Theor Appl Genet (2004) 108:558–566
DOI 10.1007/s00122-003-1462-2

ORIGINAL PAPER

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Comparative mapping between *Quercus* and *Castanea* using simple-sequence repeats (SSRs)

Received: 9 April 2003 / Accepted: 13 August 2003 / Published online: 16 October 2003

Abstract Simple sequence repeat (SSR) markers from *Quercus* and *Castanea* were used for comparative mapping between *Quercus robur* (L.) and *Castanea sativa* (Mill.). We tested the transferability of SSRs developed in *Quercus* to *Castanea* and vice-versa. In total, 47% (25) of the *Quercus* SSRs and 63% (19) of the *Castanea* SSRs showed a strong amplification product in the non-source species. From these 44 putative comparative anchor tags, 19 (15 from *Quercus* and 4 from *Castanea*) were integrated in two previously established genetic linkage maps for the two genera. SSR loci were sequenced to confirm the orthology of the markers. The combined information from both genetic mapping and sequence analysis were used to determine the homeology between seven linkage groups, aligned on the basis of pairs or triplets of common markers, while two additional groups were matched using a single microsatellite marker. Orthologous loci identified between *Q. robur* and *C. sativa* will be useful as anchor loci for comparative mapping studies within the Fagaceae family.

Communicated by D.B. Neale

This paper is dedicated to the memory of Paulo Costa

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Introduction

Comparative mapping relies on mapping orthologous loci in two or more species and comparing their position along homeologous linkage groups. With the availability of genetic linkage maps, comparative mapping studies among phylogenetically related plants have been widely developed in the last decade (Nadeau and Sankoff 1998). Transferring genetic and molecular information from one species to another, as well as discovering the main processes of genome evolution, are two promising prospects in developing comparative mapping studies. In crops such studies showed that gene content (synteny), marker order (co-linearity) and quantitative trait loci (QTLs) position are often conserved among phylogenetically related species (Paterson et al. 2000; Schmidt 2000). Grasses are a well known example, where extensive comparative mapping studies suggested that cultivated cereals can be considered as a single genetic system. Therefore, resources available in the small rice genome can be exploited in analysing large genomes such as wheat (Devos and Gale 2000; Freeling 2001).

As far as forest trees are concerned a few examples in pine (Devey et al. 1999; Sewell et al. 1999; Brown et al. 2001; Chagné et al. 2003), eucalypt (Marques et al. 2002) and poplar (Cervera et al. 2001) have reported the transferability of genetic and molecular information among closely related species within the same genus. RFLP, microsatellite and EST markers were successfully used to identify orthologous loci and align homeologous linkage groups. A common set of comparative anchor tags would allow the establishment of consensus maps in which it would be possible to merge the information from QTL studies carried out in different pedigrees, environments and phylogenetically related species. The transferability of genetic and molecular information from either one pedigree to another or across closely related species would provide a tool to exploit pre-existing pedigrees and QTL studies. This would be very useful in forest trees where the creation of a pedigree is a costly and time-demanding task.

Quercus robur L. and *Castanea sativa* Mill. are two major broadleaf species in Europe belonging to the Fagaceae family. Both genera are distributed throughout the Northern hemisphere in Asia, Europe and North America. Recent phylogenetic analyses have demonstrated a close relationship between the two genera (Manos and Steele 1997; Manos et al. 2001) and two genetic linkage maps based on molecular markers have been published (Barreneche et al. 1998; Casasoli et al. 2001). Both species have the same number of chromosomes (2n=2*x*=24) and also similar genetic and physical genome sizes: 1,200 cM (Kosambi) and 0.94 pg/C, for *Q. robur*, and 1,050 cM and 0.81 pg/C, for *C. sativa* (Zoldos et al. 1998; Brown CS and Siljak-Yakovlev S, personal communication). In addition, QTLs for adaptive traits relating to bud phenology and water use efficiency are currently being mapped in both species (Saintagne et al., unpublished results; Casasoli et al., unpublished results). Comparative mapping between these two species would provide the means to investigate the correspondence of QTLs across the two genera and the opportunity to identify homeologous chromosomal regions affecting important adaptive traits within the Fagaceae family.

Microsatellite markers have already been used for comparative mapping in forest trees (Marques et al. 2002). Despite the decrease of cross-amplification efficiency with evolutionary distance, microsatellite markers can be cross-transferred in phylogenetically related species as shown in the Citrineae (Kijas et al. 1995), Brassicaceae (Van Treuren et al. 1997; Plieske and Struss 2001), *Eucalyptus* (Byrne et al. 1996), Gramineae (Guyomarc'h et al. 2002; Matsuoka et al. 2002), Fagaceae (Steinkellner et al. 1997b), Asteraceae (Whitton et al. 1997), Leguminoseae (Peakall et al. 1998), Pinaceae (Fisher et al. 1998; Karhu et al. 2000; Kutil and Williams 2001; Rajora et al. 2001; Sheperd et al. 2002), Cucurbitaceae (Danin-Poleg et al. 2000), Vitaceae (Di Gaspero et al. 2000; Rossetto et al. 2002) and Rosaceae (Yamamoto et al. 2001; Dirlewanger et al. 2002). The cross-transferability of microsatellite markers showed contrasting results depending largely on the evolutionary distance and the complexity of the genome. Nevertheless, it is widely accepted that the successful cross-amplification of an SSR locus does not prove the maintenance of the repeat motif in the non-source species. The lack of the repeat motif, as well as modifications both at the repeat and the flanking regions, clearly suggest the need for caution in using the same microsatellite loci across different taxa (Peakall et al. 1998).

In this study we used two sets of microsatellites specifically designed in species belonging to the *Quercus* and *Castanea* genera in order to: (1) test their cross-genera transferability between *Q. robur* and *C. sativa*; (2) integrate them into available genetic linkage maps; and (3) compare their map position and evaluate their usefulness for comparative mapping within the Fagaceae family.

Materials and methods

Plant material

Two mapping populations (full-sib progenies) were used to test and map microsatellite markers in the pedunculate oak (*Q. robur* L.) and the European chestnut (*C. sativa* Mill.). The DNA extraction procedures have been previously described in Barreneche et al. (1998) and Casasoli et al. (2001).

Microsatellites

Primer information for *Quercus* microsatellites was obtained from Dow et al. (1995), Dow and Ashley (1996), Steinkellner et al. (1997a), Kampfer et al. (1998), Lexer (1999), and Aldrich et al. (2002), while *Castanea* microsatellites were developed by Buck et al. (2003) and Marinoni et al. (2003). PCR reactions were performed in a final volume of 20 μl containing 2 μl of PCR buffer 10x (Invitrogen, Carlsbad, Calif., USA), 2.0 mM of $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, 5% DMSO, 5–10 ng of genomic DNA and 0.5 U of *Taq* polymerase (Invitrogen, Carlsbad, Calif., USA). Amplifications were run in a GeneAmp PCR System 9600 (Perkin Elmer, Norwalk, Conn., USA) under the following conditions: 10 min denaturation at 95°C, 26 cycles of 50 s at 94°C, 45 s at the annealing temperature recommended for the source-species amplification, 1 min and 30 s at 72°C, with a final extension at 72°C for 8 min (Botta et al. 1999). When microsatellites were tested in the non-source species, the amount of DNA template was increased up to 10 times compared to that used for source species, and the annealing temperature was lowered from 3° to 5°C. For evaluation of cross-genus amplification, microsatellites of the source species were amplified as a positive control. After testing the microsatellite markers on the two parents and six F_1 individuals, informative microsatellites were selected for mapping. Amplified microsatellites were run on 6% denaturing polyacrylamide gels, in a Li-cor automatic DNA sequencer (model 4000 and 4000L IRD40, Li-cor Inc, Lincoln, Neb., USA) or were silver stained as described in Streiff et al. (1998).

5S rDNA

The nuclear 5S ribosomal DNA locus (5S rDNA) was amplified in chestnut using two universal plant primers (F: *5′-TGGGAAGTC-CTCGTGTTGCA-3′*, R: *5′-ATTAGTGCTGGTATGATCGC-3′*) already used to map the same locus in oak (Barreneche et al. 1998). The locus was amplified in a 25-μl reaction containing 10 mM of Tris HCl pH 8.3, 50 mM of KCl, 1.5 mM of $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, 100 μg/ml of BSA, 0.5 ng of genomic DNA and 0.75 U of *Taq* polymerase (Roche, Basel, Switzerland) with the following PCR profile: 3 min denaturation at 94°C, 30 cycles of 1 min at 94°C, 1 min at 65°C, 2 min at 72°C, with a final extension at 72°C for 10 min. An amplified fragment of 290 bp was cloned and sequenced showing significant homeology with a non-transcribed region of the 5SrDNA gene. An informative-size polymorphism for the chestnut mapping population was revealed in this region by using a 6% non-denaturing polyacrylamide gel visualized by silver staining, providing an additional comparative anchor tag.

Genetic mapping

A total of 96 individuals from each progeny were genotyped with informative microsatellites that were integrated in both framework linkage maps (Barreneche et al. 1998; Casasoli et al. 2001). MAPMAKER V 2.0 (Lander et al. 1987) and JOINMAP V 1.3 (Stam 1993) were used for linkage analysis using a LOD threshold of 6.0 as a grouping criterion to map the new microsatellite markers. JOINMAP software was used to map microsatellites showing an intercross configuration, i.e. segregating 1:2:1, whereas

test-cross loci segregating 1:1 and 1:1:1:1 were mapped by MAPMAKER.

Cloning and sequencing

In both species, one allele from the 17 mapped microsatellite loci was cloned (TOPO TA-cloning kit, Invitrogen, Carlsbad, Calif., USA) and sequenced in both strands using the ABI prism DNA sequencing kit (Dye Primer Cycle Sequencing Core kit, PE Biosystems, Warrington, UK) according to the manufacturer's instructions. *Quercus* and *Castanea* sequences were aligned by means of ALIGNn software (http://www.infobiogen.fr/services/analyseq/cgi-bin/alignn_in.pl).

Results and discussion

Cross-genus transferability of microsatellite markers

Two sets of microsatellite markers developed either in *Quercus* or *Castanea* were tested for amplification in *Q. robur* and *C. sativa* (Table 1). In both cases, the same PCR conditions used in the source species were initially used in the non-source species. Microsatellites showing very light, multi-band and unclear profiles were discarded from further analyses. Slight modifications on DNA concentration and annealing temperature were performed for loci cross-amplifying in the non-source species and showing a weak amplification product similar in size to the locus detected in the source species.

Most of the oak microsatellites have been previously integrated into a genetic linkage map of *Q. robur* (Barreneche et al. 1998; Barreneche et al. in preparation). The 53 oak primer pairs were tested in chestnut. As summarized in Fig. 1a, 37 (70%) of the oak SSRs tested gave a positive amplification in chestnut (scored as 1 or 2 in Table 1), 25 (47%) showed a clear and strong PCR product (scored as 2 in Table 1) and 16 (30%) segregated in the chestnut mapping population. All 35 SSR primer pairs were amplified and segregated in oak mapping progeny as they were previously selected to meet this requirement. The set of 30 chestnut microsatellites was tested in both oak and chestnut mapping populations. As expected, all loci amplified in chestnut and 21 (70%) of them segregated in the mapping population (Fig. 1b). Twenty five (83%) also gave an amplification product in oak, 19 (63%) gave a clear and strong amplification band, and five (17%) segregated in the oak mapping population. These results proved that, although EST markers have been recently shown to be ideal markers for comparative mapping studies (Brown et al. 2001), microsatellites still remain attractive for cross-taxa amplification (Powell et al. 1996). As expected from previous studies in the Fagaceae (Steinkellner et al. 1997b) we confirmed the transferability between *Quercus* and *Castanea*, but we also found that the cross-genus amplification efficiency was significantly different between the two sets of microsatellites.

Eight out of 30 chestnut microsatellites tested in oak contain tri-nucleotide repeats, whereas the oak microsatellites are mainly di-nucleotide repeats. Among the SSR loci cross-amplifying (scored as 2 in Table 1) in *Q. robur* and *C. sativa*, five segregated in the oak progeny, and 16 segregated in the chestnut progeny. A decrease of informative content was observed for chestnut SSRs when transferred to oak. It is worth observing that 12 out of the 19 chestnut microsatellites amplifying in oak, contained either tri-nucleotide or imperfect and compound repeat motifs in chestnut. Most of them were either monomorphic or did not segregate in the oak pedigree (because the two parents were homozygotes). In contrast, among the seven imperfect oak microsatellites tested in chestnut (Table 1), only two clearly amplified: QrZAG4 and QrZAG101; the former being mapped in chestnut, the latter being monomorphic in the parents of the chestnut progeny.

According to previous studies (Wang et al. 1994; Young et al. 2000; Kutil and Williams 2001; Morgante et al. 2002; Scotti et al. 2002), tri-nucleotide SSRs seemed to be better candidates for cross-transferability, whereas SSR loci containing imperfect and compound repeats proved to be less transferable across taxa (Kutil and Williams 2001). In addition, the tri-nucleotide repeats are frequently associated with functional regions of the genome, often clustered in regulatory genes (Young et al. 2000). Thus, serving a functional role in eukaryotic genomes, they are more likely to be conserved across taxa, even though less polymorphic (Kutil and Williams

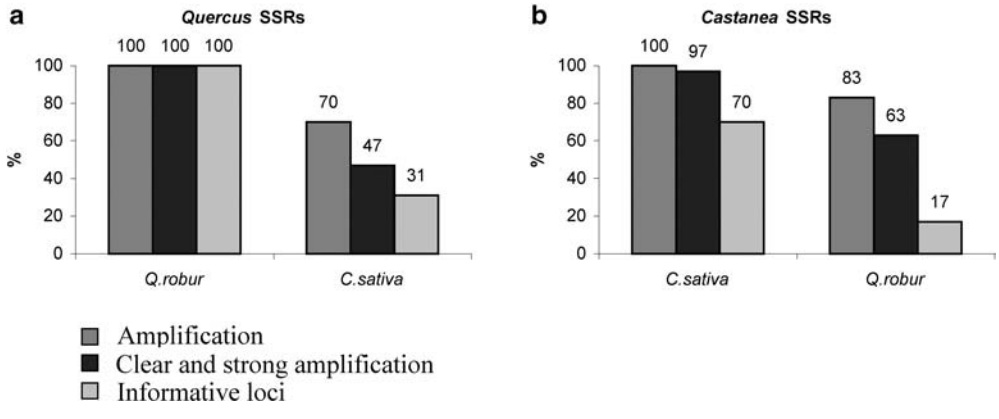

Fig. 1a, b Efficiency of SSR cross-transferability. Percentage of *Quercus* (**a**) and *Castanea* (**b**) microsatellite amplification and informative loci in the mapping progenies

Table 1 List of SSR loci tested. Amplification and segregation results

Locus[a]	Repeat motif	Amplification in *Q. robur* [b]	Amplification in *C. sativa* [b]	Segregation pattern-linkage group in *Q. robur* [c]	Segregation pattern-linkage group in *C. sativa* [c]
QpZAG1/5	$(GT)_{16}(GA)_9$	2	0	1:1:1:1-LG7	
QpZAG3/62	$(AG)_4$	2	0	1:1:1:1-LG10	
QpZAG3/64	$(AG)_{21}$	2	2	1:2:1-LG2	Not informative (M)
QpZAG9*	$(AG)_{12}$	2	2	1:1:1:1-LG7	1:1:1:1-LG5
QpZAG15	$(AG)_{23}$	2	2	1:2:1-LG9	1:1:1:1-LG9
QpZAG16	$(AG)_{19}$	2	2	1:1:1:1-LG6	Not informative (M)
QpZAG36	$(AG)_{19}$	2	2	1:1:1:1-LG2	1:1:1:1-LG1
QpZAG46	$(AG)_{13}$	2	0	1:1:1:1-LG2	
QpZAG58**	$(GA)_{34}$	2	2	1:1:1:1-LG5	1:1-LG4(L)1:1-LG10(Sb)
QpZAG102	$(AG)_5AA(AG)_{13}$	2	0	1:1:1:1-LG9	
QpZAG104	$(AG)_{16}AT(GA)_3$	2	1	1:1-LG2	
QpZAG108	$(AG)_{13}$	2	1	1:1:1:1-LG2	
QpZAG110	$(AG)_{15}$	2	2	1:1:1:1-LG8	1:1-LG7
QpZAG119	$(GA)_{24}$	2	2	1:1:1:1-LG2	1:1:1:1-LG1
QrZAG2**	$(TC)_{22}$	2	0	1:1:1:1-LG10	
QrZAG4	$(GA)_{37}GT(GA)_7$	2	2	1:1-LG11	1:2:1-LG3
QrZAG5*/**	$(TC)_{23}$	2	2	1:1:1:1-LG8(S) LG6(L)	1:1-LG2
QrZAG7	$(TC)_{17}$	2	2	1:1:1:1-LG2	1:1:1:1-LG1
QrZAG8	$(TC)_{31}$	2	1	1:1-LG12	
QrZAG11	$(TC)_{22}$	2	2	1:1:1:1-LG10	1:1:1:1-LG10
QrZAG15	$(GA)_{15}$	2	0	1:1:1:1-LG9	
QrZAG18**	$(GA)_{41}$	2	0	1:1-LG11	
QrZAG19	$(GA)_7$	2	0	1:1-LG9	
QrZAG20	$(TC)_{18}$	2	2	1:1-LG1	1:1:1:1-LG6
QrZAG25	$(GA)_{32}$	2	1	1:1:1:1-LG10	
QrZAG30	$(GA)_{26}$	2	0	1:1-LG12	
QrZAG31**	$(GA)_{31}$	2	2	1:1:1:1-LG9	1:1-LG9(S) 1:1-LG6(Lb)
QrZAG39	$(TC)_{34}$	2	0	1:1:1:1-LG5	
QrZAG49	$(GA)_{22}$	2	0	1:1:1:1-LG7	
QrZAG58	$(GA)_{20}$	2	0	1:1:1:1-LG2	
QrZAG65	$(TC)_{21}(TA)_{10}$	2	1	1:1:1:1-LG10	
QrZAG73	$(TC)_{43}$	2	1	1:1-LG5	
QrZAG74	$(GA)_{23}$	2	1	1:1:1:1-LG1	
QrZAG87	$(TC)_{20}$	2	1	1:1:1:1-LG2	
QrZAG90	$(GA)_{34}$	2	1	1:1-LG9	
QrZAG96	$(TC)_{20}$	2	2	1:1-LG10	1:1-LG10
QrZAG101	$(TC)_{20}(AC)_{15}$	2	2	1:1-LG1	Not informative (M)
QrZAG102	$(GA)_{29}$	2	0	1:1-LG3	
QrZAG103	$(TC)_{15}$	2	1	1:1-LG10	
QrZAG108	$(GA)_{19}(GGGA)_3$	2	0	1:1:1:1-LG7	
QrZAG111**	$(TC)_{19}$	2	0	1:1:1:1-LG11	
QrZAG112	$(GA)_{32}$	2	2	1:1-LG12	Not informative (P)
QrZAG121**	$(GA)_{23}$	2	2	1:1:1:1-LG9	Not informative (P)
MSQ4	$(GA)_{17}$	2	2	1:1:1:1-LG4	Not informative (M)
MSQ13	$(GA)_{14}$	2	2	1:1:1:1-LG6	Not informative (M)
MSQ16	-	2	1	1:1:1:1-LG9	
MicJ-AG22	-	2	2	1:1:1:1-LG9	Not informative (M)
quru-GA-0A01	$(GA)_{11}$	2	2	1:1-LG8	1:1:1:1-LG7
quru-GA-0C11	$(GA)_{15}$	2	2	1:1:1:1-LG1	1:1:1:1-LG6
quru-GA-0C19	$(GA)_{18}$	2	1	1:1-LG8	Not informative
quru-GA-0M05	$(GA)_{20}$	2	0	1:1:1:1-LG10	
quru-GA-0M07	$(GA)_{19}$	2	2	1:1:1:1-LG9	Not mapped yet
quru-GA-1F02	$(GA)_{15}$	2	2	1:1:1:1-LG6	Not informative (M)
EMCs1**	$(GGC)_3GGTTGC(GGC)_3$	2	2	1:1-LG7	1:1-LG11
EMCs2**	$(GGC)_7$	2	2	Not informative (M)	1:1-LG6
EMCs4**	$(GCG)_7$	2	2	Not informative (M)	1:1-LG7
EMCs10	$(CA)_8$	2	2	Not informative (M)	Not informative (P)
EMCs11	$(GGC)_6$	1	2		1:1:1:1-LG5
EMCs12	$(CCA)_5$	2	1	Not informative (M)	
EMCs13	$(CAG)_{10}$	2	2	1:1-LG1	Not informative (M)
EMCs14	$(GAG)_6/(GGC)_6$	2	2	Not informative (M)	1:2:1-LG5
EMCs15	$(CCA)_9$	2	2	1:1-LG9	1:1-LG9
EMCs17	$(CCAA)_5$	0	2		Not informative (P)
EMCs22	$(GA)_{19}$	1	2		1:1-LG2
EMCs32	$(GA)_{18}$	1	2		1:1:1:1-LG12
EMCs38	$(GA)_{31}$	1	2		1:1-LG4
CsCAT 1**	$(TG)_5TA(TG)_{24}$	1	2		1:1-LG8

Table 1 (continued)

Locus[a]	Repeat motif	Amplification in *Q. robur* [b]	Amplification in *C. sativa* [b]	Segregation pattern-linkage group in *Q. robur* [c]	Segregation pattern-linkage group in *C. sativa* [c]
CsCAT 2	$(AG)_{16}$	0	2		1:1:1:1-LG10
CsCAT 3	$(AG)_{20}$	0	2		1:1:1:1-LG12
CsCAT 5**	$(GA)_{20}$	2	2	Not informative (P)	1:1:1:1-LG8(S)-1:2:1-LG3(L)
CsCAT 6	$(AC)_{24}AT(AC)_4$	2	2	Not informative (P)	1:1:1:1-LG1
CsCAT 7**	$(TG)_8CG(TG)_4$	2	2	Not informative (P)	1:1-LG6
CsCAT 8	$(GT)_7(GA)_{20}$	0	2		1:1-LG6
CsCAT 13	$(CT)_{14}$	0	2		Not informative (P)
CsCAT 14	$(CA)_{22}$	2	2	1:1-LG4	1:1:1:1-LG2
CsCAT 15	$(TC)_{12}$	2	2	Not informative (P)	1:1:1:1-LG8
CsCAT 16	$(TC)_{20}$	2	2	Not informative (M)	1:1-LG6
CsCAT 17*	$(CA)_{19}A(CA)_2AA(CA)_3$	2	2	1:1-LG10	1:1:1:1-LG2
CsCAT 20	$(AC)_4(AT)_3A(AT)_3$	2	2	Not informative (M)	Not informative (M)
CsCAT 27	$(AC)_4$	2	2	Not informative (M)	Not informative (M)
CsCAT 32	$(AC)_4TC(AC)_3$	2	2	not informative (P)	Not informative (P)
CsCAT 39	$(AG)_{39}$	2	2	Not informative (P)	Not informative (P)
CsCAT 41	$(AG)_{20}$	1	2		1:1:1:1-LG8

[a] *Oak* SSRs: Qr (*Q. robur*) and Qp (*Q. petraea*) loci (Steinkellner et al. 1997a, Kampfer et al. 1998, Lexer 1999); MSQ loci (*Q.macrocarpa*) (Dow et al. 1995, Dow and Ashley 1996); MicJ-AG22 locus (Kawahara, personal communication); quru loci (*Q. rubra*) (Aldrich et al. 2002). *Chestnut (Castanea sativa)* SSRs: EMCs loci (Buck et al. 2003); CsCAT loci (Marinoni et al. 2003)
[b] 2: positive amplification, 1: light or unclear amplification, 0: no amplification
* Size of QpZAG9, QrZAG5 and CsCAT17 loci does not correspond in oak and chestnut
** QrZAG2, QrZAG5, QrZAG18, QrZAG111, QrZAG121 in oak, and EMCs1, EMCs2, EMCs4, CsCAT1, CsCAT5, CsCAT7, QpZAG58, QrZAG31 in chestnut, showed two or three amplification levels
[c] Not informative (P): locus showing polymorphic alleles in female and male parents but the parental configuration is not informative (M): monomorphic locus, homozygous parents for the same allele

2001; Rajora et al. 2001; Sheperd et al. 2002). This could explain the higher amplification efficiency observed for chestnut microsatellites. A possible explanation for imperfect and compound repeats lowering cross-transferability can be drawn from an intriguing hypothesis by Taylor et al. (1999). Taylor and co-workers hypothesized that interruptions within the repeat motif are responsible for its monomorphism and shortening, two processes which will lead to the "death" of a SSR motif. A first interruption of the repeat motif would stabilize the SSR motif because the slipped-strand mispairing will occur more infrequently; this could explain the lower polymorphism at these loci. Later deletions at the repeat motif would cause the shortening and thus the "death" of the microsatellite; this possibly could explain the lower conservation of the locus across species as well as the lower polymorphism (Kruglyak et al. 1998; Kutil and Williams 2001).

SSR mapping and linkage groups alignment

Microsatellite loci exhibiting Mendelian segregation in both species were integrated into *Q. robur* and *C. sativa* genetic linkage maps. Figure 2 shows all SSR loci mapped in both species and their position along the aligned linkage groups. Sixty SSR loci were distributed over the 12 oak linkage groups. Most of the microsatellite loci have been already mapped in the *Q. robur* linkage map (Barreneche et al. 1998; Barreneche et al., in preparation). Here five new microsatellites, cross-transferred from chestnut, were integrated into the oak map: EMCs1 (Q7), EMCs13 (Q1), EMCs15 (Q9), CsCAT14 (Q4) and CsCAT17 (Q10). Thirty nine microsatellite loci were integrated into the *C. sativa* genetic linkage map (Fig. 2), 22 of which were specifically developed in chestnut, and 17 were transferred from oak. At least one microsatellite locus was mapped on each linkage group of both species, confirming the wide distribution of SSR loci throughout their genomes. Due to their random distribution throughout plant genomes, microsatellites can be efficiently exploited in macro-synteny projects providing anchor loci to align linkage groups. Among the SSR loci analysed, 19 loci were mapped in both *Q. robur* and *C. sativa*. These loci, in addition to *Dia* (Diaphorase), previously mapped in oak (Barreneche et al. 1998) and chestnut (Casasoli et al. 2001) and the 5SrDNA loci, made it possible to perform a preliminary mapping comparison between the two species. As shown in Fig. 2, seven homeologous groups were detected on the basis of pairs or triplets of common markers. Two linkage groups were matched using a single but orthologous (based on sequence information) microsatellite marker. In two cases three common loci were mapped in homeologous linkage groups. In both cases the order of the markers was conserved (Q2-C1, Q9-C9 see Fig. 2). This corresponding order observed among triplets of microsatellites supports the usefulness of these markers as anchor loci for comparative mapping within the Fagaceae family.

In the source species, most of the microsatellites showed a unique amplification product. In some cases, two or three co-segregating amplification products were

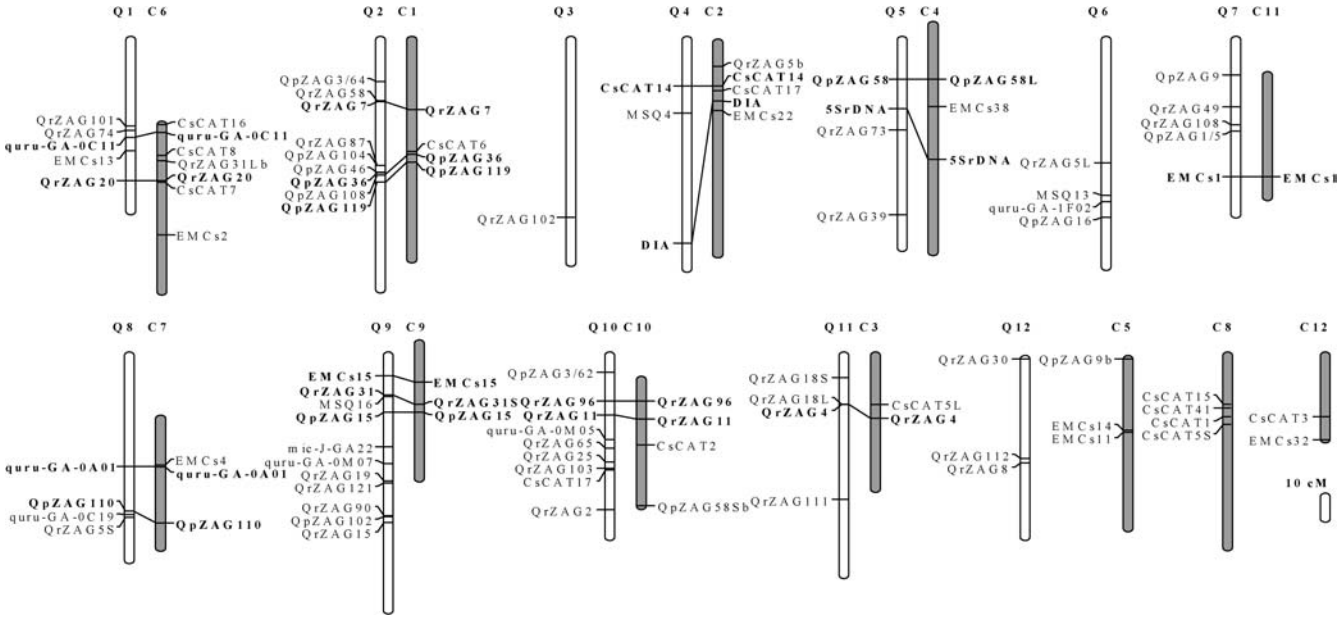

Fig. 2 Microsatellite markers integrated into *Q. robur* and *C. sativa* genetic linkage maps. *Open and solid bars* denote oak and chestnut linkage groups, respectively. Linkage groups are named as in Barreneche et al. (1998) and Casasoli et al. (2001). Oak linkage groups are taken as a reference and arranged in sequence from Q1 to Q12. Nine chestnut linkage groups, aligned with the corresponding oak linkage groups, are reported on the right. The three remaining chestnut linkage groups are reported after oak linkage groups. The figure was drawn using MapChart software (Voorrips 2002)

observed (QrZAG2, QrZAG18, QrZAG111, and QrZAG121 in oak, and EMCs1, EMCs2, EMCs4, CsCAT1 and CsCAT7 in chestnut). A different segregation pattern between the two amplification products was observed for QrZAG5 in oak and CsCAT5 in chestnut. In general, cross-amplified loci showed a single band corresponding to the size range of the source allele. Nevertheless, primer pairs for QpZAG9, QrZAG5 and CsCAT17 loci in chestnut and oak amplified fragments of different size. None of the two products amplified in oak by QrZAG5 primer pairs corresponded to the product amplified in chestnut. These three loci did not map in corresponding linkage groups in the two species. Therefore, they were called QpZAG9b, QrZAG5b and CsCAT17b in the non-source species. Furthermore QpZAG58 and QrZAG31 showed an additional band in chestnut not present in the source species. Both in oak and in chestnut, the SSR primer pairs producing two fragments that did not co-segregate amplified two unlinked loci and they were marked as "large" (L) and "small" (S) in relation to the band size on the gel. Thus, QrZAG5S (Q8) corresponded to smaller fragments amplified by these primer pairs and QrZAG5L (Q6) to larger fragments.

A similar situation was found in chestnut for CsCAT5: CsCAT5L mapped on the linkage group C3, whereas CsCAT5S was on C8. As for the QpZAG58 and QrZAG31 microsatellites, two independent loci were identified in chestnut. QpZAG58L and QrZAG31S mapped on linkage groups C4 and Q9, respectively. Both groups corresponded to the Q5 and Q9 oak groups, where the putative ortholgous microsatellites were located. On the other hand, QpZAG58S and QrZAG31L mapped on C10 and C6 chestnut groups, and were called b to point out the lack of orthology with the source species.

These interesting mapping results provide evidence that microsatellite loci can occur as gene families and that the discovery of paralogous loci is possible when the same microsatellites are amplified in different taxa. Multi-copy microsatellites have been already observed (Fisher et al. 1998; Peakall et al. 1998; Karhu et al. 2000). Moreover, Röder et al. (1998) reported that microsatellites can amplify non-homologous loci in different genomes. These microsatellites can represent a drawback in using SSR markers for comparative mapping, and sequence analysis would then be necessary to demonstrate orthology. Nevertheless, multi-copy microsatellites provide particular insights in the evolution processes of these markers. Obtaining two distinct loci by using the same primer pairs could also be advantageous in mapping studies from the practical point of view.

SSR sequence comparison

The nucleotide sequences of 17 SSR loci, mapped in both species, was compared. One allele from each locus that amplified in the non-source species was cloned and sequenced. The sequence was then aligned with that of the source species. Table 2 shows the results obtained when priming sites, flanking regions and repeat motifs were compared. Fourteen microsatellites showed corresponding allele size, conserved priming sites and a high sequence identity at the flanking regions (from 86.3% to

Table 2 Sequence analysis of microsatellite loci mapped in *Q. robur* and *C. sativa*

Locus	Allele size in oak (bp)[a]	Allele size in chestnut (bp)[a]	Sequence identity at the flanking regions (%)[b]	Linkage group in oak and chestnut	Repeat motif in the source and non-source species
QpZAG9	192 (182–210)	274	65.2	Q7≠C5	$(AG)_{11}\rightarrow(AG)_3AAGAGT(GA)_9$ [c]
QpZAG15	136 (108–152)	113	91.1	Q9≡C9	$(AG)_{23}\rightarrow(AG)_{12}$
QpZAG36	226 (210–236)	209	97.4	Q2≡C1	$(GA)_{17}\rightarrow(GA)_9$
QpZAG58	158 (150–210)	175[d]	94.4	Q5≡C4[d]	$(GA)_{35}\rightarrow G(A)_5G(A)_4(GA)_{16}(N)_{29}(GA)_4AA(GA)_2$
QpZAG110	221 (206–252)	222	95.7	Q8≡C7	$(GA)_{15}\rightarrow(GA)_{18}$
QpZAG119	84 (64–98)	98	92.5	Q2≡C1	$(GA)_{24}\rightarrow(AT)_3(GA)_{27}$
QrZAG4	191 (116–158)	116	88.3	Q11≡C3	$(GA)_{37}GT(GA)_7\rightarrow(GA)_7CA(GA)_3$
QrZAG5	248 (225–244)	142	45.2[e]	Q8≠C2	$(TC)_{23}\rightarrow(GAAAA)_3$
QrZAG7	149 (115–153)	124	86.3	Q2≡C1	$(TC)_{17}\rightarrow(TC)_{10}$
QrZAG11	273 (238–267)	260	93.1	Q10≡C10	$(TC)_{22}\rightarrow(TC)_{17}$
QrZAG20	179 (160–200)	176	97.2	Q1≡C6	$(TC)_{18}\rightarrow(TC)_{17}$
QrZAG31	190 (150–190)	145[f]	86.3	Q9≡C9[f]	$(GA)_{31}\rightarrow(GA)_3$
QrZAG96	172 (135–194)	166	94.8	Q10≡C10	$(TC)_{20}\rightarrow(TC)_{16}$
EMCs1	168	183 (170–190)	94.2	Q7≡C11	$(GGC)_3GGTTGC(GGC)_3\rightarrow(GGC)_3$
EMCs15	86	95 (90–110)	95.6	Q9≡C9	$(CCA)_9\rightarrow(CCA)_7$
CsCAT14	153	165 (135–163)	86.4	Q4≡C2	$(CA)_{22}\rightarrow(CA)_{14}(TA)_7$
CsCAT17	114	152 (145–189)	77.4	Q10≠C2	$(CA)_{19}A(CA)_2AA(CA)_3\rightarrow(CA)_4A(CA)_2AACA$

[a] Size of the sequenced allele and size range in the source species (from literature) are reported
[b] Sequence identity at the priming sites has been always observed
[c] In addition to this repeat another SSR motif, $(TAA)_6$, was present in chestnut
[d] An additional fragment of 99 bp was present in chestnut; it has been demonstrated to be an independent locus mapping on the C10
[e] Overall sequence identity
[f] An additional fragment of 184 bp was present in chestnut; it has been demonstrated to be an independent locus mapping on the C6

97.4%). In addition, in most cases, the source species repeat motif was present in the non-source species. This result is strong evidence that these loci are orthologous and that they can be used as reliable anchor tags for comparative mapping studies within the Fagaceae family.

However, the overall picture that can be drawn from the sequencing comparisons (Table 2) reflects the complexity of microsatellite evolution. Caution is therefore needed when using microsatellites transferred among related taxa, especially for population genetics studies. Despite the high sequence identity at the flanking regions (from 86.3% to 97.4%) observed for 14 loci mapped in corresponding linkage groups, the repeat motif in the non-source species was in some cases shortened and/or modified (imperfect and/or compound). Although further analyses, like sequencing alleles in a broad spectrum of populations, are needed to confirm the shortening or modification of the repeat motif in the non-source species, this is a clear trend already observed in several studies (Van Treunen et al. 1997; Peakall et al. 1998; Di Gaspero et al. 2000; Kutil and Williams 2001). In four cases (QrZAG4, QrZAG7, QrZAG31 and CsCAT14) short 7-bp, 13-bp, 10-bp and 4-bp deletions, respectively, were present at the flanking regions in the non-source species. Except for QrZAG4, the deletion was just at one end of the microsatellite motif and accounted for the lower sequence identity between oak and chestnut. This result suggests that polymorphism in the non-source species can arise not only from the repeat motif but also from mutations at the flanking regions. Homoplasy due to indels in the flanking regions can occur. Therefore, these loci have to be considered carefully for comparisons among related taxa. Molecular mechanisms, which are involved in microsatellite evolution, are quite variable depending on the nature of the repeat motif (Orti et al. 1997). Mutation rates can differ between simple and complex microsatellites as well as between the repeat motif and the flanking regions, and also slippage rates vary between taxonomic groups (Kruglyak et al. 1998). Therefore, estimations of population genetics parameters can be biased by assuming only the simple stepwise mutation model for the evolution of microsatellite loci (Van Treunen et al. 1997; Karhu et al. 2000).

Examining the persistence and changes of the microsatellite loci transferred across taxa will probably help to shed light on the molecular evolution of these loci. QpZAG58 and QrZAG31 can be considered as two interesting cases. In chestnut both loci amplified two fragments, each identifying a different locus: QpZAG58L (C4), QpZAG58Sb (C10) and QrZAG31Lb (C6), QrZAG31S (C9). Alleles from these four loci were cloned and sequenced. QpZAG58L and QrZAG31S showed homeology with the corresponding loci in oak. Despite the conservation of primer sequences, QpZAG58Sb and QrZAG31Lb loci showed indels and a lower sequence homology at the flanking regions (78% and 54%, respectively) when compared with both QpZAG58, QrZAG31 oak loci and QpZAG58L, QrZAG31S chestnut loci. As for the repeat motif, the perfect di-nucleotide repeat present in oak at locus QpZAG58 was shortened and interrupted in the orthologous chestnut locus, whereas locus QpZAG58Sb contained a mono-nucleotide stretch: $(A)_9$ and $(A)_{11}$ in the lower and higher allele, respectively. The QrZAG31Lb locus in the sequenced allele showed a shorter motif than the locus QrZAG31S, orthologous to the oak one. It is

likely that both loci were involved in duplication events after the divergence between *Quercus* and *Castanea* genera with the duplicated locus evolving independently from the original one. The repeat motif changed from a perfect di-nucleotide stretch in oak to an imperfect and interrupted motif in the QpZAG58L orthologous locus in chestnut, to a short mono-nucleotide repeat in QpZAG58Sb. The locus QrZAG31, which already exhibited a short motif in chestnut, was shortened further in the duplicated locus, probably not representing an actual microsatellite in chestnut.

Three microsatellite loci (QpZAG9, QrZAG5 and CsCAT17), which amplified fragments of different size and did not map in corresponding linkage groups, showed the lowest sequence identity at the flanking regions: 65.2%, 45.2% and 77.4%, respectively. QpZAG9 was 191 bp and 276 bp in length in oak and chestnut, respectively. A large insertion was present in chestnut and the repeat motif was shorter and imperfect. Within the insertion in the chestnut allele, an additional stretch of $(TAA)_6$ was present, which was not found in oak. The QrZAG5 locus was 247 bp in length in oak and 142 bp in chestnut; a penta-nucleotide repeat was present in chestnut. The sizes of the CsCAT17 locus were 114 bp and 152 bp in oak and chestnut, respectively. Despite the difference in size due to the shorter repeat motif in oak, the sequence identity at the flanking regions was quite high (77,4%). QpZAG9, QrZAG5 and CsCAT17 might represent paralogous loci.

Conclusions and perspectives

This study is the first attempt towards a comparative genetic mapping analysis between *Q. robur* and *C. sativa*. Thanks to the genetic linkage maps established for both species and the cross-genera transferability of microsatellite markers, nine out of the 12 linkage groups could be aligned. Our findings suggest a conservation of macrosynteny between *Q. robur* and *C. sativa*. Microsatellite markers, mapped both in *Q. robur* and *C. sativa*, are the first anchor loci available for comparative mapping within the Fagaceae family. In addition to these markers, ESTs will soon be integrated in both linkage maps in order to align the 12 linkage groups present in the two species and to provide a reliable consensus framework for comparative QTL mapping. Furthermore, analyses at the DNA sequence level of common microsatellites across different species belonging to the Fagaceae family will enable insights on the molecular evolution of SSR motifs. Our results support the idea that microsatellites behave heterogeneously depending on the nature of the repeat motif. The level of polymorphism and conservation across species seem to be related to the type of repeat motif. Consequently, as suggested by Peakall et al. (1998), it may be possible to find specific SSR loci to address different evolutionary questions across species.

Acknowledgements We thank S. Mariette for helpful suggestions and fruitful discussions concerning microsatellite analysis; D. Chagné for critical comments on this manuscript; J. Romero-Severson for sharing the *Quercus rubra* primers (quru primers). This work was supported by the EU research project "CASCADE"(EVK2-CT-1999–00006). The authors declare that the experiments conducted for this publication comply with the current laws of France, Italy and UK.

References

Aldrich PR, Michler CH, Sun W, Romero-Severson J (2002) Microsatellite markers for Northern red oak (Fagaceae: *Quercus rubra*). Mol Ecol Notes 2:472–474

Barreneche T, Bodenes C, Lexer C, Trontin JF, Fluch S, Streiff R, Plomion C, Roussel G, Steinkellner H, Burg K, Favre JM, Glössl J, Kremer A (1998) A genetic linkage map of *Quercus robur* L. (pedunculate oak) based on RAPD, SCAR, microsatellite, minisatellite, isozyme and 5S rDNA markers. Theor Appl Genet 97:1090–1103

Botta R, Akkak A, Marioni D, Bounous G, Kampfer S, Steinkellner H, Lexer C (1999) Evaluation of microsatellite markers for characterizing chestnut cultivars. Acta Hort 494:277–282

Brown GR, Kadel III EE, Bassoni DL, Kiehne KL, Temesgen B, van Buijtenen JP, Sewell MM, Marshall KA, Neale DB (2001) Anchored reference loci in loblolly pine (*Pinus taeda* L.) for integrating pine genomics. Genetics 159:799–809

Buck E, Hadonou M, James C, Blakesley D, Russell K (2003) Isolation and characterisation of polymorphic microsatellites in European chestnut (*Castanea sativa* Mill.). Mol Ecol Notes 3:239–241

Byrne M, Marquez-Garcia MI, Uren T, Smith DS, Moran GF (1996) Conservation and genetic diversity of microsatellite loci in the genus *Eucalyptus*. Aust J Bot 44:331–341

Casasoli M, Mattioni C, Cherubini M, Villani F (2001) A genetic linkage map of European chestnut (*Castanea sativa* Mill.) based on RAPD, ISSR and isozyme markers. Theor Appl Genet 102:1190–1199

Cervera MT, Storme V, Ivens B, Gusmão J, Liu BH, Hostyn V, Slycken JV, van Montagu M, Boerjan W (2001) Dense genetic linkage maps of three populus species (*Populus deltoids*, *P.nigra* and *P. trichocarpa*) based on AFLP and microsatellite markers. Genetics 158:787–809

Chagné D, Brown G, Lalanne C, Madur D, Pot D, Neale D, Plomion C (2003) Comparative genome and QTL mapping between maritime and loblolly pines. Mol Breed (in press)

Danin-Poleg Y, Reis N, Baudracco-Arnas S, Pitrat M, Staub JE, Oliver M, Arus P, deVicente CM, Katzir N (2000) Simple sequence repeats in *Cucumis* mapping and map merging. Genome 43:963–974

Devey ME, Sewell MM, Uren TL, Neale DB (1999) Comparative mapping in loblolly and radiata pine using RFLP and microsatellite markers. Theor Appl Genet 99:656–662

Devos KM, Gale MD (2000) Genome relationships: the grass model in current research. Plant Cell 12:637–646

Di Gaspero G, Peterlunger E, Testolin R, Edwards KJ, Cipriani G (2000) Conservation of microsatellite loci within the genus *Vitis*. Theor Appl Genet 101:301–308

Dirlewanger E, Cosson P, Tavaud M, Aranzana MJ, Poizat C, Zanetto A, Arus P, Laigret F (2002) Development of microsatellite markers in peach (*Prunus persica* L. Batsch) and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor Appl Genet 105:127–138

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

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

Fisher PJ, Richardson TE, Gardner RC (1998) Characteristics of single- and multi-copy microsatellites from *Pinus radiata*. Theor Appl Genet 96:969–979

Freeling M (2001) Grasses as a single genetic system. Reassessment 2001. Plant Physiol 125:1191–1197

Guyomarc'h H, Sourdille P, Charmet G, Edwards KJ, Bernard M (2002) Characterisation of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D-genome of bread wheat. Theor Appl Genet 104:1164–1172

Karhu A, Dieterich JH, Savolainen O (2000) Rapid expansion of microsatellite sequences in Pines. Mol Biol Evol 17:259–265

Kampfer S, Lexer C, Glössl J, Steinkellner H (1998) Characterization of (GA)*n* microsatellite loci from *Quercus robur*. Hereditas 129:183–186

Kijas JMH, Fowler JCS, Thomas MR (1995) An evaluation of sequence tagged microsatellite site markers for genetic analysis within *Citrus* and related species. Genome 38:349–355

Kruglyak S, Durrett RT, Schug MD, Aquadro CF (1998) Equilibrium distributions of microsatellite repeat length resulting from a balance between slippage events and point mutations. Proc Natl Acad Sci USA 95:10774–10778

Kutil BL, Williams CG (2001) Triplet-repeat microsatellite shared among hard and soft pines. J Hered 92:327–332

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

Lexer 1999 Application of microsatellite markers to genetic map construction and genetic analysis of forest seed material in Oaks (*Quercus* spp). PhD Thesis, Universität für Bodenkultur, Vienna, Austria

Manos PS, Steele KP (1997) Phylogenetic analysis of "higher" Hamamelidaea based on plastid sequence data. Am J Bot 84:1407–1419

Manos PS, Zhe-Kun Zhou, Cannon CH (2001) Systematics of Fagaceae: phylogenetic tests of reproductive trait evolution. Int J Plant Sci 162:1361–1379

Marinoni D, Akkak A, Bounous G, Edwards KJ, Botta R (2003) Development and characterization of microsatellite markers in *Castanea sativa* (Mill). Mol Breed 11:127–136

Marques CM, Brondani RPV, Grattapaglia D, Sederoff R (2002) Conservation and synteny of SSR loci and QTLs for vegetative propagation in four *Eucalyptus* species. Theor Appl Genet 105:474–478

Matsuoka Y, Mitchell SE, Kresovich S, Goodman M, Doebley J (2002) Microsatellites in *Zea*-variability, patterns of mutations, and use for evolutionary studies. Theor Appl Genet 104:436–450

Morgante M, Hanafey M, Powell W (2002) Microsatellites are preferentially associated with non-repetitive DNA in plant genomes. Nature Genet 30:194–200

Nadeau JH, Sankoff D (1998) Counting on comparative maps. Trends Genet 14:495–501

Orti G, Pearse DE, Avise JC (1997) Phylogenetic assessment of length variation at a microsatellite locus. Proc Natl Acad Sci USA 94:10745–10749

Paterson AH, Bowers JE, Burow MD, Draye X, Elsik CG, Jiang CX, Katsar CS, Lan TH, Lin YR, Ming R, Wright RJ (2000) Comparative genomics of plant chromosomes. Plant Cell 12:1523–1539

Peakall R, Gilmore S, Keys W, Morgante M, Rafalski A (1998) Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants. Mol Biol Evol 15:1275–1287

Plieske J, Struss D (2001) Microsatellite markers for genome analysis in *Brassica*. I. Development in *Brassica napus* and abundance in *Brassicaceae* species. Theor Appl Genet 102:689–694

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci 1:215–222

Rajora OP, Rahman MH, Dayanandan S, Mosseler A (2001) Isolation, characterization, inheritance and linkage of microsatellite DNA markers in white spruce (*Picea glauca*) and their usefulness in other spruce species. Mol Gen Genet 264:871–882

Röder MS, Korzun V, Wendehake K, Planschke J, Tixier M, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rossetto M, McNally J, Henry RJ (2002) Evaluating the potential of SSR flanking regions for examining taxonomic relationships in the Vitaceae. Theor Appl Genet 104:61–66

Schmidt R (2000) Synteny: recent advances and future prospects. Curr Plant Biol 3:97–102

Scotti I, Magni F, Paglia GP, Morgante M (2002) Trinucleotide microsatellites in Norway spruce (*Picea abies*): their features and the development of molecular markers. Theor Appl Genet 106:40–50

Sewell MM, Sherman BK, Neale DB (1999) A consensus map for loblolly pine (*Pinus taeda* L.). I. Construction and integration of individual linkage maps from two outbred three-generation pedigrees. Genetics 151:321–330

Sheperd M, Cross M, Maguire TL, Dieters MJ, Williams CG, Henry RJ (2002) Transpecific microsatellites for hard pines. Theor Appl Genet 104:819–827

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

Taylor JS, Durkin JMH, Breden F (1999) The death of a microsatellite: a phylogenetic perspective on microsatellite interruptions. Mol Biol Evol 16:567–572

Van Treuren R, Kuittinen H, Kärkkäinen K, Baena-Gonzalez E, Savolainen O (1997) Evolution of microsatellites in *Arabis petraea* and *Arabis lyrata*, outcrossing relatives of *Arabidopsis thaliana*. Mol Biol Evol 14:220–229

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Wang Z, Weber JL, Zhong G, Tanksley SD (1994) Survey of plant short tandem DNA repeats. Theor Appl Genet 88:1-6

Whitton J, Rieseberg LH, Ungerer MC (1997) Microsatellite loci are not conserved across the Asteraceae. Mol Biol Evol 14:204–209

Yamamoto T, Kimura T, Sawamura Y, Kotobuki K, Ban Y, Hayashi T, Matsuta N (2001) SSRs isolated from apple can identify polymorphism and genetic diversity in pear. Theor Appl Genet 102:865–870

Young ET, Sloan JS, van Riper K (2000) Trinucleotide repeats are clustered in regulatory genes in *Saccharomyces cerevisiae*. Genetics 154:1053–1068

Zoldos V, Papes D, Brown SC, Panaud O, Siljak-Yakovlev S (1998) Genome size and base composition of seven *Quercus* species: inter- and intra-population variation. Genome 41:162–168