High variability of chloroplast DNA in three Mediterranean evergreen oaks indicates complex evolutionary history

P Jiménez¹, U López de Heredia¹, C Collada², Z Lorenzo¹ and L Gil¹

¹Unidad de Anatomía, Fisiología y Genética, ETSI Montes, Universidad Politécnica de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain; ²Departamento de Biotecnología, ETSI Montes, Universidad Politécnica de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain

Chloroplast DNA variation was studied in three evergreen *Quercus* species (*Q. suber* L., *Q. ilex* L. and *Q. coccifera* L.) from the Western Mediterranean Basin using PCR-RFLP. We studied five primer pair/enzyme combinations, four of them previously used in other European *Quercus*, obtaining a large number of haplotypes (81) grouped in three main types (*suber* type, *ilex-coccifera I* type and *ilex-coccifera II* type). Such level of haplotype diversity is higher than previously reported for the genus. Remarkable differences in haplotype richness between species have been found.

Q. ilex and *Q. coccifera* usually share the same haplotypes, while a number of *Q. suber* populations possesses variants of the *ilex-coccifera I* type. This fact is interpreted as a result of genetic introgression between *Q. suber* and *Q. ilex*. Reproductive factors that could determine this exchange are discussed, as well as the influence of different species histories on the present structure of evergreen *Quercus* in the Western Mediterranean Basin.

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Introduction

Mediterranean evergreen *Quercus* species are a group with overlapping habitats, which often leads to their consideration as an homogeneous entity in botanical, biogeographical or paleohistorical studies. In the Western Mediterranean Basin, *Q. ilex* (holm oak), *Q. suber* (cork oak), and *Q. coccifera* (kermes oak) are the dominant broadleaved species. The three species are sympatric in many areas, but some differences in their ecological requirements produce distinct responses to environmental conditions and hence different evolutionary histories. Previous studies have shown differences in their genetic variation patterns at both nuclear and cytoplasmic levels (Michaud *et al*, 1995; Toumi and Lumaret, 1998, 2001; Manos *et al*, 1999; Belahbib *et al*, 2001; Lumaret *et al*, 2002).

Most studies on genetic variation of forest trees have been carried out using nuclear markers. The chloroplast genome typically shows low intraspecific variation, although in the last decade, the development of new markers has allowed the detection of enough variation to assess phylogeographic patterns. Many such studies in *Quercus* species have used the PCR-RFLP technique (Whittemore and Schaal, 1991; Ferris *et al*, 1993; Petit *et al*, 2002; Olalde *et al*, 2002), producing a large amount of work in different species with a similar methodology,

which allows comparison between studies. Although cpDNA diversity has been studied in *Q. ilex* and *Q. suber* (Belahbib *et al*, 2001; Lumaret *et al*, 2002), no work has been published to date on *Q. coccifera*.

The present work investigates the cytoplasmic variation of the sclerophyllous *Quercus* species in the Western Mediterranean Basin and the relationships among species. This report focuses on the description and comparison of PCR-RFLP patterns for *Q. suber*, *Q. ilex* and *Q. coccifera*, discussing possible causes for the differences found.

Material and methods

A total of 2313 individuals of *Q. ilex* (1118, 121 populations), *Q. suber* (859, 90 populations) and *Q. coccifera* (401, 46 populations) covering the Western Mediterranean range was sampled (Table 1). The sampling was more intense in the Iberian Peninsula than in other regions. In each stand, adult leaves were collected from 5 to 10 nonadjacent trees per species (mean = 9.2). The DNA was extracted using a modified protocol of Doyle and Doyle (1990) following Dumolin *et al* (1995).

We used five pairs of primers to amplify the following chloroplast fragments: trnC[tRNA-Cys(GCA)]-trnD[tRNA-Asp(GUC)] (CD), trnT[tRNA-Thr(GUG)]-trnF[tRNA-Phe(UGU)] (TF), trnD[tRNA-Asp(GUC)]-trnT[tRNA-Thr(GGU)] (DT), psaA[PSI (P700 apoproteine A1)]-trnS[tRNA-Ser(GGA)] (AS) and trnS[tRNA-Ser(GCU)]-trnR[tRNA-Arg(UCU)] (SR) (Taberlet *et al*, 1991; Demesure *et al*, 1995; Dumolin-Lapègue *et al*, 1997a; Grivet *et al*, 2001). Each amplified fragment was digested

Correspondence: L. Gil, Unidad de Anatomía, Fisiología y Genética, ETSI Montes, Universidad Politécnica de Madrid s/n, 28040 Madrid, Spain.

E-mail: lgil@montes.upm.es

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with one restriction enzyme to avoid the risk of counting the same mutation twice (Dumolin-Lapègue *et al*, 1997b). Table 2 shows PCR conditions and the enzymes used. Digestion products were separated by electrophoresis in 8% polyacrylamide gels (18 × 24 cm) using Tris-borate—EDTA buffer 1 × at 500 V for 240 min. Fragments were revealed with a silver staining method in order to check polymorphisms. Fragment sizes were estimated using a 1 kb ladder (Gibco).

Data were scored as multistate characters as in Dumolin-Lapègue *et al* (1997b) and Petit *et al* (1997): each polymorphic restriction fragment is considered a character and the states are the different sizes of this fragment. The length variants were noted from 1 to 6, 9 and 0 being reserved for restriction site mutations (9 means the appearance of a new restriction point and, consequently, two new bands instead of the expected one; and 0 the disappearance of a restriction point implying a new band with size equal to the sum of two missing bands). The numbers increase from the highest to the lowest molecular weight fragments to ease the notation, but this does not imply any mutational sequence.

Each individual's haplotype was a combination of the variants present in each restriction fragment for the five cpDNA amplified regions. A matrix of mutational differences between haplotypes was calculated to produce a minimum length spanning network of haplotypes using the R Ape 1.0 package (Paradis *et al*, 2003). This method is used as an alternative to Wagner parsimony analysis to connect the haplotypes by direct links having the smallest possible length (Prim, 1957), but conveys better the relationships between haplotypes (Excoffier and Smouse, 1994). Nodes of the network represent the haplotypes, while length of the branches is proportional to the number of mutations between them (Prim, 1957).

Table 1 Number of populations sampled in each country and species

	<i>Q. ilex</i>	<i>Q. suber</i>	<i>Q. coccifera</i>
Spain	96	61	35
Portugal	2	10	3
France			
Continental	10	2	8
Corsica	5	1	—
Italy			
Peninsula	1	2	—
Sardinia	—	2	—
Sicily	3	3	—
Malta	4	—	—
Tunisia	—	2	—
Algeria	—	1	—
Morocco	—	6	—
Total	121	90	46

Table 2 PCR and digestion conditions

Fragment/enzyme	PCR conditions	Digestion
DT/ <i>TaqI</i>	94°C 45 s/58°C 4 min/72°C 2 min] × 30 cycles	65°C 3–4 h
CD/ <i>TaqI</i>	94°C 45 s/54.5°C 45 s/72°C 2 min] × 30 cycles	65°C 3–4 h
AS/ <i>HinfI</i>	94°C 45 s/57°C 4 min/72°C 2 min] × 37 cycles	37°C O/N
SR/ <i>HinfI</i>	94°C 45 s/56°C 2 min/72°C 2 min] × 30 cycles	37°C O/N
TF/ <i>HinfI</i>	94°C 45 s/56°C 2 min/72°C 2 min] × 30 cycles	37°C O/N

Results

The polymorphic fragments and the size of the variants are indicated in Table 3. A total of 23 polymorphic regions with consistent and repeatable variation have been scored. The number of variants for each polymorphic fragment ranged between 2 and 6, most of them being length variants derived from insertion–deletions, except for five mutations involving recognition points. The most variable fragments for the three species are SR and AS, two regions in which microsatellite regions have been described in white oaks (Grivet *et al*, 2001). Another variable fragment, DT, also bears at least five (A)*n* repeats in both white (Deguilloux *et al*, 2003) and evergreen oaks (work in progress).

Combination of variants in the 23 polymorphic fragments has led to the definition of 81 different haplotypes (description of haplotypes is shown in Appendix A1). The minimum length spanning network obtained for the pool of samples (Figure 1) reflects the relationships between them. As expected in such complex data, some homoplasy may occur. In fact, connections to central haplotypes through missing ones present some uncertainty (ie haplotypes 7, 67, 72). Despite the possible homoplasy, the existence of two peripheral

Table 3 Polymorphic fragments and their variants

Polymorphic fragments	Size of the variants (bp)
DT- <i>TaqI</i>	753(1), 722(2), 699(3) , 669(4), 558+109(9) ^a
DT- <i>TaqI</i> 3	361(1), 358(2), ^a 349(3) , 335(4), 331(5)
DT- <i>TaqI</i> 4	225(1), 223(2) , 217(3)
CD- <i>TaqI</i>	1743(1), 1307(2) , 942(3), 891(4) ^a
CD- <i>TaqI</i> 2	833(1), 780(2)
CD- <i>TaqI</i> 3	523(1) , 512(2), 506(3) ^b
CD- <i>TaqI</i> 6	277(1) , 269(2), 202+66(9), absent in <i>Q. suber</i>
CD- <i>TaqI</i> 10	70(1), 57(2) , 43(3) ^b
AS- <i>HinfI</i> 1	852(1), 806(2) , 771(3), 767(4) ^b
AS- <i>HinfI</i> 2	611(1), 578(2)
AS- <i>HinfI</i> 3	577(1) , 512(2), 597–75(0)
AS- <i>HinfI</i> 4	473(1), 457(2), 412(3)
AS- <i>HinfI</i> 6	299(1), 291(2), 268(3), 261(4) , 254(5), 240(6)
AS- <i>HinfI</i> 8	183(1), 114(2)
SR- <i>HinfI</i> 1	578(1), 528(2)
SR- <i>HinfI</i> 2	335(1), 326(2) , ^b 324(3), ^b 317(4), 421–132(0)
SR- <i>HinfI</i> 3	316(1), 307(2), 296(3)
SR- <i>HinfI</i> 4	296(1), 293(2), 288(3) , 286(4), 185+115(9) ^b
SR- <i>HinfI</i> 5	268(1) , 263(2), 241(3), ^b 228(4), ^b 520–202(0)
TF- <i>HinfI</i> 1	487(1), ^b 479(2), ^a 468(3)
TF- <i>HinfI</i> 3	278(1) , 270(2), 266(3) ^b
TF- <i>HinfI</i> 4	268(1), 261(2) , 254(3), 249(4), ^b 243(5) ^a
TF- <i>HinfI</i> 5	263(1), 163+74(9)

Number in parentheses indicates the code of each one. The most frequent variant for each fragment is in bold.

^aVariants restricted to *ilex-coccifera* II type.

^bVariants restricted to *suber* type.

Figure 1 Minimum spanning network of the 81 haplotypes present in the three species. Black dots represent missing or unsampled haplotypes.

groups of haplotypes with a large distance (9 and 12 mutations, respectively) from the main cluster and without intermediate types is clear. As the geographic survey is very extensive, with no important gaps, we consider that these three groups represent divergent lineages: *suber* type (for H1–H4, occurring exclusively in *Q. suber* individuals), *ilex-coccifera I* (H5–H74) and *ilex-coccifera II* (H75–H81) types. Separation between species is not complete, because about 40% of *Q. suber* populations show the *ilex-coccifera I* type, and *Q. coccifera* trees show the *suber* type and no *Q. suber* show the *ilex-coccifera II* type (haplotype frequencies for each species is shown in Appendix A1).

Differences between the *suber* and *ilex-coccifera I* and *II* types are due to the number of polymorphic restriction fragments and to the amount and length of variants in each. Five polymorphic fragments were identified for the *suber* type, each showing two length variants. Alternatively, for the *ilex-coccifera I* type there were 22 variable fragments, and six for the *ilex-coccifera II* type. As a consequence, the number of haplotypes generated for the *ilex-coccifera I* type (70) is significantly higher than the one for the *suber* type (4) or for the *ilex-coccifera II* type (7).

Discussion

The present study documents cpDNA variation in five widely used chloroplast fragments in the three main evergreen *Quercus* species of the Western Mediterranean range. Four of these fragments have been analysed using the same technique in other European *Quercus* species (Petit *et al*, 2002), so comparison between the results can be performed. Restriction patterns are quite different in deciduous and in evergreen *Quercus* (Petit *et al*, 2002) and no common haplotypes for both groups have been detected. When comparing the interspecific cpDNA variation of both groups, an extensive sharing of cpDNA haplotypes was noted between deciduous *Quercus* species (Petit *et al*, 2002).

Our haplotype network for evergreen *Quercus* (Figure 1) indicates the existence of three groups, one of which can be considered as specific of *Q. suber*. Within this lineage, haplotypes 2–4 are very close, while haplotype 1 has diverged by two mutations from them. This one is the most frequent haplotype found in *Q. suber*, with an Iberian-Moroccan distribution (the most intensively sampled area), and it is separated from the Middle Mediterranean range, where haplotypes 2–4 occur (data in preparation). The network also shows the mean pairwise distance between *suber* and the two *ilex-coccifera* lineages. This result confirms the work of Manos *et al* (1999) on the phylogeny of the genus with nuclear and chloroplast markers, and of Belahbib *et al* (2001) on the phylogeography of *Q. ilex* and *Q. suber* in Morocco. Both report the separation between the two taxa. However, our data point out that these lineages do not completely correspond with a separation between species, as some of the *Q. suber* samples occur in *ilex-coccifera* types. Asymmetry is evident, since no *Q. ilex* shows a *suber* haplotype, in contrast to the extensive sharing found in deciduous oaks. Haplotype sharing between *Q. suber* and *Q. ilex* has also been reported for Moroccan sympatric populations (Belahbib *et al*, 2001) and it is interpreted by the authors as interspecific genetic exchanges. In the present work, the absence in *Q. suber* of haplotypes from the divergent *ilex-coccifera II* type is noteworthy, even though some mixed populations were sampled in the regions where this lineage appears. Further research should clarify this point, as well as the existence of some limits to interspecific exchange.

Hybridisation between *Q. ilex* and *Q. suber* occurs in nature, although it is not a frequent event. Capture of unexpected chloroplast haplotypes by hybridisation and introgression has been proposed as the most likely explanation for the sharing of cytoplasmic genes both in deciduous and evergreen oaks (Belahbib *et al*, 2001; Petit *et al*, 2002) as well as in other species (Smith and Sytsma, 1990; Palmé and Vendramin, 2002). Asymmetric hybridisation has been confirmed by Boavida *et al* (2001), who described postpollination barriers in *Q. suber* to interspecific crosses with *Q. ilex*, *Q. coccifera*, *Q. faginea* and *Q. robur*. These authors also report higher success rate in the interspecific crosses for *Q. suber* acting as pollen donor rather than as female parent due to differential growth of the pollen tubes of both species. Phenological differences also favour this direction of hybridisation (Varela and Valdivieso, 1995; Elena-Rosselló and de la Cruz, 1998). Since both species are protandrous and *Q. ilex* flowers earlier, early cork oak male flowers can pollinate late female holm oaks. This interpretation is supported by the discovery of *ilex-coccifera I* haplotypes in *Q. suber* individuals, and the absence of the opposite situation.

No such data are available for *Q. coccifera*; so, in spite of the presence of some common haplotypes, no mechanism for introgression with *Q. suber* can be confirmed. On the other hand, almost complete sharing of haplotypes between *Q. ilex* and *Q. coccifera* has been observed, which could be due to an incomplete lineage sorting or hybridisation, which may occur in the same way as in deciduous *Quercus* (Petit *et al*, 2002) since no particular *Q. coccifera* lineage has been found.

The presence of possible homoplasmy would mainly have an effect in the *ilex-coccifera I* lineage where the high

number of haplotypes confuses the relationships between them, and causes difficulty in the recognition of subgroups. However, it does not affect the divergence between *ilex-coccifera* I and II lineages. This divergence is also clear in the geographic distribution of each lineage: lineage I is found in the Iberian Peninsula, southern France and Morocco, while lineage II is restricted to the Central Mediterranean Basin and some points of south-eastern Spain and Balearic Islands (data in preparation). Lineage II would correspond to the cytotypes 3–10 described by Lumaret et al (2002) for *Q. ilex* using classic RFLPs.

The strong haplotypic richness obtained for *ilex-coccifera* I type is remarkable, since it is much higher than levels previously reported for other species of the genus (Petit et al, 2002; Olalde et al, 2002). Considering the low mutation rate reported for the chloroplast genome (Wolfe et al, 1987; Provan et al, 1999), the high diversity found, particularly in Iberian *Q. ilex* (*ilex-coccifera* I), is most likely explained by the ancient presence of the species, which would have allowed the accumulation of this high number of mutations. Furthermore, glaciations might not have involved the extinction of most haplotypes, as the persistence of numerous populations is possible. A similar situation is found in Asian stone oaks (*Lithocarpus*) (Cannon and Manos, 2003), which show a high level of chloroplast sequence variation, explained by the authors as a result of the continuous presence of populations in southeast Asia. This could be the case for *Q. ilex* and, to a lesser extent, *Q. coccifera*, which is able to inhabit very different ecosystems due to their great plasticity (Barbero et al, 1992). In addition, their ability to resprout after a major perturbation could favour the persistence of the species when climatic conditions are worse, and therefore the maintenance of strong cpDNA variation. Conversely, the thermophilous character and strict soil requirements of *Q. suber* (it can only live in acid or decarbonated soils) make it less resilient to strong environmental changes and probably caused a great range reduction during glacial ages.

In conclusion, these new data, along with previous reports, indicate that evergreen Mediterranean oaks present remarkable levels of cpDNA variation, sometimes due to interspecific hybridisation. Our results suggest that the action of several factors (interspecific exchanges, population size fluctuations, migration) have caused complex evolutionary histories. Differences in cytoplasmic variability are also found between *Q. suber* and *Q. ilex*–*Q. coccifera*, which are interpreted as a consequence of their different responses to environmental changes. Future work should focus on discerning the geographic structure of this variation to study the history of the species in depth. Chloroplast and nuclear data should also be compared in order to assess the possible reflection of cytoplasmic introgression in the nuclear genome, as well as the correspondence between both kinds of marker.

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Appendix A1

A description of haplotypes is presented in Table A1.

Table A1 Haplotypes detected in the survey

Hapl	DT-TaqI			CD-TaqI					AS-HinfI						SR-HinfI					TF-HinfI					Frequency		
	1	3	4	1	2	3	6	10	1	2	3	4	6	8	1	2	3	4	5	1	3	4	5	Qs	Qi	Qc	
1	4	1	2	4	2	3	0	3	3	2	1	1	2	2	2	2	3	9	3	1	3	4	9	58.91	0	0	
2	4	1	2	3	2	3	0	3	4	2	1	1	2	2	2	2	3	9	4	1	3	4	9	0.81	0	0	
3	4	1	2	3	2	3	0	3	3	2	1	1	2	2	2	3	3	9	4	1	3	4	9	5.70	0	0	
4	4	1	2	3	2	3	0	3	3	2	1	1	2	2	2	3	3	9	4	1	3	4	9	6.64	0	0	
5	3	1	2	3	2	1	1	2	2	2	1	3	6	2	2	2	3	2	4	3	1	2	9	0	0	2.49	
6	3	1	2	3	2	1	1	2	2	2	1	3	5	2	2	2	3	2	1	3	1	2	9	0	2.24	0	
7	4	3	2	3	2	1	1	2	2	2	1	3	5	2	2	2	3	2	0	3	1	2	9	0.12	0	0	
8	3	1	2	2	2	1	1	2	2	2	1	3	5	2	2	2	3	2	1	3	1	2	9	0	0.63	0	
9	2	1	2	2	2	1	1	2	2	2	1	3	5	2	2	2	3	2	0	3	1	2	9	0	0.89	0	
10	3	4	2	2	2	1	1	2	2	2	1	3	5	2	2	1	3	2	1	3	1	2	9	0	0.63	0	
11	3	4	2	2	2	1	1	2	2	2	1	3	5	2	2	2	3	2	4	3	1	2	9	0	0	1.25	
12	4	1	2	2	2	1	1	2	2	2	1	3	4	2	2	2	3	2	1	3	1	2	9	0	0.09	0	
13	4	1	2	2	2	1	1	2	2	2	1	3	4	2	2	2	3	1	1	3	1	2	9	0	2.95	0.75	
14	4	1	2	2	2	1	1	2	2	2	1	3	4	2	2	2	3	3	1	3	1	2	9	0	0.45	0	
15	3	5	2	2	2	1	1	2	2	2	1	3	5	1	2	2	3	2	1	3	1	2	9	0.81	0.54	0	
16	3	5	2	2	2	1	1	2	2	2	1	3	5	1	2	2	3	1	1	3	1	2	9	0	0.09	0	
17	3	5	2	2	2	1	1	2	2	1	2	3	4	1	2	2	3	2	1	3	1	2	9	0	2.15	0	
18	3	1	2	2	2	1	1	2	2	1	2	3	4	1	2	2	3	2	1	3	1	2	9	0	0.27	0	
19	3	5	2	2	2	1	1	1	2	2	1	2	3	4	1	2	2	3	2	2	3	1	2	9	0	0.09	0
20	3	5	2	2	2	1	1	1	2	1	2	3	4	1	2	2	3	2	2	3	1	1	9	0	4.92	2.00	
21	3	3	2	2	2	1	1	2	2	2	1	3	5	2	2	2	3	2	1	3	1	2	9	0	0.89	0	
22	3	3	2	2	2	1	1	2	2	2	1	3	6	2	2	2	3	2	1	3	1	2	9	0	0.45	0	
23	3	3	2	2	2	1	1	2	2	2	1	3	5	2	2	2	2	2	1	3	1	2	9	0.35	8.05	6.73	
24	3	3	3	2	2	1	1	2	2	2	1	3	1	2	2	2	2	2	1	3	1	2	9	0	0	0.50	
25	3	3	2	2	2	1	1	2	2	2	1	3	5	2	2	2	3	1	1	3	1	2	9	0.70	3.49	3.49	
26	3	3	2	2	2	1	9	2	2	2	1	3	5	2	2	2	3	1	1	3	1	2	9	0.35	0	1.25	
27	2	3	2	2	2	1	1	2	2	2	1	3	5	2	2	2	3	1	1	3	1	2	9	0	0	1.75	
28	2	3	2	2	2	1	9	2	2	2	1	3	5	2	2	2	3	1	1	3	1	2	9	1.16	0.27	1.25	
29	2	3	2	2	2	1	1	2	2	2	1	3	4	2	2	2	3	2	1	3	1	2	9	0	0.27	0	
30	2	3	2	2	2	1	1	2	2	2	1	3	4	2	2	4	3	2	1	3	1	2	9	2.10	0	0	
31	2	3	2	2	2	1	1	2	2	2	1	3	4	2	2	4	3	2	2	3	1	2	9	0.23	0	0	
32	2	1	2	2	2	1	1	2	2	2	1	3	4	2	2	4	3	2	4	3	1	2	9	0	0	2.49	
33	3	3	2	2	2	1	1	2	2	2	1	3	4	2	2	4	3	2	1	3	1	2	9	0	0.09	0	
34	3	3	1	2	2	1	1	2	2	2	1	3	4	2	2	4	3	2	1	3	1	2	9	0	0.36	8.48	
35	3	3	2	3	2	1	1	2	2	2	1	3	4	2	2	4	3	2	1	3	1	2	9	0.23	0.45	1.25	
36	3	3	2	2	2	1	1	2	2	2	2	3	4	2	2	4	3	2	1	3	1	2	9	1.63	0.36	0	
37	3	3	2	2	2	1	1	2	2	2	2	3	4	2	2	1	3	2	1	3	1	2	9	0	8.05	10.97	
38	3	3	2	2	2	1	1	2	2	2	2	3	4	2	1	1	3	2	1	3	1	2	9	0	0.27	0	
39	2	3	2	2	2	1	1	2	2	2	2	3	4	2	1	1	3	2	1	3	1	2	9	0	0.09	0	
40	2	3	2	2	2	1	1	2	2	2	2	3	3	2	1	1	3	2	1	3	1	2	9	0.12	6.35	3.24	

Table A1 Continued

Hapl	DT-TaqI			CD-TaqI					AS-HinfI						SR-HinfI					TF-HinfI				Frequency			
	1	3	4	1	2	3	6	10	1	2	3	4	6	8	1	2	3	4	5	1	3	4	5	Qs	Qi	Qc	
41	2	3	2	2	2	1	1	2	2	2	2	3	4	2	1	1	3	1	1	3	1	2	9	0	0.09	0	
42	3	3	2	2	2	1	1	2	2	2	2	2	4	2	2	1	3	2	1	3	1	2	9	1.05	0.72	0	
43	1	3	2	2	2	1	1	2	2	2	2	2	4	2	2	1	3	2	1	3	1	2	9	0	0.18	0	
44	3	3	2	2	2	1	1	2	2	2	2	2	4	2	2	1	3	2	4	3	1	2	9	0	0.09	0	
45	3	3	2	2	2	1	1	2	2	2	2	2	4	2	2	2	3	2	1	3	1	2	9	0	0	2.00	
46	2	3	2	2	2	1	1	2	2	2	2	2	4	2	2	1	3	2	1	3	1	2	9	0	0.89	0	
47	3	3	2	2	2	1	1	2	2	2	1	3	4	2	2	4	3	1	1	3	1	2	9	1.51	0	0	
48	3	3	2	2	2	1	1	2	2	2	2	3	4	2	2	4	3	1	1	3	1	2	9	0.47	0.98	0	
49	3	3	2	2	2	1	1	2	2	2	1	2	4	2	2	4	3	2	1	3	1	2	9	1.16	2.86	4.99	
50	3	3	2	2	2	1	2	2	2	2	2	2	4	2	2	4	3	2	1	3	1	2	9	0	0.18	0	
51	2	3	2	3	2	1	1	2	2	2	1	3	4	2	2	2	3	2	1	3	2	2	9	0.23	0	0	
52	3	3	2	3	2	1	1	2	2	2	1	3	4	2	2	2	3	2	1	3	2	2	9	0	4.11	2.74	
53	2	3	2	3	2	1	1	2	2	2	2	3	4	2	2	0	3	2	1	3	2	2	9	0	0.09	0	
54	2	3	2	3	2	1	1	2	2	2	2	3	4	2	2	4	3	2	1	3	2	2	9	0	0.89	0	
55	2	3	2	3	2	1	1	2	2	2	2	3	1	2	2	0	3	2	1	3	2	2	9	0	1.07	2.49	
56	2	3	2	2	2	1	2	2	2	2	1	3	4	2	2	2	3	2	1	3	1	1	9	0	0.09	0	
57	2	3	2	2	2	1	2	2	2	2	1	3	4	2	2	1	3	2	1	3	1	1	9	0.35	1.97	0	
58	3	5	2	2	2	1	2	2	2	2	1	3	4	2	2	2	3	2	1	3	1	1	9	0	0.18	0	
59	3	5	2	2	2	1	1	2	2	2	2	1	3	4	2	2	2	3	2	1	3	1	1	9	3.49	13.95	9.98
60	3	5	2	2	2	1	2	2	2	2	1	3	4	2	2	1	3	2	1	3	1	1	9	0.47	0.18	0	
61	3	5	2	2	2	2	1	2	2	2	1	3	4	2	2	1	3	2	1	3	1	1	9	0	0.18	0	
62	3	4	2	2	2	1	2	2	2	2	1	3	4	2	2	2	3	2	1	3	1	1	9	0	0.09	0	
63	3	4	2	2	2	1	2	2	2	2	2	3	4	2	2	2	3	2	1	3	1	1	9	0	0.09	0	
64	3	4	2	2	2	1	2	2	2	2	2	3	4	2	2	2	3	2	1	3	1	2	9	0	0.09	0	
65	3	5	2	2	2	1	2	2	2	2	2	3	4	2	2	2	3	2	1	3	1	1	9	0	0.09	0	
66	3	5	2	2	2	1	2	2	2	2	2	2	4	2	2	2	3	2	1	3	1	1	9	0.35	0	0	
67	2	3	2	2	2	1	1	2	3	2	2	3	4	2	2	2	3	2	4	3	1	2	9	0.08	0.09	2.24	
68	2	3	2	2	2	1	1	2	1	2	2	3	4	2	2	1	3	2	4	3	1	2	9	0	1.16	0	
69	2	3	2	2	2	1	2	2	1	2	2	3	4	2	2	1	3	2	1	3	1	2	9	0	0.63	2.24	
70	2	3	2	2	2	2	1	2	1	2	2	3	4	2	2	1	3	2	1	3	1	2	9	0	0.18	0	
71	2	3	2	2	2	2	1	2	1	2	2	3	4	2	2	2	3	2	1	3	1	1	9	0	8.86	5.49	
72	2	3	2	2	1	1	1	2	2	2	0	3	4	2	2	2	3	2	4	3	1	2	1	0	0.36	0	
73	2	3	2	2	1	1	1	2	2	2	0	3	4	2	2	2	3	2	4	3	1	1	1	0.51	0.45	0	
74	3	3	2	2	1	1	1	2	2	2	0	3	4	2	2	2	3	2	4	3	1	2	1	0	0.09	0	
75	9	2	2	1	2	1	2	2	2	2	1	3	3	2	2	4	3	1	4	2	2	3	9	0.47	0.89	0	
76	9	2	2	1	2	1	2	2	2	2	2	3	3	2	2	4	3	1	4	2	2	3	9	0	0.27	9.48	
77	9	2	2	1	2	1	2	2	2	2	2	3	3	2	2	4	3	1	4	2	2	5	9	0	0	2.49	
78	9	2	2	1	2	1	2	2	2	2	1	3	3	2	2	4	3	1	4	2	2	5	9	0	3.67	0	
79	9	2	2	1	2	1	2	2	2	2	1	3	3	2	2	4	1	1	4	2	2	3	9	0	2.50	4.49	
80	9	2	2	1	2	1	2	2	2	2	1	3	3	2	2	4	1	4	4	2	2	3	9	0	5.10	0	
81	9	2	2	1	2	1	2	2	2	2	2	3	3	2	2	3	1	1	4	2	2	3	9	0	1.25	3.24	

For each haplotype, length variants (see Table 2) observed in each polymorphic fragment (DT1, DT3, etc) are listed. At the end of each line, haplotype frequency (%) is indicated (Qs: *Q. suber*; Qi: *Q. ilex*; Qc: *Q. coccifera*).