Plant Syst. Evol. 247: 165–175 (2004)
DOI 10.1007/s00606-004-0166-7

Plant Systematics and Evolution
Printed in Austria

Molecular evidence of hybridisation between the endemic *Phoenix canariensis* and the widespread *P. dactylifera* with Random Amplified Polymorphic DNA (RAPD) markers

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Received March 20, 2003; accepted February 24, 2004
Published online: June 11, 2004

Abstract. We used RAPD markers to test whether morphologically intermediate individuals between the Canarian endemic *Phoenix canariensis* and the widespread *P. dactylifera* correspond to hybrids. Consistent with previous allozyme evidence, the scarcity of appropriate RAPD markers to distinguish *P. dactylifera* and *P. canariensis* indicated a close genetic relationship among these species. Only two of the 54 ten-mer primers (*OPM*-8 and *OPK*-14) tested in 221 individuals from 7 localities in different islands enabled us to unambiguously identify both species. While *P. canariensis* possesses two exclusive monomorphic bands of 1000 bp and 750 bp (for *OPM*-8 and *OPM*-14, respectively), *P. dactylifera* is characterised by two bands of 900 bp and 950 bp for the same primers. The additivity of these taxon-specific bands in the individuals that were morphologically intermediate provided, for the first time, firm evidence for their hybrid origin. Because these hybridisation capabilities pose clear threats to the survival of the endemic *P. canariensis* and some individuals that had been morphologically characterised as pure *P. canariensis* revealed later a hybrid nature in the RAPD analysis, we suggest that RAPD markers be used to estimate the possible incidence of introgression in the scarce extant natural populations of *P. canariensis*. This procedure will provide a straightforward means to select target populations to implement the "in situ" conservation strategies suggested previously on the basis of allozyme research.

Key words: Canarian date palm, Canary Islands, date palm, hybrids, hybrid detection, plant conservation, *Phoenix canariensis*, *Phoenix dactylifera*, RAPD.

Introduction

Phoenix canariensis is an endemic palm species from the Canary Islands that co-occurs with its widespread congener *Phoenix dactylifera* in many stands as a consequence of the artificial planting of the latter. *P. dactylifera* were planted by the Canarian farmers for use and enhanced exploitation. Nowadays, both species are planted by ornamental reasons in all the world, inclusive in the Canary Islands. Although this practice increases the economical yielding of palm cultivars in the islands, the unwitting introduction of the widespread species poses two important problems for the conservation of the endemic one. First, presumably as a consequence of the phylogenetic

closeness between both taxa, juveniles of both species are indistinguishable (Sosa et al. 1998). Second, *P. canariensis* and *P. dactylifera* are thought to cross very easily in nature (Kunkel and Kunkel 1974, Hodel 1995, Corner 1966, Barrow 1998, Morici 1998). Indeed, based only on morphological features, a number of authors have argued that intermediate morphological forms between the endemic species and the widespread *P. dactylifera* are hybrid individuals (Kunkel and Kunkel 1974, Montesinos-Barrera 1979, Niebla-Tome 1990, Del Cañizo 1991, Morici 1998). However, there is neither experimental proof of such inter-specific breeding capabilities, nor any molecular evidence to support these claims. Therefore, it is difficult at this stage of knowledge to determine whether the intermediate individuals are real hybrids, morphological variants or ecotypes.

Assessment of inter-specific hybridisation and introgression between species or subspecies is important for the implementation of appropriate genetic conservation strategies. An efficient management of natural genetic resources needs to identify and conserve the remaining unique populations and to evaluate the extent to which they are endangered by the introduction of alien species (Novak and Mack 1993, Largiader and Scholl 1996), that pose a clear threat to the genetic integrity of the endemic populations through hybridisation and eventual outbreeding depression. Therefore, the lack of knowledge about the nature, origin and purity of the *P. canariensis* populations has stood in the way of the conservation efforts for this Canarian endemic.

Morphological evidence bolsters the hypothesis that hybridisation and introgression might be occurring between *P. canariensis* and *P. dactylifera*, with the putative hybrids having a wide range of phenotypic variation. Previous isozyme studies with these species (González-Pérez 2001) support a recent speciation of *P. canariensis* from an ancestor closely related to *P. dactylifera*. However, consistent with this inferred phylogenetic closeness, isozymes failed to provide monomorphic molecular markers to unambiguously differentiate these two species and their putative hybrids.

During the last decade, several novel DNA-markers have emerged which have been rapidly integrated into the arsenal of common routine laboratory tools available for genome analysis. Since their introduction, Random Amplified Polymorphic DNA (RAPD) markers (Williams et al. 1990) have become very popular (mainly because of the ease of their analysis) and have been used for a variety of purposes in plant genetics: cultivar identification (Cabrita et al. 2001), parentage determination (Elisiário et al. 1999), genetic relationships evaluation (Nicese et al. 1998), estimation of population genetic variability (Sales et al. 2001), and identification of inter-specific hybrids (Crawford et al. 1993, Soltis and Soltis 1993, Bailey et al. 1995, Perron and Bousquet 1997, Roelfos et al. 1997, Urbanska et al. 1997, Neuffer et al. 1999, Koontz et al. 2001, Caraway et al. 2001).

RAPD analysis (Williams et al. 1990) overcomes many of the limitations of allozymes. Unlike allozymes, RAPDs have high mutation rates and are thus adequate to detect differences among closely related species. In addition, RAPD analysis requires only small amounts of template DNA, does not need prior DNA sequence information and is simple and quick to perform. By using single, short, arbitrary primers, this technique is capable of scanning a genome for many priming sites that are close enough to allow efficient amplification. In general, RAPD amplification results in DNA fragments that are inherited as Mendelian dominant characters (Williams et al. 1990). However, 15 to 25% of RAPD loci have been shown to display codominant inheritance patterns in some taxa (Echt et al. 1992, Fritsch et al. 1993, Rieseberg and Ellstrand 1993). On the other hand, RAPD markers are theoretically dispersed throughout the genome, and it is likely that most of them are not associated with genes. Therefore, RAPD analysis may provide an adequate tool to unambiguously characterize *P. canariensis* populations and, together with taxonomic

and morphological data, help achieve a more comprehensive understanding of genetic relationships between this endemic and its widespread congener *P. dactylifera*.

The aim of the present study was to evaluate the suitability of RAPD markers to differentiate unambiguously *P. canariensis* from *P. dactylifera* and to identify eventual hybrids between these two species.

Materials and methods

Plant material. *Phoenix canariensis* and *P. dactylifera* are diploid (n = 18), long-lived, dioecious arborescent monocotyledons that are obligate outcrossers. While *P. canariensis* is strictly endemic of the Canary islands, *P. dactylifera* distributes from the West of Asia to the North of Africa and its cultivation is common all through the temperate areas of the planet. *Phoenix canariensis* inhabits altitudinal ranges between 30 to 1000 m, including different habitats, and possesses a considerable degree of morphological variation that makes it difficult to circumscribe the species taxonomically. Although the canarian endemic and the widespread species have been argued to cross very easily (Kunkel and Kunkel 1974, Hodel 1995, Corner 1966, Morici 1998, Barrow 1998), a hybrid between them has not been described as of yet. At the present level of knowledge, the distinction between a hybrid individual and an ecotype is very difficult. In this context, we used morphological features to distinguish three kinds of populations in the Canaries (González-Pérez 2001): i) *P. canariensis* natural populations, (ii) *P. dactylifera* cultivated populations, and (iii) mixed stands, that displayed *P. canariensis*, *P. dactylifera*, and a continuous range of morphologically intermediate plants (hybrids and/or ecotypes). All mixed stands in the Canaries were originally natural populations of *P. canariensis* where the widespread congeners were introduced artificially. If these mixed stands are hybrid zones, rarely will they represent a single generation; rather, they will probably include individuals of both the segregating generations and the parental species (Rieseberg and Ellstrand 1993).

Sampling. Leaves from 221 individuals the height of which ranged from 1 to 10 meter were collected in seven localities (Fig. 1, Table 1) and subjected to RAPD amplification. According to the morphological characterisation described above, three of these populations were *P. canariensis* (Acusa, Tamargada and Mirca), two *P. dactylifera* (Elche and Gran Tarajal) and the other two were mixed stands (Tafira and Maspalomas). We also included a population from Elche, since the adult date palm specimens introduced in the Canary Islands over 15-50 years ago were mostly brought from this locality in peninsular Spain (Morici 1998).

DNA extraction. The leaves sampled were crushed with liquid nitrogen using a sterile mortar and pestle until we obtained a fine-grained powder. DNA extraction was carried out following the Dellaporta et al. (1983) method as modified by Corniquel and Mercier (1994). After ethidium bromide staining, DNA was quantified in agarose gels by using known qualities of calf thymus DNA (Amersham Pharmacia Biotech) as concentration gauges. A dilution test was carried out to determine the optimal amount of DNA for amplification.

RAPD amplifications and electrophoresis. Amplification reactions were set in a 25 µl final volume of reaction mixture containing 18.4 µl H_2O, 2.5 µl 10x TBE buffer (0.89 M Tris-Borate and 0.5 M EDTA), 1.5 µl $MgCl_2$ (25 mM), 0.4 µl dNTPs (10mM of each dATP, dGTP, dCTP, and dTTP), 1 µl of primer (0.1 × 0.5 O.D. units; Operon Technologies), 0.2 µl unit Taq polymerase (5 units/µl; BIOTAQ DNA polymerase, Bioline) and 10–20 ng of genomic DNA. Amplifications were carried out in an Eppendorf Mastercycler Gradient, programmed for 45 cycles with the following temperature profile: 30 sec at 94 °C, 30 sec at 36 °C, and 1 min at 72 °C. The initial and final steps were at 94 °C for 1.5 min, and 72 °C for 10 sec, respectively. RAPD products were resolved through a 1.8% agarose gel run at 200 V (60 mA) for 3 hours in 1x TBE buffer. A 100 Base-Pair DNA ladder was added as a molecular ruler. DNA was stained with ethidium bromide (0.5 µg/ml) and photographed under UV light (λ = 302 nm) using a digital camera Kodak DC 40. Scoring was carried out conservatively, excluding some markers that we considered unreliable. The presence or absence of each band was recorded for each individual and given a value of 1 or 0 depending on its presence or absence, respectively. Bands of identical size were assumed to be homologous across the individuals where they were detected, and bands of different sizes were

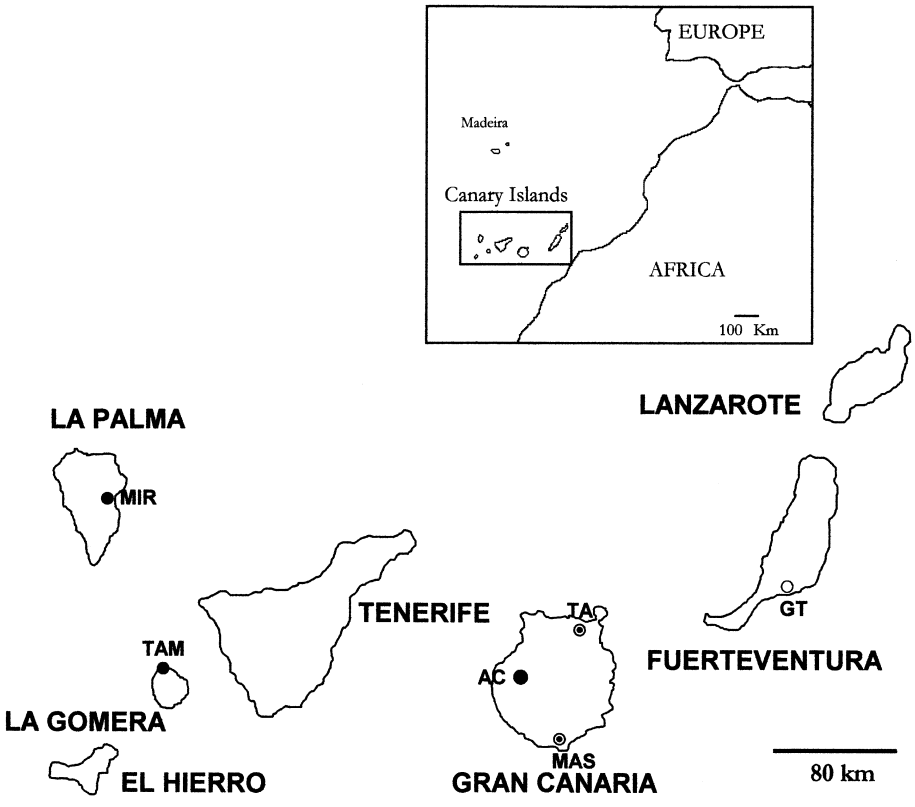

Fig. 1. Map of the Canary Islands showing *Phoenix* populations sampled. Symbols are as follows: (●) *Phoenix canariensis*, (⊙) hybrid or mixed stands, (O) *Phoenix dactylifera*

assumed to represent separate genetic loci. Markers that were inconsistently amplified in repeated RAPD reactions were not included in the subsequent data analysis. We improved reproducibility of the PCR products by maintaining standardised conditions with regard to all possible sources of variation. Thus, we always used the same PCR machine, the same Taq polymerase and treated samples of both species in separate amplification plates. In addition, we repeated some runs to confirm reproducibility and did not find any differences among replicate runs.

Control samples containing all reaction material except DNA were used to test that no self-amplification or DNA contamination occurred. Primers were initially screened to identify well-amplified molecular markers for both *Phoenix* species. RAPD profiles were photographed with a digital camera and visualised using the Kodak digital Science 1D software. We considered bands as species-specific if they occurred in at least 90% of the plants examined in one pure *Phoenix* species but not in the other.

Data analysis. We only used amplification products that were clearly present or absent through all experiments for the data analysis. This approach reduced the influence of non-reproducible, artifactual bands that might bias our analyses. Individuals with a substantial number of missing data were excluded from the analysis.

A multivariate representation of individuals from each population sampled was carried out by subjecting presence/absence of RAPD fragments (Table 2) to Principal Component Analysis (PCA) using the software SPSS 11.0.1 (SPSS Inc. Chicago, IL, USA).

Results

RAPD markers. Out of 54 primers tested (Operon Technologies; kits *OPK*, *OPL, OPM* and *OPN*), only 26 gave fragment patterns that could be consistently scored across all populations of *P. canariensis* and *P. dactylifera*. These primers originated between 1 and 3

Table 1. *Phoenix* populations analysed and its morphological characterisation. N: sample size. % *Phoenix* sp.: percentage of individuals that could not be characterised morphologically

Locality	Population	Code	N	Individual characterisation			Population characterisation
				% *P. canariensis*	% *P. dactylifera*	% *Phoenix* sp.	
Gran Canaria	Acusa	AC	25	100.0	0.0	0.0	*Phoenix canariensis*
	Tafira	TA	46	72.2	5.5	22.2	Mixed/Hybrid stand
	Maspalomas	MAS	34	14.7	64.7	20.6	Mixed/Hybrid stand
Fuerteventura	Gran Tarajal	GT	17	0.0	90.5	9.6	*Phoenix dactylifera*
La Gomera	Tamargada	TAM	41	76.9	0.0	23.1	*Phoenix canariensis*
La Palma	Mirca	MIR	18	70.4	0.0	29.6	*Phoenix canariensis*
Elche	Hort del Gat.	EL	40	0.0	100.0	0.0	*Phoenix dactylifera*

RAPD products each, giving a total of 48 fragments that ranged in size from 300 bp to 1600 bp (Table 2).

Under the criterion of marker specificity stated in the Methods, only two primers (*OPM*-8 and *OPK*-14) gave diagnostic fragments of 900 bp and 1000 bp (*OPM*-8) and 750 bp and 950 bp (*OPM*-14) that discriminate unambiguously between *P. canariensis* (*OPM*-8-1000 and *OPK*-14-750) and *P. dactylifera* (*OPM*-8-900 and *OPK*-14-950) samples (Fig. 2). The apparent homogeneity of these markers in all the individuals that could be ascribed to either species on morphological grounds suggests that their eventual recombination could be used to support the occurrence of interspecific hybridisation.

It is important to note that we also found a few individuals that were ascribed to *P. canariensis* under morphological considerations, but were identified as hybrids by means of RAPD analysis. These "cryptic hybrids" had a predominant incidence in the Tafira population (TA-2, TA-3, TA-5, TA-6, TA-14, TA-15, TA-16 and TA-18). However, three cryptic hybrids were detected in the pure *P. canariensis* population of Mirca, in La Palma (MIR-2, MIR-13 and MIR-18).

On the other hand, we detected fragments that were common within a species and that appeared in hybrid individuals, too. In this sense, two fragments generated from *OPM-8* primer (*OPM*-8-450 and *OPM*-8-550) were common within *P. dactylifera* populations and hybrid individuals from mixed/hybrid. While *OPK*-14-350 and *OPK*-14-650 were exclusive within *P. canariensis* populations and hybrid individuals from mixed/hybrid stands. In addition, a common fragment (*OPK*-14-420) within *Phoenix canariensis* and *P. dactylifera* individuals analysed were detected.

Data analysis. Because of their dominant nature, the RAPD patterns were treated strictly as taxonomic markers, Therefore, no population genetic inferences were drawn from these data.

The two first Principal Components accounted for 62.31% of the total variance

Table 2. RAPD products obtained for the 26 primers that gave reproducible bands in the two species of *Phoenix* studied. N = number of fragments, a: fragment exclusive from *P. canariensis*, b: fragment exclusive from *P. dactylifera*

Primer	Sequence (5′ to 3′)	N	Fragment size (bp)
OPM-2	GTTGGTGGCT	1	600
OPM-4	GGCGGTTGTC	3	650, 850, 1200
OPM-7	CCGTGACTCA	2	800, 950
OPM-8	TCTGTTCCCC	2	450, 500, 550, 900[b], 1000[a]
OPM-17	TCAGTCCGGG	2	850, 1000
OPM-19	CCTTCAGGCA	2	900, >1600
OPN-1	CTCACGTTGG	1	1000
OPN-3	GGTACTCCCC	3	450, 750, 1100
OPN-*5*	ACTGAACGCC	2	650, 1200
OPN-6	GAGACGCACA	2	500, 850
OPN-7	CAGCCCAGAG	3	300, 750, 1300
OPN-10	ACAACTGGGG	2	500, 1000
OPN-11	TCGCCGCAAA	3	750, 850, 1300
OPN-12	CACAGACACC	3	1000, 1200, 1600
OPN-14	TCGTGCGGGT	1	800
OPN-17	CATTGGGGAG	1	650
OPN-19	GTCCGTACTG	3	600, 1300, 1400
OPN-20	GGTGCTCCGT	2	1300, 1400
OPK-1	CATTCGAGCC	1	600
OPK-2	GTCTCCGCAA	1	600
OPK-3	CCAGCTTAGG	1	1300
OPK-5	TCTGTCGAGG	1	1000
OPK-11	AATGCCCCAG	1	650
OPK-13	GGTTGTACCC	2	600, 1000
OPK-14	CCCGCTACAC	2	350, 420, 450, 650, 750[a], 950[b], >1600
OPK-15	CTCCTGCCAA	1	650

(Fig. 3) and separated distinctly the Canary palm individuals from the date palm individuals. In the multivariate space defined by PCA, the putative hybrids were mostly ordinated between *P. canariensis* and *P. dactylifera* individuals, albeit much closer to the Canarian endemic. There was a slight intermixing of the hybrids within *P. canariensis* samples, indicating that they are more similar to the Canarian date palm than to *P. dactylifera* in terms of RAPD markers. Surprisingly, there were only one individual (MAS-13) from Maspalomas population (mixed/hybrid stands) and none from Tafira population (mixed/hybrid stands) clustered with *P. dactylifera* individuals. In addition, MAS-13 was the only one individual from the mixed/hybrid stands (Maspalomas and Tafira populations) that presented the two species-specific molecular markers described for *P. dactylifera* (*OPM*-8-900 and *OPK*-14-950).

Discussion

RAPD markers. Molecular data would support the hypothesis of a hybrid origin of morphologically intermediate individuals if unique markers found in the putative parents were additive in the putative hybrids. Hybrid origin could be refuted if (1) there were no combinations of the parental markers in the putative hybrids and/or (2) many unique markers were observed in the parents and the putative hybrid (Koontz et al. 2001). The allozyme data for *P. canariensis* and *P. dactylifera* (González-Pérez 2001) were equivocal with regard to the possible hybrid origin of the

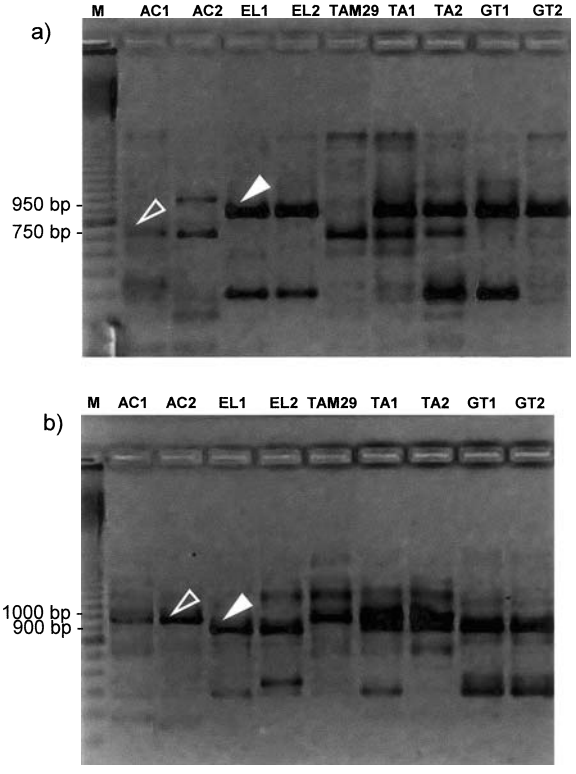

Fig. 2. a) RAPD profile of 13 samples of *Phoenix canariensis* (lanes AC1, AC2, and TAM29), *P. dactylifera* (lanes EL1, EL2, GT1, and GT2) and hybrids (lanes TA1 and TA2) generated by the primer *OPK*-14. The open and white arrowheads indicate the molecular markers of *P. canariensis* (750 bp) and *P. dactylifera* (950 bp), respectively. **b)** RAPD profile of 13 samples of *P. canariensis* (lanes AC1, AC2, and TAM29), *P. dactylifera* (lanes EL1, EL2, GT1 and GT2) and a hybrids (lanes TA1, and TA2) generated by the primer *OPM*-8. Open and white arrowheads indicate the molecular marker of *P. canariensis* (1000 bp) and *P. dactylifera* (900 bp), respectively. Numbers on the left represent molecular weights of the DNA ladder, in bp (lane M)

morphologically intermediate individuals because they did not reveal any differences that were monomorphic in either putative parental species. According to Gallez and Gottlieb (1982), this finding would indicate a close relationship among these *Phoenix* species, but could not refute or support the existence of hybridisation.

By contrast, the presence of few unique RAPD markers in each pure species and their additivity in the morphologically intermediate individuals does indicate hybridisation between *P. canariensis* and *P. dactylifera* in the Canaries. The fact that only two of the 54 primers tested (8.3% of all RAPD fragments analysed in this study) provided suitable molecular markers for the identification of *P. canariensis* and *P. dactylifera* converges with previous allozyme evidence in 19 Canarian populations of these species (Gonzalez-Perez 2001) to suggest a very close relationship between them.

Evidence for introgression (Kunkel and Kunkel 1974, Naranjo-Rodriguez 1999) was consistently found in the mixed populations analysed (Maspalomas and Tafira), where pure *P. canariensis* and *P. dactylifera* individuals with species-specific RAPD markers co-occur with morphologically intermediate individuals where these markers are combined (Table 1; Fig. 3). The intermediate position of the *Phoenix* individuals from these two mixed populations relative to the pure monospecific populations in the PCA analysis (Fig. 3) can be construed as the result of a combination of intermediate, parental and extreme character states in these mixed populations. Moreover, the particular combination of characters differs among individuals within Maspalomas and Tafira populations. This lack of character coherence, recently considered to be the rule in plant hybrids (Rieseberg and Ellstrand 1993), may have two causes in these Canarian mixed populations of *Phoenix*. First, hybridisation may have led to a disruption of a co-adapted gene pool, thus reducing developmental stability (Rieseberg and Ellstrand 1993, Rieseberg 1995, Møller and Swaddle 1997) and producing a more variable phenotype (e.g. Levin 1970). And second, increased morphological variability may simply reflect genetic differences generated by recombination segregation.

In regard to this "cryptic hybrids" in the population from Mirca, morphologically characterised as pure *P. canariensis* population, this finding is surprising, not only because *P. dactylifera* individuals were not observed within this population, but also because this

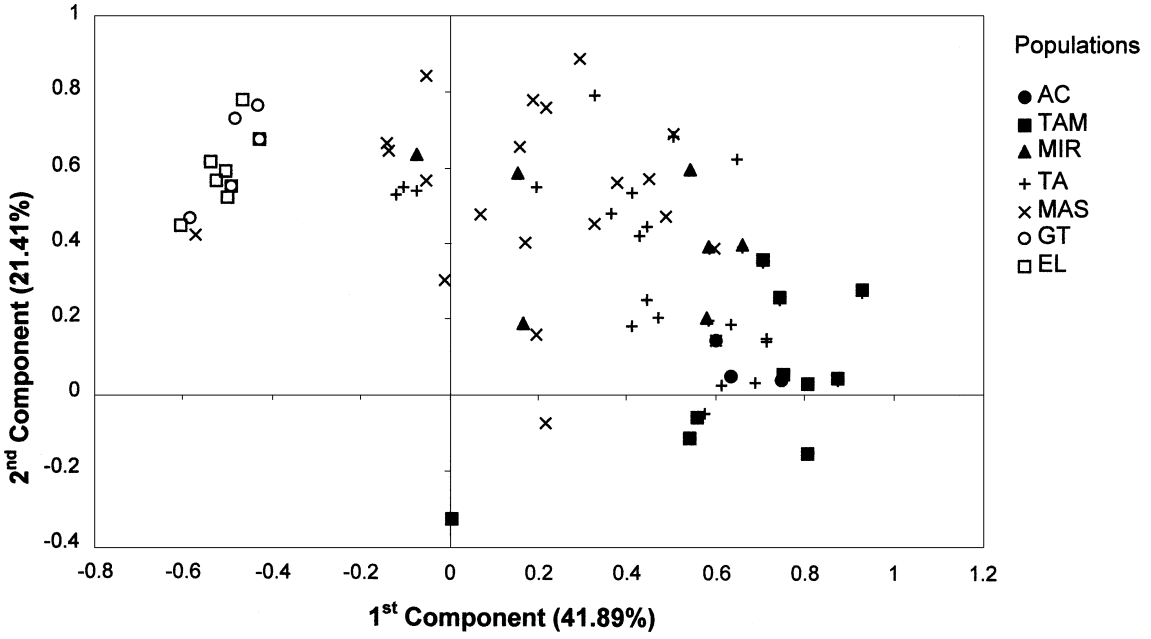

Fig. 3. Principal component analysis based on the correlation matrix of presence/absence of RAPD fragments. Values within brackets are the percent of total variation explained by the corresponding component

widespread species has not been described in that island in the nature (Izquierdo et al. 2001). We suggest that an outside source of *P. dactylifera* from artificial planting should be contaminating this Canarian date palm population; unfortunately, it is not uncommon to see *P. dactylifera* specimens planted in many gardens of the Canary Islands (Jaime O'Shanahan, personal communication).

The three individuals from La Palma notwithstanding, assignments to the wrong species only have a remarkable incidence in populations where *P. canariensis* and *P. dactylifera* coexist. A high degree of gene exchange between the two species can be the more plausible explanation for these incorrect taxonomic ascriptions. If gene flow occurs between a hybrid and one individual of the parental species, then the segregating generations will be mostly advanced backcrosses and have multi-locus associations typical of the most compatible parents (Rieseberg and Ellstrand 1993). Besides, hybrids are a mosaic of parental and intermediate characters rather than solely intermediate ones. Consistent with these expectations, the cryptic hybrids detected in *Phoenix are* quite intermixed in the multivariate representations (Fig. 3) that were built with all the RAPD fragments scored. Therefore, the incongruence between morphological and molecular features may be due to the blurring of most genetic differences by the recurrent action of interbreeding and gene exchange between the two *Phoenix* species through the generations. Therefore, our data support the presence of different hybrid generations and hybridisation events in the populations, as well as a high fertility of the hybrid seed.

P. dactylifera from Elche (Spain) were planted over 3.000 years ago by Phoeniceans (Kyburz 1995), and therefore it could be consider a representative sample of this species. Overall, assuming that the investigated populations are a representative sample of *P. canariensis* and *P. dactylifera*, we can conclude that RAPD markers distinguish these species genetically and provide, for the first time, convincing evidence of inter-specific hybridisation between them. In addition, the fact that the diagnostic markers found show a strictly additive behaviour in the putative

hybrids strongly suggests that hybrid individuals are of recent origin (Rieseberg et al. 1989, Koontz et al. 2001). However, we would have to increase the number of analysed populations, mainly *P. dactylifera* from North of Africa, to guarantee the specificity of the found markers

Conservation implications. Inter-specific mating between a endemic species and a common one will have one of two consequences relevant to conservation biology (Ellstrand and Elam 1993): i) if hybrid progeny and progeny from advanced hybridisation are vigorous and fertile, the endemic species is at risk from genetic assimilation; ii) if hybrid progeny are sterile or have reduced vigour, then the species is at risk from outbreeding depression.

As we have shown in this work, even populations that are characterised morphologically as pure *P. canariensis* may contain hybrids as identified by RAPD markers, thereby indicating the existence of gene flow from planted *P. dactylifera* individuals. Thus, the discriminative molecular tool provided by RAPDs should be used in the first place to estimate the possible incidence of hybridisation in the scarce extant natural populations of *P. canariensis*. Consistent with previous conservation indications given in González-Pérez (2001), target populations for designing multiple interconnected preserves to facilitate gene flow and buffer the eventual action of inbreeding and genetic drift should be those that the molecular markers identify as pure *P. canariensis*.

Further, despite the genetic closeness found between *P. canariensis* and *P. dactylifera,* introduction and/or transplanting of the widespread species (or of hybrid individuals) in the natural populations of the endemic species should be avoided to guarantee the genetic purity of the natural *P. canariensis* populations. Parallel to this, efforts should be made to prevent cultivated *P. dactylifera* from establishing near any of the remaining wild *P. canariensis* populations with a view to diminish the incidence of natural hybridisation between both species.

We thank 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). The authors wish to thank Jaime O'Shanahan for his wise comments about *Phoenix canariensis*. This research was funded by the Gobierno de Canarias (94-2614). A PhD Research Fellowship (Direccion General de Universidades, Gobierno de Canarias) to Gonzalez-Perez. is also acknowledged.

References

Bailey J. P., Child L. E., Wade M. (1995) Assessment of the genetic variation and spread of British populations of *Fallopia japonica* and its hybrid *Fallopia x bohemica*. In: Pysek P., Pranch K., Rejmonek M., Wade M. (eds.) Plant invasions: general aspects and special problems. SPB Academic Publishing, Amsterdam, pp. 140–150.

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

Cabrita L. F., Aksoy U., Hepaksoy S., Leitão J. M. (2001) Suitable of isozyme RAPD and AFLP markers to asses genetic differences and relatedness among fig (*Ficus carica* L.) clones. Sci. Hort. 87: 261–273.

Caraway V., Carr G. D., Morden C. W. (2001) Assessment of hybridization and introgression in lava-colonizing Hawaiian *Dubautia* (Asteraceae: Madiinae) using RAPD markers. Amer. J. Bot. 88(9): 1688–1694.

Corner E. J. H. (1966) The natural history of palms. University of California Press, Berkeley.

Corniquel B., Mercier L. (1994) Date palm (*Phoenix dactylifera* L.) cultivars identification by RFLP and RAPD. Plant Sci. 101: 163–172.

Crawford D. J., Brauner S., Cosner M. B., Stuessy T.F. (1993) Use of RAPD markers to document the origin of the intergeneric hybrid x *Margyracaena skottsbergii* (Rosaceae) on the Juan Fernandez Islands. Amer. J. Bot. 80: 89–92.

Del Cañizo J. A. (1991) PALMERAS. Ediciones Mundi-Prensa.

Dellaporta S. L., Wood J., Hicks J. B. (1983) A plant DNA minipreparation, version II. Plant. Mol. Biol. Rep. 1: 19–21

Echt C. S., Erdahl L. A., McCoy T. J. (1992) Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. Genome 35: 84–87.

Elisiário P. J., Justo E. M., Leitao J. M. (1999) Identification of mandarin hybrids by isozyme and RAPD analysis Sci. Hort. 81: 287–299.

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

Fritsch P. F., Hanson M. A., Spore C. D., Pack P. E., Rieseberg L. H. (1993) Constancy of primer amplification strength among distantly related flowering plant taxa. Plant Mol. Biol. Rep. 11: 10–20.

Gallez G. P., Gottlieb L. D. (1982) Genetic evidence for the hybrid origin of the diploid plant *Stephanomeria diegensis*. Evolution 36: 1158–1167.

González-Pérez M. A. (2001) Caracterización molecular de la palmera canaria (*Phoenix canariensis*) como base para su conservación. Ph. D. thesis, University of Las Palmas de Gran Canaria. Canary Islands. Spain.

Hodel D. R. (1995) *Phoenix:* The date palms. Palm J. 122: 14–36.

Izquierdo I., Martin J. L., Zurita N., Arechavaleta M. (2001) Lista de especies silvestres de Canarias (hongos, plantas y animales terrestres). Consejeria de Politica Territorial y Medio Ambiente del Gobierno de Canarias.

Koontz J. A., Soltis P. S., Brunsfeld S. J. (2001) Genetic diversity and tests of the hybrid origin of the endangered yellow larkspur. Conserv. Biol 15(6): 1608–1618.

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

Kyburz R. (1995) Palms in Europe: The Palms of Elche. Principes 39 (4): 215–218.

Largiader C. R., Scholl A. (1996) Genetic introgression between native and introduced brown trout *Salmo trutta* L. populations in the Rhone River Basin. Mol. Ecol. 5: 417–426.

Levin D. A. (1970) Development instability in species and hybrids of *Liatris*. Evolution 24: 613–624.

Møller A. P., Swaddle J. P. (1997) Asymetric, development/stability, and evolution. Oxford University Press, Oxford.

Montesinos-Barrera J. (1979) La Palmera Canaria. Aspectos botánicos y culturales. Aguayro 111.

Morici C. (1998) *Phoenix* in the wild. Principes 42 (2): pp. 85–93.

Naranjo-Rodriguez R. (1999) Maspalomas Espacio Natural. Ayuntamiento de San Bartolomé de Tirajana. Canary Islands, Spain.

Neuffer B., Auge H., Mesch H., Amarell U., Brandl R. (1999) Spread of violets in polluted pine forests: morphological and molecular evidence for the ecological importance of interspecific hybridization. Mol. Ecol. 8: 365–377

Nicese F., Hormaza J. I., McGranahan G. H. (1998) Molecular characterization and genetic relatedness among walnut (*Juglans regie* L.) genotypes based on RAPD markers. Euphytica 101(2): 199–206.

Niebla-Tome J. E. (1990) *Phoenix canariensis*. La Palmera Canaria. Agricultura 59: 522–527.

Nogales M. (2000) Seed dispersal by common ravens *Corvus corax* among islands habitats (Canarian Archipelago). Ecoscience 6(1): 56–61.

Novak S. J., Mack R. N. (1993) Genetic variation in *Bromus tectorum* (Poaceae): comparison between native and introduced populations. Heredity 71: 167–176.

Perron M., Bousquet J. (1997) Natural hybridisation between black spruce and red spruce. Mol. Ecol. 6: 725–734.

Rieseberg L. H. (1995) The role of hybridization in evolution: old wine in new skins. Amer. J. Bot. 82, 944–953.

Rieseberg L. H., Ellstrand N. C. (1993) What can molecular and morphological markers tell us about plant hybridisation?. Critical Reviews in Plant Sci. 12: 213–241.

Rieseberg L. H., Zona S., Aberbom L., Martin T. D. (1989) Hybridization in the island endemic, Catalina Mahogany. Conserv. Biol. 3(1):52–58.

Roelfos D., van Velzen J., Kuperus P., Bachmann K. (1997) Molecular evidence for an extinct parent of the tetraploid species *Microseris acuminata* and *M. campestris*. Mol. Ecol. 6. 641–649.

Rohlf F. J. (1993) NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 1.80. Analysis System, Version 1.80.; Applied Biostatistics, Steauket, New York.

Sales E., Nebauer S. G., Mus M., Segura J. (2001) Population genetic study in the Balearic endemic plant species *Digitalis minor* (Scrophulariaceae) using RAPD markers. Amer. J. Bot. 88(10): 1750–1759.

Santana S. A. (1992) Paisajes históricos de Gran Canaria. Cabildo Insular de Gran Canaria.

Soltis D. E., Soltis P. S. (1993) Molecular data and the dynamic nature of polyploidy. Crit. Rev. Plant Sci. 12: 243-273.

Sosa P. A., Bouza N., Cabrera-Pérez M. A., Luque A. (1998) Genetic variation in *Phoenix canariensis* and P. *dactylifera* (Arecaceae) populations of Gran Canaria using isozyme electrophoresis. Bol. Mus. Mun. Funchal 5: 443–338.

Urbanska K. M., Hurka H., Landolt E., Neuffer B,, Mummenhoff K. (1997) Hybridization and evolution in *Cardamine* (Brassicaceae) at Urnerboden Central Switzerland: biosystematic and molecular evidence. Plant Syst. Evol. 20: 233–256.

Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A., Tingey S. V. (1990) DNA polymorphisms amplified by arbitrary primers are useful genetic markers. Nucleic Acids Res. 18: 6531–6535.

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