Comparison of Quantitative Trait Loci for Adaptive Traits Between Oak and Chestnut Based on an Expressed Sequence Tag Consensus Map

Manuela Casasoli,[*,1] **Jeremy Derory,**[*,1] **Caroline Morera-Dutrey,**[*] **Oliver Brendel,**[†] **Ilga Porth,**[‡] **Jean-Marc Guehl,**[†] **Fiorella Villani**[§] **and Antoine Kremer**[*,2]

*INRA, UMR Biodiversité Gènes et Ecosystèmes, 33612 Cestas Cedex, France, †INRA-UHP, UMR Ecologie et Ecophysiologie Forestières, Centre de Nancy 54280 Champenoux, France, §CNR, Istituto di Biologia Agroambientale e Forestale, 05010 Porano (TR), Italy and ‡ARC Seibersdorf Research, Biotechnology Unit, A-2444 Seibersdorf, Austria

ABSTRACT

A comparative genetic and QTL mapping was performed between *Quercus robur* L. and *Castanea sativa* Mill., two major forest tree species belonging to the Fagaceae family. Oak EST-derived markers (STSs) were used to align the 12 linkage groups of the two species. Fifty-one and 45 STSs were mapped in oak and chestnut, respectively. These STSs, added to SSR markers previously mapped in both species, provided a total number of 55 orthologous molecular markers for comparative mapping within the Fagaceae family. Homeologous genomic regions identified between oak and chestnut allowed us to compare QTL positions for three important adaptive traits. Colocation of the QTL controlling the timing of bud burst was significant between the two species. However, conservation of QTL for height growth was not supported by statistical tests. No QTL for carbon isotope discrimination was conserved between the two species. Putative candidate genes for bud burst can be identified on the basis of colocations between EST-derived markers and QTL.

THE genetic basis and evolution of adaptive traits that evolve in response to selection are still largely unknown. Because of the prominent neo-Darwinian view that pointed out the major role of mutations with small effects (infinitesimal model) (FISHER 1930), the study of the genetic basis of adaptation has received little attention until recently (ORR and COYNE 1992). At the same time, BARTON and TURELLI (1989) reviewed theories and experimental results on evolutionary quantitative genetics. Most of the quantitative traits can evolve in response to selection because the additive variance represents a significant part of their phenotypic variance. Nevertheless, the number of loci involved, the magnitude of their effects, the type of gene action (additivity, dominance, epistasis, and pleiotropy), and the existence of genotype-by-environment interaction effect remain unknown for many traits of adaptive significance. In particular, the number of their underlying loci and the magnitude of the allelic effects are key factors of the evolution of adaptive traits. ORR and COYNE (1992) showed that Fisher's model was incomplete and that mutations with large effects were sometimes involved in adaptation. Indeed, on the basis of ORR's (1998) recent theoretical work, adaptation seems to involve many loci of small and moderate effect but also a few loci of large effect, giving rise to an L-shaped distribution of factors fixed during adaptive evolution. Moreover, from an evolutionary point of view, in a complex organism, adaptation would occur mainly with mutations of intermediate effects that permit it to achieve an appropriate tradeoff between an acceptable probability of fixation and an acceptable probability to be favorable (ORR 2000).

Quantitative trait locus (QTL) studies were expected to provide new insights into fundamental questions regarding the genetic basis of quantitative traits and adaptation (MITCHELL-OLDS 1995). Despite some biases in QTL analysis, such as the underestimation of the number of loci involved and the overestimation of their effects, important clues on quantitative genetic variation were obtained in animal and plant QTL studies. An L-shaped distribution of QTL effects was often observed and cloning of some QTL clearly showed that individual genes with large effects could be responsible for a proportion of quantitative variation, according to Orr's model. At the same time, several interacting polymorphisms in coding and noncoding regions of a single gene, as well as epistatic interactions among alleles at different loci, seemed to be of great importance in determining quantitative variation. Moreover, mutation and balancing selection were shown to play a major role in the maintenance of quantitative trait variation (BARTON and KEIGHTLEY 2002). Especially in the case of adaptation, QTL studies have been applied to elucidate some of the basic questions: number, effect, and stability of loci involved (WU 1998; HURME *et al.* 2000; JERMSTAD

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[1]These authors contributed equally to this work.

[2]*Corresponding author:* INRA, UMR BIOGECO Equipe de Génétique, 69 Rte. d'Arcachon, 33612 Cestas Cedex, France. E-mail: antoine.kremer@pierroton.inra.fr

et al. 2003; Verhoeven *et al.* 2004). Furthermore, comparative QTL mapping for adaptive traits among populations and species can lead to the identification of genomic regions conserved over a long evolutionary period and therefore potentially harboring genes of great importance for the adaptive process. At the same time, nonconserved QTL could be responsible for local adaptation or interspecific differentiation.

We compared the genetic architecture of several important adaptive traits in two widely distributed forest tree species in Europe (oaks and chestnut). A long-term project aimed at identifying genomic regions and genes responsible for different adaptive traits in forest trees has been initiated in European white oaks (*Quercus robur* L. and *Q. petraea* L.) and European chestnut (*Castanea sativa* Mill.). Both Quercus and Castanea genera belong to the Fagaceae family and are phylogenetically closely related, and the two genera diverged ∼60 million years ago (Manos and Steele 1997; Manos *et al.* 2001). Both genera are of great ecological and economical importance in Europe and offer an interesting example of natural (both Quercus and Castanea), cultivated (Castanea), and naturally hybridizing species (*Q. robur* and *Q. petraea*). QTL for three important adaptive traits, *i.e.*, the timing of bud burst, carbon isotope discrimination, and height growth, were detected in *Q. robur* and *C. sativa* (Casasoli *et al.* 2004; Scotti-Saintagne *et al.* 2004; O. Brendel, unpublished results) and their location on the genetic map is compared in this article.

To compare QTL positions between Quercus and Castanea, linkage groups were aligned to identify orthologous genomic regions. In previous work, seven linkage groups were aligned between these two species using orthologous microsatellite markers and two others were tentatively matched on the basis of a single common marker (Barreneche *et al.* 2004). Recent availability of expressed sequence tag (EST) sequence information in oak species (Porth *et al.* 2005a,b; J. Derory, P. Leger, V. Garcia, J. Schaeffer, M. T. Hauser, F. Salin, C. Luschnig, C. Plomion, J. Glössl and A. Kremer, unpublished results) gave us the opportunity to exploit this resource for comparative mapping. Indeed, EST-derived markers are ideal anchor points for this purpose. They are coding sequences and, consequently, usually easily transferable between species. A high number of ESTs are often available and they can represent expressional and/or functional candidate genes. Moreover, ESTs from J. Derory, P. Leger, V. Garcia, J. Schaeffer, M. T. Hauser, F. Salin, C. Luschnig, C. Plomion, J. Glössl and A. Kremer (unpublished results) were developed within the framework of a transcriptomic analysis of bud burst in *Q. petraea*. Therefore, colocations between these ESTs and QTL controlling timing of bud burst could provide putative candidate genes for this trait. The long-term objective of this project is to provide molecular tools for monitoring adaptive variation in natural populations of forest trees as a starting point to understand the genetic mechanisms of adaptive differentiation within and between populations and species.

The aims of this work were to: (1) align the 12 linkage groups of *Q. robur* and *C. sativa* genetic maps using EST-derived markers in addition to the previous existing orthologous markers and (2) compare the number, effect, and position of QTL controlling three different adaptive traits between the two species. Finally we intended to use the comparison of QTL mapping in the two genera to identify positional candidate genes for the phenotypic traits analyzed.

MATERIALS AND METHODS

EST resources: A total of 92 ESTs, developed in oak either by Porth *et al.* (2005a) or by J. Derory, P. Leger, V. Garcia, J. Schaeffer, M. T. Hauser, F. Salin, C. Luschnig, C. Plomion, J. Glössl and A. Kremer (unpublished results), were used in this work. Ten ESTs from Porth *et al.*, already mapped in oak (Porth *et al.* 2005b), were amplified and mapped in chestnut. In addition to these already developed sequence-tagged sites (STSs), 82 EST sequences from the database at http://cbi.labri.fr/outils/SAM/COMPLETE/index.php, project "*Quercus petraea* bud ESTs" (J. Derory, P. Leger, V. Garcia, J. Schaeffer, M. T. Hauser, F. Salin, C. Luschnig, C. Plomion, J. Glössl and A. Kremer, unpublished results), were used to develop molecular markers for mapping both in oak and in chestnut.

Amplification of EST-derived markers (STSs): ESTs from Porth *et al.* (2005a,b) were amplified in chestnut using primer sequences developed by these authors. About 100 EST sequences from Derory *et al.* (2005), corresponding mainly to proteins with known functions, were used to recover homologous sequences by using the BLAST procedure (http://www.ncbi.nlm.nih.gov/blast/) (Altschul *et al.* 1990). These homologous sequences were aligned with the corresponding oak EST (CLUSTALW, http://www.ebi.ac.uk/clustalw/) to identify the most conserved regions. Primers were designed using the Primer3 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi), following three main criteria: (1) primer sequences were selected in a conserved region of the DNA fragment; (2) the expected size of the amplified fragment was fixed between 100 and 450 bp; and (3) the melting temperature was fixed between 55° and 60°. Following these criteria, 82 primer pairs were designed. Both amplified and mapped ESTs, primer sequences, and expected fragment sizes are reported in Table 1. For five of these ESTs (07B10, 08A01, 08B04, 08C11, and 08G04) primers pairs had been already designed in oak by J. Derory, P. Leger, V. Garcia, J. Schaeffer, M. T. Hauser, F. Salin, C. Luschnig, C. Plomion, J. Glössl and A. Kremer (unpublished results) and used in this work. Molecular markers derived from amplification of genomic DNA using primers designed on an EST are referred to from now on as STSs.

A touchdown PCR protocol (Don *et al.* 1991) was used to amplify the STS markers. The reaction mixture (12.5 μl) contained: 1.25 μl of 10× PCR buffer (Invitrogen, Carlsbad, CA), 2 mм of $MgCl_2$, 200 μм of each dNTP, 0.2 μм of each primer, 100 μg/ml of BSA, 5–10 ng of genomic DNA, and 0.4 unit of *Taq* polymerase (Invitrogen). Amplifications were performed using a GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, CT) under the following conditions: 10 min at 94°; 10 touchdown cycles of 45 sec at 94°, 45 sec at the annealing temperature decreasing 0.5° each cycle; 1 min at 72°; and

finally 25 or 30 cycles of 45 sec at 94°, 45 sec at the annealing temperature, 1 min at 72°, and a final extension of 10 min at 72°. PCR conditions were optimized to obtain a single amplified band. Annealing temperature and number of cycles for each STS are reported in Table 1. Quality and size of the amplified products were checked on a 1.5% agarose gel.

STS polymorphism detection, mapping, and sequencing: The 10 ESTs developed by PORTH *et al.* (2005a,b), had been previously mapped in oak using SNP genotyping techniques (SYVÄNEN 2001). Denaturing gradient gel electrophoresis (DGGE) (MYERS *et al.* 1987) and single-strand conformation polymorphism (SSCP) (ORITA *et al.* 1989) methods were used to detect polymorphisms in the STS markers developed in this study. Experimental procedures were performed according to TEMESGEN *et al.* (2001) and PLOMION *et al.* (1999) for DGGE and SSCP, respectively. The parents of the oak and chestnut mapping populations and six F_1 individuals of each progeny were first screened using the SSCP technique. If an informative polymorphism was detected, then this technique was used to map the STS; otherwise DGGE was tested to reveal polymorphism. If no informative polymorphism was detected using DGGE, then the STS marker has not been mapped.

Mapping populations and data corresponded to those used in the original *Q. robur* (BARRENECHE *et al.* 1998) and *C. sativa* (CASASOLI *et al.* 2001) genetic linkage maps. A subsample of 57 individuals was genotyped with informative STS markers. MapMaker V.2 (LANDER *et al.* 1987) and JoinMap V.3 (VAN OIJEN and VOORRIPS 2001) software were used for linkage analysis using a LOD threshold of 6.0 as a grouping criterion and the KOSAMBI (1944) function to estimate genetic distances.

STS markers were sequenced in oak and chestnut. Amplified fragments were directly sequenced using the Amersham (Arlington Heights, IL) DYEnamic ET terminator sequencing kit and revealed with the MegaBACE automated capillary sequencer (Molecular Dynamics, Sunnyvale, CA). Sequences were then aligned with the original EST and between the two species using the ALIGNn software (http://www.infobiogen. fr/services/analyseq/cgi-bin/alignn_in.pl).

Construction of *Q. robur* and *C. sativa* consensus linkage maps: Two consensus genetic maps were constructed in *Q. robur* and *C. sativa* to merge marker and linkage information from different mapping experiments in a single map for each species. Some of the markers that were contained in the two parental QTL framework maps (CASASOLI *et al.* 2004; SCOTTI-SAINTAGNE *et al.* 2004), all SSR markers mapped in both species (BARRENECHE *et al.* 2004), and STSs mapped in this study were merged in these new consensus maps. Since the two original oak and chestnut linkage maps (BARRENECHE *et al.* 1998; CASASOLI *et al.*, 2001) were used to select a subsample of evenly distributed markers for QTL analysis and to map SSR and STS markers, a single consensus genetic map could be constructed for each species from original data. In both species, we used the following strategy to construct the consensus map:

1. The MapMaker software was used to establish a framework map for each parent using the "ripple" option (LOD > 2.0) to optimize the marker order. In most of the cases, the optimized marker order corresponded to that obtained in the QTL map. This map was constructed using a higher number of F_1 individuals; therefore the statistical support for marker order is also higher.
2. The JoinMap software was then used to join female and male maps by means of 3:1, 1:2:1, and 1:1:1:1 segregating markers. As the information content of 1:1/3:1 and 1:1/1:2:1 marker couples was quite low (RITTER *et al.* 1990) and marker density was higher than that in the framework map, we used the "fixed-order" option to optimize the marker order in the consensus map. If, after adding 3:1 and 1:2:1 segregating

markers, the marker order in the consensus map corresponded to that obtained using MapMaker, then the fixed-order option was not used. On the other hand, if some incongruities occurred, then the order obtained with Map-Maker was fixed prior to adding all the markers.

Merging QTL information on the consensus map and comparative QTL mapping: The BioMercator software (ARCADE *et al.* 2004) was used for integrating QTL and marker information in the consensus map of the two species separately. By using a homothetic projection process, this software permitted us to merge marker and QTL information from independent experiments. In our case, the consensus and QTL maps shared several markers. Therefore, QTL position and confidence intervals were projected from the QTL to the consensus map by means of the common loci.

To compare QTL positions between *Q. robur* and *C. sativa*, common intervals were identified using orthologous markers on the two consensus maps. Orthologous markers used were either SSRs described in a previous article (BARRENECHE *et al.* 2004) or STSs developed within the frame of this study. The orthology of the SSRs was verified by sequencing and aligning their flanking regions (BARRENECHE *et al.* 2004). In addition to SSRs and STSs, one isozyme locus and one 5SrDNA locus were also used as orthologous markers. A common interval was defined as the region on a linkage group bearing the same orthologous markers at its extremities. A common interval was comparable between the two species if it was at least 5 cM long in both species. Below this genetic distance, marker order was unreliable for markers genotyped on 57 individuals. When more than one orthologous marker were mapped at the end of one common interval, then the marker genotyped using a higher number of individuals or being more informative was chosen as the limit of the interval. For instance, the marker EMCs11 genotyped on 96 F_1 individuals was chosen in comparison to EST Cons126 genotyped on 57 F_1 individuals on the group C5, or EST 06E10 segregating 1:1 was chosen in comparison to EST Cons105 segregating 1:3, in the linkage groups Q12–C12 (Figure 1). After having identified common intervals between oak and chestnut consensus maps, unique QTL were defined for each species separately. In each species, more QTL for the same trait were often mapped in the same genomic region (for instance, QTL for the same trait detected in the two parental maps or during two different years). These QTL were considered as a unique QTL if its most probable position (*i.e.*, the highest LOD score position given by the composite interval mapping and the bootstrap) (CASASOLI *et al.* 2004; SCOTTI-SAINTAGNE *et al.* 2004) was included in the same interval identified by orthologous markers. If the most probable position of the QTL was located between two different intervals, then the QTL was localized in the interval with other QTL for the same trait or containing the larger length of the confidence interval. This is a practical definition of unique QTL at the intraspecific level to perform comparative QTL mapping between the two species. Finally, unique QTL were considered as conserved between the two species if they mapped in the same common interval. The probability that QTL colocations between the two species occurred by chance was calculated according to LIN *et al.* (1995) with slight modifications as described in FELTUS *et al.* (2003). In short, the probability p that the QTL colocations between the two species occurred by chance is given by

$$p = \frac{\binom{1}{m}\binom{n-1}{s-m}}{\binom{n}{s}},$$

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where n is the total number of common intervals compared (34 in our case), l is the number of QTL in the species exhibiting the largest number of QTL, s is the number of QTL in the second species, and m is the number of QTL colocalized between the two species.

RESULTS

STS amplification: STS markers from PORTH *et al.* (2005a) were previously amplified in oak; thus they were optimized in chestnut and all succeeded in amplifying (10/10). As far as STS markers from J. DERORY, P. LEGER, V. GARCIA, J. SCHAEFFER, M. T. HAUSER, F. SALIN, C. LUSCHNIG, C. PLOMION, J. GLÖSSL and A. KREMER (unpublished results) are concerned, 73% (60/82) and 70% (57/82) amplified a single and strong band in oak and chestnut, respectively. Only four STSs amplified in oak and one amplified in chestnut did not succeed in amplifying in the other species or exhibited a multiband pattern. Primer pairs resulting in multiband patterns for both species were discarded from further analyses. Only successfully amplified and mapped STSs were reported in Table 1, *i.e.*, overall, 49 sequences. Expected size of the amplified fragments, based on the EST sequence, varied from 115 to 454 bp. A size ranging from 120 to 1600 bp was obtained for these STS markers (Table 1). Eighteen (37%) out of the 49 STSs developed in this work gave a fragment of the expected size in both species, whereas 24 (49%) STS markers gave products longer than predicted and, therefore, it is expected that they contain intronic sequences. Only seven STSs (14%) amplified a fragment of different size in oak and chestnut.

STS polymorphism detection and mapping: For mapping STS markers developed in this work, SSCP and DGGE techniques were used to detect informative polymorphisms in oak and chestnut full-sib families. The 10 ESTs from PORTH *et al.* (2005b) were all informative and consequently mapped in chestnut. Among the 61 STSs from J. DERORY, P. LEGER, V. GARCIA, J. SCHAEFFER, M. T. HAUSER, F. SALIN, C. LUSCHNIG, C. PLOMION, J. GLÖSSL and A. KREMER (unpublished results) properly amplified in, at least, one of the two species, 70% (43/61) and 61% (37/61) were informative in oak and chestnut full-sib families, respectively. The SSCP technique revealed most of the polymorphisms and was used mainly to map these markers. In six cases, DGGE revealed polymorphisms that could not be detected by SSCP. Twenty-one STS markers were not mapped for one of the two species because no polymorphisms were detected or SSCP and/ or DGGE run conditions could not be optimized (Table 1).

Fifty-one and 45 STS markers were mapped in oak and chestnut, respectively (Figure 1). Six STS markers were mapped with a LOD threshold fixed between 4.0 and 6.0 (Table 1). Nevertheless, their linkage with more than one marker on the same linkage group supported their map location. In all other cases, LOD > 6.0, confirming that 57 individuals were enough for mapping

new markers on the oak and chestnut linkage maps. The two oak parental framework maps covered 852.6 cM (female) and 876.0 cM (male). The two chestnut parental maps covered 863.4 cM (female) and 866.6 cM (male). These framework maps were used as starting points to construct the two oak and chestnut consensus maps as previously described.

The two consensus maps are available on-line at http:// www.pierroton.inra.fr/biogeco/genetique/recherches/ Oak-map/index.html. Figure 1 shows linkage consensus groups of both species. For clarity reasons, only a sub-sample of markers was represented in Figure 1 to easily identify common genetic intervals between the two species.

Comparative STS mapping: With a total number of 55 orthologous markers mapped in both species (18 SSRs, 1 isozyme, 1 5SrDNA, and 35 STSs), the 12 linkage groups could be aligned. Thirty-seven STS markers could be mapped in both *Q. robur* and *C. sativa*. In most of the cases, the sequence analysis of the amplified fragments confirmed that the amplification product corresponded to the EST sequence and homology between oak and chestnut was verified (Table 1). In five cases, the low quality of sequences did not permit us to draw any conclusions. These results confirmed the orthology between STS markers mapped in oak and chestnut. Homeologous linkage groups contained from 2 to 7 common anchor markers. Marker order was conserved in most of the cases. Five inversions, corresponding to true local inversions or caused by mapping errors (*e.g.*, Q1/C6 and Q2/C1), were observed between tightly linked markers (Figure 1). Among them, in four cases, markers involved were separated by a distance <5 cM and the STS Cons38 in the group C1 was a marker linked with a LOD value of 5.0; therefore the statistical support for its position was low.

In comparison with the map constructed by BARRENECHE *et al.* (2004) for oak, two microsatellite markers already mapped in chestnut were integrated into the oak map: EMCs11 (Q7) and CsCAT15 (Q3). EMCs11 was mapped in the same F_1 full-sib family used in this work (T. BARRENECHE, personal communication), whereas CsCAT15 was mapped in an interspecific cross between *Q. robur* and *Q. petraea* (A. KREMER, personal communication). In this last case, linkage groups of the interspecific cross were aligned to those of the intraspecific cross used in this work; thus linkage information for the microsatellite CsCAT15 could be recovered. One important difference was pointed out in comparison to the results obtained in BARRENECHE *et al.* (2004). Linkage group Q7 was previously matched with linkage group C11 on the basis of the EMCs1 marker. Due to the new orthologous markers mapped in this work, the groups Q7 and C11 did not seem to be homeologous linkage groups. The new pairs of homeologous linkage groups were Q6–C11 and Q7–C5 (Figure 1). Microsatellite EMCs1 amplified several fragments of

TABLE 1

Characteristics of the STSs used in this study: sequences of the primers pairs, PCR conditions, expected and observed fragment sizes, sequence homology between Quercus and Castanea, mapping method, and linkage group in both species

EST name[a]	Accession no.	Forward primer (5′→3′)	Reverse primer (5′→3′)	Expected size (bp)[b]	PCR protocol (Q-C)[c]	Observed size	Identity[d]	Mapping method	Linkage group Q-C[e]
IT11	CF369263	Porth et al. (2005b)	Porth et al. (2005b)	555	Ref.-5	555	99	Ref.-Q/SSCP-C	10-10
IT12	CF369264	Porth et al. (2005b)	Porth et al. (2005b)	522	Ref.-5	522	93.5*	Ref.-Q/SSCP-C	NM-3
IT21	CF369266	Porth et al. (2005b)	Porth et al. (2005b)	338	Ref.-5	770	94	Ref.-Q/SSCP-C	1-6
IT25	CF369268	Porth et al. (2005b)	Porth et al. (2005b)	187	Ref.-3	187	97.2	Ref.-Q/SSCP-C	6-11
IT57	CF369273	Porth et al. (2005b)	Porth et al. (2005b)	282	Ref.-5	282	93.5	Ref.-Q/SSCP-C	4-2
IT62	CF369274	Porth et al. (2005b)	Porth et al. (2005b)	346	Ref.-5	705Q 600C	91.9	Ref.-Q/SSCP-C	10-10
2T11	CF369278	Porth et al. (2005b)	Porth et al. (2005b)	397	Ref.-5	397Q 500C	89*	Ref.-Q/SSCP-C	NM-8
2T3	CF369283	Porth et al. (2005b)	Porth et al. (2005b)	284	Ref.-3	630	94.3	Ref.-Q/SSCP-C	10-10
2T13	CF369280	Porth et al. (2005b)	Porth et al. (2005b)	334	Ref.-5	334	94.5	Ref.-Q/SSCP-C	11-3
2T32	CF369284	Porth et al. (2005b)	Porth et al. (2005b)	386	Ref.-5	608Q 500C	93	Ref.-Q/SSCP-C	2-1
01A03	CR627501	GGTATGGGAGCTGGTCGTAA	AGAGAGCCAACAGGGAGACA	382	1-1	500	94.3	SSCP	7-5
01E07	CR627526	AAGCTCTAGACCTCGCTTGC	GGCTGGCAGCCTGCCTTGC	145	1-1	400Q 145C	86	SSCP	5 E-4
02F02	CR627566	ACTGGAAATCGCTCAGGACT	GCTCGATATCCTTGGGCATA	164	6-6	164	86.3	SSCP	1-6
02G03	CR627575	ACGCTCCAAAACTTGGTCAG	CGGGCAGGTACTTAATGAGG	206	1-1	700	90.3	SSCP	3-8
06B07	CR627724	GTCCTCCAATTCGCCATTTA	ATGGGAACAGTTAGCCGATG	307	1-1	307	93.3	SSCP	8-7
06E10	CR627745	TGGTGGAACCTTAGCTGACC	AAGGATGACATGGCCTCAAC	333	1-1	700	94.5	SSCP-C DGGE-Q	12-12
07A08	CR627771	AGCTGGGTGGAGAGAGTGAA	TGCGGGTATAGATGGGGTTA	341	7-7	700	86.1*	SSCP	2-NI
07A09	CR926157	AGCAGCCAAGGTGAAAAAGA	TCAATGGCTGCTGAAGTGAG	252	1-1	300	95.7	DGGE	5-4
07B10	CR627781	∫	∫	360	See ref.	360		SSCP	8-NA
07C03	CR627785	GATGGCAAGGTTTTGCAGAC	TCTCTTCTGGCTGGCTTTTC	285	1-1	285	96.6*	DGGE-C	NI-4
08A01	CR627918	∫	∫	210	See ref.	500		SSCP	3 E-NM
08A03	CR627920	GCTGCAATGTGAAGTGGTGT	TGGGAGGAAGTGGAAGAATG	454	1-1	454	94.1*	SSCP	12 E-NI
08B04	CR627933	∫	∫	327	See ref.	327		SSCP	9-NM
08C05	CR627943	AAACTCCTGTCATCCGCAAC	CCCGGATTTGGTTAGGAAGT	213	2-2	213	95.5*	SSCP	2-NI
08C11	CR627947	∫	∫	316		316	94.4	SSCP	2-1
08D11	CR627958	GGTACTTCATTCCCACAGCAA	GGCTCCAATGTTGCTCCTTA	343	8-8	700	88.4*	SSCP	11-3
08G04	CR627986	∫	∫	393	See ref.	1000		SSCP	11-NA
Cons 13	CR627506	CGGCGAGGTACACAAATCTAA	AACGGCACCGTAAGGTTCTT	301	1-2	301	89.5-93.1	SSCP	1-6
Cons 14	CR627508	TTGTGGTAACCAGTTCGACTCT	AGTTGTTGGCGGTGCTTT	243	1-1	1200	—	SSCP	5-4
Cons 19	CR627517	GAAGCACAACAATGTTATCCTAA	CGGGCAGGTACAATAGGA	178	1-2	300	81.3	SSCP-Q DGGE-C	9.2/4
Cons 21	CR627523	GAATCGCCAAGTGAAACCAT	GGCGGTTCTTCAGAACGATA	333	1-1	550	89.3	SSCP	2-1
Cons 30	CR627541	GGGGATTCATTGCCACTTCT	CATACTTTGCATCGCCAACA	424	1-1	1400Q 1500C	93.1	SSCP	4-2
Cons 33	CR627568	GAGAAAACCACATAGAGATCAGATG	GCTCCGTAGCGCAGAAAGAAG	153	3-3	200Q 250C	95.3	SSCP	12 E-12
Cons 38	CR627606	AAGCTTCCCATGTTTCGATG	CTCGCACTTGACGCCTTGTGT	123	5-5	123	91.7	DGGE	2-1 E
Cons 41	CR627646	CAAATCTGATCCCGCAAAT	GCCATTGACACAAAGAACCA	443	1-1	500	90.7*	SSCP	NI-1
Cons 46	CR627952	GCCGAGGTACAAAAATCCAT	ACCATGAGACGCCAAGTGGAA	215	NA-2	800C	—	SSCP	NA-9
Cons 48	CR627721	TCAACACCAACCAACACC	CAGGTACCACGAAGGTCACA	191	4-4	191Q 400C	—	DGGE-Q	6-NI
Cons 58	CR627732	CCAATTCTCTTACTGGCAAGG	GCTTTGGGATGATGTTTTGG	255	1-1	500	92.7	SSCP	5-4

(continued)

TABLE 1

(Continued)

EST name[a]	Accession no.	Forward primer (5' → 3')	Reverse primer (5' → 3')	Expected size (bp)[b]	PCR protocol (Q-C)[c]	Observed size	Identity[d]	Mapping method	Linkage group Q-C[e]
Cons 61	CR627776	ATTTCGAAGCTCGACTGCAT	GTCCATTGCAGATGGCTTTT	260	1-NA	1600Q	95.7*	SSCP	6-NA
Cons 68	CR627777	GAAGTTGCTTCTGGGGATCA	GGCTCGTACGTGAACTCCTC	244	5-NA	500Q	92.9*	SSCP	1-NA
Cons 72	CR627907	AGGAGCCCTCCTTTAGCAGT	CCCGCAGTTAAAAAGGCATA	312	1-1	1000Q 800C	90.9*	SSCP	10-NI
Cons 74	CR627801	AACCTTGCTAAGGCCCCTCTC	CATCAGAAGGTCCCAGTGCT	137	3-3	137	86.7	SSCP	9-9
Cons 75	CR627924	GGCAGGTACCTCCATGGTTA	TCAATGCTGGCTTCTTTGTG	257	6-6	600	88*	SSCP	NI-8
Cons 86	CR627976	CCAGATATCAGGAAAACCAAGC	CAAGCATGAAGCACCACTCAAC	270	3-NA	600	—	SSCP	8-NM
Cons 90	CR628018	ATAGCACCGCACTTGCACTT	TGCAAACTTAACCCAATCACC	188	3-1	300Q 1200C	—	SSCP	2-7
Cons 104	CR627823	GCTTGCAGGGTGTTAGTGTG	TCCTAATACAACATTGTCTTCCTCA	250	5-5	250	95.4	SSCP	3-8
Cons 105	CR627826	GCTGCACGAGTGTTCTCTGT	GAATGGCGTGTCAAACATTG	185	1-1	600	95.4	SSCP	12-12
Cons 106	CR627828	GGTGGGGTGAGCAGTAGAAA	GGATGTTGGTGTTGCTGTTG	326	1-1	326	91.2*	SSCP	NI-1
Cons 107	CR627830	TCTGGTGGTGAAATCATGGA	GAAGTGAACACCACCCGTTT	272	1-1	900	92.6	SSCP	11-3
Cons 109	CR627834	GGTCACAGGTGCAGTTGGAT	CTGCGGAACTGGCTGTAAGT	194	1-1	1200	100*	SSCP	7-5
Cons 110	CR627835	AGGGGTGATATTGCACTTGG	GCAGTGACCTTGTGTAAGATGG	219	6-6	219	92.6	SSCP	9-9
Cons 111	CR627837	TTCTTTTCGCCTCATTCCTC	GCTGGCGTTTAAAATTATTTGC	219	1-3	219Q 600C	89.5*	SSCP	12-NI
Cons 112	CR627839	ATGGCTCGTACCAAGCAAAC	GGGCAGGTACTTTCGGATCT	171	1-1	171	93.4*	SSCP	5-4
Cons 126	CR628009	ACCCTTACCCTGCGACTTCT	TGCTCAAGAGGCTGTGAAGA	238	1-1	400	94.7	SSCP	7-5
Cons 127	CR628014	CAAACCTCCCATTCCACATC	CCCAAGGTCAGCACAAACTT	289	6-6	289	94.3	SSCP	6-11
Cons 129	CR628019	GCAGTGCTCTCCACAAACCT	GGTGGCAAGCACCAACTAAT	120	3-3	120	—	SSCP	6-NI
Cons 129	CR628021	TCCTCCTTCCAGCACCTCTA	TTATGGCAGGCTTGGGTTAG	210	1-1	500	79.6	SSCP	9 [E]-9
Cons 130	CR628241	CCATTGAGCTCCTTCTCAGC	CTTCAACAGGTGCAGCAAAG	190	6-6	190	91.9*	SSCP	2-NI
Cons 135	CR628167	TGCCCTTCTTACCAGCTCCTC	GAAAGAGGGAGACCACGTCA	115	1-1	200	100*	SSCP	NI-1

[a] STSs 08A01, 07B10, 08B04, Cons 86, and 08G04 have not been sequenced.

[b] The expected sizes were based on the knowledge of the EST sequence and primer design. The observed sizes were approximate because they were based on an electrophoresis on an agarose gel. The unmapped amplified ESTs were either noninformative or mapping methods (SSCP or DGGE) have not been successfully optimized.

[c] The protocol PCR1 corresponded to the one described in MATERIALS AND METHODS: 10 touchdown cycles from 58° to 53°, followed by 25 cycles at 53°. Protocol 2: 55°–50° / 25 cycles at 50°. Protocol 3: 55°–50° / 30 cycles at 50°. Protocol 4: 65°–60° / 30 cycles at 60°. Protocol 5: 58°–53° / 30 cycles at 53°. Protocol 6: 60°–55° / 30 cycles at 55°. Protocol 7: 68°–63° / 25 cycles at 63°. Protocol 8: 67°–62° / 30 cycles at 62°. NA, nonamplified. Ref., reference listed in columns 3 and 4.

[d] Except for 01E07 and Cons 129, all STS sequences matched the same gene in both species using a BLASTX procedure. If STS was mapped or sequence was available for only one species, alignment was done with the original oak EST (*). In five cases, sequence reaction did not work (—).

[e] We used a LOD threshold ≥6.0 to map STSs except for those marked with E, for which 4.0 < LOD score < 6.0. NI, noninformative; NM, nonmapped.

[f] J. DERORY, P. LEGER, V. GARCIA, J. SCHAEFFER, M. T. HAUSER, F. SALIN, C. LUSCHNIG, C. PLOMION, J. GLÖSSL and A. KREMER (unpublished results).
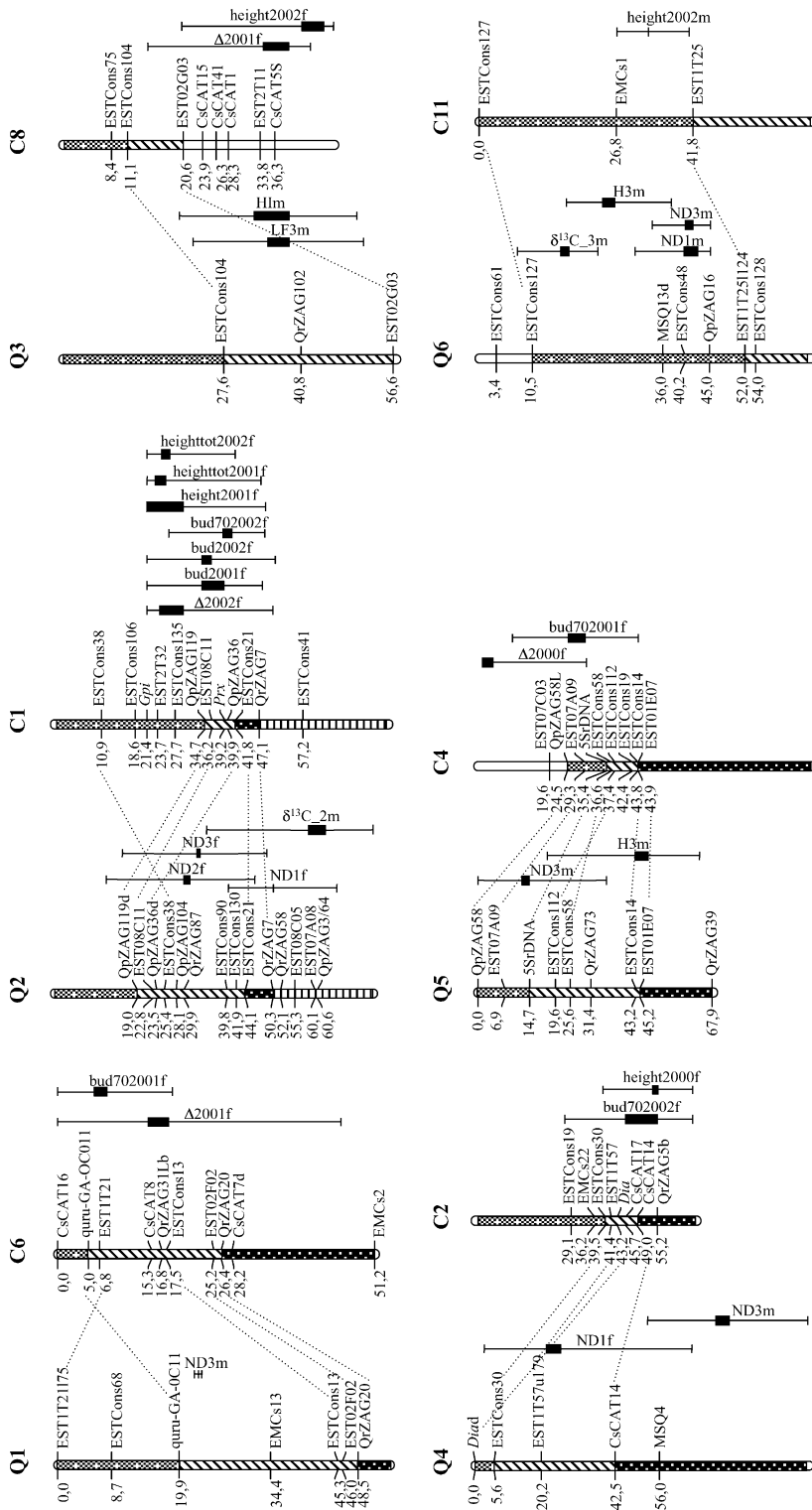
FIGURE 1.—Homeologous linkage groups between *Q. robur* (Q) and *C. sativa* (C). Linkage groups were named as in BARRENECHE *et al.* (1998) and CASASOLI *et al.* (2001). Oak linkage groups were taken as a reference and ordered from Q1 to Q12. Orthologous markers mapped in this work and previously by BARRENECHE *et al.* (2004) were represented. Markers EST2T32, EST2T3, EST1T11, EST2T13, and CsCAT15 were not represented in oak linkage groups because they were mapped in a different mapping population as explained in the text. Marker ESTS08A01 was mapped in linkage group Q3 but it was not represented because its introduction perturbed marker order. This marker was orthologous to the EST Cons75 mapped in the homeologous linkage group C8. Common intervals identified by orthologous markers were filled with corresponding backgrounds in both oak and chestnut linkage groups. These homeologous genomic regions were used to identify corresponding QTL for adaptive traits in both species: each QTL is represented on the right of the linkage group by its confidence interval (95% confidence intervals, solid lines) and by the most probable position (rectangles on solid lines; see MATERIALS AND METHODS). As previously described in MATERIALS AND METHODS, the most probable position of the QTL was considered to identify unique QTL at the intraspecific level and, therefore, to perform comparative QTL mapping between the two species (*i.e.*, QTL were considered conserved between oak and chestnut if their most probable position was included in the same homeologous interval identified by orthologous markers). QTL were represented and named as in CASASOLI *et al.* (2004), SCOTTI-SAINTAGNE *et al.* (2004), and O. BRENDEL (unpublished results). For oak QTL, ND was used to indicate bud burst QTL (number of days until the plant reached the phenotypic stage scored), and NF (number of flushes), LF (mean flush length), HI (total height increment), and H (total height) were used to indicate height growth QTL; $\delta^{13}C\_1$ was used to indicate QTL for carbon isotope discrimination. Numbers 1, 2, and 3 following QTL names corresponded to three independent phenotypic assessments (SCOTTI-SAINTAGNE *et al.* 2004). For chestnut QTL, bud and bud70 were used to name bud burst QTL (bud corresponded to the date of the first observed unfolded leaf; bud70 corresponded to the date when 70% of the tree buds showed an unfolded leaf; height (annual height increment) and heighttot (total plant height) were used to name total height growth QTL; Δ was used to indicate QTL for carbon isotope discrimination. Numbers 2000, 2001, and 2002 following QTL names corresponded to three independent phenotypic assessments (CASASOLI *et al.* 2004). Both in oak and in chestnut QTL names are followed by f (female) or m (male) to indicate the parental linkage map where QTL were originally detected. The figure was drawn using MapChart software (VOORRIPS 2002).
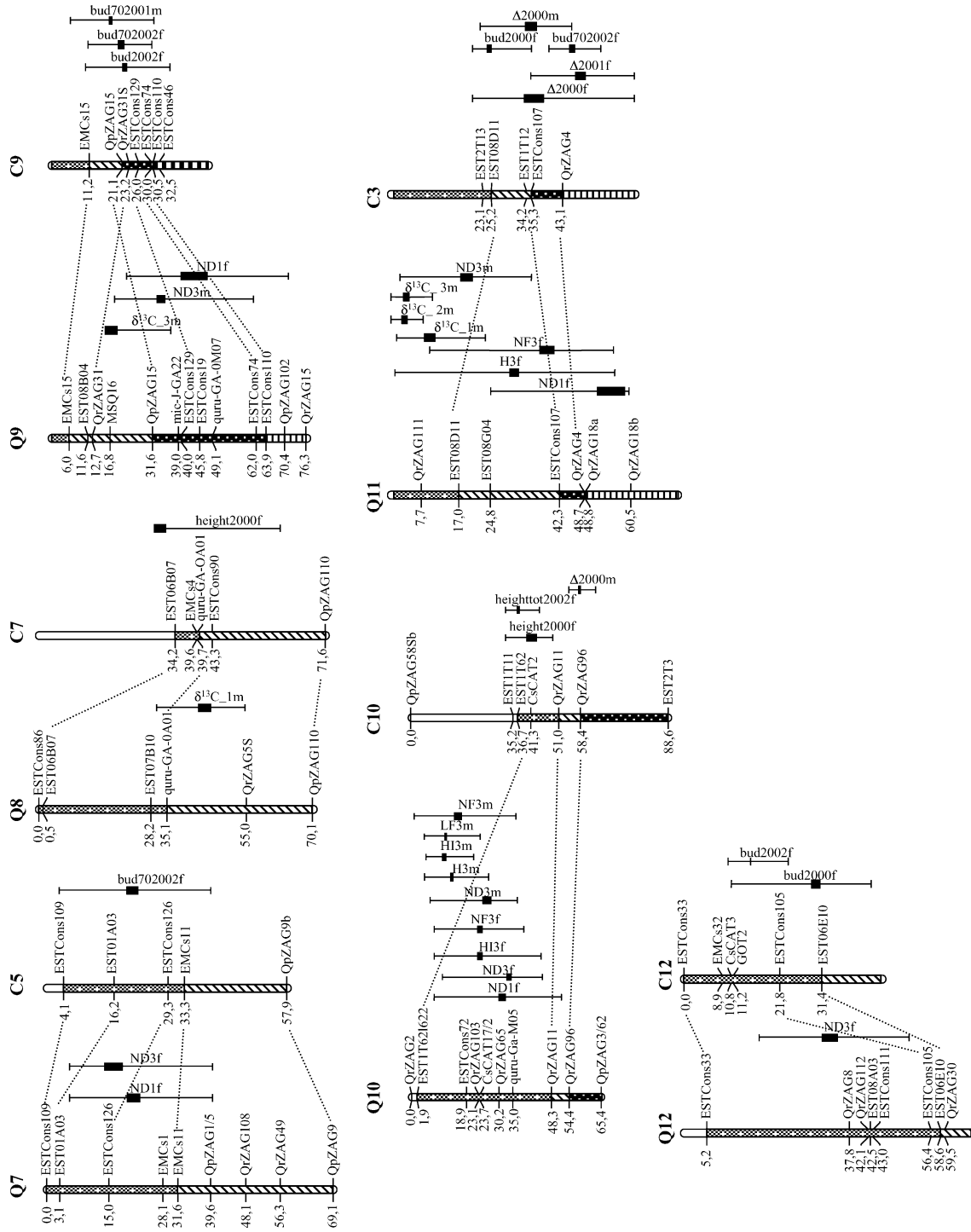
FIGURE 1.—*Continued.*

TABLE 2

Number and mean PEV values of QTL detected in *Q. robur* and *C. sativa*, respectively, are reported for each homeologous linkage group pair

	Q1-C6	Q2-C1	Q3-C8	Q4-C2	Q5-C4	Q6-C11	Q7-C5	Q8-C7	Q9-C9	Q10-C10	Q11-C3	Q12-C12
					Bud burst							
QTL no.	1-1	3-3	0-0	2-1	1-1	2-0	2-1	0-0	2-3	3-0	2-2	1-2
PEV (%)	10.1-8.8	8.4-9.1	0.0-0.0	7.0-8.5	4.8-10.3	8.0-0.0	8.7-6.7	0.0-0.0	5.4-9.0	7.4-0.0	8.2-7.6	5.3-7.1
					Carbon isotope discrimination							
QTL no.	0-1	1-1	0-1	0-0	0-1	1-0	0-0	1-0	1-0	0-1	3-3	0-0
PEV (%)	0.0-7.3	12.7-12.2	0.0-8.8	0.0-0.0	0.0-10.1	4.4-0.0	0.0-0.0	15.8-0.0	4.9-0.0	0.0-10.4	26.0-8.7	0.0-0.0
					Height growth							
QTL no.	0-0	0-3	2-1	0-1	1-0	1-1	0-0	0-1	0-0	6-2	2-0	0-0
PEV (%)	0.0-0.0	0.0-9.0	10.3-17.0	0.0-9.6	6.7-0.0	6.4-11.5	0.0-0.0	0.0-11.8	0.0-0.0	14.1-7.6	7.1-0.0	0.0-0.0

Average PEV value among QTL for the same trait mapped on the same linkage group for each species is shown. In the column headings, the first number corresponds to the average value of Quercus, and the second corresponds to the average value of Castanea. Q, Quercus; C, Castanea.

different size in chestnut (BARRENECHE *et al.* 2004). In the interspecific oak cross, this marker was mapped in a different linkage group than in the intraspecific oak cross (A. KREMER, personal communication). It is therefore possible that fragments of different size correspond to paralogous loci as already shown in similar cases (BARRENECHE *et al.* 2004).

In two cases (EST Cons19 and EST Cons90, Table 1 and Figure 1), STS markers did not locate in homeologous linkage groups between oak and chestnut. Sequence data confirmed that EST Cons19 corresponded to the gene for the ribosomal protein L13. The marker EST Cons90 was developed from an EST corresponding to a metallothionein-like protein type 3 (MT-3) and two fragments of different size were amplified in the two species. In the two cases, we were dealing with gene families and several independent loci corresponding to different gene family members of these proteins were mapped in plant species (BARAKAT *et al.* 2001; GUO *et al.* 2003).

Merging of *Q. robur* and *C. sativa* QTL studies on the consensus map and comparison of QTL position: QTL data were obtained from CASASOLI *et al.* (2004), SCOTTI-SAINTAGNE *et al.* (2004), and O. BRENDEL (unpublished results). QTL results for timing of bud burst, carbon isotope discrimination, and height growth were calculated at the 5% genomewise significance level within each species (Table 2). For each pair of homeologous linkage groups, the total number of QTL detected and the mean phenotypic explained variance (PEV) values were reported for each species. For a given trait in a given species, QTL were detected separately within each experiment (3 different years in Castanea, three different sites in Quercus). The total number of QTL contributing to a trait was then reduced to the number of unique QTL. Unique QTL, as explained in MATERIALS

AND METHODS, are either QTL expressed in different years but located within the same interval defined by orthologous markers or QTL expressed in only 1 year.

In total, there were 19 and 14 individual QTL for the timing of bud burst in oak and chestnut, respectively. Concerning carbon isotope discrimination, 7 and 8 QTL in oak and chestnut, respectively, were detected over a 3-year period. Finally, height growth was measured in oak only during 1 year using four different measurements; thus the number of detected QTL per trait and year can be compared. A mean value of 3 QTL per trait was detected in oak. In chestnut, the mean value per trait and year was 1.5.

For the timing of bud burst, the average PEV values varied from 4.8 to 10.1% in oak and from 6.7 to 10.3% in chestnut. PEV values for carbon isotope discrimination varied from 4.4 to 26.0% and from 7.3 to 12.2% in oak and chestnut, respectively. Finally, concerning height growth, average PEV values varied from 6.4 to 14.1% in oak and from 7.6 to 17.0% in chestnut. PEV values are comparable between two different QTL experiences if the numbers of individuals used for their estimation are similar (BEAVIS 1995). Despite the higher number of F_1 individuals in the oak progeny in comparison to the chestnut one (278 *vs.* 186), the mean values of individuals used for QTL mapping were 165 and 147 in oak and chestnut, respectively. Therefore, PEV values were generally comparable. In both species, more QTL with either low or moderate rather than high effect were detected (CASASOLI *et al.* 2004; SCOTTI-SAINTAGNE *et al.* 2004; O. BRENDEL, unpublished results).

A total number of 34 common intervals were identified between the oak and chestnut genetic linkage maps by means of the orthologous markers. Following the previously described criteria to declare unique QTL, 13 and 10 unique QTL were identified for timing of bud

TABLE 3

Number of unique and colocated QTL detected in *Q. robur* and *C. sativa*, respectively

	Q1-C6	Q2-C1	Q3-C8	Q4-C2	Q5-C4	Q6-C11	Q7-C5	Q8-C7	Q9-C9	Q10-C10	Q11-C3	Q12-C12	Total Q-C	Feltus test
Bud burst														
Unique QTL in Q-C/colocalized QTL	1-1/1	2-1/1	0-0/0	2-1/1	1-1/1	1-1/0	1-1/1	0-0/0	1-2/1	1-0/0	2-2/2	1-1/1	13-10/9	0.000211
Carbon isotope discrimination														
Unique QTL in Q-C/colocalized QTL	0-1/0	1-1/0	0-1/0	0-1/0	0-1/0	1-0/0	0-0/0	1-0/0	1-0/0	0-1/0	1-2/0	0-0/0	5-7/0	—
Height growth														
Unique QTL in Q-C/colocalized QTL	0-0/0	0-1/1	1-1/0	0-1/0	1-0/0	1-1/1	0-1/0	0-1/0	0-0/0	1-1/1	1-0/0	0-0/0	5-6/2	0.193668

In each entry the numbers represent the number of unique QTL in Quercus–the number of unique QTL in Castanea/the number of colocated QTL.

burst, 5 and 7 unique QTL were identified for carbon isotope discrimination, and, finally, 5 and 6 unique QTL for height growth were identified in chestnut and oak, respectively (Table 3). Among these unique QTL, 9 controlling timing of bud burst and 2 controlling height growth were colocated between the two species (Table 3 and Figure 1). No QTL involved in carbon isotope discrimination was colocated in the oak and chestnut map. Following Lin *et al.* (1995), the probability to obtain these colocations by chance is $P = 0.0002$ in the case of timing of bud burst and $P = 0.1937$ in the case of height growth.

DISCUSSION

EST-derived markers—a powerful tool for comparative mapping studies: The availability of gene fragment sequences, such as ESTs, has recently increased due to the broad development of sequencing program and gene expression studies. Transcriptome analyses in plants gave rise to an enormous potential for studying plant physiology and development (Alba *et al.* 2004) and made available gene sequences through EST databases. In the case of nonmodel species, such as most of the forest trees, this is of particular value for genetic and molecular studies. Usefulness of EST sequences was pointed out in conifer species. Given their high sequence conservation between species, ESTs were shown to be of great interest for comparative mapping studies in pine species (Brown *et al.* 2001). In addition, some ESTs colocating with QTL for wood quality were considered as putative candidate genes for this trait (Brown *et al.* 2003; Chagné *et al.* 2003). Our results confirmed both the efficiency of EST-derived markers for comparative mapping studies and their high potential to identify candidate genes.

STS amplification results clearly showed that a high proportion (70%) of markers developed from ESTs were amplified in oak and easily cross-amplified in chestnut. The location of the primers in the most conserved regions of the EST sequences and the amplification of coding regions were most likely the reasons for this high amplification efficiency. Roughly, 50% of the STSs amplified larger fragments than expected on the basis of the EST sequence. This result could be explained by the occurrence of intronic sequences. The presence of introns increases the chance to detect informative polymorphisms for mapping, but it could also represent a drawback. Because of the lower PCR efficiency, larger fragments were often difficult to amplify. Therefore, considering the possible presence of introns in amplified fragments, it seemed judicious to design primer pairs for amplifying fragments of a quite small size (100–400 bp).

Several approaches were available to detect mutations in amplified DNA fragments (Ahmadian 2001). Among

the different techniques, SSCP and DGGE were preferred in this work, for several reasons. They did not need any previous sequence characterization of the amplified fragment, the cost was significantly lower than that of SNP genotyping approaches, and they required only equipment readily available in molecular biology laboratories. Moreover, several polymorphisms could sometimes be revealed simultaneously, giving us the possibility to obtain codominant and full informative molecular markers. In our case, SSCP and DGGE techniques represented very efficient approaches for mapping EST-derived markers. We decided to screen amplified STS fragments, first using SSCP. It has been shown that 90% of the mutations present in a given DNA fragment can be detected using this technique (NATARAJ *et al.* 1999). Using SSCP and DGGE, informative polymorphisms for mapping have been detected in 70% (43/61) and 66% (47/71) of the total number of ESTs in oak and chestnut, respectively. These results show very clearly that ESTs are an important source of molecular markers for comparative mapping studies.

Alignment of the 12 *Q. robur* and *C. sativa* linkage groups: Mapping of 55 orthologous molecular markers into the *Q. robur* and *C. sativa* genetic maps permitted us to align the 12 linkage groups of both species. Macrosynteny and macrocolinearity were well conserved between the two species (Figure 1). No major macrorearrangements were observed and inversions between tightly linked markers were probably due to mapping errors or real inversions. Comparative mapping studies in plant species showed that microrearrangements occurred more frequently than macrorearrangements during plant genome evolution (BENNETZEN 2000). Density of common orthologous anchor markers between the oak and chestnut genomes is still very low. Thus, rearrangements between these two genomes still remain undetected. It is interesting to point out that new duplications and microrearrangements between rice and maize were only recently identified using sequence data (PATERSON *et al.* 2004; SALSE *et al.* 2004) and these authors adopted a more critical view of the well-known "single genetic system" model in the Poaceae family (BENNETZEN and FREELING 1993; GALE and DEVOS 1998). Thus, our results concerning oak and chestnut genomes are still very preliminary.

Anyway, despite the lack of exhaustive data, much evidence suggests that genome organization and structure are quite conserved within the Fagaceae family. Physical (0.94 pg/C and 0.81 pg/C in oak and chestnut, respectively) and genetic (1200 and 1050 cM in oak and chestnut, respectively) genome sizes are conserved between *Q. robur* and *C. sativa*. The two species share the same haploid number of chromosomes ($2n = 2x = 24$) and our findings suggest that the 12 linkage groups are homeologous, showing both conserved macrosynteny and macrocolinearity. Moreover, gene structure seems

to be conserved as demonstrated by amplification of similar STS fragment sizes even in the presence of intronic sequences. These preliminary results suggest a stable genome that probably, taking into account the physical genome size, contains a low proportion of repeated sequences. Orthologous markers developed in this study and previously by BARRENECHE *et al.* (2004) provide molecular tools of utmost importance to investigate genome organization and evolution within the Fagaceae family.

Comparative QTL mapping for adaptive traits in *Quercus* and *Castanea*: Comparative QTL mapping has been often used to discover evolutionary conserved genomic regions controlling quantitative traits (PATERSON *et al.* 1995). However, heterogeneous sampling strategies can hamper the comparative analysis of QTL, as well as the small population sizes, which are used in most of the QTL detection experiments, can introduce important biases in QTL results (BARTON and KEIGHTLEY 2002). In our comparative QTL analysis, for instance, some important drawbacks have to be considered: first, QTL studies in oak (SCOTTI-SAINTAGNE *et al.* 2004; O. BRENDEL, unpublished results) and chestnut (CASASOLI *et al.* 2004) were based on only one full-sib family in each species; hence only a subsample of QTL existing in natural populations and controlling the three adaptive traits under study was considered here. Second, because of the relatively low population size, QTL of low effect remained mostly undetected and PEV values were overestimated (BEAVIS 1995). Moreover, confidence intervals of QTL positions were quite large, leading to an imprecise location of QTL on the linkage group. Despite these limitations, and because similar sampling strategies were used in both species, we were able to draw important conclusions from the comparative analysis that was discussed here. A first interesting observation concerned the overall conservation of genetic architecture of adaptive traits between oak and chestnut in terms of QTL number and contribution to the phenotypic variance. In both species, similar numbers of QTL were detected for the three investigated adaptive traits, with a higher number of QTL for the timing of bud burst and a lower number for carbon isotope discrimination and height growth.

For the three traits and in both species, QTL of low and moderate effects were more frequent than those of large effect. Overall, these results were consistent with Orr's model of the L-shaped distribution of factors fixed during adaptation and a similar trend was observed in both species. Therefore, the presence of more QTL of low and moderate than large effect appears to be a general characteristic of the genetics of adaptive traits. However, differences among the traits can also be observed. The higher number of QTL detected for the timing of bud burst and in comparison to growth traits was already reported in forest trees (WENG *et al.* 2002; JERMSTAD *et al.* 2003). One possible explanation is that timing of bud burst is under a moderate to strong

genetic control, often showing high heritability values (Howe *et al.* 2003). On the contrary, height growth is under low to moderate genetic control and, in the case of juvenile height growth, genetic control changes from one developmental state to another (Wu *et al.* 2002). Taking into account that QTL detection power depends on the trait heritability, QTL for timing of bud burst would be more efficiently detected than QTL for height growth, as heritability of bud burst is rather high in *Q. robur* (Scotti-Saintagne *et al.* 2004).

Conservation of genomic regions controlling bud burst: Similarities and differences among the three studied adaptive traits show clearly when we compare the position of the detected QTL between *Q. robur* and *C. sativa*. A highly statistically significant number of colocations for QTL controlling the timing of bud burst was observed between oak and chestnut, whereas QTL colocations for height growth seemed to occur by chance. No colocation was observed for carbon isotope discrimination. It is interesting to point out that homeologous linkage groups Q11 and C3 contained a QTL for carbon isotope discrimination detected several times, explaining a high phenotypic variance for this trait in both species, but the position was not conserved.

The conservation of QTL for timing of bud burst between oak and chestnut deserves particular attention, especially when taking into account the difference with the two other adaptive traits analyzed. As shown by Scotti-Saintagne *et al.* (2004) year–year genetic correlations for bud burst are significant. Therefore, QTL for this last trait were more stable across independent experiments in both oak and chestnut than QTL for growth or carbon isotope discrimination (Casasoli *et al.* 2004; Scotti-Saintagne *et al.* 2004; O. Brendel, unpublished results). This could partially explain the higher conservation of QTL for bud burst between these two species. Timing of bud burst is probably a less complex trait than carbon isotope discrimination or growth, as less physiological processes contribute to the phenotypic expression of the trait. Timing of bud burst in forest trees is mostly influenced by temperature (Howe *et al.* 2003). Contrastingly, different and numerous environmental factors could affect both carbon isotope discrimination (Brugnoli and Farquhar 2000) and growth (Kirschbaum 2000), and therefore a high number of physiological processes are involved in their phenotypic expression. This implies that QTL detected for these traits can vary among experiments depending on the environmental factor predominantly acting on the physiological component involved. QTL detection under controlled experimental conditions could help to verify this hypothesis.

Another possible explanation, for the difference in QTL conservation among the three adaptive traits analyzed, could be in the mechanisms responsible for phenotypic plasticity. Using a QTL approach, Wu (1998) concluded that phenotypic plasticity of growth in Po-

pulus depends mostly on gene regulation. Genes and molecular mechanisms responsible for phenotypic plasticity are poorly known. Two hypotheses have been proposed: on the one hand, identical alleles expressing different effects depending on the environment might determine varying phenotypes of the same genotype ("allelic sensitivity"); on the other hand, regulating genes might turn on or off other genes depending on the environment ("gene regulation") (Via *et al.* 1995). If phenotypic plasticity of carbon isotope discrimination and growth is governed mostly by gene regulation, and phenotypic plasticity of timing of bud burst is dominated by allelic sensitivity, then the result of our QTL comparative mapping could be explained in terms of these mechanisms: the same loci would be involved mostly in the case of bud burst, whereas different loci would be involved mostly in carbon isotope discrimination and height growth. Taking into account the difference in trait complexity and in the number of environmental factors acting on these traits, this explanation could be likely. Nevertheless, no experimental data can support this hypothesis.

Our results also raised the issue of the maintenance of polymorphism at the same loci in Quercus and Castanea. In both species, many loci controlling bud burst were heterozygotes in the parental individuals of the controlled cross used for QTL detection. Although timing of bud burst is under strong natural selection in forest species, a high level of within-population variation is maintained (Howe *et al.* 2003) in these species. Our results suggested that these selection pressures were acting on the same genomic regions through mechanisms able to maintain diversity over long evolutionary times (balancing, disruptive, or frequency-dependent selection). At least in oaks, it has been shown that coevolution of defoliating insects and oaks may be responsible for wide within-population variation of bud burst (Tikkanen and Tiitto 2003). Maintenance of a high level of heterozygote loci would fit well with the hypothesis of a phenotypic plasticity mainly based on allelic sensitivity. In this case, the high level of within-population variation would be an evolutionary strategy to keep a high level of phenotypic plasticity. Conservation of QTL position does not imply that genes responsible for the QTL are the same. In the Poaceae family, Doust *et al.* (2004) showed that *tb*1 gene, playing a major role in the genetic control of branching in maize, was only poorly involved in foxtail millet, despite conservation of QTL and gene positions. Other genes located in the same chromosomal region could be better candidates in the case of foxtail millet. Thus, despite the striking conservation of QTL controlling timing of bud burst in *Q. robur* and *C. sativa*, this work did not allow us to make any conclusions about correspondence of loci controlling this trait between the two species.

ESTs that colocate with evolutionarily conserved QTL for timing of bud burst may represent potential

candidate genes. Examples of ESTs to be considered are cons58 (coding for auxin-repressed protein) and 08C11 (coding for a metal nicotianamine transporter, YSL1), which are located in conserved QTL of bud burst. However, given the large confidence interval of QTL position, additional arguments will be needed to refine the final choice of candidate genes.

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