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Genetic variability in some species of the genus *Quercus* (*Fagaceae*)

Abstract

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Due to its frequent hybridisation and introgressions in the Iberian Peninsula, the genus *Quercus*, comprises several taxonomic problems. After revising the morphology of different species in central (*Quercus broteroi, Q. faginea* and *Q. pyrenaica*) and southern Iberian Peninsula (*Q. broteroi, Q. canariensis, Q. lusitanica, Q. pyrenaica* and *Q. broteroi* × *Q. canariensis*), molecular biology techniques are used to find out their possible relationships. This work presents a preliminary study of the variability in several groups of *Quercus* using RFLPs.

Introduction

In the Iberian Peninsula there are twelve *Quercus* species including deciduous and perennial populations (Franco 1990; Rivas-Martínez & Sáenz Laín 1991; Galán de Mera 1995; Achhal 2002). These species present identification problems due to high polymorphism and frequent interspecific hybridisation and introgressions (Bellarosa & al. 1990).

For these reasons, several techniques have been employed in the study of their relationships: morphological characteristics, electronic microscopy (Penas & al. 1994; Llamas & al. 1995; Bussotti & Grossoni 1997), chemio-taxonomical characteristics (Knops & Jensen 1980; Mariani Colombo & al. 1983) and biochemical markers (Afzal-Rafii 1988; Lumaret & al. 1988; Collada & al. 1988). In the last decade, molecular biology techniques have also been introduced to resolve taxonomical problems and phylogeographical aspects (Dumolin-Lapègue & al. 1997, Samuel & al. 1998; Manos & al. 1999; Lumaret & al. 2002; Olalde & al. 2002; Petit & al. 2002a; Petit & al. 2002b).

The analysis of cpDNA variability is a powerful tool in phylogenetic studies and their relationships with the geographical distribution of haplotypes. However cpDNA is very conserved in plants (Wolfe & al. 1987), its variations provide an opportunity for phylogeny reconstruction at the population level. In addition, its maternal inheritance allows us to analyse the dispersal routes (Dumolin & al. 1995).

In the genus *Quercus*, cpDNA analysis has been used in perennial (Collada & al. 2001) and deciduous species (Dumolin-Lapègue & al. 1997; Herrán & al. 1999; Lumaret & al.

2002; Olalde & al. 2002; Petit & al. 2002a; Petit & al. 2002b). However, some interesting aspects remain to be resolved in the subgenus *Quercus*, such as the identity of the pair *Q. faginea* Lam. - *Q. broteroi* (Coutinho) A. Camus, in whose communities, some biogeographical units in the Iberian Peninsula are based (Rivas-Martínez & al. 2001). The position of the hybrids of *Q. canariensis* Willd. and *Q. faginea* - *Q. broteroi* and the occurrence of hybrids between *Q. lusitanica* Lam. and *Q. pyrenaica* Willd. is also unclear. Thus, the aim of this study is to explain how the genetic flow in some species has occurred between two possible well defined biogeographical units: the Sistema Central and its surroundings and the Andalusian mountains separated by the Guadalquivir valley.

Materials and methods

1. Plant material. Leaves were sampled from populations of *Quercus faginea* (Ciudad Real, Guadalajara, Madrid), *Q. broteroi* (Cáceres, Ciudad Real), *Q. lusitanica*, *Q. canariensis*, *Q. broteroi* × *Q. canariensis* (*Q.* ×*marianica*) (Cádiz) and *Q. pyrenaica* (Cáceres, Cádiz, Guadalajara, Madrid). Vouchers were deposited in the USP herbarium (Fig. 1).
Q. faginea - Q. broteroi. A total of 117 trees of *Q. faginea* (Ciudad Real, Guadalajara, Madrid) and 40 of *Q. broteroi* (Cáceres, Ciudad Real, Madrid) were sampled.

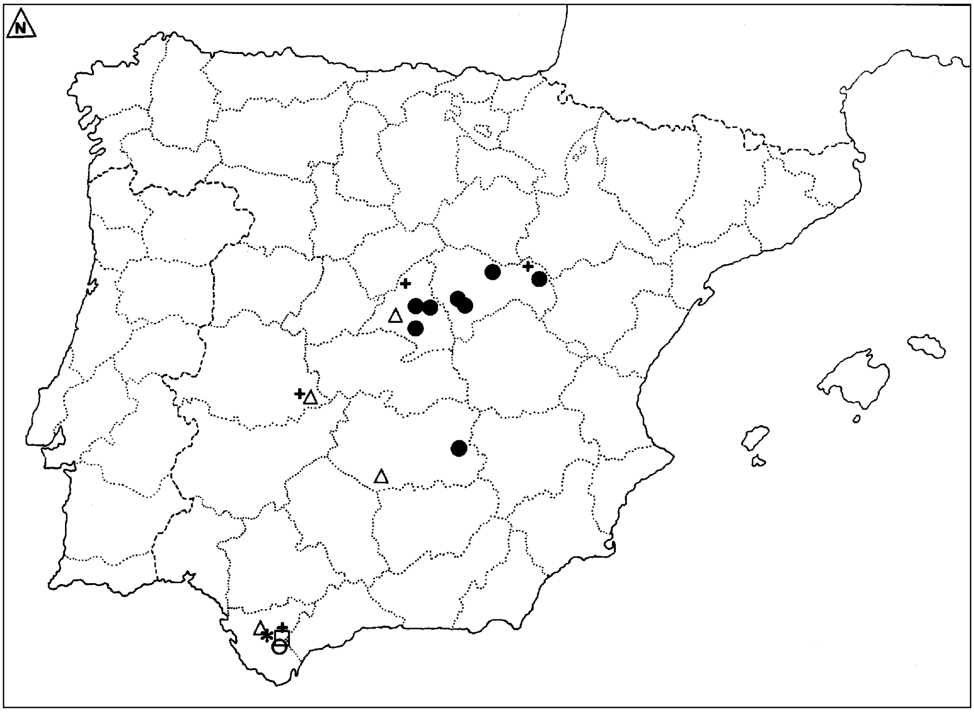

Fig. 1. Locations where samples were collected. (*Q. faginea* ●, *Q. broteroi* △, *Q. canariensis* ○, *Q.* × *marianica* *, *Q. pyrenaica* + and *Q. lusitanica* □).

Q. canariensis - Q. ×marianica – Q. broteroi. In Cádiz leaves from 5 trees of *Q. canariensis*, from 5 trees of *Q. ×marianica* and from 5 trees of *Q. broteroi* were collected.
Q. lusitanica - Q. pyrenaica. In Cádiz a total of 8 individuals of *Q. pyrenaica* were sampled, 5 individuals of *Q. lusitanica* and 3 individuals of arborescent *Q. lusitanica*. In addition, 2 individuals of *Q. pyrenaica* from Madrid, two individuals from Guadalajara and one individual from Cáceres were studied.

2. DNA extraction. DNA from all sampled species was extracted using the FastDNA Kit (Bio 101) and was purified with QIAquick PCR Purification kit (QIAGEN).

3. PCR-RFLP methods. PCR amplifications were performed in 25 µl reactions containing 1 µl DNA extracted, 2 units Taq DNA polymerase (Roche), 2.5 µl Taq DNA polymerase buffer, 1 µl deoxynucleotide 1 mM, 0.25 mM of trnD primer and 0.25 mM of trnT primer (Demesure & al. 1995). An initial denaturation for 4 min at 94ºC was followed by 35 cycles of denaturation (45 s at 92ºC), annealing (45 s at 59.1 ºC) and extension (3 min at 72ºC) with a final extension for 10 min at 72ºC. A negative control was included in each amplification.

The PCR product (15 ml) was digested with 1 U of Taq I following the manufacturer's instructions (Roche). Restriction fragments were separated in 8% polyacrylamide gels at 65 V for 14-15 h at room temperature. After electrophoresis, gels were stained with silver nitrate.

4. Genetic data analysis. Haplotypes are defined as different combinations of variants of length in the different polymorphic fragments.

Results and discussion

1. Morphology

The main morphological characteristics of the species studied are described in Table 1.

2. Molecular study

Q. faginea - Q. broteroi. Only one pattern of digestion was found in all the trees (Fig. 2) after digestion of the product DT. Therefore, the genetic composition of the analysed populations was monomorphic. In this digestion, *Q. suber* was used as an outgroup showing a different pattern of digestion.

Unlike that found by Rivas-Martínez & Sáenz Laín (1991), *Q. faginea* and *Q. broteroi* seem to be ecological forms of one species, edaphically indifferent, as their haplotypes are very similar throughout the territories of central Spain. This stable situation is uncommon in other species of the subgenus *Quercus*, and is also uncommon in other territories in the Iberian Peninsula (Olalde & al. 2002; Petit & al. 2002b).
Q. canariensis - Q. ×marianica - Q. broteroi. The patterns of digestion are shown in Fig. 3. In the Aljibe mountains (Cádiz), there are different haplotypes belonging to each species. *Q. broteroi* shows the same pattern as in *Q. faginea* from the Sistema Central. The upper restriction fragments and restriction fragments around 300 bp, identify *Q. canariensis* from *Q. ×marianica*. The pattern of *Q. ×marianica* is similar to *Q. broteroi*, although

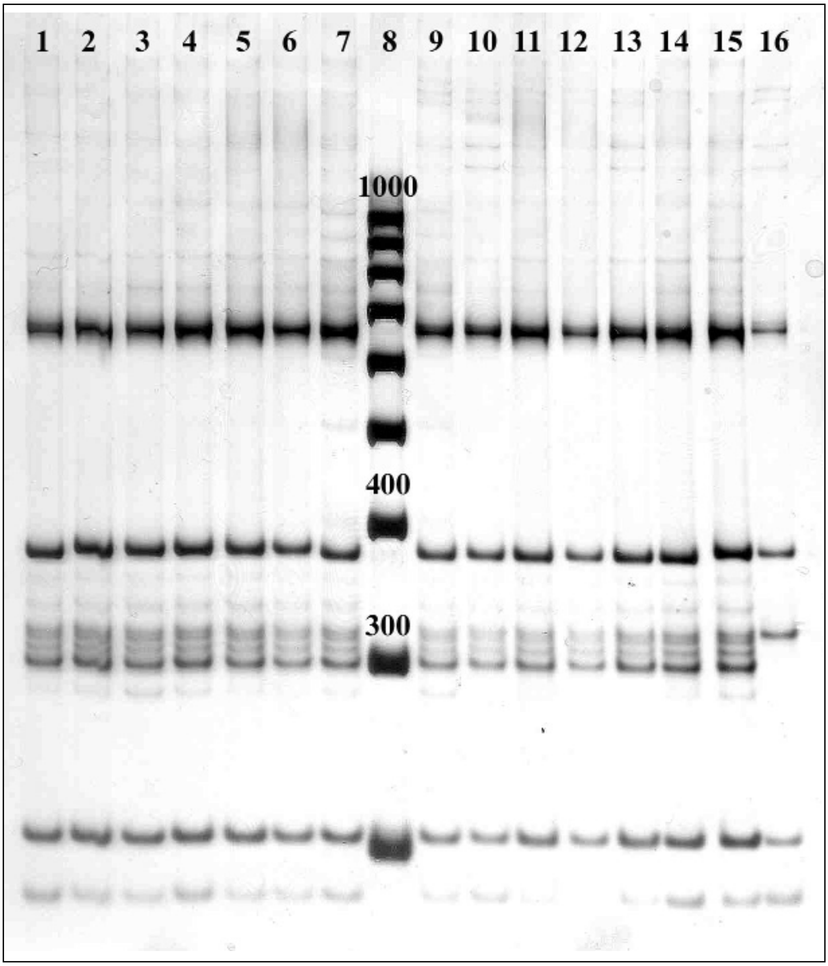

Fig. 2. Digestion pattern: *Quercus broteroi* (1, 2, 3, 4, 5, 6, 7), weight marker of 1000 bp (8), *Q. faginea* (9, 10, 11, 12, 13, 14, 15) and *Q. suber* (16).

morphological characteristics are clearly identifiable in the hybrid. Following Dumolin & al. (1995) the direction of crossing is from *Q. canariensis* to *Q. broteroi*.

On the other hand, Olalde & al. (2002) distinguish different haplotypes of *Q. canariensis* in the south of Spain that are probably hybrids. This aspect coincides with the morphology of the hybrids of *Q. faginea* (Sáenz de Rivas 1968; Sáenz de Rivas & Rivas-Martínez 1971).

Q. pyrenaica - *Q. lusitanica.* The patterns of digestion are shown in Fig. 4. *Q. pyrenaica* is a quite uniform species as is shown in the pattern of digestion and in previous studies (Olalde & al. 2002). However, *Q. lusitanica* exhibits introgression in the Aljibe mountains (Cádiz). Thus, the patterns of lanes 9, 12, 13, 14 and 16 are more similar to *Q. pyrenaica* than *Q. lusitanica* (lanes 10, 11, 15).

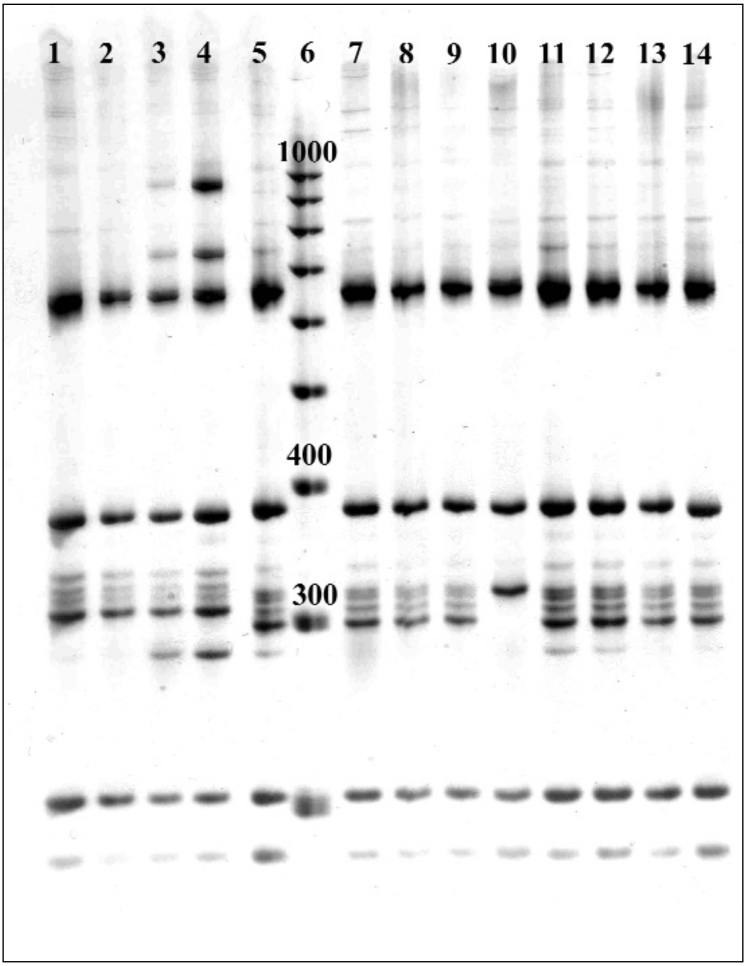

Fig. 3. Digestion pattern: *Quercus canariensis* (1, 2, 3, 4, 5), weight marker of 1000 bp (6), *Q.* ×*marianica* (7, 8, 9, 10) and *Q. broteroi* (11, 12, 13, 14).

The pattern of lane 7 is more similar to the *Q. lusitanica* haplotype. Cross direction could be occurring on both sides. This aspect demonstrates the existence of hybrid specimens between the two species. This situation has not previously been described in the Iberian Peninsula.

Conclusions

According to the studied haplotypes, *Q. faginea* appears to be the same species as *Q. broteroi*. *Q. faginea* – *Q. canariensis* and *Q. lusitanica* – *Q. pyrenaica* show introgressions in southern Spain. From a geographical viewpoint, populations studied exhibit more uni-

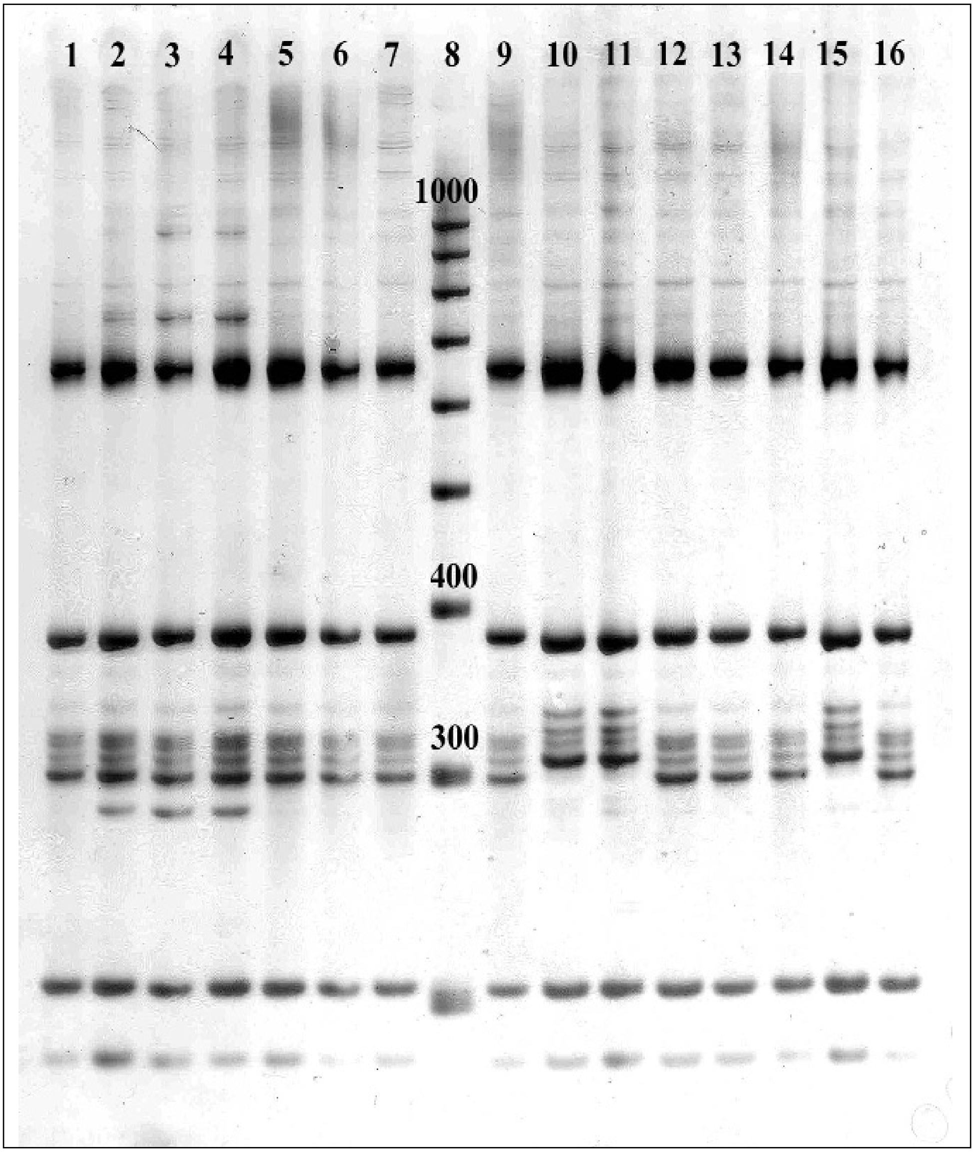

Fig. 4. Digestion pattern: *Q. pyrenaica* (1, 2, 3, 4, 5, 6), *Quercus* sp. (7), weight marker of 1000 bp (8), *Q. lusitanica* no arborescent (9, 10, 11, 12, 13) and *Q. lusitanica* arborescent (14, 15, 16).

formity of haplotypes in the central than in the southern Iberian Peninsula. One explanation may be that the Guadalquivir valley is probably a biogeographical border that separates genetic patterns between the central Iberian Peninsula and the Betic mountains of southern Spain.

Table 1. Morphological characteristics of the studied species of *Quercus*.

	Q. faginea	*Q. broteroi*	*Q. lusitanica*	*Q. pyrenaica*	*Q. canariensis*	*Q. ×marianica*
Leaves	Dentate - sawed	Crenated -dentate	Dentate – lobed, entire base	Pinnatifid	Sinuate -crenate	Mucronate - spinescent
Foliar size	3-9 × 1.5-5 cm	5-15 × 2.5-9 cm	2.5-12 × 1.2-5 cm	5-18 × 2.2-10 cm	5-20 × 2.5 -11 cm	5-18 × 2.5-10 cm
Foliar indument	Stellate 250 - 350 µm	Stellate 400 - 450 µm	Short stellate hairs	Large stellate hairs	Floccose, eventually glabrescent >420 µm	Stellate >420 µm

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