Figure 2), separated the Canary palm populations (AC, LS, RC, ANC, TAM, HY, VP, MIR and ZU) from the date palm populations (HM, GT, PB and EL). In the multivariate space defined by PCA, the mixed populations are much closer to *P. canariensis*, whereas the population of *P. theophrasti* is next to those of *P. dactylifera*. The values of genetic differentiation coefficient (F_{ST}) between pairs of *P. canariensis* populations (Table 3) ranged from $F_{ST} = 0.003$, between Vegaipala (VP) and Las Hayas (HY), to $F_{ST} = 0.652$, between VP and ANC. VP and HY, both from La Gomera, showed substantial genetic differences with respect to the remaining *P. canariensis* populations. In fact, the genetic differentiation among Canarian date

Table 1 Basic genetic variability estimators at 18 loci for the surveyed *Phoenix* populations

Population	*Island*	n	A	P	*He (SD)*	*Ho (SD)*	F_{IS}
Phoenix canariensis							
Acusa (AC)	GC	25	1.72	61.1	0.168 (0.075)	0.169 (0.048)	0.00***
La Sorrueda (LS)	GC	45	1.78	55.6	0.216 (0.056)	0.187 (0.070)	0.14***
Rambla de Castro (RC)	TE	24	1.89	50.0	0.244 (0.063)	0.249 (0.084)	−0.02***
La Culata (ANC)	TE	10	1.25	18.8	0.102 (0.056)	0.156 (0.088)	−0.60*
Tamargada (TAM)	GO	41	1.53	41.2	0.111 (0.049)	0.153 (0.082)	−0.39***
Las Hayas (HY)	GO	40	1.65	47.1	0.142 (0.057)	0.165 (0.084)	−0.12***
Vegaipala (VP)	GO	41	1.29	23.5	0.099 (0.049)	0.165 (0.090)	−0.68***
Mirca (MIR)	LP	27	1.69	43.8	0.165 (0.062)	0.174 (0.084)	−0.05***
Zumacal (ZU)	LP	22	1.53	35.3	0.174 (0.064)	0.191 (0.085)	−0.10***
Mean		**30.55**	**1.59**	**41.8**	**0.158**	**0.179**	
Mixed populations							
Barranco Angostura (BA)	GC	21	1.71	58.8	0.265 (0.062)	0.268 (0.089)	−0.01***
Fataga (FA)	GC	41	2.00	66.7	0.261 (0.060)	0.230 (0.073)	0.12***
Tafira (TA)	GC	46	2.06	66.7	0.246 (0.054)	0.240 (0.076)	0.02***
Maspalomas (MAS)	GC	35	2.17	72.2	0.325 (0.054)	0.281 (0.073)	0.14***
Río Palma (RP)	FV	38	2.11	72.2	0.211 (0.050)	0.200 (0.075)	0.05***
Haría (HA)	LZ	42	2.06	66.7	0.216 (0.053)	0.216 (0.080)	0.01***
Maguez (MAG)	LZ	34	1.71	58.8	0.252 (0.060)	0.240 (0.084)	0.05***
Mean		**36.71**	**1.97**	**66.0**	**0.254**	**0.239**	
Phoenix dactylifera							
Hospital Materno (HM)	GC	10	2.00	66.7	0.289 (0.059)	0.253 (0.081)	0.16***
Pasito Blanco (PB)	GC	27	1.78	50.0	0.217 (0.057)	0.194 (0.075)	0.11***
Gran Tarajal (GT)	FV	42	2.06	64.7	0.336 (0.066)	0.301 (0.091)	0.11***
Elche (EL)	—	40	1.94	58.8	0.267 (0.064)	0.288 (0.089)	−0.08***
Mean		**29.75**	**1.95**	**60.1**	**0.277**	**0.259**	

n = sample size, A = average number of alleles per locus; P = percentage of polymorphic loci; Ho = observed heterozygosity; He = expected heterozygosity, SD = standard error. F_{IS} = fixation index, *$P<0.05$; ***$P<0.001$. Island codes are GC: Gran Canaria, TE: Tenerife, GO: Gomera, LP: La Palma, FV: Fuerteventura, LZ: Lanzarote. Letters in brackets after the population names are the population codes. Bold numeric values are mean values.

Table 2 Genetic diversity statistics (Nei, 1973) based on allele frequencies at the 18 loci surveyed for the three kinds of Canarian *Phoenix* populations examined

	H_T	H_S	D_{ST}	G_{ST}	% H_S
P. canariensis	0.199	0.150	0.050	0.249	75.12
Mixed populations	0.291	0.249	0.042	0.144	85.59
P. dactylifera	0.341	0.271	0.070	0.205	79.48

H_T = total gene diversity, H_S = average gene diversity within populations, D_{ST} = average gene diversity among populations, G_{ST} = gene diversity among populations, relative to H_T, $\%H_S$ = per-percentage of genetic diversity within populations.

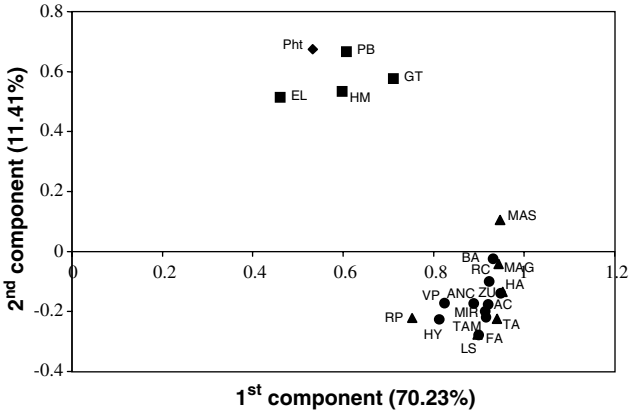

Figure 2 Principal component analysis based on the correlation matrix of the allele frequencies of *Phoenix* populations. (●) *P. canariensis*, (▲) mixed populations, (■) *P. dactylifera*, (◆) *P. theophrasti*. Values within brackets are the per cent of total variation explained by the corresponding component.

palm populations decreased considerably ($F_{ST}=0.170$) when HY and (VP) populations were excluded from analysis (Figure 3). On the whole, genetic differentiation within *P. canariensis* was relatively high ($F_{ST}=0.287$) when compared to the values recorded for species sharing the same ecological and life history traits (Hamrick and Godt, 1990).

Mixed populations showed genetic differentiation values ranging from 0.012 to 0.379, exhibiting a moderate F_{ST} value ($F_{ST}=0.152$) among populations (Wright, 1978). As regards *P. dactylifera*, F_{ST} ranged from 0.129 to 0.391, showing a considerably higher genetic differentiation coefficient among populations ($F_{ST}=0.252$).

The F_{ST} values revealed a close genetic relationship between *P. canariensis* populations and the mixed populations (Table 3). The values of F_{ST} obtained by comparing *P. dactylifera* with *P. canariensis* ($F_{ST}=0.442$) or *P. dactylifera* with the mixed populations ($F_{ST}=0.307$) were higher than those corresponding to the comparison

Table 3 Genetic differentiation coefficient values (F_{ST}) (above the diagonal) and Nei's (1972) genetic identities (below the diagonal) for all pairwise combinations between the 20 *Phoenix* populations analysed

	Phoenix canariensis									*Mixed populations*							*Phoenix dactylifera*			
	AC	*LS*	*RC*	*ANC*	*TAM*	*HY*	*VP*	*MIR*	*ZU*	*BA*	*HA*	*MAG*	*TA*	*FA*	*RP*	*MAS*	*GT*	*HM*	*PB*	*EL*
AC		0.093	0.207	0.116	0.309	0.492	0.551	0.107	0.169	0.172	0.138	0.168	0.106	0.101	0.437	0.174	0.406	0.501	0.547	0.559
LS	0.975		0.218	0.102	0.261	0.461	0.504	0.117	0.172	0.110	0.103	0.182	0.054	0.016	0.277	0.164	0.395	0.465	0.543	0.545
RC	0.932	0.923		0.161	0.228	0.314	0.373	0.092	0.070	0.193	0.088	0.141	0.110	0.174	0.433	0.089	0.288	0.437	0.420	0.469
ANC	0.967	0.967	0.942		0.403	0.614	0.652	0.032	0.135	0.218	0.067	0.222	0.101	0.122	0.501	0.153	0.405	0.544	0.580	0.583
TAM	0.938	0.94	0.948	0.929		0.439	0.456	0.145	0.154	0.348	0.254	0.255	0.185	0.223	0.480	0.316	0.506	0.672	0.668	0.657
HY	0.851	0.842	0.914	0.842	0.921		0.003	0.425	0.233	0.496	0.426	0.320	0.375	0.409	0.587	0.375	0.518	0.693	0.686	0.659
VP	0.845	0.834	0.908	0.834	0.920	0.999		0.488	0.292	0.554	0.475	0.366	0.419	0.455	0.620	0.421	0.547	0.722	0.701	0.685
MIR	0.975	0.970	0.972	0.982	0.977	0.897	0.892		0.038	0.184	0.062	0.173	0.066	0.104	0.450	0.158	0.411	0.548	0.565	0.576
ZU	0.957	0.950	0.975	0.963	0.975	0.956	0.952	0.987		0.226	0.096	0.138	0.100	0.147	0.445	0.156	0.392	0.531	0.556	0.565
BA	0.945	0.963	0.915	0.919	0.910	0.811	0.800	0.943	0.918		0.122	0.135	0.109	0.065	0.231	0.081	0.268	0.348	0.431	0.431
HA	0.962	0.970	0.970	0.978	0.947	0.876	0.867	0.982	0.972	0.957		0.153	0.059	0.095	0.379	0.099	0.382	0.491	0.510	0.540
MAG	0.945	0.937	0.942	0.913	0.936	0.903	0.899	0.945	0.952	0.942	0.946		0.153	0.153	0.320	0.081	0.253	0.391	0.395	0.476
TA	0.968	0.982	0.959	0.963	0.958	0.875	0.867	0.981	0.968	0.958	0.98	0.942		0.012	0.305	0.131	0.356	0.455	0.481	0.513
FA	0.968	0.992	0.929	0.954	0.945	0.848	0.840	0.968	0.949	0.971	0.968	0.938	0.992		0.231	0.131	0.337	0.415	0.487	0.491
RP	0.819	0.905	0.776	0.778	0.843	0.742	0.734	0.818	0.809	0.912	0.850	0.861	0.876	0.913		0.323	0.393	0.498	0.619	0.536
MAS	0.932	0.936	0.957	0.930	0.893	0.850	0.842	0.942	0.936	0.958	0.962	0.961	0.945	0.940	0.836		0.160	0.257	0.250	0.377
GT	0.745	0.762	0.812	0.708	0.694	0.672	0.666	0.741	0.737	0.827	0.765	0.850	00.772	0.782	0.744	0.900		0.155	0.129	0.226
HM	0.724	0.738	0.707	0.686	0.605	0.578	0.568	0.687	0.673	0.792	0.714	0.754	0.705	0.739	0.693	0.838	0.894		0.327	0.337
PB	0.698	0.670	0.785	0.690	0.602	0.602	0.594	0.698	0.691	0.766	0.737	0.797	0.717	0.704	0.535	0.882	0.940	0.841		0.391
EL	0.569	0.586	0.649	0.508	0.477	0.464	0.455	0.553	0.538	0.695	0.593	0.634	0.596	0.618	0.592	0.731	0.864	0.789	0.784	

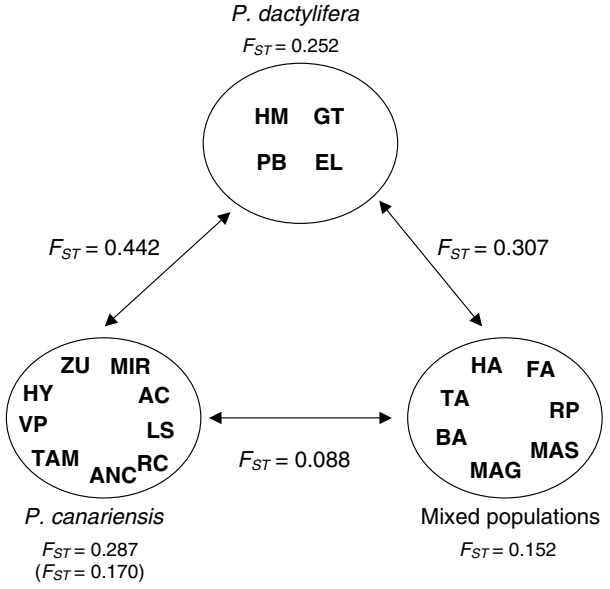

Figure 3 Genetic differentiation coefficient (F_{ST}) within and among *P. canariensis*, *P. dactylifera* and mixed populations. The value of F_{ST} in brackets is that for *P. canariensis* when Las Hayas (HY) and Vegaipala (VP) populations are excluded from the analysis.

of the mixed populations with *P. canariensis* ($F_{ST} = 0.088$; Figure 3).

Nei's (1972) genetic identities between *P. canariensis* population pairs (Table 3) ranged from $I = 0.847$ to $I = 0.999$ (average $I = 0.935$). The identities between the Canarian date palm populations and the mixed populations were also high (average $I = 0.916$). Genetic identities between pairs of *P. dactylifera* populations were slightly lower, spanning from $I = 0.806$ to $I = 0.942$ (average $I = 0.864$) and indicating a moderate level of genetic differentiation among populations.

Discussion

Allozyme diversity within and among populations

A high degree of genetic variation was found in *P. canariensis* populations ($A = 1.59$, $P = 41.8$), similar to the values expected for monocotyledons ($A = 1.66$, $P = 40.3$) and for species with sexual reproduction ($A = 1.53$, $P = 34.9$), but higher than that for endemic species ($A = 1.39$, $P = 26.3$) (Hamrick and Godt, 1990). Average levels of expected heterozygosity, the more integrative measure, in *P. canariensis* (He = 0.158), were higher than those found in most other species within the Arecaceae. Thus, although Eguiarte *et al*. (1992) found a similar value to *P. canariensis*' in the rain forest palm *Astrocaryum mexicanum* (He = 0.153), lower values were described in *Carpentaria acuminata*, (He = 0.143), *Ptychosperma bleeseri* (He = 0.006) and *Pinanga tenella* (He = 0.133) (Shapcott, 1998a, b, 1999) and in *Washingtonia filifera* (He = 0.008) (McClenaghand and Beauchamp, 1986). This result agrees with other studies that show higher genetic diversity levels in Canarian endemic plants than in those distributed in other oceanic islands (Francisco-Ortega *et al*, 2000; Batista *et al*, 2001; Batista and Sosa, 2002; Bouza *et al*, 2002). On the whole, the three groups of populations exhibited higher genetic variability values than species with similar life history traits (monocotyledons, endemics and species with sexual reproduction). However, genetic variation levels detected in the *P. dactylifera* populations analysed was lower than described by other authors (eg, Bennaceur *et al*, 1991) in date palm cultivars.

Earlier workers predicted that hybrid taxa would be more variable genetically and have greater evolutionary potential than their parental species because they would combine the alleles of both parents (Rieseberg, 1997). In our case, mixed populations exhibited higher levels of genetic diversity than *P. canariensis* and slightly higher

than *P. dactylifera* populations (Table 1). The number of individuals of each *Phoenix* species notwithstanding, there are other factors that might be influencing diversity levels in the mixed populations. These might include the degree of genetic differentiation between both species, the breeding system, the possible hybridisation between *P. canariensis* and *P. dactylifera*, and historical contingency.

Most genetic variation in *P. canariensis* is maintained within populations ($\%H_s = 75.12$), as expected for predominantly outcrossing species (Hamrick and Godt, 1990). A similar percentage of genetic variation was maintained within populations in *P. dactylifera* ($\%H_s = 79.48$). The probable reason for the high intrapopulational gene diversity exhibited by the mixed populations ($\%H_s = 85.59$) is the heterogeneous genetic makeups that coexist in these disturbed populations.

Population genetic structure

On the whole, none of the *P. canariensis* populations analysed was found to conform to Hardy–Weinberg proportions. Except for Acusa and La Sorrueda, all the Canarian date palm populations showed a heterozygote excess (Table 1). A heterozygosity excess can be the possible result of random stochastic events or the consequence of balancing selection promoting high heterozygosity (Linhart *et al*, 1981; Waser, 1987; Eguiarte *et al*, 1992). Although we do not have conclusive data to discard either possibility, it is highly improbable that most *P. canariensis* populations have an excess of heterozygotes by drift alone.

Populations from Acusa and La Sorrueda showed a heterozygosity deficit. This was not significant in Acusa, where deviations from Hardy–Weinberg equilibrium might be due mainly to stochastic factors associated with its small size ($N = 25$). In La Sorrueda, the distribution of specimens in several clumps considerably separated in space (100–300 m) argues for the incidence of the Wahlund effect as the more likely explanation of the heterozygosity deficit observed, whereby heterozygosity reduces if the clumps sampled have diverged sufficiently (Elseth and Baumgardner, 1981).

Contrary to the general pattern for *P. canariensis*, all mixed populations showed a heterozygosity deficit save for Barranco Angostura, which exhibited a slight heterozygosity excess. Small heterozygosity deficit in outbreeding species are often a consequence of biparental inbreeding, especially in small populations or in those exhibiting spatial genetic structure (Sampson *et al*, 1988).

Owing to the cultivated condition of the *P. dactylifera* populations, it is not surprising that they do not conform to Hardy–Weinberg equilibrium proportions.

Genetic relationships within among species

The high level of allozyme similarity found among *P. canariensis* and *P. dactylifera* populations fits the values expected for closely related species (Gottlieb, 1981). Considering the extensive distribution of *P. dactylifera* in the Mediterranean area, it is feasible that *P. canariensis* is recently derived from a common ancestor closely related to *P. dactylifera*. The hypothesis of a recent divergence from *P. dactylifera* gains additional support from the fact that *P. canariensis* has less allozyme variation than *P. dactylifera*, its allelic makeup is a subset of that found in *P. dactylifera*, with no unique alleles, and there is a high genetic identity between both taxa; all of these features are characteristic of recently derived species (Purdy and Bayer, 1996). Consistent with the values of isozyme variation in *P. canariensis*, recent speciation from a widespread progenitor has been suggested as one of the causes of high genetic variation in endemic plant species (Loveless and Hamrick, 1988; Pleasants and Wendel, 1989). Thus, the isozyme data bolster Morici's (1998) morphological argument of a close phylogenetic relationship of *P. canariensis* with *P. dactylifera*. However, the high levels of genetic variation detected in *P. canariensis* populations indicate that more than one colonisation event could have occurred, as suggested by Francisco-Ortega *et al* (2000) for different Canarian endemics.

In close agreement with previous evidence based on morphology (Barrow, 1998), *P. theophrasti* is closer to *P. dactylifera* than to *P. canariensis* in the multivariate representation (Figure 2).

Unfortunately, we could not obtain specific and unambiguous isozyme molecular markers to differentiate *P. canariensis* and *P. dactylifera* individuals. Only four exclusive alleles were detected in *P. dactylifera* (*Mdh1*-c, *Mdh1*-e, *Pgm2*-c and *Pgm2*-e). However, given that none of these was taxon specific or even monomorphic, our expectations of having a marker of taxonomic circumscription at this molecular level of analysis are not met. The lack of an isozyme marker further supports a recent speciation of *P. canariensis* from an ancestor similar to *P. dactylifera*. Of the 43 alleles, 39 were common to both *Phoenix* species analysed, and this furnishes evidence of their shared evolutionary history. In a recent survey, the sharing of 14 of the 25 total alleles detected in five Canarian taxa of *Cistus* (Batista *et al*, 2001) was used to support the close phylogenetic relationship among them. Similarly, a close relatedness between two *Brighamia* species from Hawaii was inferred from their sharing of only six alleles out of 22 (Gemmill *et al*, 1998).

The frequency of the alleles shared exclusively by *P. dactylifera* and the mixed populations can help us estimate the degree with which date palm individuals were introduced in the latter (Gallagher *et al*, 1997). In all cases, these shared alleles are poorly represented in the mixed populations (with frequencies from 0.000 to 0.319), thereby suggesting that a relatively low number of *P. dactylifera* specimens were introduced. This argument is cogent with F_{ST} values, which imply a much closer genetic relationship between *P. canariensis* and the mixed populations than between either of these and *P. dactylifera* (Figure 3).

Given that we did not detect different monomorphic alleles in *P. canariensis* and *P. dactylifera* that were present in heterozygosity in individuals with intermediate morphological traits, this allozyme survey cannot substantiate the existence of putative hybrid individuals in nature. Although molecular markers represent a powerful tool for identifying hybrid taxa, even this approach can generate ambiguous results (Rieseberg, 1997). A taxon can share molecular markers with related taxa due to the joint retention of alleles following speciation from a polymorphic ancestor (symplesiomorphy). This phenomenon has also been referred to as lineage sorting when discussed in the context of gene lineage data (Avise, 1994; Rieseberg, 1997). Besides, the likelihood of finding exclusive molecular markers decreases as the

time since divergence reduces. Consequently, it is usually easier to reject the hypothesis of hybrid origin than to confirm it with molecular data sets.

The PCA (Figure 2) separated *P. dactylifera* and *P. canariensis* clearly, and showed a closer relationship between the latter species and the mixed populations. In general, the percentage of *P. canariensiś* individuals in the mixed populations outnumbers that of *P. dactyliferá*s, which probably contributes further the closer genetic relationship between *P. canariensis* and the mixed populations.

Since hybrids are a mosaic of parental and intermediate characters (Rieseberg and Ellstrand, 1993), it is possible that some of the individuals that we characterised as morphologically intermediate are in fact different ecotypes of *P. canariensis*. Also, morphologically intermediate individuals may be hybrid progeny corresponding to F_2 or later generations that have lost *P. dactylifera* alleles by backcrossing to pure *P. canariensis*. As most gene flow occurs between the hybrid and a single parent (*P. canariensis*), the segregating generations will be mostly advanced generation backcrosses and have multilocus associations typical of the most compatible parent (*P. canariensis*), as suggested earlier (Rieseberg *et al*, 1989; Arnold *et al*, 1991; Nason *et al*, 1992; Rieseberg and Ellstrand, 1993). In addition, selection against recombinants should be intense in hybrid zones; therefore, the surviving individuals would be those that retained the ecological traits of one parent, such as backcrosses (Anderson, 1998). As our data do not offer an insight on whether hybridisation between *P. canariensis* and *P. dactylifera* occurs, we cannot discard any of the two explanations.

Populations of *P. dactylifera* have not been described in La Gomera (Izquierdo *et al*, 2001) and, therefore, a hybrid origin of the highly differentiated VP and HY seems improbable. Hence, until additional studies of these populations are carried out, it seems safer to construe their high genetic differentiation mainly as the result of genetic drift in combination with a long history of isolation.

The higher degree of genetic differentiation detected within the Canarian range of *P. dactylifera* ($F_{ST} = 0.252$) probably reflects low levels of gene flow in combination with different geographical origins of the introduced date palms.

Conservation implications

One of the purposes of this work was to use the information regarding the degree and distribution of genetic variability in *P. canariensis* for the implementation of conservation strategies. Fortunately, the high levels of genetic diversity present in *P. canariensis* are encouraging for conservation efforts because they should help buffer the effects of selection and potential inbreeding in the populations (Travis *et al*, 1996). As most of the high genetic variability in *P. canariensis* is maintained within populations, avoiding fragmentation to prevent genetic variability loss through a cessation of interpopulation genetic interchange must be a crucial commitment for '*in situ*' conservation strategies. As it is not possible to design a reserve that includes all the populations of *P. canariensis* in any given island of occurrence, establishing multiple small ecological reserves for this species should be most effective at buffering inbreeding and genetic drift (Hawkes *et al*, 1997), especially if these reserves are managed in a coordinated way to facilitate gene flow. Ideally, these reserves should include the more polymorphic populations of *P. canariensis*, as this strategy will enhance the potential of surviving environmental change (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987). According to this criterion, populations Rambla de Castro (RC; Ho = 0.249, He = 0.244) and La Sorrueda (LS; Ho = 0.187, He = 0.216) would be the best-suited management targets in Tenerife and Gran Canaria, respectively.

Genetic conservation strategies based on seed and germplasm collection and preservation in gene banks are necessary for the *ex situ* conservation of any endangered plant. The population at RC is the best candidate for sampling for this purpose, as it shows the higher values of the basic indicators of allozyme variation.

At this stage, transplanting between two different populations must be strongly discouraged for two important reasons. First, we can neither ascribe the morphologically intermediate individuals unambiguously to *P. canariensis* or *P. dactylifera*, nor differentiate between juvenile individuals of either species. And second, the mixing of genetically distinct populations of *P. canariensis* may pose the risk of outbreeding depression, whereby a reduction in fitness arises due to a loss of local adaptations or the break-up of coadapted gene complexes (Storfer, 1999).

Although outbreeding depression commonly manifests through the decrease in the value of fitness-related traits such as fruit production, survivorship or seed germination, it often results in a heterozygosity loss in the outbred populations (Fenster and Galloway, 2000). At odds with this prediction, the mixed populations (where outbreeding could have proceeded for several generations) display much higher average heterozygosity values (He = 0.254, Ho = 0.239) than *P. canariensis* (He = 0.158, Ho = 0.179). Hence, our isozyme data do not provide evidence that historical transplanting of *P. dactylifera* specimens into *P. canariensis*' populations compromised population survival. However, we must bear in mind that the effects of outbreeding depression might take a long time to manifest and may even be preceded by several generations of heterosis. Remarkably, Fenster and Galloway (2000) detect a significant heterozygosity loss and a decrease in five fitness-related traits in *Chamaecrista fasciculata* (Fabaceae) after only three generations of outbred crosses, where the first generation far outperformed either parental line. Thus, having adequate demographic data for the mixed *Phoenix* populations would be crucial to detecting outbreeding depression.

Hybridisation has recently been shown to have both beneficial and harmful consequences for the conservation of plant diversity, leading to increased diversity in some instances and to possible extinction of populations or species by genetic swamping in other. This occurs when a locally rare species loses its genetic integrity and becomes assimilated into a locally common species as a result of repeated events of hybridisation and introgression (Rieseberg, 1991; Ellstrand, 1992; Ellstrand *et al*, 1999).

One of the aims of *P. canariensis* conservation should be the search for a molecular marker able to discriminate

unambiguously *P. canariensis* and *P. dactylifera* individuals from their putative hybrids. Since we have not been able to find these molecular markers through isozyme electrophoresis, and bearing in mind that the mutation rate of isozyme markers is usually lower than that of DNA markers (Li, 1997; Yan *et al*, 1999), DNA markers will be the best candidates for differentiating among individuals belonging to both species.

Acknowledgements

We thank the Gobierno de Canarias for financial support through its 'Programa de Becas de postgrado para la realización de tesis doctorales'. We thank Dr Michel Ferry for providing samples of *Phoenix dactylifera* from the Hort del Gat, Research Station on Date Palm and Arid Land Farming Systems in Elche (Spain). We also thank Joan Pedrola-Monfort for kindly sending leaves of *Phoenix theophrasti*. We are grateful to all the people who provided helpful field assistance. This paper is dedicated to Jaime O'Shanahan for his great interest in this work and his defense of the Canarian date palm. This work was funded by Viceconsejeria de Educación, Gobierno de Canarias Ref. 93/163 and 94/2614. We thank A Stephens for English corrections.

References

Allphin L, Windham MT, Harper KT (1998). Genetic diversity and gene flow in the endangered dwarf bear poppy, *Arctomecon humilis* (Papaveraceae). *Am J Bot* **85**: 1251–1261.

Anderson E (1998). Hybridization of the habitat. *Evolution* **2**: 1–9.

Arnold Ml, Bucker CM, Robinson JJ (1991). Pollen mediated introgression and hybrid speciation in Louisiana irises. *Proc Natl Acad USA* **88**: 1398–1402.

Avise JC (1994). *Molecular Markers, Natural History and Evolution*. Chapman & Hall: New York.

Barrow SC (1998). A monograph of *Phoenix* L. (Palmae: Coryphoideae). *Kew Bull* **53**: 513–575.

Batista F, Bañares A, Caujapé-Castells J, Carque E, Marrero-Gómez M, Sosa PA (2001). Allozyme diversity in three endemic species of *Cistus* (Cistaceae) from the Canary Islands: intraspecific comparisons and implications for genetic conservation. *Am J Bot* **88**: 1582–1592.

Batista F, Sosa PA (2002). Allozyme diversity in natural populations of *Viola palmensis* Webb & Berth. (Violaceae) from La Palma (Canary Islands): implications for conservation genetics. *Ann Bot* **90**: 725–733.

Bendiab K, Baaziz M, Majourhat K (1998). Preliminary date palm cultivar composition of Moroccan palm groves as revealed by leaf isoezymes phenotypes. *Bioch Syst Ecol* **26**: 71–82.

Bennaceur M, Lanaud C, Chevallier MH, Bounaga N (1991). Genetic diversity of the date palm (*Phoenix dactylifera* L.) from Algeria revealed by enzyme markers. *Plant Breed* **107**: 56–69.

BOC (1991). Orden de 20 Febrero: protección de especies de la flora vascular silvestre de las Islas Canarias. *Bol Canarias* **35**: 1324–1334.

Booij I, Monfort S, Ferry M (1995). Characterization of thirteen date palm (*Phoenix dactylifera*) cultivars by enzymes electrophoresis using the PhastSystem. *J Plant Physiol* **145**: 62–66.

Bouza N, Caujapé-Castells J, González-Pérez MA, Batista F, Sosa PA (2002). Population structure and genetic diversity of two endangered endemic species of the Canarian Laurel Forest: *Dorycnium spectabile* (Fabaceae) and *Isoplexis chalcantha* (Scrophulariaceae). *Int J Plant Sci* **163**: 619–630.

Caujapé-Castells J, Pedrola-Monfort J, Membrives N (1999). Contrasting patterns of genetic structure in the South African species *Androcymbium bellum, A. guttatum, and A. pulchrum* (Colchicaceae). *Bioch Syst Ecol* **27**: 591–605.

Chabaud B (1882). Le Phoenix canariensis. *Provence Agric Hortic Ilustr* **19**: 293–297.

Charlesworth D, Charlesworth B (1987). Inbreeding depression and its evolutionary consequences. *Ann Rev Ecol Syst* **18**: 237–268.

Clayton JW, Tretiak DN (1972). Amine citrate buffer for pH control in starch gel electrophoresis. *J Fish Res Board Can* **29**: 1169–1172.

Eguiarte LE, Perez-Nasser N, Piñero D (1992). Genetic structure, outcrossing rate and heterosis in *Astrocaryum mexicanum* (tropical palm): implications for evolution and conservation. *Heredity* **69**: 217–228.

Elisiário PJ, Justo EM, Leitao JM (1999). Identification of mandarin hybrids by isozyme and RAPD analysis. *Sci Hortic* **81**: 287–299.

Ellstrand NC (1992). Gene flow by pollen: implications for plant conservation. *Oikos* **63**: 77–86.

Ellstrand NC, Prentice HC, Hancock JF (1999). Gene flow and introgression from domesticated plants into their wild relatives. *Ann Rev Ecol Syst* **30**: 539–563.

Elseth GD, Baumgardner KD (1981). *Population Biology*. Van Nostrand: New York.

Fenster CB, Galloway LF (2000). Population differentiation in an annual legume: genetic architecture. *Evolution* **54**: 1157–1172.

Francisco-Ortega F, Santos-Guerra A, Kim SC, Crawford DJ (2000). Plant genetic diversity in the Canary Islands: a conservation perspective. *Am J Bot* **87**: 909–919.

Gallagher KG, Schierenbeck KA, D'Antonio CM (1997). Hybridization and introgression in *Carpobrotus* spp. (Aizoaceae) in California. II. Allozyme evidence. *Am J Bot* **84**: 905–911.

Gemmill CEC, Ranker TA, Ragone D, Perlman SP, Wood KR (1998). Conservation genetics of the endangered endemic Hawaiian genus *Brighamia* (Campanulaceae). *Am J Bot* **85**: 528–539.

Godt MJW, Hamrick JL (1998). Allozyme diversity in the endangered pitcher plant *Sarracenia rubra* ssp. *alabamensis* (Sarraceniaceae) and its close relative *S. rubra* ssp. *rubra*. *Am J Bot* **85**: 802–810.

González-Pérez, MA (2001). Caracterización molecular de la palmera canaria (*Phoenix canariensis*) como base para su conservación (PhD. Thesis). Las Palmas de Gran Canaria. Canary Islands. University of Las Palmas de Gran Canaria: Spain.

Gottlieb LD (1981). Electrophoretic evidence and plant populations. *Progr Phytochem* **7**: 1–46.

Guo SW, Thompson EA (1992). Performing the exact test of Hardy–Weinberg proportions for multiple alleles. *Biometrics* **48**: 361–372.

Hamrick JL, Godt MJW (1990). Allozyme diversity in plant species. Brown HD, Clegg MT, Kahler AL, Weir BS (eds) *Plant Populations Genetics, Breeding and Genetic Resources*. Sinauer: Sunderland, MA.

Hawkes JG, Maxted N, Zohary D (1997). Reserve design. lang2057In: Maxted N, Ford-Lloyd BV, Hawkes JG (eds) *Plant Genetic Conservation*. Chapman and Hall: London, pp 132–143.

Hodel DR (1995). *Phoenix*: the date palms. *Palm J* **122**: 14–36.

Izquierdo I, Martín JL, Zurita N, Arechavaleta M (2001). Lista de especies silvestres de Canarias (hongos, plantas y animales terrestres). Consejería de Política Territorial y Medio Ambiente del Gobierno de Canarias.

Kunkel G, Kunkel MA (1974). Flora de Gran Canaria. Arboles y arbustos arboreos. Las Palmas de Gran Canaria: Excmo. Cabildo Insular de Gran Canaria, Vol, **1**: 4–5.

Lande R, Schemske DW (1985). The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **29**: 24–40.

Lewis PO (1993). *GeneStat-PC 3.31. Raleigh*. North Carolina State University: North Carolina.
Li WH (1997). *Molecular Evolution*. Sinauer Publishers: Sunderland, MA.
Linhart YB, Mitton JB, Sturgeon KB, Davis ML (1981). Genetic variation in space and time in a population of ponderosa pine. *Heredity* **46**: 407–426.
Loveless MD, Hamrick JL (1988). Genetic organization and evolutionary history in two North American species of *Cirsium*. *Evolution* **42**: 254–265.
McClenaghand LR, Beauchamp AC (1986). Low genetic differentiation among isolated populations of the California fan palm (*Washingtonia filifera*). *Evolution* **40**: 315–322.
Morici C (1998). *Phoenix* in the wild. *Principes* **42**: 85–89.
Nason JD, Ellstrand NC, Arnold Ml (1992). Patterns of hybridization and introgression in populations of oaks, manzanitas, and irises. *Am J Bot* **79**: 101–111.
Nei M (1972). Genetic distance between populations. *Am Nat* **106**: 283–292.
Nei M (1973). Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci USA* **70**: 3321–3323.
Pleasants JM, Wendel JF (1989). Genetic diversity in a clonal narrow endemic, *Erythronium propullens*, and its progenitor, *Erythronium albidum*. *Am J Bot* **76**: 1136–1151.
Purdy BG, Bayer RJ (1996). Genetic variation in populations of the endemic *Achillea millefolium* ssp. *megacephala* from the Athabasca sand dunes and the widespread ssp. *lanulosa* in western North America. *Can J Bot* **74**: 1138–1146.
Raymond M, Rousset F (1997). *GENEPOP. Version 3.1. Laboratorie de Génétique et Environnement. Montpellier*. Université de Montpellier II.: France.
Rieseberg LH (1991). Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. *Am J Bot* **78**: 1218–1237.
Rieseberg LH (1997). Hybrid origins of plant species. *Ann Rev Ecol Syst* **28**: 359–389.
Rieseberg LH, Ellstrand NC (1993). What can molecular and morphological markers tell us about plant hybridization? *Crit Rev Plant Sci* **12**: 213–241.
Rieseberg LH, Zona S, Aberbom L, Martin TD (1989). Hybridization in the island endemic, Catalina Mahogany. *Conserv Biol* **3**: 52–58.
Sampson JF, Hopper SD, Jones SH (1988). Genetic diversity and the conservation of *Eucalyptus crucis* Maiden. *Aust J Bot* **36**: 447–460.
Santana SA, Toledo RJM (1997). Introducción y dispersión de *Phoenix dactylifera* en el Archipiélago Canario: elementos de discusión. *Second International Symposium on Ornamental Palms and other Monocts from the Tropics. Canary Islands, Spain*.
Schnabel A, Laushman RH, Hamrick JL (1991). Comparative genetic structure of two co-occurring tree species: *Maclura pomifera* (Moraceae) and *Gleditsia triacanthos* (Leguminoseae). *Heredity* **67**: 357–364.
Shapcott A (1995). The spatial genetic structure in natural populations of the Australian temperate rainforest tree *Atherosperma moscatum* (Labill.) (Monimiaceae). *Heredity* **74**: 28–38.
Shapcott A (1998a). The patterns of genetic diversity in *Carpentaria acuminata* (Arecaceae), and rainforest history in northern Australia. *Mol Ecol* **7**: 833–847.
Shapcott A (1998b). The genetics of *Ptychosperma bleeseri* a rare palm from the Northern Territory, Australia. *Biol Conserv* **85**: 203–209.
Shapcott A (1999). Comparison of the population genetics and densities of five *Pinanga* palm species at Kuala Belalong, Brunei. *Mol Ecol* **8**: 1641–1654.
Sosa PA, Bouza N, Cabrera-Pérez MA, Luque A (1998). Genetic variation in *Phoenix canariensis* and *P. dactylifera* (Arecaceae) populations of Gran Canaria using isoezyme electroforesis. *Bol Mus Mun Funchal* **5**: 443.
Storfer A (1999). Geneflow and endangered species translocations: a topic revisited. *Biol Conserv* **87**: 173–180.
Stuber CW, Goodman MM, Johnson FM (1977). Genetic control and racial variation of β-glucosidase isozymes in maize (*Zea mays* L.). *Biochem Genet* **15**: 383–394.
Swofford DL, Selander RB (1981). *BIOSYS-1: a Computer Program for the Analysis of Allelic Variation in Genetics. Realese 1. User's manual*. University of Illinois: Urbana, Ill.
Torres AM, Tisserat B (1980). Leaf isozymes as genetic markers in date palms. *Am J Bot* **67**: 162–167.
Travis SE, Maschinski J, Keim P (1996). An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically endangered plant, using AFLP markers. *Mol Ecol* **5**: 735–745.
Waser NM (1987). Spatial genetic heterogeneity in a population of the montane perennial plant *Delphinium nelsonii*. *Heredity* **58**: 249–256.
Weir BS (1990). *Genetic Data Analysis*. Sinauer Publishers: Sunderland, MA.
Wendel F, Weeden F (1989). Visualization and interpretation of plant isozymes. In: Soltis D, Soltis P (eds) *Isozyme in Plant Biology*. Dioscorides Press: Portland.
Wright S (1978). *Evolution and the Genetics of Populations, Vol 4: Variability within and among Natural Populations*. University of Chicago Press: Chicago.
Yan G, Romero-Severson J, Walton M, Chadee DD, Severson DW (1999). Population genetics of the yellow fever mosquito in Trinidad: comparisons of AFLP and RFLP markers. *Mol Ecol* **8**: 951–964.