Genetic Resources and Crop Evolution **52:** 97–109, 2005. © Springer 2005

Contrasted genetic diversity and differentiation among Mediterranean populations of *Ficus carica* L.: A study using mtDNA RFLP

B. Khadari[1,2,*], C. Grout[1,3], S. Santoni[3] and F. Kjellberg[4]

*[1]Conservatoire Botanique National Méditerranéen de Porquerolles, Parc National de Port-Cros, Castel Ste Claire, F 83418 Hyères cedex, France; [2]UMR Biologie du Développement des Espèces Pérennes Cultivées (BEPC), INRA. 2, place Viala. 34 060 Montpellier cedex 1, France; [3]UMR Diversité et Génome des Plantes Cultivées, INRA. 2, place Viala, 34 060 Montpellier cedex 1, France; [4]Centre d'Ecologie Fonctionnelle et Evolutive, CNRS, 1919 route de Mende 34293 Montpellier Cedex 5, France; *Author for correspondence (UMR Biologie du Développement des Espèces Pérennes Cultivées (BEPC), INRA. 2, place Viala. 34 060 Montpellier cedex 1, France; e-mail: Khadari@ensam.inra.fr; phone: +33 4 99 61 23 70; fax: +33 4 67 04 54 15)*

Received 6 February 2003; accepted in revised form 13 June 2003

Key words: Common fig, Domestication, *Ficus carica* L., Genetic structure, mtDNA RFLP, Natural population, Subspontaneous forms

Abstract

Patterns of mitochondrial DNA (mtDNA) variation revealed by RFLP were investigated for 63 individuals of the common fig, *Ficus carica* L., in 15 supposedly natural populations throughout the Mediterranean basin. Fifteen haplotypes were detected using one restriction enzyme (*Hind*III) and four probes (*atpα*, *coxIII*, *nad3rpsl2* and *rps12*). Mitochondrial diversity within populations varied from monomorphic to entirely polymorphic and population differentiation was high ($F_{ST} = 0.323$, $P < 10^{-5}$). Seven groups of populations were defined on the basis of genetic and geographic proximity and lead to significant pairwise F_{ST} estimates except for the Corsican group which was similar to the Moroccan one. Fig populations were structured into three clusters: Balearic, West and East Mediterranean gene pools. The low diversity and strong differentiation of the Balearic populations strongly supports an ancient origin and the presence of natural populations in this area before domestication. Significant genetic differentiation between the West and East Mediterranean probably also reflects a diversification of the common fig over the Mediterranean basin preceding domestication. In contrast, Italian island populations seem to result from introduced cultivated fig since they present continental haplotypes. Our study represents a first mtDNA polymorphism survey and these indications should be confirmed by analysing local cultivated forms from the Baleares and from Italian islands and further natural populations from the East Mediterranean.

Introduction

In order to understand the genetic structure and the evolutionary history of domesticated species it is necessary to be able to distinguish natural populations of the crop's wild relatives from subspontaneous or feral forms (introgressed natural populations or naturalised from previously cultivated forms). Such distinction is possible for several herbaceous species because cultivated forms present distinctive phenotypic traits that have become fixed by selection during the process of domestication. For instance, Spanish *Medicago sativa* populations could be clearly separated into three types, intermediate, introgressed and wild type, using phenotypic data and this was confirmed

by the use of genetic markers (Jenczewski et al. 1999a, b). For Mediterranean fruit species, distinction between natural and subspontaneous populations is more difficult for several reasons. First, because they are long lived species, few generations separate extant cultivars from their wild ancestors, limiting the amplitude of trait selection, especially as genotypes of domesticated forms were fixed early due to vegetative propagation by cuttings. Second, the distribution of wild and cultivated forms is most often sympatric favouring gene exchange between them ever since domestication. Third, cultivated forms have been distributed by various human migrations into different Mediterranean areas. As a consequence, naturalised forms previously cultivated and local populations introgressed by introduced cultivated forms could obscure the original genetic structuring of natural populations.

The common fig, *Ficus carica* L., is one of the early domesticated (7000–8000 B.P.; Zohary and Hopf 2000) long lived perennial Mediterranean fruit species. Charred fig pips show that the common fig was present early in the Near East (9900–9500 BP; Kislev 1997) and more recently in the West Mediterranean area (about 6500 BP; Sarpaki 1995). According to the hypothesis of Zohary and Hopf (2000), the common fig was mainly domesticated in the East Mediterranean and selected forms were distributed from there to different areas, particularly into the West Mediterranean area. However, the original distribution of the common fig before domestication is unknown especially with respect to whether it was present in the Western part of the Mediterranean. Genetic structuring analysis among supposedly natural populations using neutral molecular markers should provide insights to clarify fig evolution before the domestication period as has been the case for the olive tree (Lumaret and Ouazzani 2001; Besnard et al. 2001, 2002a).

Nuclear polymorphism data on tree species has shown that they present a high level of genetic diversity within populations and a low differentiation between populations (Hamrick et al. 1992; Le Corre et al. 1997). The observed low differentiation of fig nuclear genes (Valdeyron et al. 1985) could be explained as well by this low differentiation as by the occurrence of subspontaneous populations. Hence nuclear polymorphism may be of limited value to provide insights on fig genetic structuring over the Mediterranean region. In contrast, analyses based on cytoplasmic DNA polymorphism usually give a completely different pattern: a high level of differentiation among populations presenting a clear geographic structure (McCauley 1995; Dumolin-Lapègue et al. 1997; Tomaru et al. 1998; Sinclair et al. 1998). Contrasting genetic patterns between organellar and nuclear genes is due to several factors. First, cytoplasmic polymorphism is relatively low and maternally inherited in most angiosperms allowing the study of seed-mediated dispersal which corresponds to limited gene flow. Second, compared to a diploid genome, drift is twice as strong for a haploid genome leading to stronger genetic differentiation among populations.

Intraspecific cpDNA variation is generally very low because of its slow rate of evolution (Palmer 1987) but population studies have shown that the variation is geographically structured (Dumolin-Lapègue et al. 1997). In contrast to cpDNA, mtDNA variation due to its structural rearrangements can be easily revealed by RFLP using mtDNA genes as probes (Sinclair et al. 1998; Tomaru et al. 1998). MtDNA polymorphism can be useful for genetic studies at the intraspecific level particularly for assessing the distribution of genetic diversity within and among populations.

In the present study, we examined the mtDNA variation revealed by RFLP and the genetic structure of 15 natural fig populations distributed throughout the Mediterranean basin. The principal aim of this paper is to give a first insight on the level of fig genetic diversity and on population differentiation. The second aim is to detect whether the common fig was present in the West Mediterranean before the domestication period. Our results were interpreted with regard to the biogeographic events related to the last glaciations (Hewitt 1996; Taberlet et al. 1998). We discuss also the ability to distinguish natural populations from subspontaneous forms.

Materials and methods

Plant material

We sampled 63 fig trees from 15 populations representing different Mediterranean areas (Table 1). With 11 populations *versus* 4, the West was more

Table 1. Location and sample size of the 15 populations of *Ficus carica*.

Population number	Country or island	Locality	Sample size	Group of populations
1	France	Hérault	5	I
2	France	Perpignan	5	I
3	Spain	Badajoz	4	I
4	Corsica, France	Flum Altu	5	II
5	Corsica, France	Poggio di Nazza	4	II
6	Sicily, Italy	Ustica	5	III
7	Pantelleria, Italy	Pantelleria	3	III
8	Lebanon	Beirut	3	IV
9	Lebanon	Tyr	3	IV
10	Morocco	Bni Ider (Rif)	5	V
11	Morocco	Akchour-Talembote (Rif)	5	V
12	Baleares, Spain	Mallorca (1)	5	VI
13	Baleares, Spain	Mallorca (3)	4	VI
14	Syria	Saint Siméon	3	VII
15	Turkey	Manisa	4	VII

represented than the East Mediterranean. The number of individuals analysed per population ranged from 3 to 5 which corresponds, according to the model of Pons and Petit (1995), to the optimal sampling for measuring the genetic differentiation at haploid loci. The sampled trees within population were separated by 40 to 1000 m while populations were at least 20 km distant. All populations were sampled in natural sites distant from areas of fig cultivation and, when feasible, the sex ratio of the population was checked to be 1 : 1 in order to avoid collection of previously cultivated individuals corresponding to female trees. Despite these precautions, we considered the sampled populations as 'presumably natural populations'.

Molecular analysis

Total DNA was extracted according to Tai and Tanksley (1990) from fresh leaves. The dried DNA was removed in 2.4 mL of Caesium chloride solution with a refractive index value of 1.397. Ten μg of ethidium bromide and Triton (1% final concentration) were added. This solution was centrifuged at 200 000 *g* for 20 h. The DNA band was recovered with a syringe. The volume was adjusted to 5 mL with water and DNA was precipitated by the addition of cold ethanol (15 mL). The DNA pellet was recovered and washed with 75% ethanol, dried and dissolved in 0.2 mL of 1 $\times$ TE.

Five μg of total DNA was digested with restriction endonucleases. Restriction fragments were separated on 1% agarose gel electrophoresis and transferred onto a charged Nylon membrane (Appligene-Oncor) using alkaline transfer with a transblotter. Probes were radiolabelled by random priming [20 μCi of ^{32}P αdCTP (3000 Ci/mmol) (1Ci = 37 Gbq) per probe]. Membranes were hybridised overnight at 65 °C in 7% SDS, 0.5 M $NaHPO_4$ (pH 7.2) buffer and then washed three times in 40 mM $NaHPO_4$ (pH 7.2), 0.1% SDS (20 min at 65 °C).

Four restriction enzymes, *BamH*I, *Dra*I, *EcoR*I and *Hind*III, and eight mitochondrial probes were used in pairwise combinations to search for polymorphisms: the ATPase sub-unit α gene (*atp*α) of sunflower ATP synthetase (Schuster and Brennicke 1986), the ATPase subunit 9 gene (*atp9*) of maize ATP synthetase (Dewey et al. 1985), two different fragments of the NADH dehydrogenase gene, subunit 1 (*nad1*) from *Arabidopsis* (Wissinger et al. 1991), subunit 3 (*nad3*) from tobacco (Lelandais et al. 1996), a fragment containing the ribosomal protein s12 gene and the NADH dehydrogenase subunit 3 gene (*nadrps12*) from *Oenothera* (Schuster et al. 1990), the ribosomal protein s12 (*rps12*) from Tobacco (Vitart et al. 1992), a fragment containing 18S ribosomal RNA gene from *Oenothera* (Brennicke et al. 1985) and a fragment containing cytochrome oxidase subunits (*coxIII*) from *Oenothera* (Hiesel et al. 1987).

Screening of the enzyme/probe combinations revealing polymorphism were performed on six fig

trees originating from six different populations, namely populations n° 1, 6, 8, 10, 12 and 14 (Table 1). These samples were selected to cover the whole collected range and the most insular situation in order to maximise the likelihood of covering the whole range of genetic diversity in the studied populations.

Data analyses

For each fig sample, the mitotype was defined by combining the patterns detected with the four enzyme/probe combinations used. In a first phase, we considered the mitochondrial genome as a single locus and the different mitotypes as alleles. Unbiased haplotype diversity statistics (Nei 1978) corrected for haploid data were calculated using the Genetix 4.0 software (Belkhir 1999) as follows: $H_T = H_S + D_{ST}$, and $G_{ST} = D_{ST}/H_T$, where H_T is the diversity in the total population, H_S and D_{ST} are the average diversities within and between populations, respectively, and G_{ST} is the relative extent of differentiation among populations. Using the Genepop 3.1b software package (Raymond and Rousset 1995a), we estimated population differentiation by computing pairwise F_{ST} and within group F_{ST} and we assessed the significance of population differentiation using exact tests (Raymond and Rousset 1995b). In addition, differentiation among population was also estimated by G_{ST} to allow comparison with published data on other species.

In a second phase, in order to build a phenetic tree, each enzyme/probe combination that gave polymorphic restriction fragment phenotypes was considered as an independent locus, each phenotype constituting an allele. Based on the frequency of these phenotypes, a matrix of unbiased genetic identities (Nei 1978) for pairwise populations was computed by the Genetix 4.0 software. A phenetic analysis was constructed by the neighbor-joining method (Saitou and Nei 1987) to determine genetic relationships among populations using the Phylip program (Felsenstein 1995).

Results

mtDNA polymorphism

The preliminary survey of the restriction fragment patterns in 6 samples from 6 different Mediterranean areas with 32 enzyme/probe combinations showed that 4 probes failed to produce any polymorphism, while the 4 remaining probes failed to reveal any informative polymorphism using *BamH*I, *Dra*I and *Eco*RI. Polymorphism was detected with *Hind* III and 4 probes (*atpα*, *coxIII*, *nadrps12* and *rpl2*). These 4 enzyme/probe combinations were used in the full survey of the 63 individuals and revealed 9 different polymorphic restriction fragments ranging in size from 3.4 to 16 kb (Figure 1). The number of polymorphic restriction fragments obtained with each of the 4 enzyme/probe combinations, *Hind*III/*rpl12*, *Hind*III/*atpα*, *Hind*III/*nad3rpsl2* and *Hind*III/*coxIII* was 3, 4, 1 and 1, respectively. For the *Hind*III/*rpl12* and *Hind*III/*atpα* combinations, 4 other polymorphic restriction fragments were detected in another data set (unpublished data on some isolated individuals and some cultivars). Combining the patterns obtained with the 4 enzyme/probe combinations, 15 different mitotypes (designated as 1 to 15) were defined among the 63 fig trees analysed (Table 2). Patterns of these mitotypes were characterised by 1 to 4 variants. The restriction fragment *nad3rps12*-16 was unique to mitotype 3, and 3 variants (*rpl12*-6.1, *rpl12*-5.6 and *atpα*-5.7) were shared by only 2 mitotypes (Table 2). The most similar mitotypes differed by only one variant band whereas the most distant were distinguished by 6 variants.

Among the 15 mitotypes defined in the full survey of 63 fig trees, 8 were observed in a single individual. Out of these, mitotypes 9 and 15 were observed in 2 and 4 individuals, respectively, in a preliminary extended data set including some isolated individuals and some cultivars (unpublished data). Seven different mitotypes designated as 1 to 7 were detected in 55 individuals belonging to the 15 populations studied. The most frequent was mitotype 1 which represents 36% of the plants analysed whereas the other mitotypes were identified in 2 to 9 plants (Table 2). Mitotype 1 was detected in 10 populations and was particularly abundant in Italian, Moroccan and Spanish populations (Figure 2). In contrast, mitotype 3 was specific to Balearic populations and almost fixed. Finally, mitotypes 2 and 5 characterised populations from the West Mediterranean whereas mitotypes 4, 6 and 7 were more frequently detected in Eastern populations (8, 9, 14 and 15).

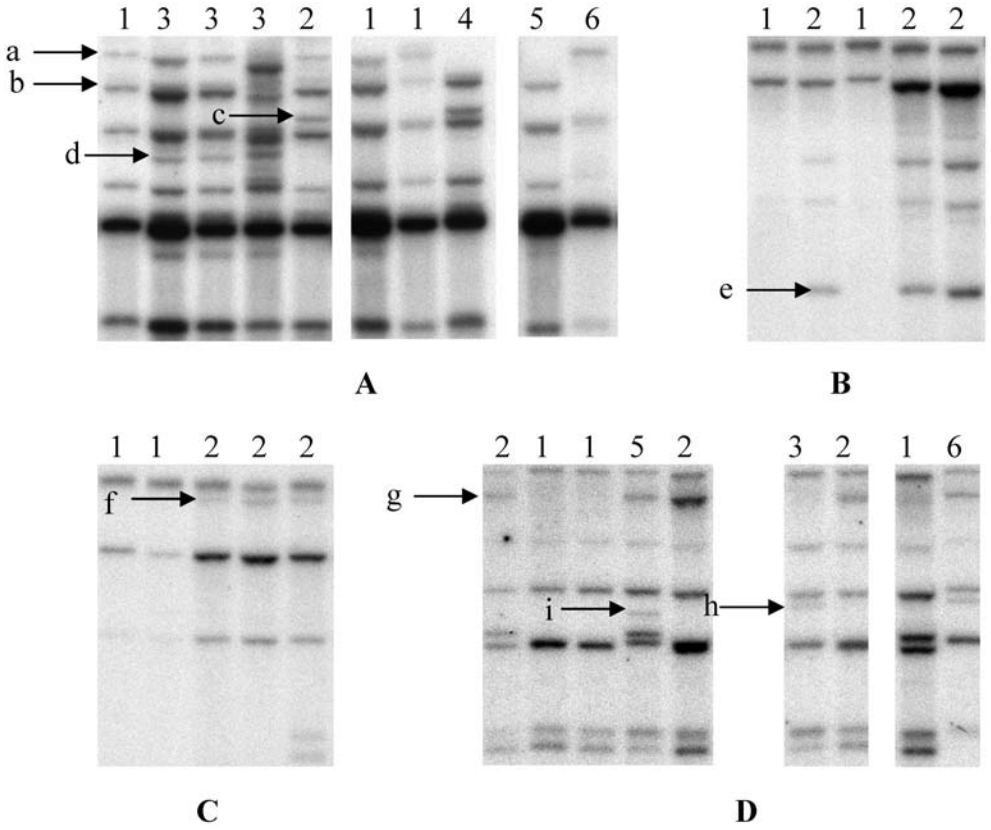


Figure 1. Southern hybridation patterns of HindIII fragments with the atpα (A), coxIII (B), nad3rps12 (C) and rpl12 (D). Each arrow indicates a polymorphic band with the following size (kb), a (12.3), b (9.4), c (7.4), d (5.7), e (4), f (16), g (10), i (6.1) and h (5.6). The numbers above the lanes correspond to the pattern number as reported in Table 2.

Table 2. Frequency of the 15 mitotypes defined according to the restriction patterns revealed on the 63 individuals (15 populations) using the 4 enzyme/probe combinations. Depending on the enzyme/probe combination, the different restriction patterns are designated by number 1 to 6 (see Figure 1).

	Mitotype														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Enzyme/probe combination	Restriction fragment patterns														
HindIII/*rpl2*	1	1	1	2	1	1	3	2	1	1	2	4	5	6	1
HindIII/*atpα*	1	2	2	1	1	3	1	3	2	4	2	4	5	6	6
HindIII/*nadrps12*	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1
HindIII/*coxIII*	1	1	2	1	2	1	1	1	2	2	1	1	2	1	1
Number of occurrence in the whole data set	23	9	8	6	4	3	2	1	1	1	1	1	1	1	1

Diversity within populations and differentiation among populations

Mitochondrial diversity in the total population was high ($H_T = 0.833$, Table 3) and the average diversity within populations was relatively important ($H_S = 0.579$). Diversity within populations differed greatly (ranging from 0 to 0.889). Three populations were monomorphic (6, 9 and 12, Figure 2) while four populations were entirely

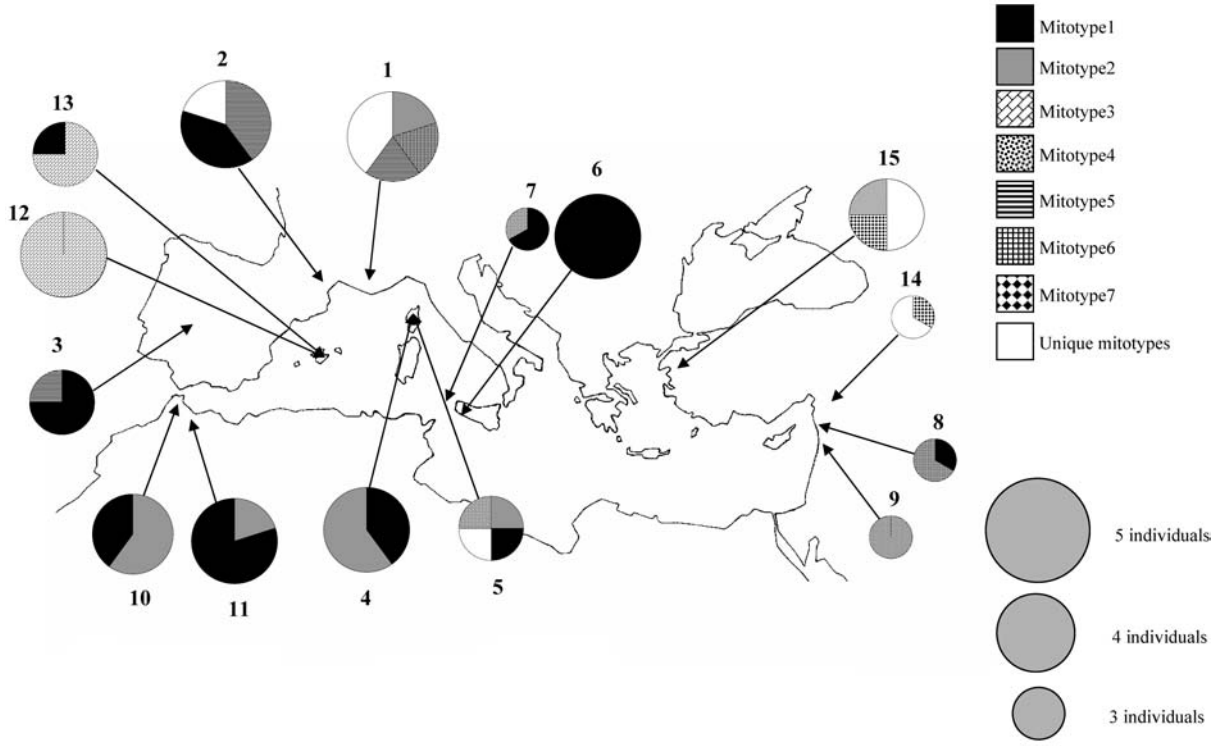


Figure 2. Distribution of the mitotypes in natural fig populations. Populations are numbered as in Table 1. Eight mitotypes were only observed once and are grouped as "unique mitotypes" (white symbol).

Table 3. Mitotype diversity within and among populations and groups of populations in *Ficus carica*.

	H_T	H_S	G_{st}	F_{st}
Population level	0.833	0.579	0.305	0.323***
Group of populations level	0.830	0.608	0.267	0.284***

***$P < 10^{-3}$.

polymorphic, each individual displaying a different mitotype (1, 5, 14 and 15). The other populations had 2 or 3 mitotypes (Figure 2).

Population differentiation was important since it accounted for one third of total mitochondrial diversity as indicated by the estimates of both Gst (0.305) and Fst (0.323, $P < 10^{-5}$, Table 3). Pairwise Fst estimates among all the studied populations showed different levels of differentiation (Table 4). Except for Lebanese populations, no significant differentiation was observed between populations sampled within the same country or island, i.e. Balearic, Moroccan or Corsican populations. The population from Sicily was also similar to the nearby population from Pantelleria. In contrast, Balearic populations were significantly differentiated from all other populations, except for the Pantelleria population (Table 4), lack of significance probably resulting from to low sample size for Pantelleria. The Lebanese population n° 8 was significantly differentiated from the other Lebanese population n° 9 and from the Syrian population but not differentiated from several West Mediterranean populations (1, 2, 3, 5, 7 and 11). Except for populations from Corsica (population n° 5), Lebanon (population n° 8), and Pantelleria, significant differentiation was observed between Eastern populations (n° 9, 14 and 15) and Western populations (Table 4).

Differentiation and relationships between groups of populations

Samples were pooled into groups of populations as indicated in Table 1 on the basis of geographic proximity and/or genetic similarity. For each of the Corsican, Italian, Moroccan and Balearic groups (II, III, V and VI), populations were sampled from the same geographic area and were

Table 4. Pairwise Fst estimates among populations of *Ficus carica.*

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	0.022													
3	0.191**	−0.096												
4	0.091**	0.167**	0.209**											
5	−0.053 ns	0.006 ns	0.077 ns	−0.053 ns										
6	0.500***	0.333 ns	0.062 ns	0.500**	0.394**									
7	0.137**	−0.014 ns	−0.152 ns	0.145 ns	−0.130 ns	0.189 ns								
8	0.008 ns	0.140 ns	0.236 ns	0.277**	0.071 ns	0.634**	0.143 ns							
9	0.388**	0.502***	0.707***	0.620***	0.025 ns	1.000***	0.500 ns	0.667**						
10	0.090**	0.167**	0.209**	−0.250 ns	−0.053 ns	0.500**	0.145 ns	0.277**	0.620***					
11	0.271***	0.118 ns	−0.119 ns	0.107 ns	0.093 ns	0.000 ns	−0.104 ns	0.311 ns	0.742***	0.107 ns				
12	0.500***	0.600***	0.781***	0.752***	0.552***	1.000***	0.764***	0.764***	1.000***	0.700***	0.800***			
13	0.231***	0.264**	0.385**	0.384**	0.200**	0.706**	0.313 ns	0.376**	0.707***	0.384**	0.443**	0.062 ns		
14	0.000 ns	0.118**	0.282**	0.240***	0.000 ns	0.634***	0.167**	0.167**	0.500**	0.240***	0.366***	0.634***	0.282**	
15	0.000 ns	0.107***	0.250***	0.215**	−0.067 ns	0.552***	0.071 ns	0.147**	0.250 ns	0.215***	0.325***	0.552***	0.250***	−0.091 ns

***: $P < 0.001$, **: $P < 0.01$, ns: non-significant.

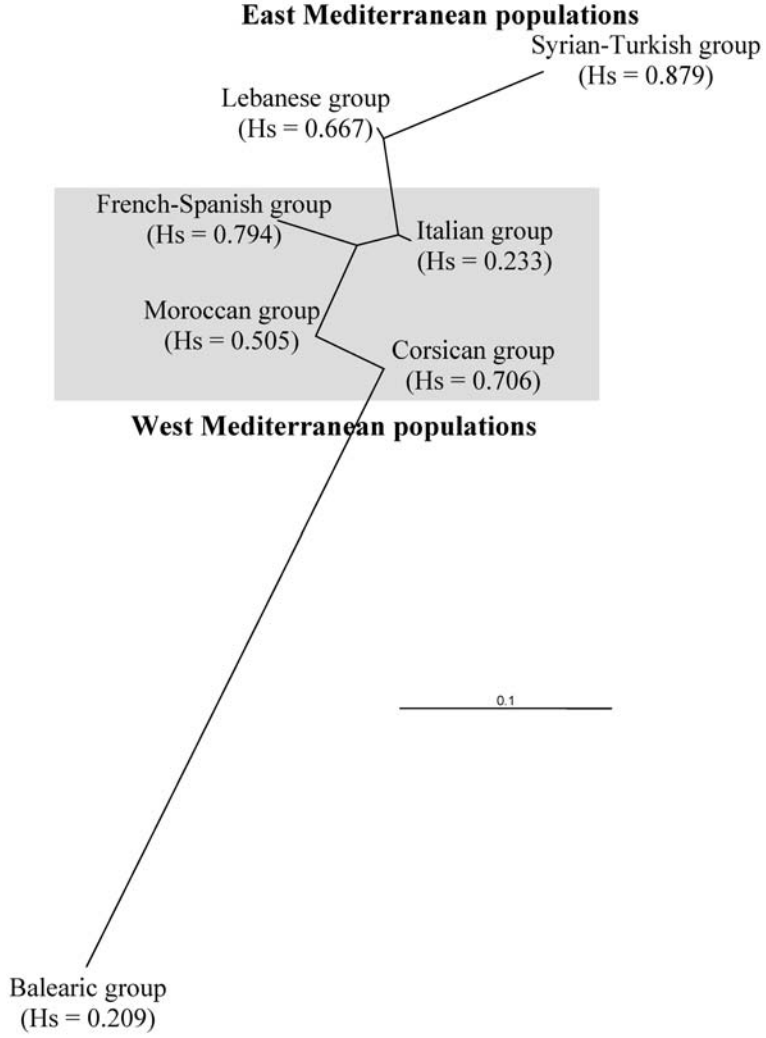


Figure 3. Neighbor-joining phenogram using distance estimated by (1 – unbiased genetic identity) among group of populations of *Ficus carica*.

also not significantly different. In contrast, populations sampled over larger geographic scales (i.e. from France and Spain) were pooled into group I and group VII because of the lack of differentiation within each enlarged group, while the Lebanese group (IV) included two significantly different populations but sampled from the same area.

The average diversity within groups of populations was higher than the value obtained at population level (Hs = 0.608, Table 3). The Balearic and Italian groups had the lowest values of mitochondrial diversity (Hs = 0.209 and 0.233 respectively, Figure 3), while the most diversified groups were the Syrian–Turkish and French–Spanish groups (Hs = 0.879 and 0.794 respectively, Figure 3). The genetic differentiation between the 7 groups of populations was important but slightly decreased in comparison to values obtained at population level (Fst = 0.284, $P < 10^{-5}$, Table 3). Pairwise Fst estimates among all groups of populations were statistically significant, except for group II which was not significantly differentiated from group IV (Table 5).

Relationships between groups of populations were illustrated by a Neighbor-joining phenogram using unbiased genetic identities based on the

Table 5. Pairwise Fst estimates (upper value) and distance estimated by 1 – unbiased genetic identity (lower value) among groups of populations of *Ficus carica*.

Group of populations	I	II	III	IV	V	VI
II	0.071***					
	0.092					
III	0.177**	0.270**				
	0.064	0.079				
IV	0.143***	0.169***	0.408***			
	0.116	0.080	0.054			
V	0.095**	−0.030 ns	0.163**	0.312***		
	0.058	0.009	0.042	0.100		
VI	0.416***	0.495***	0.739***	0.553***	0.589***	
	0.428	0.272	0.429	0.483	0.284	
VII	0.119***	0.140***	0.403***	0.089***	0.276***	0.443***
	0.185	0.145	0.138	0.087	0.169	0.579

***: $P < 0.001$, **: $P < 0.01$, ns: non-significant.

frequencies of *atpα*, *coxIII*, *nadrps12* and *rpl2* phenotypes (Nei 1978; Figure 3). The pairwise genetic identities ranged from 0.009 to 0.578 and averaged 0.185 ± 0.164 (SE) showing different levels of genetic relationships (Table 5 and Figure 3). The Balearic group was very distant from the other groups with pairwise genetic distances ranging from 0.272 to 0.578. Among the groups without the Balearic one, the most distinct one was the Syrian–Turkish group followed by the Lebanese group (Table 5 and Figure 3). The pairwise genetic distances among the French–Spanish, Corsican, Italian and Moroccan groups were lower than the average value (0.185) ranging from 0.009 to 0.092. The Neighbor-joining phenogram indicated that the groups of populations could be structured into 3 clusters: the Balearic cluster (A), cluster B including the French–Spanish, Corsican, Italian and Moroccan groups (West Mediterranean populations) and cluster C corresponding to the Syrian–Turkish and Lebanese groups (East Mediterranean populations, Figure 3). The genetic differentiation among these 3 clusters was important and statistically significant (Fst = 0.307, $P < 10^{-4}$). When the Balearic group was removed from the data set, the West Mediterranean populations were significantly differentiated from the East Mediterranean populations with the value of Fst decreased (Fst = 0.171, $P < 10^{-4}$). These results were due to a strong differentiation between the Balearic group and the other populations.

Discussion

Before interpreting the significance of our results, we shall discuss the validity of mtDNA markers used. RFLP analysis using mt DNA genes as probes has been applied in several studies assuming that the fragments detected correspond to the mitochondrial genome (Sinclair et al. 1998; Tomaru et al. 1998). Despite the drastic selection of reproducible and polymorphic restriction fragments, we should verify if the polymorphism detected represents mtDNA and not nuclear DNA. The ultimate proof that the markers correspond to mtDNA is the demonstration that the polymorphism is maternally inherited like in most angiosperms (Corriveau and Coleman 1988; Chat et al. 1999; Besnard et al. 2000). Inheritance of mtDNA has not been investigated in our study but a number of observations point to the fact that we are studying mtDNA. First, each of the four probes used corresponds to one well defined mitochondrial gene (*atpα*, *coxIII*, *nadrps12* and *rpl2*; see 'Material and methods') and probes cloned from rRNA and tRNA genes were not used because of the sequence homology with nuclear DNA (Brennicke et al. 1985). Second, patterns of restriction fragment analysis showed several bands of different size and no double bands as would be expected for nuclear DNA RFLP (see Figure 1). Lastly, the high level of genetic differentiation obtained among fig populations (about one

third of total diversity) is inconsistent with nuclear DNA polymorphism since woody long-lived perennial species like the common fig show a lower level of genetic differentiation among populations for nuclear genes (Fst = 0.084 on average; Hamrick et al. 1992). These observations strongly support that the polymorphism examined in our study is mitochondrial and not nuclear.

Despite the limited number of mtDNA markers used, 15 distinct mitotypes were identified in fig populations. Compared to other woody perennial species, the level of polymorphism was however lower than detected in *Theobroma cacao* (44 mitotypes among 177 cocoa clones; Laurent et al. 1993) and in *Hevea brasiliensis* (212 mitotypes among 397 wild and cultivated accessions; Luo et al. 1995). But it was higher than obtained in *Fagus crenata* (8 mitotypes; Tomaru et al. 1998) and in olive tree, *Olea europaea* (4 mitotypes; Besnard et al. 2002b). These contrasting mitochondrial polymorphisms are species dependant but are also due to the scoring methodology and the selection of restriction fragments as mtDNA markers, our methodology being closest to that used on *Fagus* and *Olea*. Compared to data on *Fagus crenata* (Tomaru et al. 1998) and *Olea europaea* (Besnard et al. 2002b), mitochondrial polymorphism detected in fig populations was increased by the occurrence of 8 mitotypes identified in only one individual. Detection of unique mitotypes was probably due to the limited sampling of the study populations since 2 of the unique mitotypes were identified in several individuals in another data set (unpublished data). Compared to previous studies in plants (Belhassen et al. 1993; Ronfort et al. 995; Tomaru et al. 1998; Muller et al. 2001), the level of polymorphism observed in fig populations is consistent with the high rate of structural rearrangement which characterises mtDNA variation (Palmer 1992).

Mitochondrial diversity among fig populations was high ($H_T = 0.833$) and similar to values obtained for *Fagus* populations (Tomaru et al. 1998) but higher than the diversity observed among olive populations (Besnard et al. 2002b). Contrary to the low diversity within *Fagus* populations ($H_S = 0.031$, Tomaru et al. 1998), the diversity within fig populations was often large and particularly very contrasted among populations (ranging from monomorphic to entirely polymorphic populations). Based on both detected polymorphism and diversity observed within populations, the sampling strategy applied in our study (3 to 5 individuals per population) seems to be insufficient to clarify the genetic structure of fig populations especially as populations sampled in the same country or island were genetically similar, i.e. Balearic, Moroccan or Corsican populations. Our sampling methodology was adopted according to the model of Pons and Petit (1995) which showed that the most efficient strategy for the accurate measurement of genetic differentiation at haploid loci is to sample many populations rather than many individuals per population. Such sampling is best suited for the estimation of differentiation using chloroplast DNA polymorphism because of the low level of variation (Dumolin-Lapègue et al. 1997). However, due to the high level of mtDNA variation, increasing the number of individuals analysed per population would allow to better clarify the genetic structure of fig populations as in the case of the *Fagus* study (Tomaru et al. 1998). For these reasons, samples were pooled into groups of populations taking into account the lack of differentiation between populations or similar geographic origin.

Genetic analysis at the level of population groups showed similar results to the analysis at the population level as defined in our study, but with more accurate assessment of genetic diversity partitioning among and within fig populations. In fact, contrasting diversity and genetic differentiation observed among fig populations were confirmed and genetic structuring defined three gene pools: Balearic, West and East Mediterranean populations. The lack of genetic differentiation within the French–Spanish group and within the Syrian–Turkish group indicates that a relevant spatial scale for population cytoplasmic differentiation on the mainland is of the order of several hundred kilometres as is the case for temperate forest species (Dumolin-Lapègue et al. 1997). This observation will have to be confirmed by analysing more fig trees from Spain and particularly from Syria and Turkey. Our study shows that in the common fig, 10 to 20 individuals should be sampled over a large spatial scale (from 100 to 200 km) to assess accurately the genetic distribution of mitotypes among and within fig populations. Nevertheless, the biogeographic significance of our mtDNA RFLP data can be interpreted at the level of the population groups.

Populations from the Balearic and Italian islands were characterised by two common features: insular situation and low mitochondrial diversity. However, in contrast to the populations from Sicily and Pantelleria that share continental mitotypes, the Balearic populations form a strongly differentiated gene pool which cannot be explained by recent colonisation events because of the specific mitotype. Low diversity and strong differentiation in insular populations fit with the model of genetic drift under reduced size during numerous generations. Hence the most likely scenario is that the Balearic populations are the result from an old colonisation and have survived locally during the last glaciations as seems to be the case for a series of other species (Huntley and Birks 1983; Hewitt 1996; Taberlet et al. 1998). The genetic differentiation suggests that the Balearic populations did not contribute to the recolonisation of the Western Mediterranean after the last glaciation. In contrast, populations from Sicily and Pantelleria could originate from recent events due to human introduction of plant material from distant areas and would hence correspond to subspontaneous populations (a naturalised form of previously cultivated fig or hybrid forms) as is the case for *Olea europaea* (Besnard et al. 2002a). Until these hypotheses are confirmed by analysing further populations and locally cultivated forms from the Balearic islands and from Italian islands, our study points at an important concern for the understanding the evolution of domesticated Mediterranean woody species: distinguishing real natural populations from introgressed natural populations and from subspontaneous or feral forms.

Our data strongly support the hypothesis that the Balearic populations got established before fig domestication (7000–8000 BP; Zohary and Hopf 2000) and argue in favour of fig populations in the West Mediterranean being also present before domestication following the same pattern as in *Olea europaea* (Terral and Arnold-Simard 1996; Besnard et al. 2002b). Moreover, the differentiation between Western and Eastern populations probably reflects fig evolution before domestication suggesting that most of the sampled populations were natural. The Corsican populations presenting both specific mitotypes and mitotypes shared with the continent suggest an intermediate situation between the Balearic and the Italian islands situation. This could result from a mixture of ancient local populations with recent human introductions. Since fig domestication, all Mediterranean areas have been strongly affected by human activities. Therefore, even sampling fig populations in natural sites distant from cultivated areas with a 1 : 1 sex ratio and surveying for the lack of domestication traits such as the first parthenocarpic crop (Zohary and Hopf 2000), our sampling represented natural populations but also introgressed ones. Mitotypes shared by distant populations from the West and East Mediterranean (populations from Lebanon and Italian or Corsican islands) strongly support the partly introgressed nature of these populations as was also the case for the olive tree (Besnard et al. 2002a).

This first survey of mt DNA polymorphism shows a contrasting diversity within populations and different levels of genetic differentiation among populations. Despite widespread cultivation and probable diffusion of cultivars over large distances, and despite probable introgression of some natural populations, our study suggests the presence of true natural populations in both the West and East Mediterranean. This is probably because the natural habitat of the common fig is in cliffs where competition with other plants is limited while it is mainly cultivated in flatter areas with deeper soils (Khadari and Kjellberg, pers. obs.). These first results show that: (1) it should be feasible to understand the biogeographic history of the common fig using genetic markers in a more thorough study; and (2) it should be feasible to determine whether West Mediterranean maternal lines contributed to the extant stock of cultivars.

Acknowledgements

We wish to thank all the colleagues who provided fig tree samples for this study, namely: Prof. U. Aksoy, Dr A. Al Ibrahim, Prof. M. Ater, Prof. S. Ragusa Di Chiara, Dr K. Elias, Dr S. Gözlekci, Dr D. Oro, and Dr R. Rivoal. We are particularly indebted to J.P. Roger and P. Baud for providing us with cuttings fig samples and fresh leaves. During the whole study, the encouragement and help of Dr F. Boillot and Prof. F. Dosba were decisive. Comments on an earlier version of the manuscript from B. Fady, M.H. Muller and J. Ronfort were greatly appreciated. This research was supported by the CBNMP (Conserva-toire Botanique

National Méditerranéen de Porquerolles) and by funding from the BRG (Bureau des Ressources Génétiques, Paris).

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