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A genetic linkage map of *Quercus robur* L. (pedunculate oak) based on RAPD, SCAR, microsatellite, minisatellite, isozyme and 5S rDNA markers

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Abstract A genetic map of Pedunculate oak (*Quercus robur*) was constructed based on one 5S rDNA, 271 RAPD, ten SCAR, 18 microsatellite, one minisatellite, and six isozyme markers. A total of 94 individuals from a full-sib family was genotyped. Two maps, including 307 markers, were constructed according to the "two-way pseudo-testcross" mapping strategy. Testcross markers segregating in the 1 : 1 ratio were first used to establish separate maternal (893.2 cM, 12 linkage groups) and paternal (921.7 cM, 12 linkage groups) maps. Both maps provided 85–90% genome coverage. Homologies between the male and female linkage groups were then identified based on 74 intercross markers segregating in the 3 : 1, 1 : 2 : 1 and 1 : 1 : 1 : 1 ratios (RAPDs, SCARs, SSRs, 5S rDNA and isozymes) in the hybrid progeny. In each map, approximately 18% of the studied markers showed segregation distortion. More than 60% of the skewed markers were due to an excess of heterozygote genotypes. This map will be used for: (1) studying the molecular organisation of genomic regions involved in inter- and intraspecific differentiation in oaks and (2) identification of QTLs for adaptive traits.

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Introduction

Depending on the authors the number of existing oak species varies between 300 and 600. They are spread over the northern hemisphere (Europe, Africa, North America and Asia) from boreal zones to semi-arid areas. Pedunculate oak (*Quercus robur* L.) is one of the most important and widespread oak species in Europe. It belongs to the white oak section (*Lepidobalanus*) and is distributed throughout Europe from central Spain to the Urals. According to Camus (1954) the *Lepidobalanus* section is the richest with respect to numbers and diversity and comprises more than 150 species spread over all four continents.

Quercus robur L. has a broad ecological range, from very acid to chalky and from moist to mesophilic soils, but it prefers to grow on neutral and moist soils (Becker and Levy 1990). Genetic studies in pedunculate oak have mostly concentrated on population genetics, provenance research and the inheritance of economically important traits (Kremer et al. 1993, for a review).

Cytogenetic investigations have been restricted to karyotypic analysis. *Quercus robur* L. is a diploid species ($2n = 2x = 24$), although in some cases supernumerary chromosomes (B type) have been reported (Ohri and Ahuja 1990; Besendorfer et al. 1996). Natural triploid individuals have been described (Johnsson 1946; Butorina 1993) that usually are larger than diploid trees. Compared to data available on other woody angiosperms (e.g. *Populus*, *Eucalyptus*, *Acacia*, *Pyrus*, *Fraxinus*) the physical genome size of *Q. robur* is larger (1.88 pg/2C) (Zoldoš et al. 1998), although its size is among the lower values found in plants (Favre

and Arnould 1996; Favre and Brown 1996). Segregation studies with gene markers have been conducted in *Quercus robur* with isozymes (Zanetto et al. 1996), SCARs (sequence characterised amplified regions, Bodénès et al. 1996) and microsatellites (Steinkellner et al. 1997a). However, due to the small number of loci analysed in each of these studies, no linkage groups could be constructed except with isozymes when two linkage groups were identified (Zanetto et al. 1996).

Our objective was to construct a genetic map with all earlier developed markers (isozymes, SCARs, microsatellites, minisatellites, 5S rDNA). In order to locate these markers in the genome, we added randomly distributed markers using amplification with arbitrarily designed primers [random amplified polymorphic DNA (RAPD) Williams et al. 1990]. The pedigree used for the map construction is a full-sib F_1 progeny of *Quercus robur* L. Linkage groups were assembled according to the "two-way pseudo-testcross" method described by Grattapaglia and Sederoff (1994).

To our knowledge, this is the first reported linkage map in the genus *Quercus*, and in the *Fagaceae* family as well. Some of the markers used in the construction of the map have been reported to be applicable to other oak species: isozymes in *Q. petraea* and *Q pubescens* (Müller-Starck et al. 1996), microsatellites in other sections of the genus *Quercus* (Steinkellner et al. 1997b). Because oaks of a given botanical section usually exhibit high genetic similarities (Kremer and Petit 1993), we expect that the present map will provide a useful tool for various applications in population and quantitative genetics in different white oak species.

Materials and methods

Plant material

Segregation data from a two-generation full-sib pedigree including 94 individuals were used to construct two genetic maps, one for each parental tree. The male parent originated from near Arcachon (southwest of France), and the female parent was located on the Forestry Research Station in Pierroton (southwest of France). The controlled cross was made in the spring of 1992 according to the techniques described in Zanetto et al. (1996). About 400 acorns were collected in pollination bags, of which 94 were used for map construction. Preliminary segregation analysis of the 94 offspring with a few isozymic loci did not detect any acorn resulting from fertilisation with foreign pollen (Zanetto et al. 1996).

Marker analysis

Genomic DNA was extracted from leaves according to Saghai-Maroof et al. (1984) with some minor modifications described by Moreau et al. (1994).

Procedures used to study different markers are summarized in Table 1. Specific primer sequences and annealing temperatures for the SCAR, microsatellite, minisatellite and 5S rDNA markers are indicated in Table 2.

Data analysis

After segregation was scored for each marker, Mendelian segregation was tested in the offspring using Chi-square goodness-of-fit tests. Linkage analysis of the markers was performed using a combination of MAPMAKER (Lander et al. 1987) and JOINMAP (Stam 1993) software (see Results section). The Kosambi (Kosambi 1944) mapping function was used to convert recombination frequencies to map distances in centiMorgans.

Genome length, E(G), of both parental maps was estimated under the assumption of random marker distribution according to method "3" of Chakravarti et al. (1991): $G = 2MX/K$, where $M = n(n-1)/2$ is the number of informative meioses (n is the number of loci analysed), X is the maximum observed map distance among the locus pairs above a threshold LOD, Z, and K is the number of locus pairs having LOD values at or above Z. The values tested for Z were 3, 4 and 5. The values of X and K were obtained using the "LODs" function of MAPMAKER with the maximum recombination fraction set to 0.5 and minimum LOD threshold set to Z. Values for X were obtained using the Kosambi map function. These estimations were calculated by taking into consideration only pairwise comparisons between framework markers.

Results

Segregation analysis

Markers were subdivided in four different groups on the basis of the segregation patterns in the progeny:

A) Loci that were in the testcross configuration (Grattapaglia and Sederoff 1994) between the parents (heterozygous in one parent and homozygous null in the other, or vice-versa). A 1 : 1 segregation ratio was observed in the F_1 family for most RAPDs, seven SCARs (six dominant and one codominant), two isozymes, 1 microsatellite (ssrQpZAG104) and one minisatellite (Omin3).
B) Loci that were heterozygous in both parents and that segregated in a 3 : 1 ratio in the offsprings. Fifty RAPDs followed this pattern.
C) Loci that were heterozygous in both parents and that segregated in a 1 : 2 : 1 ratio. This was the case for 2 microsatellites and three isozymes.
D) Loci that were heterozygous in both parents and that segregated in a 1 : 1 : 1 : 1 ratio. Three SCARs, 15 microsatellites, one isozyme and one 5S rDNA locus followed this pattern. Markers segregating in the 1 : 1 : 1 : 1 ratio were partitioned according to the alleles contributed by the relevant parent (Jacobs et al. 1995), resulting in a marker segregating 1 : 1 in both the female and the male parent, as for category A markers.

Approximately 80% of the markers analysed in the progeny fit the expected Mendelian segregation ratios (1 : 1, 3 : 1, 1 : 2 : 1 or 1 : 1 : 1 : 1). A total of 61 markers showed segregation distortion at $\alpha = 0.05$.

Table 1 Protocols used for the molecular analysis of the different markers

Marker type	Number of markers	PCR protocol				Electrophoresis	Staining conditions	References
		Primer type	Amplification conditions					
RAPD	271	Ten-base primers Operon Technologies kits A-Z	1 step	94°C	4 min	1.8% agarose gel	Ethidium bromide	Williams et al. 1990
			35 cycles	92°C	45 s			
				40°C	45 s			
				72°C	1 min 45 s			
SCAR	10	*	1 step	94°C	3 min	1% agarose gel or 8% non-denaturating polyacrylamide gel	Ethidium bromide Silver staining	Bodénès et al. 1997
			30 cycles	94°C	45 s			
				T°C	45 s			
				72°C	1 min 30 s			
Microsatellite	18	*	1 step	95°C	6 min	6% denaturating polyacrylamide gel	Fluorescence (ALF express)	Steinkellner et al. 1997b
			30 cycles	T°C	1 min			
				72°C	30 s		Silver staining	Streiff et al. 1998
				92°C	1 min			
			1 step	72°C	8 min			
Minisatellite	1	*	1 step	94°C	4 min	2% agarose gel	Ethidium bromide	Fluch and Burg unpublished data
			30 cycles	94°C	45 s			
				50°C	45 s			
				72°C	30 s			
5S rDNA	1	*	1 step	96°C	3 min	8% non-denaturing polyacrylamide gel	Silver staining	Specht et al. 1997
			30 cycles	94°C	40 s			
				55°C	1 min			
				72°C	40 s			
Isozymes	6	–		–		11%–12% starch gel or IEF	[b]	Zanetto et al. 1996

[a] See Table 2 for specific primer sequences and annealing temperatures
[b] See Zanetto et al. (1996) for staining conditions of various enzyme systems

Table 2 Primer pairs and annealing temperatures of SCAR, microsatellite, minisatellite and 3Sr DNA markers

Locus name	Primer sequence	Annealing temperature (°C)	References
A17-700	F: GAC CGC TTG TTG GTA B: GAC CGC TTG TGA TTA	55	Bodénès et al. 1997
B11-1500	F: AGA CCC GTA GAG GAG ACA TT B: CCC GTG TAG TAT TCC ACA AA	52	Bodénès et al. 1997
B12-500	F: GTC TTC GAC TGG GGT GAA B: TCA GTC CAT TCC GAA AGA	52	Bodénès et al. 1997
B12-800	F: CCT TGA ACG CAT TAT GAC AT B: CCT TGA ACG CAG CAC AAT TC	48	Bodénès et al. 1997
F14-750	F: CAG AAG AAG CAA TGG TAA CA B: CAA CAT TTG GTG TGT CTT AG	50	Bodénès et al. 1997
I13-300	F: GTG TGG TGC AGA AAA B: ACT CCA GGT CTA TCC	52	Bodénès et al. 1997
P14-450	F: TGC AAA ACA TAA ACA TT B: AAT CCA GTG GCA AGT TTT AA	48	Bodénès et al. 1997
P17-1400	F: CGC ATG GAT ATA CTA TTC B: CTA CCT AGT GTC CTA TGG	54	Bodénès et al. 1997
R12-500	F: CGA GCG TTG ATA GCC AAT AG B: TCG GAA GCA AAA GGG TAA TT	51	Bodénès et al. 1997
U7-790	F: GCC CTA ACA AAT CAT CTC B: TAA TAT AGA AAG GGA AAG	48	Steinkellner et al. 1997a
ssrQpZAG1/5	F: GCT TGA GAG TTG AGA TTT GT B: GCA ACA CCC TTT AAC TAC CA	55	Steinkellner et al. 1997a
ssrQpZAG16	F: CTT CAC TGG CTT TTC CTC CT B: TGA AGC CCT TGT CAA CAT GC	59	Steinkellner et al. 1997a
ssrQpZAG3/64	F: TAG AAA GCC CAA AAC CAA AAC C B: CTT TTT GGA AGC CGC TTC CGT A	50	Steinkellner et al. 1997a
ssrQpZAG3/62	F: CTT GAG CAT GGA ATC CTA TG B: TCT AGA GGA GCT TTC CTT TAC AC	58	Steinkellner et al. 1997a
ssrQpZAG9	F: GCA ATT ACA GGC TAG GCT GG B: GTC TGG ACC TAG CCC TCA TG	50	Steinkellner et al. 1997a
ssrQpZAG15	F: CGA TTT GAT AAT GAC ACT ATG G B: CAT CGA CTC ATT GTT AAG CAC	50	Steinkellner et al. 1997a
ssrQpZAG36	F: GAT CAA ATT TGG AAT ATT AAG AGA G B: ACT GTG GTG GTA GTC TAA CAT GTA G	50	Steinkellner et al. 1997a
ssrQpZAG46	F: CCC CTA TTG AAG TCC TAG CCG B: TCT CCC ATG TAA GTA GCT CTG	48	Steinkellner et al. 1997a
ssrQpZAG58	F: CTG CAA GAT TCG GAC AAG CAA B: TCT TTT TTC CTA ATC TCA CCT G	50	Steinkellner et al. 1997a
ssrQpZAG102	F: CAA CAA TGA AAG GGA GAA AGC B: CGC TTT TGA CTT TCC TAC CTT	50	Steinkellner et al. 1997a
ssrQpZAG104	F: ATA GGG AGT GAG GAC TGA ATG B: GAT GGT ACA GTC GCA ACA TTC	50	Steinkellner et al. 1997a
ssrQpZAG108	F: CTA GCC ACA ATT CAG GAA CAG B: CCT CTT TTG TGA ATG ACC AAG	50	Steinkellner et al. 1997a
ssrQpZAG110	F: GGA GGC TTC CTT CAA CCT ACT B: GAT CTC TTG TGT GCT GTA TTT	48	Steinkellner et al. 1997a
ssrQpZAG119	F: GAT CAG TGA TAG TGC CTC TC B: GAT CAA CAA GCC CAA GGC AC	50	Steinkellner et al. 1997a
MSQ4	F: TCT CCT CTC CCC ATA AAC AGG B: GTT CCT CTA TCC AAT CAG TAG TGA G	50	Dow et al. 1995

Table 2 Continued

Locus name	Primer sequence	Annealing temperature (°C)	References
MSQ13	F: TGG CTG CAC CTA TGG CTC TTA G B: ACA CTC AGA CCC ACC ATT TTT CC	50	Dow et al 1995
MSQ16	F: GGA ACA ACT AGA GAG AAC CAA GTC AGG B: TTG CCT ATC CTG CC CCG TAT CAC	50	Dow and Ashley 1996
MicJ-AG22	F: CGA TTT TGA ACA CCT TCT TC B: CGA TGT GGG ATT TTG ATT TT	46	T. Kawahara (personal communication)
Omin3	F: AGA ATC GAC TTC AAT GCA AG B: CCA TTC ACA GAG GTT GTC GG	50	Fluch et al. (personal communication)
5S rDNA	F: TGG GAA GTC CTC GTG TTG CA B: ATT AGT GCT GGT ATG ATC GC	55	Specht et al. 1997

Map construction

The map construction was done in two steps. First, two separate parental maps (paternal and maternal) were constructed using markers segregating in the 1 : 1 ratio (category A and D markers) with a confidence level of $P \leq 0.01$. These "testcross" markers were grouped with the help of MAPMAKER (Lander et al. 1987) using the following criteria:

- a minimum LOD score of 3.0 for statistical acceptance of linkage.
- a maximum recombination fraction θ of 0.40.

In the initial step of map construction, the order of non-distorted markers was approximated using the "FIRST ORDER" command. From this order, a subset of markers that could be locally ordered with a likelihood ratio greater than LOD = 2 was used to establish a framework map using the "RIPPLE" command. Markers that could not meet this ordering criteria were dropped from the framework and placed as accessory markers. Accessory markers were then located beside their closest framework marker. Distorted markers that were excluded from the framework map were also placed in the established framework map as accessory markers using the "NEAR" command. These markers are indicated with a boldface asterisk in Fig. 1.

In a second step, intercross markers segregating in both parents (category B, C) in addition to category D markers already mapped in the first step were used to identify homologous counterparts between parental maps. Markers of category D were mapped using MAPMAKER. However, because MAPMAKER does not accept mixed segregation data, intercross dominant and codominant markers of category B and C were placed to an approximate location on both maps with the JOINMAP programme (Stam 1993).

Finally, a total of 307 Loci (271 RAPDs, ten SCARs, 18 microsatellites, one minisatellite, six isozymes and one 5S rDNA marker) were located on both oak linkage maps (Fig. 1). Among the 50 intercross RAPD loci (category B) only the 24 markers showing strong linkage (LOD $\geq$ 4.5) with framework markers in both maps were retained.

Female map (*Fig.* 1)

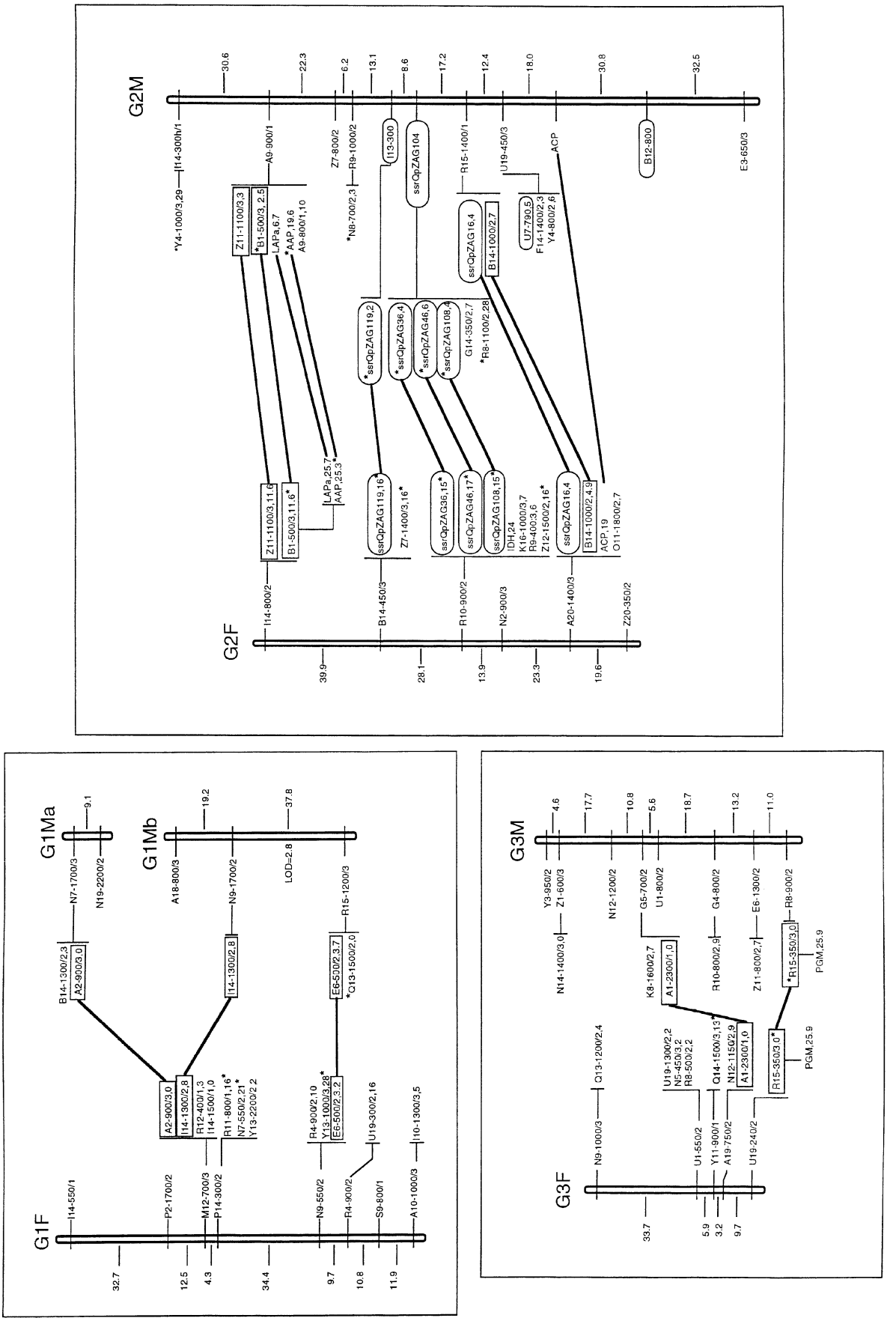
A total of 136 markers were used to establish the female map. Approximately 58% of them (79 loci) were located on the framework map. Twelve linkage groups were set up for the female map covering 893.2 cM, with an average distance between 2 framework loci of 11.30 cM. The size of the linkage groups ranged from 33 cM to 124.8 cM, with an average of 74.40 $\pm$ 16.90 cM.

Male map (*Fig.* 1)

The male map had 136 markers with 83 loci (60%) establishing the framework and covering 921.7 cM. The average distance between 2 framework loci was 11.10 cM. The framework loci were mapped into 12 linkage groups. The length of the groups ranged between 9.1 cM and 191.8 cM, with an average of 70.90 $\pm$ 27.80 cM.

Fifty-two markers in the female map and 44 markers in the male map (testcross loci), including RAPDs,

Fig. 1 Framework linkage maps of *Quercus robur* L. Female map on the *left* and male map on the *right*. Markers were mapped with a minimal LOD score of 3.0. Accessory markers (testcross and intercross types) were located beside their closest framework marker. Intercross markers (category B and C, see Results section) and some recoded testcross markers (category D) were used to identify homologous linkage groups. These markers are interconnected with *bold lines*. Intercross RAPD markers are *boxed*, and microsatellite, minisatellite, SCAR and 5S rDNA markers are *circled*. Markers displaying distorted segregation ($\alpha = 0.05$) are denoted by *boldface asterisk*


G1F
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P2-1700/2
M12-700/3
P14-300/2
N9-550/2
R4-900/2
S9-800/1
A10-1000/3
32.7
12.5
4.3
34.4
9.7
10.8
11.9
A2-900/3.0
I14-1300/2.8
R12-400/1.3
I14-1500/1.0
R11-800/1.16*
N7-550/2.21*
Y13-2200/2.2
R4-900/2.10
Y13-1000/3.28*
E6-500/2.3.2
U19-300/2,16
I10-1300/3,5
G1Ma
B14-1300/2.3
A2-900/3.0
N7-1700/3
N19-2200/2
9.1
G1Mb
A18-800/3
N9-1700/2
I14-1300/2.8
19.2
37.8
LOD=2.8
E6-500/2.3.7
R15-1200/3
*Q13-1500/2.0
G2F
I14-800/2
B14-450/3
R10-900/2
N2-900/3
A20-1400/3
Z20-350/2
39.9
28.1
13.9
23.3
19.6
Z11-1100/3.11.6
B1-500/3.11.6*
LAPa,25.7
AAP,25.3*
ssrQpZAG119.16*
Z7-1400/3.16*
ssrQpZAG119.2
ssrQpZAG36.15*
ssrQpZAG46.17*
ssrQpZAG108.15*
IDH,24
K16-1000/3,7
R9-400/3,6
Z12-1500/2,16*
ssrQpZAG36.4
ssrQpZAG46.6
ssrQpZAG108.4
G14-350/2.7
*R8-1100/2.28
ssrQpZAG16.4
B14-1000/2.4.9
ACP,19
O11-1800/2.7
G2M
*Y4-1000/3,29
I14-300h/1
A9-900/1
*N8-700/2,3
I13-300
ssrQpZAG104
R15-1400/1
U19-450/3
U7-790,5
F14-1400/2,3
Y4-800/2.6
Z7-800/2
R9-1000/2
B12-800
E3-650/3
30.6
22.3
6.2
13.1
8.6
17.2
12.4
18.0
30.8
32.5
G3F
N9-1000/3
U1-550/2
Y11-900/1
A19-750/2
U19-240/2
33.7
5.9
3.2
9.7
Q13-1200/2.4
U19-1300/2,2
N5-450/3.2
R8-500/2,2
Q14-1500/3,13*
N12-1150/2.9
A1-2300/1.0
R15-350/3.0*
PGM,25.9
G3M
N14-1400/3,0
Y3-950/2
Z1-600/3
4.6
17.7
N12-1200/2
10.8
G5-700/2
U1-800/2
5.6
18.7
K8-1600/2,7
A1-2300/1.0
R10-800/2,9
G4-800/2
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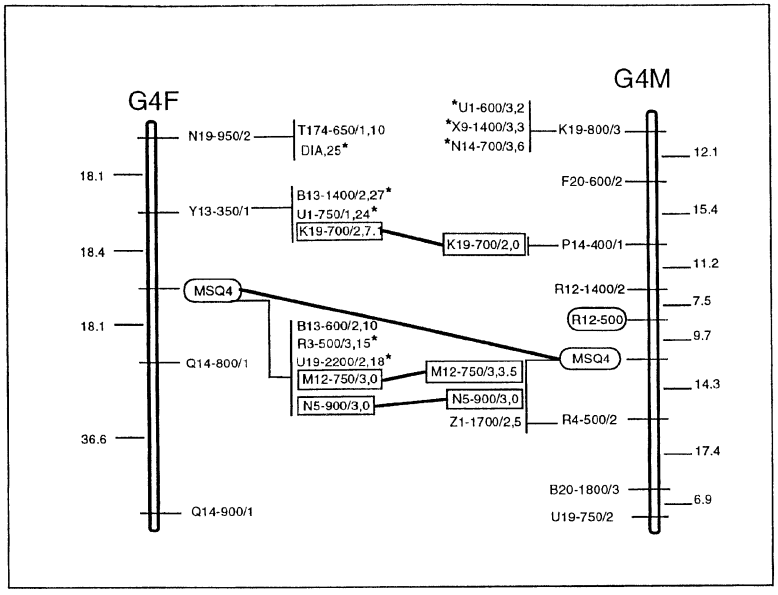

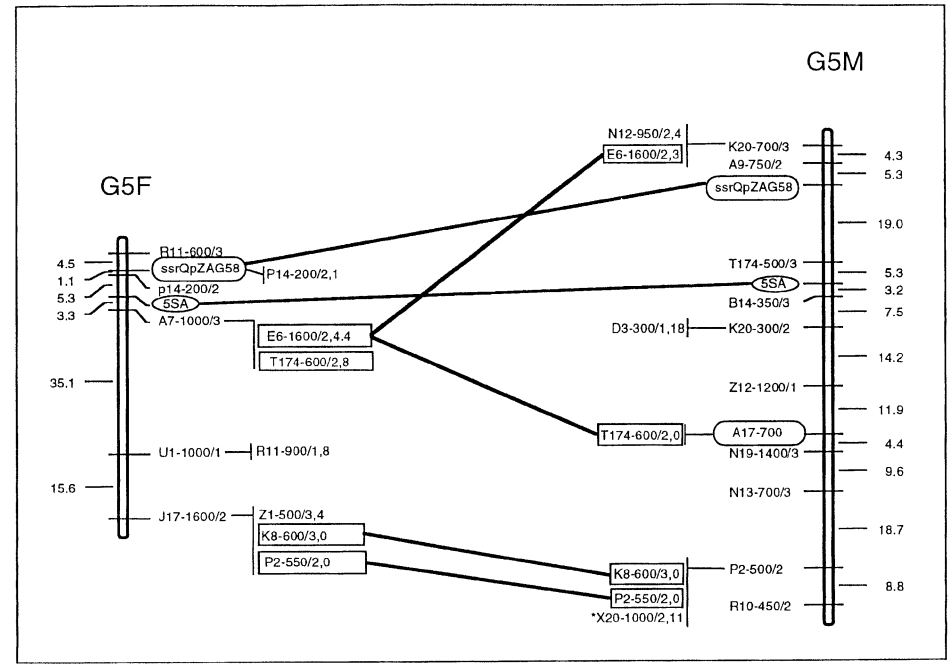

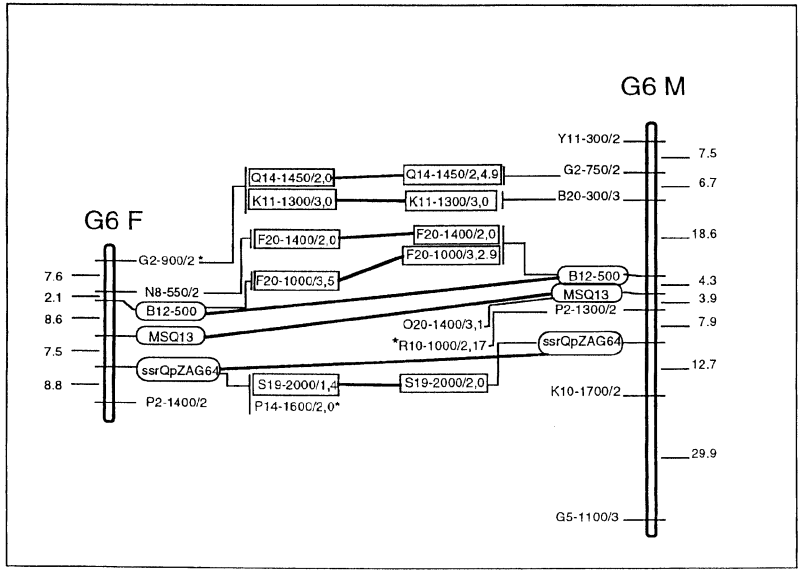

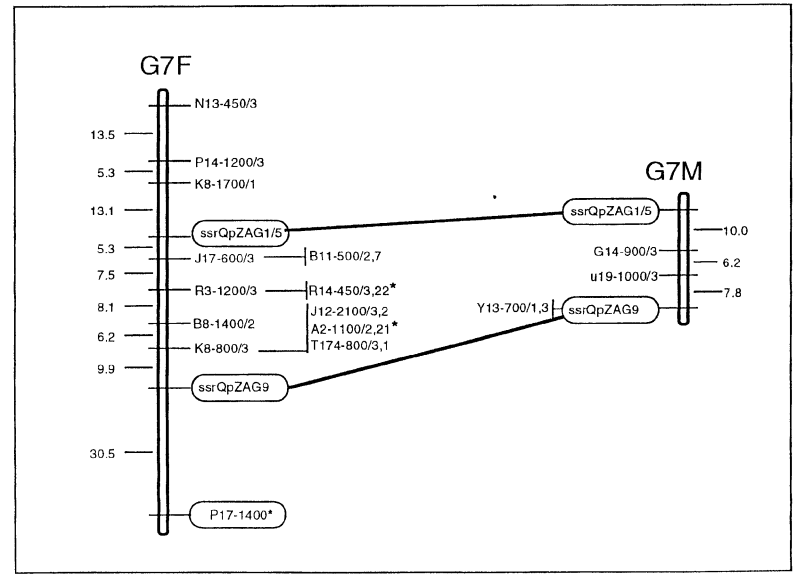

Fig. 1 Continued. (See page 1094 for legend)

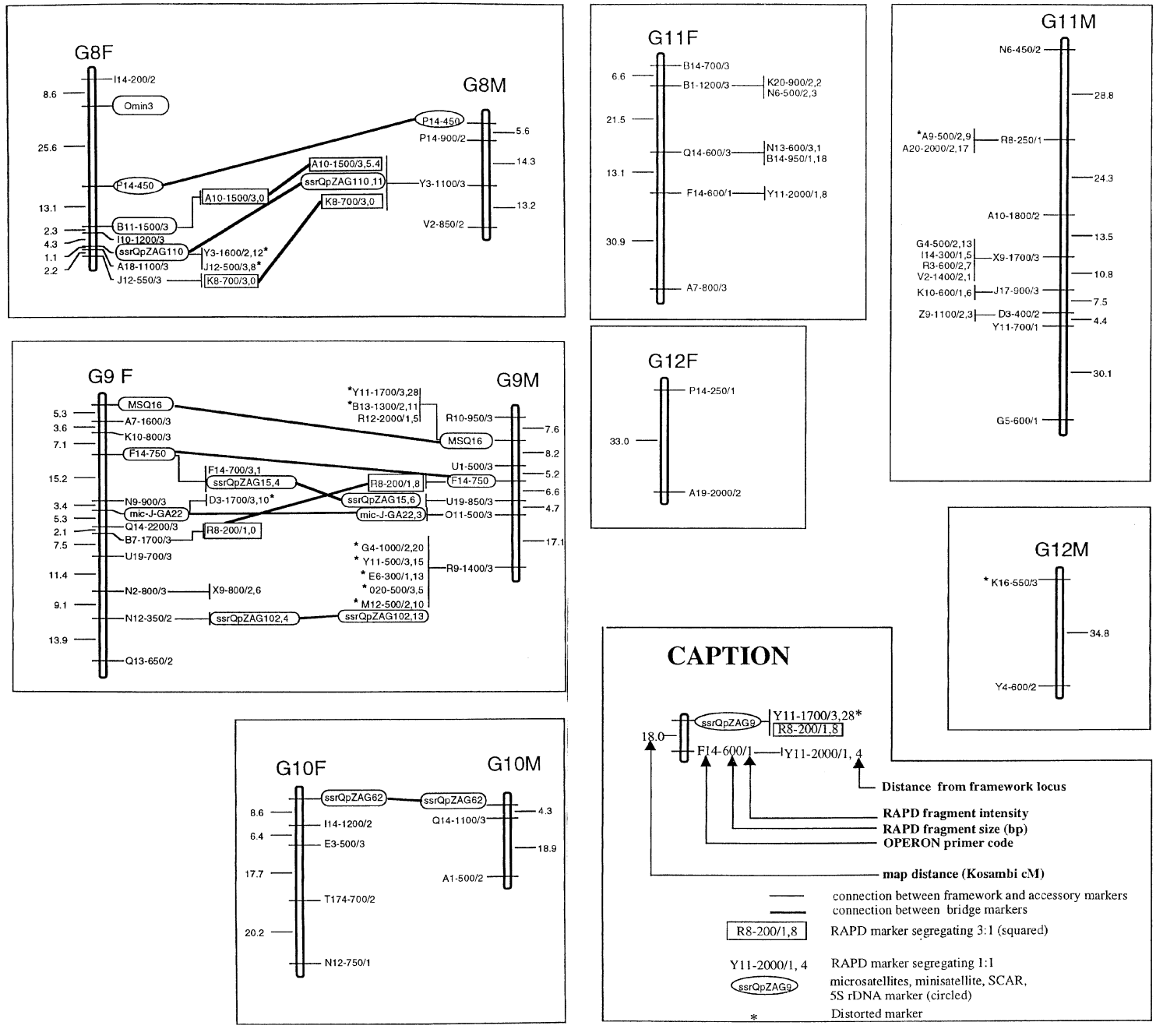

Fig. 1 Continued. (See page 1094 for legend)

SSRs, isozymes and SCARs, were used as accessory markers.

The number of linkage groups corresponded to the number of haploid chromosomes of *Quercus*: n = 12, in both maps.

Homology between parental maps

Markers segregating in both parents including intercross markers (50 RAPDs, three isozymes and 2 microsatellites) and loci segregating 1 : 1 : 1 : 1 (three SCARs, 15 microsatellites, one isozyme and one 5S rDNA marker) were used to identify homology between parental maps. These "bridge" markers are connected by bold lines in Fig. 1. Hence, ten homologous linkage groups were identified.

Because of low information content between marker-pairs corresponding to category A, B and A, C (Ritter et al. 1990), the use of codominant markers segregating 1 : 1 : 1 : 1 and therefore segregating 1 : 1 from both parents is a prerequisite for constructing an accurate combined map. Merging the male and female maps was not our primary goal but this would be possible if more microsatellites were available.

Segregation distortion

At $\alpha = 0.05$, segregation distortions from the Mendelian expectation of testcross markers (category A and D markers) were detected at 25 marker loci in the female map and 24 marker loci in the male map. The distortion level was quite similar in both parents (18%). At $\alpha = 0.01$, the number of distorted markers decreased to 13 in the female map and 11 in the male map. Sixty percent of category A-distorted RAPD markers in the male map (66% in the female) displayed an excess heterozygote genotypes.

Fifteen intercross RAPD markers showed strong deviations from Mendelian expectations, but only 2 of these were associated with a LOD score ≥ 4.5 to a framework marker in the map. Finally, a total of 61 markers (20%) showed segregation distortions at $\alpha = 0.05$. This distortion level is higher than expected by chance alone (15 distorted markers at $\alpha = 0.05$). Markers exhibiting segregation distortion were not randomly distributed in the genome. They tended to be preferentially grouped in a few clusters as shown on Fig. 1.

Genome length and map coverage

The estimated total map length (average of three values) for the female parent amounted to E(G) = 1192 cM and for the male parent to E(G) = 1235 cM. These values are the first to be reported for oak. The different estimates, obtained from a range of Z values, were very close (data not shown) and were higher than the observed genome length of 893.2 cM for the female and 921.7 cM for the male map. This indicated that the mapped loci did not provide full coverage of the oak genome. Although the number of linkage groups corresponded to the number of haploid chromosomes (n = 12), 2 small linkage groups (LG12) were identified for both parental maps. In addition, linkage group 1 of the female map was homologous to 2 unlinked linkage groups (1a and 1b) of the male map. This gap could be filled in by additional markers. Following Lange and Boehnke (1982), the minimal number, N, of randomly distributed markers required to cover a proportion of $P = 95\%$ of a genome of size of L = 1200 cM at a maximum distance between markers of 2d = 20 cM is: $N = \ln(1 - p)/\ln(1 - 2d/L) = 178$. This expectation was higher than the number of markers actually used in the initial step of map construction (136 markers for both the male and female map). According to this formula, 136 markers would provide 90% coverage for a 20-cM map, a percentage coverage that was very close to that obtained following Bishop et al. (1983) (85% for both maps).

The assumption of a random distribution of markers in the genome seemed to fit our experimental data mainly based on RAPDs. The observed and expected distributions of markers were compared for 20-cM intervals. A chi-square test for departure from a Poisson distribution was computed for six classes or groups of classes containing at least five observations. The mean of the Poisson distribution was set to the mean number of markers per 20-cM interval length. No significant departure ($\alpha = 0.01$) from the Poisson expectation was observed in this goodness-of-fit test. A random distribution of RAPD markers was also observed in maritime pine (Plomion et al. 1995).

Discussion

Pseudo-test-cross mapping in *Q. robur*

The *Q. robur* maps presented here were constructed following the "two-way pseudo-testcross" strategy. Two maps of similar size (893 cM in the female parent, 922 cM in the male parent) were obtained for each parent, each one represented by 12 linkage groups corresponding to the 12 haploid chromosomes identified in oaks (Ohri and Ahuja 1990). The two maps are furthermore equally balanced in number of framework markers, mean size of linkage groups and mean distance between framework markers. As oaks exhibit high levels of heterozygozity in natural populations (Kremer and Petit 1993; Zanetto et al. 1994), 18% of the RAPD markers were found to be heterozygous in both parents (these are referred to as intercross loci segregating in the 3 : 1 ratio) compared to the 82% that

Table 3 Map information on some woody angiosperms and annual plants

Species (chromosome number) ((pg/C))	Population type	Number of maps	Marker type	Number of markers	Observed map length	Map length coverage	Estimated map length[b]	References
Citrus (2n = 18) ((0.62 pg/C))	Intergeneric BC_1	1	RFLP isozymes	69	553 cM	–	900–1350 cM	Durham et al. 1994
	Intergeneric BC_1	1	RAPD, RFLP	312	1192 cM	70–80%		Cal et al. 1994
	Intergeneric BC_1	2	RAPD, RFLP isozymes	100	633.1 cM (female) 1503 cM (male)	– –		Luro et al. 1995
	Intergeneric F_1	1	RFLP, SSR isozymes	56	410 cM	–		Kijas et al. 1997
Eucalyptus (2n = 22) ((0, 6 pg/C))	Interspecific F_1	2	RAPD	491	1552 cM (female) 1101 cM (male)	95.80% 95.20%	1100–1650 cM	Grattapaglia and Sederoff 1994
	Interspecific F_1	2	RAPD	505	1331 cM (female) 1415 cM (male)	95–99% 95–99%		Verhaegen and Plomion 1996
	Intraspecific G_2	1	RAPD, RFLP isozymes	339	1462 cM	–		Byrne et al. 1995
Persea (2n = 24) ((0, 9 pg/C))	Interspecific F_1	2	RAPD, SSR minisatellites	90	352.6 cM	–	1200–1800 cM	Sharon et al. 1997
Malus (2n = 34) ((0.8 pg/C))	F_1	1	RAPD, RFLP isozymes	409	950 cM	–	1700–2550 cM	Hemmat et al. 1994
Populus (2n = 38) ((0.6 pg/C))	Interspecific F_1	1	RAPD, RFLP isozymes, STS	343	1261 cM	48.50%	1900–2850 cM	Bradshaw et al. 1994
Prunus (2n = 16) ((0.3 pg/C))	Interspecific F_2	1	RFLP, isozymes, morphological	118	800 cM	–	800–1200 cM	Foolad et al. 1995
Prunus persica	Interspecific F_2	1	RAPD, RFLP	65	332 cM	$< 50\%$		Rajapakse et al. 1995
	Intraspecific F_2	1	RAPD, isozymes, morphological	99	396 cM	–		Chaparro et al. 1994
	Intraspecific F_2	1	RFLP, RAPD, AFLP, IMA, morphological	270	712 cM	$\sim 100\%$		Dirlewanger et al. 1998
Prunus amygdalus	Intraspecific F_1	2	RFLP, isozymes	127	393 cM (female) 394 cM (male)	–		Viruel et al. 1995
Quercus robur L. (2n = 24) ((0.9 pg/|C))	Intraspecific F_1	2	RAPD, SCAR, SSR, isozymes 5S rDNA	307	893.2 cM (female) 921.7 cM (male)	85% 85%	1200–1800 cM	Barreneche et al. 1998 (this study)

Table 3 Continued

Species (chromosome number) ((pg/C))	Population type	Number of maps	Marker type	Number of markers	Observed map length	Map length coverage	Estimated map length[b]	References
Theobroma cacao L. (2n = 20) ((0.4 pg/C))	Intraspecific F_1	1	RAPD, RFLP, isozymes	193	759 cM	–	1000–1500 cM	Lanaud et al. 1995 (this study)
	Intraspecific BC_1	1	RAPD, RFLP	138	1068 cM	–		Crouzillat et al. 1996
Vitis (2n = 38) ((0.5 pg/C))	Interspecific F_1	2	RAPD, RFLP, isozymes	523	1196 cM (female)	–	1900–2850 cM	Lodhi et al. 1995
					1477 cM (male)	–		
Arabidopsis thaliana (2n = 10) ((0.15 pg/C))	RIL	1	RAPD, microsatellites	302	675 cM	~ 100%	500–750 cM	Reiter et al. 1992
				30				Bell et al. 1994[c]
Hordeum vulgare (2n = 14) ((5.50 pg/C))	DH	1	RAPD, RFLP, isozymes	295	1250 cM	~ 100%	700–1050 cM	Kleinhofs et al. 1993
Lycopersicon esculentum (2n = 24) ((0.98 pg/C))	F_2	1	RFLP, isozymes, morphological	1030	1276 cM	~ 100%	1200–1800 cM	Tanksley et al. 1992
Zea mays (2n = 20) ((2.5 pg/C))	RIL, F_2	1	RFLP, isozymes, molecular markers	275	1765 cM	~ 100%	1000–1500 cM	Causse et al. 1996

[a] F_1, Two-generation outbred pedigree; F_2, three-generation inbred pedigree; G_2, three-generation outbred pedigree, RIL, recombinant inbred lines; DH, doubled-haploid lines; BC_1, first-generation backcross

[b] 100–150 cM per chromosome according to Rajapakse et al. (1995)

[c] See http://cbil.humgen.upenn.edu/Natgc/SSLP_info/SSLP_map.html

segregated in a testcross configuration. Reported corresponding values in other species using the"two-way pseudo-testcross" are quite different: the number of RAPDs segregating in 3 : 1 proportions varied between 2% and 5% in *Eucalyptus* studies (Grattapaglia and Sederoff 1994; Verhaegen and Plomion 1996) and amounted to 5% in pines (Kubisiak et al. 1995). These differences can likely be imputed to the differences in levels of heterozygozity between these species. The higher proportion of heterozygote RAPD loci in both parents also allowed the assignment of homologous linkage groups of the two parents as suggested by Hemmat et al. (1994). However, homologies between groups became more evident with codominant markers: microsatellites, minisatellite, SCARs, isozymes and the 5S rDNA marker. Among the 12 linkage groups identified in the two parents, 10 pairs could clearly be associated because of the presence of several common markers. A complete merging of the two maps into one single consensus map could be accomplished only if the ordering of markers were more precisely defined and if cosegregation analysis were conducted in larger families.

Within the intraspecific F_1 cross of *Q. robur*, a large number of markers (18% in both parental maps) exhibited segregation distortion regardless of the type of marker. In other examples of "pseudo-testcross" mapping, distorted markers are present in lower proportions (9% in *Eucalyptus urophylla*, 8% in *Eucalyptus grandis* Verhaegen and Plomion 1996, 15% in *Pinus palustris* and 14% in *Pinus elliottii* Kubisiak et al. 1995). As in pines, eucalypts and pedunculate oak, there is a general trend towards a clustering of distorted markers on the linkage groups (linkage groups 2 and 4 in the female map, and linkage groups 4 and 9 in the male map, Fig. 1).

The large number of distorted markers in *Q. robur* may be related to the high genetic load observed in this species. Interestingly, the variation of diversity among eucalypts, pines and oaks as illustrated by the number of loci being heterozygous in the two parents follows the same pattern as the variation in the number of distorted loci. Although quantitative data on inbreeding depression are not available in oaks, observations of seedlings originating from control selfing crosses reveal that they experience several detrimental characters during their early development (poor germination, chlorophyll deficiency, reduced growth) (Kleinschmit and Kleinschmit 1996; and personal observations.).

Genetic map of *Q. robur* and other woody angiosperms and annual plants

To our knowledge this is the first reported genetic map of an oak species and of any member of the *Fagaceae* family. Other linkage studies based on a reduced number of isozyme loci and on several full-sib families have indicated that 4 loci (*Aap*, *Lap*, *Idh*, *Acp*) are located on the same linkage group in *Q. robur* (Zanetto et al. 1996) and *Q. petraea* (Müller-Starck et al. 1996). Interestingly these 4 loci are distributed within linkage group 2 of our genetic map (Fig. 1).

Genetic maps in other genera of broadleaved forest trees are currently only available in *Eucalyptus* (Grattapaglia and Sederoff 1994; Verhaegen and Plomion 1996; Byrne et al. 1995) and in *Populus* (Bradshaw et al. 1994). The comparison with woody angiosperms is therefore extended to fruit trees having related species in natural forests and to a few annual plants for documentary purposes (Table 3). With a few exceptions (*Eucalyptus* and *Quercus*) most existing maps of woody angiosperms cover less than 80% of the genome. Based on the data of Table 3, the physical genome size of woody angiosperms varies between 0.27 pg DNA/C (*Prunus persica*) and 0.9 pg DNA/C (*Quercus robur*), lying in the range of variation between *Arabidopsis* (0.15 pg DNA/C) and *Lycopersicon esculentum* (0.98 pg DNA/C) (Arumuganathan and Earle 1991). Most woody angiosperms have lower physical and genetic genome sizes than annual plants. *Quercus robur* has the largest physical genome among the angiosperm woody species so far investigated, but its size is not greater than the genome of tomato. Curiously, the number of chromosomes and the genetic length of the genome in tomato and oak are also of a similar magnitude. Given that 1 pg represents 0.915×10^9 bp (Bennet and Smith 1976), the physical distance per unit of genetic distance in *Q. robur* amounts to 0.77×10^6 bp/cM, which represents only 3.21 times that of *Arabidopsis thaliana* (Table 3).

Microsatellites and consensus maps in oaks and *Fagaceae*

As illustrated in our example where two maps were constructed (Fig. 1), homologies between linkage groups became obvious as codominant markers segregating in 1 : 2 : 1 or 1 : 1 : 1 : 1 or dominant markers segregating in 3 : 1 could be mapped in both parents. Because the standard error of recombination fraction between markers segregating 1 : 1 : 1 : 1 (recoded 1 : 1) and markers segregating 1 : 1 is lower than for the others (Ritter et al. 1990), their position on a linkage group is more precise. Hence, they are preferential candidates as anchor markers and for comparisons across maps.

Microsatellite primers are transportable across oak species as shown by the experiment conducted by Steinkellner et al. (1997b). Among the 18 microsatellites mapped in *Q. robur*, which were originally developed from *Q. petraea*, polymerase chain reaction (PCR) amplification and polymorphism were tested for 14 loci in other oak species and other genera of the *Fagaceae*. All amplified products for the 14 loci were polymorphic in oak species belonging to the same botanical section (*Lepidobalanus*.) The number of successful loci was

reduced to 64% in oak species of section *Cerris* and to 47% in oak species of section *Erythrobalanus*. These results are reinforced by our own experience with microsatellite loci developed from *Quercus macrocarpa* (Dow et al. 1995) and *Quercus salicina* (Kawahara T, unpublished data) and that were applied for mapping purposes in the present contribution. However, successful amplification of oak microsatellites in related genera of the family *Fagaceae* was reduced to 24% in *Fagus* and to 47% in *Castanea*. Even though the proportion of amplified fragments and the level of polymorphism decreased with increasing evolutionary distance, microsatellites can be regarded as preferential anchor and consensus markers across different oak species in the *Quercus* genus.

In the future these maps will be saturated using additional markers like amplified fragment length polymorphism (AFLP, Vos et al. 1995) and further integrated into a single consensus map when more microsatellites can be localised. This consensus map will be of great value in identifying quantitative trait loci of interest and particularly of those involved in adaptive traits of oaks.

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References

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 35 : 1439–1445

Becker M, Levy G (1990) Le point sur l'écologie comparée du chêne sessile et du chêne pédonculé. Rev For Fr 42 : 148–154

Bell CJ, Ecker JR (1994) Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics 19 : 137–144

Bennet MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Philos Trans R Soc London 216 : 179–199

Besendorfer V, Zoldoš V, Peškan T, Krnik-Rasol M, Littway T, Papeš D (1996) Identification of potential cytogenetical and biochemical markers in bioindication of common oak forest. Phyton (Horn, Austria), Special issue 36 : 139–146

Bishop DT, Cannings C, Skolnick M, Williamson JA (1983) The number of polymorphic DNA clones required to map the human genome. In: Wier BS (ed) Statistical analysis of DNA sequence data. Marcel Dekker, New York, pp 181–200

Bodénès C, Laigret F, Kremer A (1996) Inheritance and molecular variations of PCR-SSCP fragments in pedunculate oak (*Quercus robur* L.). Theor Appl Genet 93 : 348–354

Bodénès C, Joandet S, Laigret F, Kremer A (1997) Detection of genomic regions differentiating two closely related oak species *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. Heredity 78 : 433–444

Bradshaw HD Jr, Villar M, Watson BD, Otto KG, Stewart S, Stettler RF (1994) Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS, and RAPD markers. Theor Appl Genet 89 : 167–179

Butorina AK (1993) Cytogenetic study of diploid and spontaneous triploid oaks, *Quercus robur*. L. Ann Sci For 50 : 144s–150s

Byrne M, Murrell JC, Allen B, Moran GF (1995) An integrated genetic linkage map for eucalypts using RFLP, RAPD and isozyme markers. Theor Appl Genet 91 : 869–876

Cai Q, Guy CL, Moore GA (1994) Extension of the linkage map in *Citrus* using random amplified polymorphid DNA (RAPD) markers and RFLP mapping of cold-acclimation-responsive loci. Theor Appl Genet 89 : 606–614

Camus A (1954) Les Chênes – Monographie du genre *Quercus*. Editions Le Chevalier, Paris

Causse M, Santoni S. Damerval C, Maurice A, Charcosset A, Deatrick J, de Vienne D (1996) A composite map of expressed sequences in maize. Genome 39 : 418–432

Chakravarti A, Lasher LA, Reffer JE (1991) A maximum likelihood method for estimating genome length using genetic linkage data. Genetics 128 : 175–182

Chaparro JX, Werner DJ, O'Malley D, Sederoff SS (1994) Targeted mapping and linkage analysis of morphological, isozyme, and RAPD markers in peach. Theor Appl Genet 87 : 805–815

Crouzillat D, Lerceteau E, Petiard V, Morera J, Rodriguez H, Walker D, Phillips W, Ronning C, Schnell R, Osei J, Fritz P (1996) *Theobroma cacao* L.: a genetic linkage map and quantitative trait loci analysis. Theor Appl Genet 93 : 205–214

Dirlewanger E, Proner V, Parvery C, Rothan C, Guye A, Monet R (1998) Genetic linkage map of peach (*Prunus persica* (L.) Batsch) using morphological, RFLP, isoenzyme, RAPD and AFLP markers. TAG (in press)

Dow BD, Ashley MV (1996) Microsatellite analysis of seed dispersal and parentage of saplings in bur oak, *Quercus macrocarpa*. Mol Ecol 5 : 615–627

Dow BD, Ashley MV, Howe HF (1995) Characterisation of highly variable $(GA/CT)_n$ microsatellites in the bur oak, *Quercus macrocarpa*. Theor Appl Genet 91 : 137–141

Durham RE, Liou PC, Gmitter FG, Moore GA (1994) Linkage of restriction length polymorphisms and isozymes in *Citrus*. Theor Appl Genet 84 : 39–48

Favre JM, Arnould MF (1996) Nuclear DNA content and interspecific ribosomal variation between *Q. robur* and *Q. petraea*. In: Kremer A, Mühs H (eds) Inter- and intra-specific variation in European oaks: evolutionary implications and practical consequences. European Commission, EUR 16717EN, Luxembourg, pp 41–51

Favre JM, Brown S (1996) A flow cytometric evaluation of the nuclear DNA content and GC percent in genomes of European oak species. Ann Sci For 53 : 915–917

Foolad MR, Aruselkar S, Becerra V, Bliss FA (1995) A genetic map of *Prunus* based on an interspecific cross between peach and almond. Theor Appl Genet 91 : 262–269

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *E. urophyulla* using a pseudo-testcross mapping strategy and RAPD markers. Genetics 137 : 1121–1137

Hemmat M, Weeden NF, Manganaris AG, Lawson DM (1994) Molecular marker linkage map for apple. J Hered 84 : 4–11

Jacobs JME, Van Eck HJ, Arens P, Verkerck-Bakker B, te Lintel Hehhert B, Bastiaanssen HJM, El-Kharbotly A, Pereira A, Jacobsen E, Stiekema WJ (1995) A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons and classical markers. Theor Appl Genet 91 : 289–300

Johnsson H (1946) Chromosome numbers of twin plants of *Quercus robur* and *Fagus silvatica*. Hereditas 32 : 469–472

Kleinschmit JRG, Kleinschmit J (1996) Artificial hybridization between *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. In: Kremer A, Mühs H (eds) Inter- and intra-specific variation in European oaks: evolutionary implications and practical consequences. European Commission, EUR 16717EN, Luxembourg, pp 69–87

Kijas JMH, Thomas MR, Fowler JCS, Roose ML (1997) Integration of trinucleotide microsatellites into a linkage map of *Citrus*. Theor Appl Genet 94 : 701–706

Kleinhofs A, Kilian A, Shagai-Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiack JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of barley (*Hordeum vulgare*) genome. Theor Appl Genet 86: 705–712

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12: 172–175

Kremer A, Petit RJ (1993) Gene diversity in natural populations of oak species. Ann Sci For 50: 186s–202s

Kremer A, Savill PS, Steiner KC (1993) Genetics of oaks. Ann Sci For 50: 1s–472s

Kubisiak TL, Nelson CD, Nance WL, Stine M (1995) RAPD linkage mapping in a longleaf pine × slash pine F_1 family. Theor Appl Genet 90: 1119–1127

Lanaud C, Risterucci AM, N'Goran AKJ, Clement D, Flament MH, Laurent V, Falque M (1995) A genetic linkage map of *Theobroma cacao* L. Theor Appl Genet 91: 987–993

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174–181

Lange K, Boehnke M (1982) How many polymorphic genes will it take to span the human genome? Am J Hum Genet 34: 842–845

Lodhi MA, Daly MJ, Ye G-N, Weeden NF, Reisch BI (1995) A molecular marker based linkage map of *Vitis*. Genome 38: 786–794

Luro F, Lorieux M, Laigret F, Bové JM, Ollitrault P (1995) Cartographie du génome des agrumes á l'aide de marqueurs moléculaires et distortions de ségrégation. In: Bervillé A, Tersac M (eds) Techniques et utilisation des marqueurs moléculaires. INRA Editions, Paris, pp 69–82

Moreau F, Kleinschmit J, Kremer A (1994) Molecular differentiation between *Q. petraea* and *Q. robur* assessed by random amplified DNA fragments. For Genet 1: 51–64.

Müller-Starck G, Zanetto A, Kremer A, Herzog S (1996) Inheritance of isoenzymes in sessile oak (*Quercus petraea* (Matt.) Liebl.) and offspring from interspecific crosses. For Genet 3: 1–12

Ohri D, Ahuja MR (1990) Giemsa C – banded karyotype in *Quercus* L. (Oak). Silvae Genet 39: 216–219

Plomion C, O'Malley DM, Durel C-E (1995) Genomic analysis in maritime pine (*Pinus pinaster*). Comparison of two RAPDs maps using selfed and open-pollinated seeds of the same individual. Theor Appl Genet 90: 1028–1034

Rajapakse S, Belthoff LE, He G, Estager AE, Scorza R, Verde I, Ballard RE, Baird WV, Callahan A, Monet R, Abbott AG (1995) Genetic linkage mapping in peach using morphological, RFLP and RAPD markers. Theor Appl Genet 90: 503–510

Reiter RS, Williams JGK, Feldmann KA, Rafalski JA, Tingey SV, Scolnik PA (1992) Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc Natl Acad Sci USA 89: 1477–1481

Ritter E, Gebhardt C, Salamini F (1990) Estimation of recombination frequencies and construction of RFLP linkage maps in plants from crosses between heterozygous parents. Genetics 125: 645–654

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA. 81: 8014–8018

Sharon D, Cregan PB, Mhammed S, Kusharska M, Hillel J, Lahav E, Lavi U (1997) An integrated genetic linkage map of avocado. Theor Appl Genet 95: 911–922

Specht T, Szymanski M, Barciszewska MZ, Barciszewski J, Erdmann VA (1997) Compilation of 5S rRNA and 5S rRNA gene sequences. Nucleic Acids Research 25: 96–97

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JOINMAP. Plant J 3: 739–744

Steinkellner H, Fluch S, Turetschek E, Lexer C, Streiff R, Kremer A, Burg K, Glössl J (1997a) Identification and characterization of $(GA/CT)_n$ – microsatellite loci from *Quercus petraea*. Plant Mol Biol 33: 1093–1096

Steinkellner H, Lexer C, Turetschek E, Glössl J (1997b) Conservation of $(GA)_n$ microsatellite loci between *Quercus* species. Mol Ecol 6: 1189–1194

Streiff R, Labbé T, Bacilieri R, Steinkellner H, Glössl J, Kremer A (1998) Within population genetic structure in *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. assessed with isozymes and microsatellites. Mol Ecol 7: 317–328

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Miller JC, Miller L, Paterson AH, Pineda O, Röder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 32: 1141–1160

Verhaegen D, Plomion C (1996) Genetic mapping in *Eucalyptus urophylla* and *Eucalyptus grandis* using RAPD markers. Genome 39: 1051–1061

Viruel MA, Messeguer R, de Vicente MC, Garcia-Mas J, Puigdoménech P, Vargas F, Arus P (1995) A linkage map with RFLP and isozymes markers for almond. Theor Appl Genet 91: 964–971

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23: 4407–4414

Williams JGK, Kubelik AR, Livak KJ, Rafalski A, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531–6535

Zanetto A, Roussel G, Kremer A (1994) Geographic variation of inter-specific differentiation between *Quercus robur* L. and *Quercus petraea* (Matt.) For Genet 1: 111–123

Zanetto A, Kremer A, Müller-Starck G, Hattemer HH (1996) Inheritance of isozymes in Pedunculate oak (*Quercus robur* L.) J Hered 87: 364–370

Zoldoš V, Papeš D, Brown S, Panaud O, Šiljak-Yakovlev S (1998) Genome size and base composition of seven *Quercus* species: inter- and intra-population variation. Genome (in press)