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### 5 Fagaceae Trees

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## 5 Fagaceae Trees

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### 5.1 Introduction

Worldwide, there are more than 1,000 species belonging to the Fagaceae. All Fagaceae species are woody plants and are spread throughout the northern hemisphere, from the tropical to the boreal regions. The family comprises seven genera (Govaerts and Frodin 1998), and the number of species is extremely variable among genera: *Castanea* (12), *Castanopsis* (100 to 200), *Chrysolepis* (2), *Fagus* (11), *Lithocarpus* (300), *Quercus* (450 to 600), *Trigonobalanus* (3). Oaks (*Quercus*), chestnuts (*Castanea*), and beeches (*Fagus*) are widely used in forestry for wood products over the three continents (Asia, Europe, and America) and are important economic species. Consequently, they have received more attention in forest genetic research than other genera. In addition to their cultivation in

forestry, chestnuts are also used for their fruit production and have been partially domesticated for that purpose. *Castanopsis* and *Lithocarpus* are important ecological components of the Asian flora and have recently been investigated for their biological diversity (Cannon and Manos 2003). The remaining genera comprise only a very few species and for the time being have been studied mainly in botany and taxonomy.

Genetic research in Fagaceae has been restricted to the three genera of economic importance (*Castanea*, *Fagus*, and *Quercus*), although activities in phylogeny and evolutionary genetics have recently encompassed the whole family (Manos and Stanford 2001; Manos et al. 2001). Because of their long rotation times, breeding activities in the three main genera have been limited (Kremer et al. 2004). However, population differentiation has been investigated in a very large number of species, with the main aim of identifying geographic patterns or historical foot-

prints for molecular markers and phenotypic traits of forestry relevance. Population genetics has driven most of the research activities in molecular genetics and also genetic mapping, in contrast to other forest tree species where tree improvement has been the main goal. Genetic maps have been constructed in at least one species of *Quercus*, *Castanea*, and *Fagus*. Because of their low genetic divergence, it quickly became obvious that molecular markers could be easily transferred from *Quercus* to *Castanea* (and vice versa) but less easily to *Fagus*. These earlier findings led to further activities on comparative mapping across genera, especially between *Quercus* and *Castanea*.

### 5.1.1 Evolutionary Biology and Phylogeny of the Fagaceae

Fossil remains indicate that the Fagaceae appeared at the transition between the secondary and tertiary era. Remains of *Dryophyllum*, which is a fossil genus belonging to the Fagaceae, were discovered in layers belonging to the early Cretaceous (Jones 1986). Fossil remains that were unequivocally assigned to Fagaceae and dated to the Upper Eocene and Late Oligocene were found in North America (Herendeen et al. 1995) and Europe (Kvacek and Walther 1989). Differentiation of the various genera occurred during the mid Tertiary, and reported species of Fagaceae at the late Tertiary resemble already extant species. The oldest reported genera belonging to the Fagaceae occurred in Southeast Asia, where the extant species diversity is also the highest. The family originated from Southeast Asia and radiated toward Europe and America (Wen 1999; Xiang et al. 2000). Migration and major continental rearrangements contributed to disjunction and vicariance within the family, especially within *Quercus* (Manos and Stanford 2001). It is generally accepted that most major oak groups essentially evolved in the areas where they reside today (Axelrod 1983).

Phylogenetic investigations based on chloroplast or nuclear DNA data are poorly resolutive, suggesting that the differentiation into different genera was extremely rapid during the mid Tertiary (Manos and Steele 1997; Manos et al. 2001). All genera are usually clustered into a "starlike" dendrogram (polytomy), except *Fagus*, which diverged earlier from the common ancestor. However, there is a strong genomic

similarity between *Quercus*, *Castanea*, *Lithocarpus*, and *Castanopsis*. Paleontological records suggest that *Quercus* and *Castanea* separated 60 million years ago (Crepet 1989). Interspecific separation within the genera *Quercus*, *Fagus*, and *Castanea* occurred between 22 and 3 million years ago as inferred from a molecular clock based on cpDNA divergence (Manos and Stanford 2001). The reduced genetic divergence among the different genera was recently confirmed by the results obtained in transferring molecular tools and markers among genera, as it is much more difficult to transfer microsatellite markers from *Quercus* to *Fagus* than it is from *Quercus* to *Castanea* (Steinkellner et al. 1997; Barreneche et al. 2004).

### 5.1.2 Ploidy, Karyotype, and Genome Size in Fagaceae

Reported karyotype studies in *Quercus*, *Lithocarpus*, *Castanopsis*, and *Castanea* (Mehra et al. 1972), in *Quercus* (D'Emérico et al. 1995), and *Fagus* (Ohri and Ahuja 1991) indicate that the number of chromosomes within the family is remarkably stable ( $2n = 24$ ). Naturally occurring triploids have been mentioned occasionally in oaks (Butorina 1993; Naujoks et al. 1995). Extra chromosomes ( $2n = 24+1, 2$  or  $3$ ) have been reported as consequences of irregular segregation in mitoses (Zoldos et al. 1998). Otherwise, C-banding comparisons have shown that the morphology of the chromosomes of *Fagus* (Ohri and Ahuja 1991) and *Quercus* (Ohri and Ahuja 1990) are quite similar.

The DNA content is variable across genera in the Fagaceae: the 2C DNA values varying from a low of 1.11 pg in *Fagus* to a high of 2.0 pg in *Quercus* species (Table 1). GC content on the other hand appears constant across genera (40%) and is similar to most higher plants (Table 1). All values reported in Table 1 were obtained by flow cytometric analysis of interphasic nuclei and are slightly higher than earlier assessments made with the Feulgen microdensitometry method (Ohri and Ahuja 1990). The 31 species in Table 1 represent a cross-section of the Fagaceae across the northern hemisphere. The two *Fagus* species, *Fagus grandifolia* and *F. sylvatica*, were quite similar in genome size (1.27 and 1.11 pg per 2C, respectively) and are at the lower range of genome sizes among the Fagaceae, suggesting that *Fagus* has either the most rudimentary genome or the most greatly reduced genome

**Table 1.** DNA content in 31 Fagaceae species determined by flow cytometric analysis

Species	2C nuclear DNA pg (mean value)	1C nuclear DNA Mbp	GC content (%)	Reference
Genus <i>Castanea</i>				
<i>C. seguinii</i>	1.57	755	–	Arumuganathan et al.*
<i>C. sativa</i> (1)	1.61	777	–	Arumuganathan et al.
<i>C. sativa</i> (2)	1.62	–	–	Brown and Siljak-Yakovlev (pers comm)
<i>C. crenata</i>	1.65	794	–	Arumuganathan et al.
<i>C. mollissima</i>	1.65	794	–	Arumuganathan et al.
<i>C. dentata</i>	1.67	803	–	Arumuganathan et al.
Genus <i>Fagus</i>				
<i>F. grandifolia</i>	1.27	610	–	Arumuganathan et al.
<i>F. sylvatica</i>	1.11	535	40	Gallois et al. 1999
Genus <i>Quercus</i> Subgen <i>Erythrobalanus</i> **				
<i>Q. velutina</i>	1.17	565	–	Arumuganathan et al.
<i>Q. nuttallii</i>	1.39	672	–	Arumuganathan et al.
<i>Q. shumardii</i>	1.47	709	–	Arumuganathan et al.
<i>Q. nigra</i>	1.52	735	–	Arumuganathan et al.
<i>Q. rubra</i>	1.58	762	–	Arumuganathan et al.
<i>Q. palustris</i>	1.60	774	–	Arumuganathan et al.
<i>Q. coccinea</i>	1.64	791	–	Arumuganathan et al.
<i>Q. phellos</i>	1.66	799	–	Arumuganathan et al.
<i>Q. falcata</i>	1.72	832	–	Arumuganathan et al.
<i>Q. pagoda</i>	1.75	843	–	Arumuganathan et al.
<i>Q. imbricaria</i>	1.81	871	–	Arumuganathan et al.
Genus <i>Quercus</i> Subgen <i>Lepidobalanus</i> **				
<i>Q. bicolor</i>	1.35	651	–	Arumuganathan et al.
<i>Q. montana</i>	1.49	719	–	Arumuganathan et al.
<i>Q. robur</i>	1.53	740	–	Arumuganathan et al.
<i>Q. stellata</i>	1.55	745	–	Arumuganathan et al.
<i>Q. alba</i>	1.59	766	–	Arumuganathan et al.
<i>Q. macrocarpa</i>	1.62	780	–	Arumuganathan et al.
<i>Q. robur</i>	1.84	885	42	Favre and Brown 1996
<i>Q. pubescens</i>	1.86	882	42.1	Favre and Brown 1996
<i>Q. petraea</i>	1.87	901	41.7	Favre and Brown 1996
<i>Q. robur</i>	1.88	–	39.4	Zoldos et al. 1998
<i>Q. petraea</i>	1.90	–	39.8	Zoldos et al. 1998
<i>Q. pubescens</i>	1.91	–	39.7	Zoldos et al. 1998
Genus <i>Quercus</i> Subgen <i>Cerris</i> **				
<i>Q. acutissima</i>	1.42	684	–	Arumuganathan et al.
<i>Q. cerris</i>	1.91	–	40.2	Zoldos et al. 1998
<i>Q. suber</i>	1.91	–	39.7	Zoldos et al. 1998
Genus <i>Quercus</i> Subgen <i>Sclerophyllodrys</i>				
<i>Q. coccifera</i>	2.00	–	40.4	Zoldos et al. 1998
<i>Q. ilex</i>	2.00	–	39.8	Zoldos et al. 1998

\* Arumuganathan K, Schlarbaum SE, Carlson JE previously unpublished data (genome sizes are an average of three determinations of 2 to 4 individuals per species)

\*\* According to Flora Europea (<http://rbg-web2.rbge.org.uk/FE/fe.html>)

among the Fagaceae. In addition, the small genome of *Quercus velutina* at 1.17 pg per 2C is essentially the same as that of the *Fagus* species, again suggesting a basal genome size of about 1.2 pg per 2C for the Fagaceae. Among the 24 *Quercus* species presented, the range of genome sizes is essentially continuous up to a maximum of 2.0 pg per 2C in *Q. coccifera* and *Q. ilex*. We looked for any indication that the interspecific variation in the observed genome sizes followed the botanical subdivisions within *Quercus*. We used here the classification into four distinct botanical subgenera from Flora Europaea (<http://rbgweb2.rbge.org.uk/FE/fe.html>). This classification corresponds to earlier botanical descriptions of Schwarz (1964) and Camus (1936-1954) and recent molecular analyses (Manos et al. 1999; Xu et al. 2005). The species that were investigated include representatives of all four subgenera: 12 species in *Erythrobalanus* (red oaks), seven species in *Lepidobalanus* (white oaks), three species in *Cerris*, and two in *Sclerophyllodrys*. The average 2C DNA contents were 2.0 pg for subgenus *Sclerophyllodrys*, 1.75 pg for subgenus *Cerris*, 1.73 pg for subgenus *Lepidobalanus*, and 1.56 pg for subgenus *Erythrobalanus*. The two oak species with the largest genomes, *Q. coccifera* and *Q. ilex* (2.0 pg), are both evergreen species and are part of a disputed botanical subgenus (named *Sclerophyllodrys*, according to Schwarz 1964). This is intriguing, given that molecular phylogenetic analysis separates the evergreen species from the two sections of deciduous oaks (Manos and Steele 1997 and Xu et al. 2005), confirming their earlier subdivision in *Sclerophyllodrys* by Schwarz (1964).

Among the five *Castanea* species studied, genome sizes varied much less than among oaks, ranging only from 1.57 pg in *C. seguinii* to 1.67 pg per 2C in *C. dentata*. In fact DNA content varied as much within the chestnut species as between. For example, unrelated *C. seguinii* trees varied from 1.5 to 1.63 pg per 2C, while *C. sativa* varied from 1.57 to 1.65 pg per 2C (Arumuganathan et al. this study). Thus there may not be significant differences in average DNA content between *Castanea* species, and the range of average DNA content reported among species in Table 1 may just represent the natural variation in DNA content among *Castanea* individuals. The intraspecific variation in DNA content in *Quercus* was also as extensive as the amount of variation among the species. For example, the 2C DNA content varied between 1.88 pg and 2.0 pg among *Q. petraea* trees of the same populations (Zoldos et al. 1998), between

1.45 and 1.96 in *Q. pagoda*, and between 1.34 and 1.78 in *Q. macrocarpa* (Arumuganathan et al. this study). The intraspecific variation may be due in part to the occurrence of extra B chromosomes (Ohri and Ahuja 1990; Zoldos et al. 1998). While the range of DNA content among oak species appears to be greater than among chestnut and beech species, the magnitude of the differences among oak species may be related to experimental issues as well as biological ones. For example, the size estimates by Arumuganathan et al. (this study) were consistently smaller than those by Favre and Brown (1996) and Zoldos et al. (1998). One could speculate that the differences relate to the fact that Arumuganathan et al. (this study) studied primarily New World species, while the other two studies dealt exclusively with Old World species. However, the three groups report different genome sizes for *Q. robur* (1.53, 1.84, and 1.88 pg per 2C, respectively). Whether this discrepancy has a biological basis (the Arumuganathan et al. study sampled three trees of *Q. robur* "fastigiata," the "upright" horticultural variety) or resulted from experimental differences in sampling, internal size standards, and other methodologies is not clear.

In general, the genome sizes in the Fagaceae are only 3.5 to 6 times larger than the genome of *Arabidopsis* (0.32 pg; Bennett et al. 2003) and are within the size range of the sequenced rice and poplar genomes (both 1.0 pg; Brunner et al. 2004). Comparative genomics should thus be relatively efficient within the Fagaceae. Comparative genomics will lead to a better understanding of the extent to which the continuous range of DNA content is related to adaptive radiation of the species during evolution or is the result of overlapping ranges and interspecies hybridizations. Knowledge of the genome sizes reveals that genome-level comparisons between *Fagus sylvatica*, *Q. velutina*, *Q. coccifera*, and *Q. ilex* would be particularly informative and could illuminate the role of genome duplication in the evolution of the Fagaceae. When comparative genomics studies are extended to more species within the Fagaceae, it will be interesting to determine whether or not the broader range of genome sizes observed in *Quercus* relates to more extensive adaptations and specializations than exist among *Fagus* and *Castanea* species. Given the extensive natural populations of Fagaceae species that still exist across the northern hemisphere, such information will certainly provide insights into the ecology of temperate forest ecosystems.

## 5.2 Construction of Genetic Linkage Maps

### 5.2.1 Genetic Mapping in Forest Trees

PCR-based molecular markers and the two-way pseudotestcross strategy are useful tools for constructing genetic maps in forest trees (Grattapaglia and Sederoff 1994). These outbred species are characterized by long generation times, long life spans, and a high genetic load that often leads to significant inbreeding depression. Although all these elements hinder the development of the type of mapping populations normally used for genetic linkage mapping (for instance inbred lines and backcrosses), the high level of heterozygosity in forest species made two-generation full-sib pedigrees suitable populations for marker segregation analysis. Full-sib and half-sib crosses can, therefore, be used to construct single-tree genetic linkage maps thanks to dominant PCR-based molecular markers. Following this approach, called the two-way pseudotestcross strategy (Grattapaglia and Sederoff 1994), three types of segregation configurations can be obtained for dominant molecular markers in the mapping population: (1) male testcross markers, segregating in a 1:1 ratio and inherited from the male parent; (2) female testcross markers, segregating in a 1:1 ratio and inherited from the female parent; and (3) intercross markers, segregating in a 3:1 ratio and inherited from both parental trees. Male and female testcross markers are used to construct two independent single-tree genetic maps that are then aligned thanks to the intercross markers. RAPD (Williams et al. 1990) and AFLP (Vos et al. 1995) dominant molecular markers have been used most commonly to construct genetic linkage maps in forest tree species (Verhaegen and Plomion 1996; Marques et al. 1998; Arcade et al. 2000; Costa et al. 2000; Cervera et al. 2001), as their random distribution in the genome allows all chromosomes to be covered most efficiently.

The two-way pseudotestcross strategy was first applied in forest trees by Grattapaglia et al. (1995) to identify loci controlling quantitative trait loci (QTLs). In forest genetics, QTL analysis has been one of the most important applications of linkage mapping, and several studies reported successful QTL detections (Sewell and Neale 2000; van Buijtenen 2001).

### 5.2.2 Genetic Mapping Initiatives in Fagaceae

#### Oak Mapping Initiatives

**European white oaks** Starting in 1995, activities in genetic mapping were implemented in European white oaks at the INRA Research Centre in Bordeaux-Cestas (France). Motivations for genetic mapping in oaks were threefold: (1) the detection of genomic regions involved in species differentiation, (2) the detection of QTLs controlling traits of adaptive significance, and (3) the comparative analysis of genomic evolution in the Fagaceae. The whole mapping project is based on three pedigrees: one full-sib family of *Quercus robur* (3P × A4), one full-sib family of *Q. petraea* (QS28 × QS21), and one interspecific F<sub>1</sub> full-sib family *Q. robur* × *Q. petraea* (11P × QS29). An interspecific F<sub>2</sub> cross is planned as well. Given the objectives of the mapping experiments, the parents of the pedigrees were not selected for any particular criteria. The *Q. robur* parent trees originated from the southwest of France (INRA research station of Bordeaux-Cestas, and Arcachon) and the *Q. petraea* parents were from the central part of France (INRA research station of Orléans-Ardon). The controlled crosses were repeatedly done over successive years until 2004. From 200 to 1,000 seeds were obtained for each cross. The young seedlings were installed in a seedbed in a nursery, where they are raised as stool beds. Starting at age 5, the full-sibs were hedged every year at the ground level at the end of winter. Following the hedging, stump sprouts developing in spring were harvested and cut in 15- to 20-cm-long cuttings. These cuttings were then transplanted in field tests for phenotypic observations and further QTL detection. For the time being, only the *Q. robur* intraspecific cross has been fully exploited for genetic mapping and QTL detection. The clonal test of the full-sibs has now been planted in three different sites (two near Bordeaux, southwest France, and one near Nancy, northeastern France). The genetic mapping of *Q. robur* mapping was done on a sample of 94 offspring (pedigree 3P × A4), and the QTL detection on a sample of 278 offspring (replicated on average in five vegetative propagules).

Another mapping initiative for *Q. robur* was implemented in the Netherlands (ALTERRA, Wageningen). This mapping pedigree consists of 101 full-sibs (Bakker 2001). The sibs were screened by paternity analysis within an open-pollinated progeny set of 397

sibs collected on a single tree located in an urban area (Amsterdam). This tree was surrounded by three other oak trees within a radius of 10 m. One of these oak trees was selected to be the paternal tree. Paternity analysis revealed that 26% of the collected seeds were sired by this male parent. The selected seeds were germinated, grown individually in pots in a nursery (ALTERRA research station, Wageningen, the Netherlands), and measured for several morphological and physiological traits during the next 2 years (1999, 2000). The objective of this work was quite similar to the French initiative: detection of QTL controlling morphological and adaptive traits involved in species differentiation.

**American red oaks** Genetic mapping in northern red oak (*Quercus rubra* L.) was initiated at Purdue University (<http://www.genomics.purdue.edu/forestry/>; Romero-Severson 2003) and has continued at the University of Notre Dame. Using exclusion methods based on microsatellite polymorphisms (Aldrich et al. 2002, 2003a), a preliminary mapping population of 97 full-sibs was identified from the open-pollinated progeny of a single tree. The most likely male parent male was the closest conspecific. Recombination patterns revealed (Romero-Severson et al. 2003) six linkage groups (LGs) of three or more markers. A second acorn harvest from the same female parent yielded 462 full-sibs. The genetic map under construction now includes 15 microsatellites, 66 AFLP markers from the first set of progeny, and several hundred new AFLP markers from the second set of progeny. All of the potential pollen parents within 200 m of the female parent are being genotyped with all 15 microsatellite markers to eliminate any doubt over the full-sib status of the mapping population. The microsatellite markers used for genetic mapping are the same as those used for studies on interspecific gene flow (Aldrich et al. 2003b) and in northern red oak genetic diversity studies. No map has yet been published for *Q. rubra*. The long-term goal of the red oak mapping project is the detection of QTLs and genes controlling heartwood color and resistance to specific pests, specifically *Phytophthora ramorum*, the agent of sudden oak death.

### Chestnut Mapping Initiatives

**European chestnut** Starting in 1998, a genetic mapping project for European chestnut (*Castanea sativa* Mill.) was implemented using a full-sib family ob-

tained from a controlled cross performed between two highly differentiated trees originating from Turkey. Anatolia Peninsula was shown to be an important region for chestnut genetic diversity (Villani et al. 1991, 1992). As illustrated by these studies, a remarkably high level of genetic, morphological, and physiological differentiation was observed between two groups of chestnut populations coming from two phylogeographic regions, characterized by striking climatic differences: the Eurosiberian part of the peninsula in northeastern Anatolia (humid) and the Mediterranean region in western Anatolia (xeric). Common field experiments carried out at the experimental field site of Istituto di Biologia Agroambientale e Forestale, CNR (Porano, Italy), showed significant differences between these populations in growth rate, bud flush, and physiological parameters, related to the water use efficiency, allowing "drought-adapted" and "wet-adapted" ecotypes to be identified (Lauteri et al. 1997, 1999). Differences observed in the ecophysiological behavior suggested that Turkish chestnut populations are genetically adapted to contrasting environments, making them a suitable material to study the adaptive potential of this species.

The controlled cross was performed in 1998 between a female parent (Bursa) belonging to the "drought-adapted" type from western Turkey and a male parent (Hopa) belonging to the "wet-adapted" type from eastern Turkey. The parental individuals were 9 years old and were chosen according to their heterozygosity level at isozymes and high degree of variation in physiological traits. An F<sub>1</sub> full-sib family of 186 offsprings was obtained, and 96 F<sub>1</sub> individuals were used to construct two separate genetic linkage maps: a female or Bursa map and a male or Hopa map. The main objective of the project was to exploit the peculiar genetic and adaptive variation observed in these populations in order to identify the genomic regions affecting carbon isotope discrimination (related to the water use efficiency), bud phenology, and growth by means of QTL analysis.

**American and Chinese chestnuts** During the last century, American Chestnut, *Castanea dentata* (Marsch) Borkh, one of the most important timber and nut-producing tree species in eastern North America, was dramatically affected by a canker disease (chestnut blight) caused by *Cryphonectria parasitica*. American chestnut showed low levels of resistance to blight, whereas Asian chestnut



species (*Castanea crenata* (Japanese chestnut) and *C. mollissima* (Chinese chestnut) exhibited higher levels of resistance to the disease. During the 1980s an important backcross breeding program was undertaken in the USA in order to obtain selected material combining the blight resistance of Asian chestnut and good timber qualities of American chestnut (Burnham et al. 1986).

In this context, a genetic map for chestnut was constructed. The main objective of this mapping project was to identify genomic regions involved in blight resistance. In addition, the map was also used to locate loci controlling morphological traits that differentiated both species. The mapping population was  $F_2$  progeny derived from a cross between two *C. mollissima*  $\times$  *C. dentata*  $F_1$  hybrids. The female parent was the *C. mollissima* cultivar “Mahogany” and two different American chestnut trees from Roxbury, CT were used as male to create the  $F_1$  hybrids. One hundred and two  $F_2$  individuals were used for the map construction, and 185 individuals were assessed for resistance to *Cryphonectria parasitica*.

### **Beech Mapping Initiative**

A genetic mapping project for European beech (*Fagus sylvatica*) has been implemented at the University of Parma (Italy) during the last 10 years (Scalfi et al. 2004). The objective was to dissect important adaptive traits and to identify their underlying QTLs to detect genomic regions involved in important quantitative traits such as growth, phenology, and water-use efficiency. The mapping pedigree consisted of a full-sib family comprising 143 offsprings. The family was the largest in a  $4 \times 4$  diallel controlled cross performed in 1995 (Ceroni et al. 1997). The parents originated from a natural population located at high altitude in northern Italy (1,650 m altitude, just below the tree line).

### **5.2.3**

#### **Genetic Linkage Maps**

#### **for *Quercus*, *Castanea*, and *Fagus***

#### **Genetic Map of *Q. robur***

The first *Quercus* map was published in 1998 on *Q. robur* (Barreneche et al. 1998) (pedigree 3P  $\times$  A4). Using the pseudotestcross mapping strategy, two maps were constructed comprising 307 markers (271 RAPD, 10 SCARs, 18 SSRs, 1 minisatellite, 6 isozymes, and 1 ribosomal DNA marker). Both maps provided

85 to 90% coverage of the *Q. robur* genome. Segregating markers could be aligned in 12 LGs, and the map size amounted to 893.2 cM for the paternal and 921.7 cM for the female map. These maps were further upgraded by the inclusion of new SSRs (Barreneche et al. 2004) and additional AFLP and STS. The upgrading is still ongoing and to date 854 markers (271 RAPD, 457 AFLP, 10 SCAR, 59 SSR, 49 EST, 1 minisatellite, 6 isozymes, and 1 ribosomal DNA marker) have been located (Table 2).

The Dutch *Q. robur* map (pedigree A1  $\times$  A2) was also constructed using the two-way pseudotestcross strategy (Bakker 2001). Two parental maps were first established comprising 18 SSR and 343 AFLP markers. The total lengths of the maternal and paternal maps were respectively 496 and 566 cM. Thirteen LGs were obtained (for 12 chromosomes) and the two maps could be partially merged using 58 “bridge” markers (2 LGs could not be aligned). One of the paternal LGs (LG 13, 27 cM) was highly dissimilar to the other LGs in terms of marker density. This LG contained almost half (48%) of all paternal markers and 22% of the segregating (heterozygote) markers. This marker-dense LG was homologous to one of the maternal LGs that remarkably was composed exclusively of 13 segregating markers. Congruence of LGs with the French map was based on the location of SSR markers (Sect. 5.3.1). The total map length of the integrated map was 659 cM, map density being one marker per 2.4 cM for the map without taking the exceptionally dense LG 13 into account.

#### **Genetic Map of *Castanea sativa***

A first framework of the chestnut genetic linkage map was obtained with RAPD and ISSR markers (Casasoli et al. 2001). Few isozyme loci were integrated in this first version of the map. A total of 381 molecular markers segregated in the chestnut full-sib family covering a good portion of the chestnut genome (more than 70%). Intercross segregating markers allowed 11 of the 12 LGs identified to be aligned between the female and male maps. This original framework was then used to map AFLP markers and codominant locus-specific markers such as SSR- and EST-derived markers. Table 2 shows the number and type of molecular markers contained in the chestnut genetic linkage map. At present, 517 molecular markers have been mapped in chestnut covering 80% of its genome. The 12 LGs were aligned to obtain 12 consensus female and male LGs (chestnut linkage consensus groups are available at the Web site [www.pierroton.inra.fr](http://www.pierroton.inra.fr)).

**Table 2.** Summary of genetic linkage maps of *Quercus robur* and *Castanea sativa*

Species (pedigree)	Number of LGs	Total number of marker loci	RAPD, ISSR, AFLP	SSR	STS	Isozymes	% of distorted markers	Total genetic distance (cM)	Genome saturation (%)	Total size (cM)	Ref.
<i>Q. robur</i> (3P × A4)	12	854	728	59	61	6	18	950	80	1200	Barreneche et al. 2004; and this study
<i>Q. robur</i> (A1 × A2)	13	361	343	18	–	–	17.5	659	64	1035	Bakker (2001); and this study
<i>C. sativa</i> (Bursa × Hopa)	12	517	427	39	46	5	10	865	82	1050	Casoli et al. 2001; and this study
<i>C. mollissima</i> × <i>C. dentata</i> (Mahogany)	12	559	521	29	1	8	25	524	–	–	Kubisiak et al. 1997; Sisco et al. 2005
<i>F. sylvatica</i> <sup>a)</sup> (44 × 45)	12/11	138/124	128/113	10/6	9	–	23	844/971	78/82	1081/1185	Scalfi et al. 2004; Scalfi 2005

a) The two numbers indicated correspond to the map of the female and male parent

### **Genetic Map of *Castanea mollissima*/ *Castanea dentata***

The *C. mollissima*/*C. dentata* map was the first to be published in the *Fagaceae* (Kubisiak et al. 1997). At first a total of 241 markers, including 8 isozymes, 17 RFLPs, 216 RAPDs, were mapped in the F<sub>2</sub> family. Twelve LGs were identified, covering a genetic distance of 530.1 cM (corresponding to 75% of the chestnut genome). To saturate the map, additional markers were recently added to the initial map: 275 AFLP (Clark et al. 2001) and 30 STS (29 SSR and the 5SrDNA locus) (Sisco et al. 2005). To date, a total of 559 markers have been located. Relatively high levels of segregation distortion (more than 25%) have been reported in the *C. mollissima*/*C. dentata* family. Skewed segregation is a common feature in progenies resulting from interspecific crosses.

### **Genetic Map of *Fagus sylvatica***

The *Fagus* genetic linkage map was based on a total of 312 markers: 28 RAPDs, 274 AFLPs, and 10 SSRs. Two maps were constructed using the “double testcross” strategy. In the female map 132 markers were distributed in 12 LGs covering 844 cM. In the male parent only 11 LGs were identified, resulting in linkage relationships between 119 markers spanning over 971 cM (Table 2). The two maps cover about 78% and 82% of the *Fagus* genome. Using intercross markers (15 AFLP and 2 SSR) seven homologous LGs could be identified (Scalfi et al. 2004). Ten additional EST markers were then added to the map since its publication (Scalfi 2005).

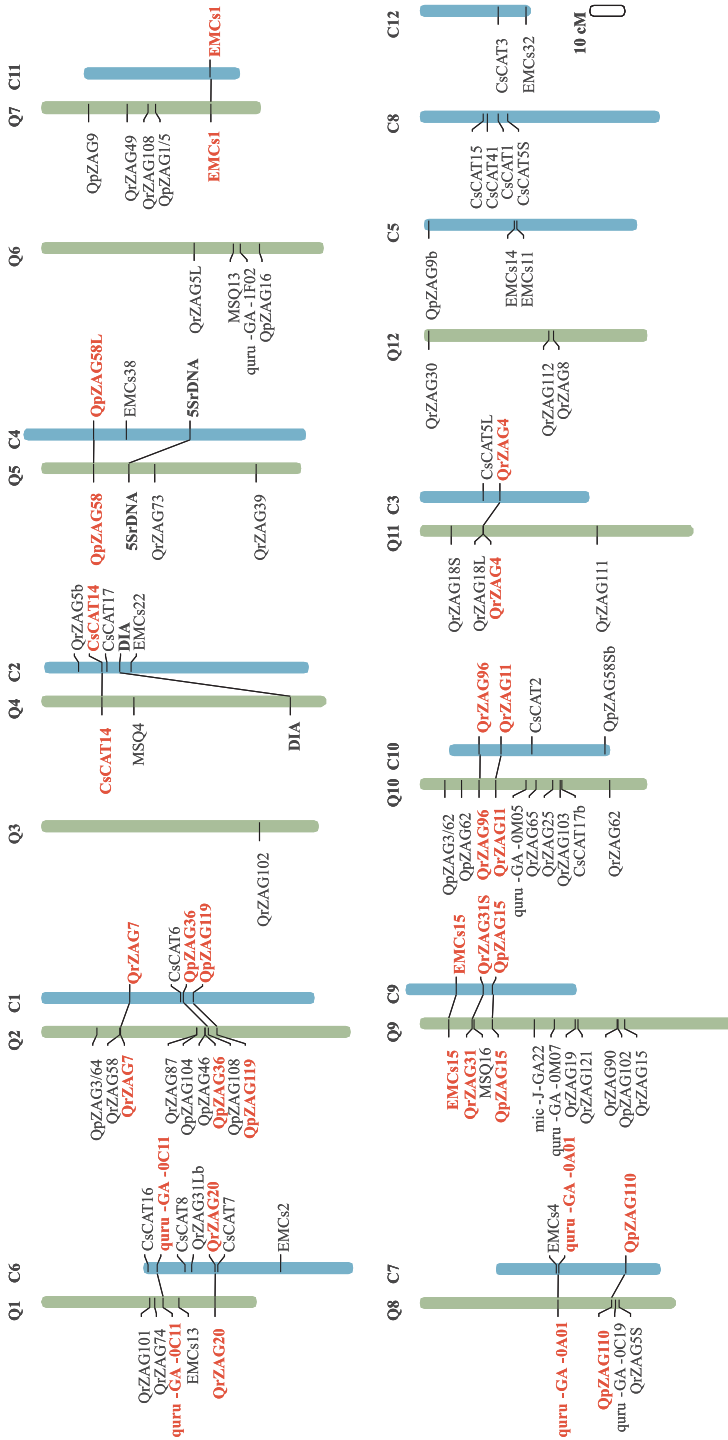
## **5.3 Comparative Mapping between *Quercus*, *Castanea*, and *Fagus***

### **5.3.1 Mapping of Microsatellites in *Quercus robur*, *Castanea sativa*, *C. mollissima*, and *C. dentata***

Microsatellite markers, which are tandemly repeated units of 2 to 6 nucleotides evenly dispersed throughout plant genomes, have been sometimes used for comparative mapping studies (Marques et al. 2002). Amplification of orthologous SSR markers across phylogenetically related species depends largely on evolutionary distance and genome complexity of com-

pared species (Powell et al. 1996). Usually, SSR cross-amplification is more efficient between closely related species with a low proportion of highly repeated sequences in their genome. Steinkellner et al. (1997) showed that microsatellite markers specifically developed in *Quercus* species were cross-amplified in chestnut. For these reasons microsatellites were supposed to be useful molecular markers for comparing *Q. robur* and *C. sativa* genetic linkage maps. To obtain orthologous markers for comparative mapping, SSR markers developed both in *Quercus* species and in *C. sativa* were therefore tested for cross-amplification and transferability between these two genera (Barreneche et al. 2004 and references therein) in a reciprocal way. We tested a total of 83 primer pairs: 53 developed in *Quercus* species and 30 in *C. sativa*. Primer pairs giving a strong amplification product were selected for mapping. Nineteen loci, 15 from oak and 4 from chestnut, were integrated into the two previously established genetic maps, allowing the first comparative mapping between LGs of the two species (Barreneche et al. 2004). Figure 1 shows the seven homeologous LGs identified by orthologous SSR and all microsatellite loci mapped in *Q. robur* and *C. sativa* genetic maps. These same SSR loci were used to align the European chestnut genetic linkage map with the *C. mollissima* × *C. dentata* interspecific map. Eleven of the 12 LGs of the two maps could be associated, nine LGs were aligned on the basis of pairs, triplets, or quadruplets of common markers, while three additional groups were matched using a single SSR marker (Sisco et al. 2005).

Overall, these findings showed that microsatellite markers could be cross-transferred between *Quercus* and *Castanea* genera and be used to recover orthologous markers for comparative mapping. Nevertheless, cross-transferability efficiency was low and the number of cross-transferred loci was not sufficient to link the 12 LGs of the two species. As expected, SSR loci were extremely useful for comparative mapping within the same genus (*Castanea*), but their cross-transferability efficiency decreased between different genera. SSR loci mapped both in *Q. robur* and *C. sativa* were sequenced in order to definitely demonstrate their orthology. Sequencing results clearly showed that both orthologous and paralogous loci could be recovered among the SSR cross-transferred between the two genera. Moreover, indels were sometimes observed within the flanking regions of the repeated motif. Therefore, although SSR loci can be cross-transferred between *Quercus* and *Cas-*



**Fig. 1.** Assignment between *Q. robur* and *C. sativa* based on orthologous microsatellites. Oak (Q, green on left, pedigree 3P × A4) and chestnut (C, light blue on right, pedigree Bursa × Hopa) LGs aligned using microsatellite markers. LGs are named as in Barreneche et al. (1998) and in Casasoli et al. (2001). Oak LGs are taken as reference and arranged in sequence from Q1 to Q12. Nine chestnut LGs, aligned with the corresponding oak LGs, are given on the right. The three remaining chestnut LGs are reported according to the oak LGs. Common orthologous SSR markers are shown in red. The EMCs1 marker was later shown to be a paralogous locus, and LG Q7 was not homeologous to LG C11 (Fig. 2). The figure, modified from Barreneche et al. (2004), was drawn using MapChart software (Voorrips 2002)

*tanea* genera, a sequence analysis is needed to demonstrate orthology and to avoid the risk of paralogy.

### 5.3.2 Mapping of EST-Derived Markers in *Q. robur* and *C. sativa*: Alignment of the 12 Linkage Groups between the Two Species

Several factors make EST (expressed sequence tag)-derived markers very useful for comparative mapping studies (Brown et al. 2001). First, ESTs are sequence fragments of coding regions; therefore sequence conservation among species is expected to be higher than that observed, for instance, in SSR loci. Second, ESTs correspond very often to genes of known function. This is of great interest because some ESTs colocalized with QTLs in a genetic linkage map may be putative positional candidate genes for a given trait. Finally, transcriptome analyses give rise to a high number of EST sequences that are the source of numerous EST-derived markers distributed throughout plant genomes. In oak, ESTs were developed by Derory et al. (2006) and Porth et al. (2005a). This gave the opportunity to exploit EST sequence information for marker design in order to complete the comparative mapping between *Q. robur* and *C. sativa* (Casasoli et al. 2006). About 100 EST sequences were selected from oak databases. Oak sequences were aligned with homologous sequences obtained from GenBank in order to design primer pairs for amplification in the most conserved regions of the sequence and assure a good cross-amplification efficiency in chestnut. A total of 82 primer pairs were designed. A proportion of about 70% produced by PCR a single and strong band both in oak and chestnut and 51 and 45 ESTs were mapped in oak and chestnut, respectively, using single strand conformation polymorphism (SSCP) and denaturing gradient gel electrophoresis (DGGE) approaches (Casasoli et al. 2006). These EST-derived markers, together with SSR markers previously mapped, provided 55 orthologous molecular markers that allowed the 12 LGs of *Q. robur* and *C. sativa* to be aligned. As shown in Fig. 2, from 2 to 7 common orthologous markers were mapped in the 12 homeologous pairs of LGs. Macrosynteny and macrocollinearity were well conserved between the two species. Few inversions, probably due to mapping errors, were observed. Although these data are still preliminary given the low number of common

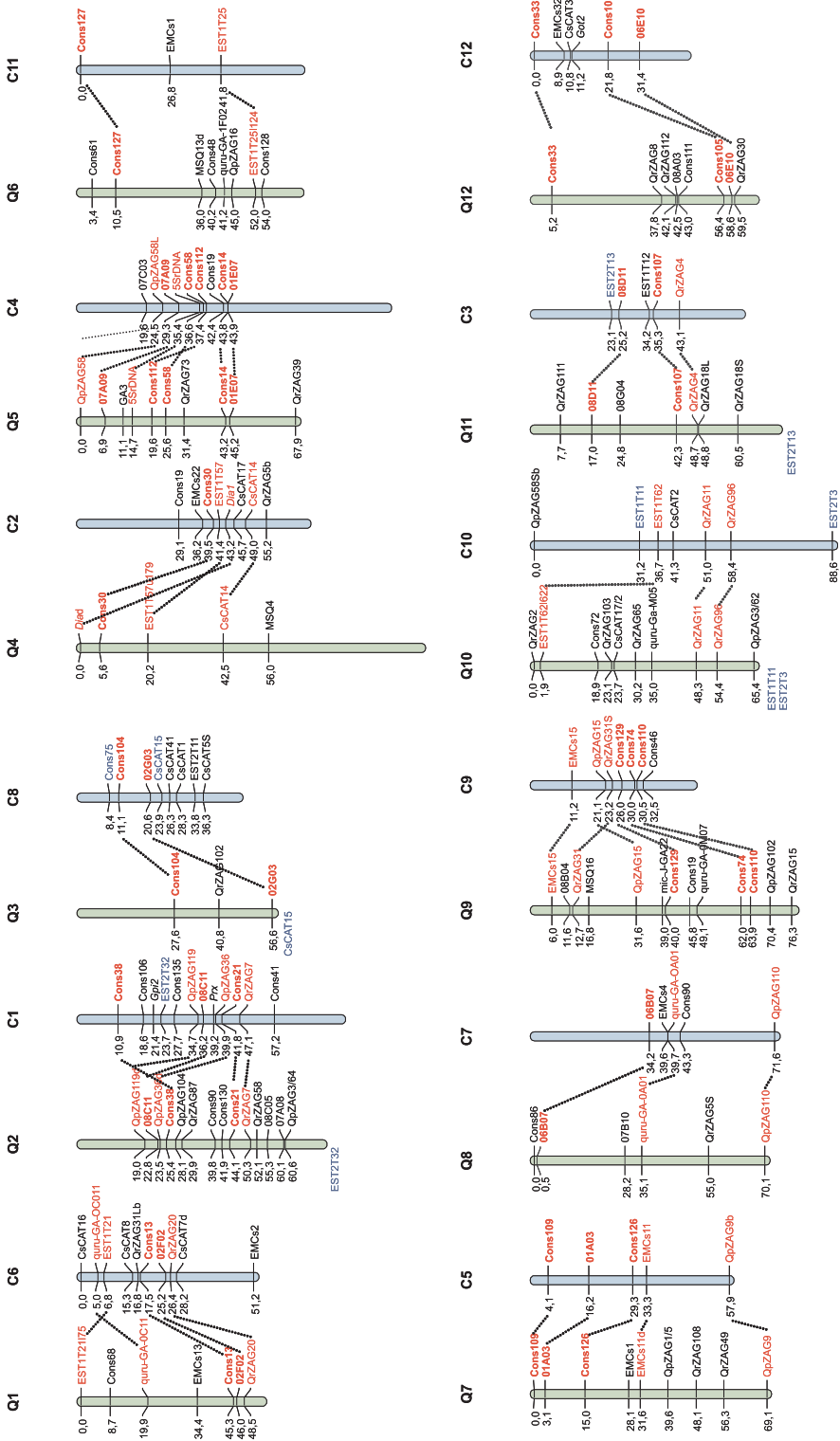
molecular markers mapped in the two species, no major chromosomal rearrangements have been identified, suggesting that oak and chestnut genomes are quite stable. Thus it appears likely that the “single genetic system” model of the grass genomes (Gale and Devos 1998) can also be applied to *Q. robur* and *C. sativa*. EST-derived markers were very easily transferred from oak to chestnut. About 50% of them contained intron-derived sequences. This increased the probability of detecting segregating polymorphisms useful for mapping in both oak and chestnut full-sib families. These markers proved to be ideal markers for comparative mapping within the Fagaceae family.

### 5.3.3 Mapping of Microsatellites and EST-Derived Markers in *Fagus sylvatica*, *Quercus robur*, and *Castanea sativa*

Success of transferability between *Fagus sylvatica*, *Quercus robur*, and *Castanea sativa* was lower. Although 86 SSR markers originally developed in other *Fagaceae* species were tested in *Fagus* (66 from *Quercus*, 20 from *Castanea*), only seven produced an interpretable banding pattern and only one marker from *Q. rubra* and one from *C. sativa* could be placed on the beech map (Scalfi 2005). One marker originally developed in *Fagus* gave good amplification also in *Quercus* and *Castanea* but was monomorphic in the crosses used for these species.

Similarly, 86 EST markers originally developed in *Quercus* were tested in beech, 46 coming from a budburst c-DNA library (Derory et al. 2006), 22 from osmotic stress response (Porth et al. 2005a), and 17 from hypoxia response cDNA-AFLP markers (C. Bodénès unpublished results). The success rate was higher than for microsatellites. In total 16 were polymorphic using various techniques (SSCP, DGGE, sequencing, CAPS, dCAPS), and 10 were finally mapped onto the beech map (Scalfi 2005).

Two markers (1T11 and 1T62) that mapped on *Quercus* and *Castanea* on LG 10 (Table 4) were mapped also on group 4 in *Fagus* with the help of a “bridge” marker (1T41): this can be considered as evidence of synteny between LG10 of *Q. robur* (3P\*A4) and *C. sativa* with LG 4 of *Fagus*. For the two markers the sequence homology of *Fagus* with *Quercus* was 82% and 43%, respectively; the lower value is due to a large insertion in the beech sequence that was not present in the cDNA of *Quercus*. Eliminating the gap,



**Fig. 2.** Comparative mapping between *Q. robur* and *C. sativa*. The 12 homeologous LGs between *Q. robur* (Q, green, pedigree 3P × A4) and *C. sativa* (C, light blue, pedigree Bursa × Hopa). The orthologous molecular markers mapped in both species are shown in red (SSRs and EST-derived markers). A subsample of molecular markers of the oak and chestnut consensus genetic linkage maps (available at [www.pierroton.inra.fr](http://www.pierroton.inra.fr)) is shown in this figure. Orthologous molecular markers mapped in a different oak cross (or showing a low mapping statistical support, Cons 75 in the oak LG Q3) are marked in blue below the LGs

the homology increased to 92%. Synteny could not be assessed for any other group since none had more than one marker mapped on it. For example, marker 2T32 mapped on LG 2 in *Quercus* was found linked to markers on LG 7-F in beech, but more than one comapping marker is needed to establish synteny.

### 5.3.4

#### Assignment of Linkage Groups Between *Quercus* and *Castanea*

Most genetic maps constructed within Fagaceae species comprised 12 LGs (Table 2). Cross-transferable SSR- and EST-derived markers made it possible to assign LGs among the four species *Q. robur*, *C. sativa*, *C. mollissima*, and *C. dentata* (Table 4). However, the assignment is still based on a limited number of markers per LG. Assignment was done by pairwise comparisons:

- Between the two *Q. robur* maps (3P × A4 and A1 × A2): 11 out of 12 LGs had at least two orthologous SSRs in common; the remaining LG was assigned by default.
- Between *Q. robur* (3P × A4) and *C. sativa* (Bursa × Hopa): between two and seven pairs of common markers (either SSR, isozymes, or EST) allowed the LGs to be assigned.
- Between the two chestnut maps (Bursa × Hopa and Mahogany): the assignment is still incomplete as only nine LGs could be assigned so far by at least two pairs of SSRs.

The results obtained so far need to be confirmed by further mapping experiments, based mainly on EST markers. They are also encouraging as suggested by the conservation of the macrosynteny and macro-collinearity that have so far been observed between the two most intensively studied species: *Q. robur* and *C. sativa*.

## 5.4

### Genes Mapped in Oaks and Chestnut

Transcriptomic investigations and differential gene expression studies were implemented recently with the main aim of identifying genes that are involved in the adaptation of oak or chestnut trees to their environment. Gene expression was monitored for different traits, or tissues:

- Bud burst in oaks
- Hypoxia in oaks
- Osmotic stress in oaks
- Juvenile and mature shoots in oaks
- Blight infection in chestnuts

Various techniques were implemented for constructing expression profiles: cDNA-AFLP, SSH, and Quantitative RT-PCR. We will briefly summarize the experiments conducted and the functions of genes that were identified. Table 3 provides a list of EST-derived markers mapped in *Q. robur* and *C. sativa*. For each EST the accession number, amplification, sequencing, and mapping results are reported.

### 5.4.1

#### Bud Burst

Candidate genes for bud burst were identified in *Q. petraea* using SSH libraries, macroarray experiments, and RT-PCR. Three subtracted libraries (SSH method) were constructed, generating 801 ESTs derived from six developmental stages of bud burst. Expression patterns of these transcripts were monitored in apical buds during bud flushing in order to identify genes differentially expressed between the quiescent and active stage of bud development. After bioinformatic processing of the ESTs, macroarray experiments revealed a total of 233 unique transcripts exhibiting differential expression during the process, and a putative function was assigned to 65% of them (Derory et al. 2006). Cell rescue/defense-, metabolism-, protein synthesis-, cell cycle-, and transcription-related transcripts were among the most regulated genes. Reverse northern and RT-PCR showed that several genes exhibited contrasting expression between quiescent and swelling buds. Among this set of 233 unique transcripts, ca. 100 were selected and tentatively amplified and mapped in oak and chestnut, as previously described. In oak and chestnut, 51 and 45 ESTs were successfully mapped, respectively, using SSCP and DGGE approaches (Casasoli et al. 2006).

### 5.4.2

#### Hypoxia

*Q. robur* and *Q. petraea* exhibit different responses to hypoxia, the first one being more tolerant to waterlogged conditions. Hypoxia-induced genes were identified from vegetative copies of the two species

**Table 3.** List of genes mapped in both *Quercus robur* and *Castanea sativa*

EST Name <sup>a</sup>	Accession number	Reference for primer sequences and PCR protocols	Expected size (bp) <sup>b</sup>	Observed size	Identity <sup>c</sup>	Linkage group Q - C <sup>d</sup>	Functional category
1T11	CF369263	Porth et al. 2005b	555	555	99	10 - 10	Unknown
1T12	CF369264	Porth et al. 2005b	522	522	93.5*	nm - 3	Metabolism
1T21	CF369266	Porth et al. 2005b	338	770	94	1 - 6	Protein synthesis
1T25	CF369268	Porth et al. 2005b	187	187	97.2	6 - 11	Unknown
1T57	CF369273	Porth et al. 2005b	282	282	93.5	4 - 2	Transcription
1T62	CF369274	Porth et al. 2005b	346	705Q 600C	91.9	10 - 10	Metabolism
2T11	CF369278	Porth et al. 2005b	397	397Q 500C	89*	nm - 8	Cell rescue, defense and virulence
2T3	CF369283	Porth et al. 2005b	284	630	94.3	10 - 10	Unclassified (plasma membrane related?)
2T13	CF369280	Porth et al. 2005b	334	334	94.5	11 - 3	Metabolism
2T32	CF369284	Porth et al. 2005b	386	608Q 500C	93	2 - 1	Protein synthesis
01A03	CR627501	Casasoli et al. 2006	382	500	94.3	7 - 5	Protein synthesis
1,00E+07	CR627526	Casasoli et al. 2006	145	400Q 145C	86	5 <sup>d</sup> - 4	Transcription
02F02	CR627566	Casasoli et al. 2006	164	164	86.3	1 - 6	Transcription
02G03	CR627575	Casasoli et al. 2006	206	700	90.3	3 - 8	Hypothetical protein
06B07	CR627724	Casasoli et al. 2006	307	307	93.3	8 - 7	Hypothetical protein
6,00E+10	CR627745	Casasoli et al. 2006	333	700	94.5	12 - 12	Protein with binding function or cofactor requirement
07°08	CR627771	Casasoli et al. 2006	341	700	86.1*	2 - ni	Hypothetical protein
07°09	CR926157	Casasoli et al. 2006	252	300	95.7	5 - 4	Cellular transport
07B10	CR627781	Derory et al. 2006	360	360		8 - na	Transcription
07C03	CR627785	Casasoli et al. 2006	285	285	96.6*	ni - 4	Hypothetical protein

<sup>a</sup> STSs 08A01, 07B10, 08B04, Cons 86, and 08G04 have not been sequenced.

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

<sup>c</sup> 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 has been done with the original oak EST (\*). In 5 cases, sequence reaction did not work (-).

<sup>d</sup> We used a LOD threshold  $\geq 6.0$  to map STS, except for those marked with e, for which  $4.0 < \text{LOD score} < 6.0$ . ni: noninformative; nm: nonmapped. Q-C: *Quercus robur* (3P × A4)-*Castanea sativa* (Bursa × Hopa)



**Table 3.** (continued)

EST Name <sup>a</sup>	Accession number	Reference for primer sequences and PCR protocols	Expected size (bp) <sup>b</sup>	Observed size	Identity <sup>c</sup>	Linkage group Q – C <sup>d</sup>	Functional category
08°01	CR627918	Derory et al. 2006	210	500		3 <sup>d</sup> – nm	Metabolism
08°03	CR627920	Casasoli et al. 2006	454	454	94.1*	12 <sup>d</sup> – ni	Protein with binding function or cofactor requirement
08B04	CR627933	Derory et al. 2006	327	327		9 – nm	Metabolism
08C05	CR627943	Casasoli et al. 2006	213	213	95.5*	2 – ni	Hypothetical protein
08C11	CR627947	Derory et al. 2006	316	316	94.4	2 – 1	Hypothetical protein
08D11	CR627958	Casasoli et al. 2006	343	700	88.4*	11 – 3	Metabolism
08G04	CR627986	Derory et al. 2006	393	1000		11 – na	Hypothetical protein
Cons 13	CR627506	Casasoli et al. 2006	301	301	89.5–93.1	1 – 6	Transcription
Cons 14	CR627508	Casasoli et al. 2006	243	1200	–	5 – 4	Protein synthesis
Cons 19	CR627517	Casasoli et al. 2006	178	300	81.3	9 – 2/4	Protein synthesis
Cons 21	CR627523	Casasoli et al. 2006	333	550	89.3	2 – 1	Protein synthesis
Cons 30	CR627541	Casasoli et al. 2006	424	1400Q 1500C	93.1	4 – 2	Hypothetical protein
Cons 33	CR627568	Casasoli et al. 2006	153	200Q 250C	95.3	12 <sup>d</sup> – 12	Hypothetical protein
Cons 38	CR627606	Casasoli et al. 2006	123	123	91.7	2 – 1 <sup>d</sup>	Energy
Cons 41	CR627646	Casasoli et al. 2006	443	500	90.7*	ni – 1	Cell rescue, defense, and virulence
Cons 46	CR627952	Casasoli et al. 2006	215	800C	–	na – 9	Cell rescue, defense, and virulence
Cons 48	CR627721	Casasoli et al. 2006	191	191Q 400C	–	6 – ni	Unknown
Cons 58	CR627732	Casasoli et al. 2006	255	500	92.7	5 – 4	Hypothetical protein
Cons 61	CR627776	Casasoli et al. 2006	260	1600Q	95.7*	6 – na	Cell rescue, defense, and virulence
Cons 68	CR627777	Casasoli et al. 2006	244	500Q	92.9*	1 – na	Metabolism
Cons 72	CR627907		312	1000Q 800C	90.9*	10 – ni	Cell cycle and DNA processing
Cons 74	CR627801	Casasoli et al. 2006	137	137	86.7	9 – 9	Cell rescue, defense, and virulence
Cons 75	CR627924	Casasoli et al. 2006	257	600	88*	ni – 8	Metabolism
Cons 86	CR627976	Casasoli et al. 2006	270	600	–	8 – nm	Unknown
Cons 90	CR628018	Casasoli et al. 2006	188	300Q 1200C	–	2 – 7	Cell rescue, defense, and virulence
Cons 104	CR627823	Casasoli et al. 2006	250	250	95.4	3 – 8	Hypothetical protein

Table 3. (continued)

EST Name <sup>a</sup>	Accession number	Reference for primer sequences and PCR protocols	Expected size (bp) <sup>b</sup>	Observed size	Identity <sup>c</sup>	Linkage group Q - C <sup>d</sup>	Functional category
Cons 105	CR627826	Casasoli et al. 2006	185	600	95.4	12 - 12	Metabolism
Cons 106	CR627828	Casasoli et al. 2006	326	326	91.2*	ni - 1	Energy
Cons 107	CR627830	Casasoli et al. 2006	272	900	92.6	11 - 3	Cell-type differentiation
Cons 109	CR627834	Casasoli et al. 2006	194	1200	100*	7 - 5	Cell rescue, defense, and virulence
Cons 110	CR627835	Casasoli et al. 2006	219	219	92.6	9 - 9	Metabolism
Cons 111	CR627837	Casasoli et al. 2006	219	219Q 600C	89.5*	12 - ni	Hypothetical protein
Cons 112	CR627839	Casasoli et al. 2006	171	171	93.4*	5 - 4	Transcription
Cons 126	CR628009	Casasoli et al. 2006	238	400	94.7	7 - 5	Protein synthesis
Cons 127	CR628014	Casasoli et al. 2006	289	289	94.3	6 - 11	Protein with binding function or cofactor requirement
Cons 128	CR628019	Casasoli et al. 2006	120	120	-	6 - ni	Energy
Cons 129	CR628021	Casasoli et al. 2006	210	500	79.6	9 <sup>d</sup> - 9	Cell rescue, defense, and virulence
Cons 130	CR628241	Casasoli et al. 2006	190	190	91.9*	2 - ni	Energy
Cons 135	CR628167	Casasoli et al. 2006	115	200	100*	ni - 1	Hypothetical protein

**Table 4.** Homologous linkage groups (LGs) in genetic maps of *Quercus robur*, *Castanea sativa*, and *C. mollissima/C. dentata*

LG in <i>Quercus robur</i> <sup>a</sup> Pedigree 3P × A4	LG in <i>Quercus robur</i> <sup>b</sup> Pedigree A1 × A2	LG in <i>Castanea sativa</i> <sup>c</sup> Pedigree Bursa × Hopa	LG in <i>C. mollissima/C. dentata</i> <sup>d</sup> Pedigree Mahogany
1	1	6	H
2	2	1	A
3*	11*	8	C
4	4	2	K
5	5	4	E
6	6	11**	B**
7	7	5	I
8	10	7	F
9	8	9	L
10	3	10	D
11	9	3	G
12	12	12	J

Assignment of linkage groups was made by comparison within the following pairs: 3P × A4 and A1 × A2, 3P × A4 and Bursa × Hopa, Bursa × Hopa and Mahogany.

\*) LG 3 in (3P × A4) and 11 in (A1 × A2) assigned by “default” (all other 11 LGs being assigned by at least 2 markers present in each species)

\*\*) LG 11 in (Bursa × Hopa) and B in (Mahogany) assigned by “default” (all other 11 linkage group being assigned by at least two markers present in each species, except for pairs 7-F and 3-G where only one marker was common.)

The numbers or letters of linkage groups (LG) correspond to the following publications:

<sup>a</sup> Barreneche et al. (1998); Barreneche et al. (2004)

<sup>b</sup> Bakker (2001) and this study

<sup>c</sup> Casasoli et al. (2001)

<sup>d</sup> Kubisiak et al. (1997); Sisco et al. (2005)

grown in hydroponic conditions. Gene expression was monitored in seedlings raised under reduced oxygen (3%) applied for 24 h. RNA was extracted from root tips before (0 h time stress) and after oxygen reduction, following the protocol of Chang et al. (1993). Stress induction was validated by measuring alcohol dehydrogenase activity. Differentially expressed fragments were obtained by cDNA-AFLP, and 170 were sequenced and compared to databanks (C. Bodénès unpublished results).

### 5.4.3 Osmotic Stress

Osmotic stress induced genes were identified in a *Q. petraea* cell line grown under moderate stress (Porth et al. 2005a). Two subtraction libraries (SSH method) were established from callus cell cultures exposed to hyperosmotic stress for 1 h (indicated as 1T) and 2 d (2T), respectively. The differentially expressed ESTs were classified according to their putative functions. At least five of these gene products were

assumed to be targets for stress tolerance in oak, e.g., betaine aldehyde dehydrogenase, two trans-acting transcription factors (one ABA-responsive, the other ABA-independent), a glutathione-S-transferase, and a heat shock cognate protein.

Seven genes were selected, based on their putative functions, to monitor their expression in vivo. Leaf tissue from hyperosmotically grown *Q. petraea* and *Q. robur* plantlets was harvested and investigated by RT-PCR at time intervals of 1, 6, 24, and 72 h. Indications of stress adaptation were found in *Q. petraea* based on up-regulation of certain genes related to protective functions, whereas in *Q. robur* down-regulation of those genes was evident (Porth et al. 2005a).

Segregating osmo-regulated loci were mapped to ten different LGs of *Quercus* (Porth et al. 2005b). By using orthologous primers, ten of the loci, including the four putatively water-stress tolerance related genes (1T57, 1T62, 2T11, and 2T13), were successfully amplified in *C. sativa*. Sequence analysis showed an identity of at least 90% (Table 3) with *Quercus*.

#### 5.4.4 Differential Expression in Juvenile and Mature Oak Shoots

A gene named QRCPE (*Quercus robur* crown preferentially expressed) that is differentially expressed between mature and juvenile-like shoots was recently discovered in oaks (Gil et al. 2003). QRCPE accumulates in ontogenetically older organs of oak trees, although it is present in zygotic and somatic embryos but absent in callus cells. The encoded protein is small, contains a predicted N-terminal hydrophobic signal peptide that targets the protein to the cell wall, and is rich in glycine and histidine residues. In *C. sativa*, the QRCPE homolog is also expressed at different levels between adult and juvenile-like tissues.

#### 5.4.5 Blight Infection in Chestnut

A cDNA clone showed similarity to a gene previously identified as encoding a cystatin. A protein shown to have antifungal activity in *C. sativa* (Pernas et al. 1998, 1999) was isolated from a cDNA library from stem tissues of *C. dentata* (Connors et al. 2001). The expression of this gene was verified by RT-PCR in healthy and diseased tissues of American chestnut (Connors et al. 2002). Amplification of a fragment of the gene in American and Chinese chestnuts and comparison of the sequences of the cloned amplification products revealed differences within the intron (SNPs or deletion). These differences could be used to locate the cystatin gene on the map of *C. mollissima*/*C. dentata* and to verify its putative colocalization with QTLs involved in blight resistance (Connors et al. 2002). However, cystatin did not map to any region known to be involved in resistance to chestnut blight.

### 5.5 QTL Detection

#### 5.5.1 Phenotypic Traits Investigated

A common objective in genetic mapping in oak, chestnut, and beech is the detection of QTLs for adaptive traits, e.g., phenotypic traits that respond strongly to natural selection, and particularly to abiotic or biotic stresses. The interest in these traits lies in the issues

raised by global change and the capacity of trees to respond to these challenges (Parmesan and Yohe 2003). This capacity depends on the level of genetic diversity for these traits and their underlying genes in natural populations. Knowledge of the genetic architecture of these traits (number and distribution of QTLs) is therefore of primary importance and has motivated research in QTL in conifers as well (Sewell and Neale 2000; van Buijtenen 2001).

In European oak, chestnut, and beech, the genetic control of three different adaptive traits, bud phenology, growth, and carbon isotope discrimination, were studied using a QTL approach (Casasoli et al. 2004; Scalfi et al. 2004; Scotti-Saintagne et al. 2004; Brendel et al. 2007). Bud phenology, growth, and carbon isotope discrimination ( $\delta$  or  $\Delta$ , which provides an indirect measure of plant water-use efficiency) are adaptive traits that show great phenotypic variation in natural populations of forest trees (Zhang and Marshall 1995; Tognetti et al. 1997; Lauteri et al. 1999; Hurme et al. 2000; Jermstad et al. 2001). Initiation and cessation of the growing seasons, defined through bud flush and bud set timing, have profound implications for adaptation of perennial plants to cold winter temperatures. Early flushing genotypes might be susceptible to spring frost damage. Likewise, bud set timing is related to the fall cold acclimation (Howe et al. 2000). Growth traits, such as annual height and diameter increments, are important components of plant vigor and biomass production, and they are profoundly influenced by abiotic and biotic stress occurrences during the growing season. In addition, they are relevant characteristics from an economic point of view and are often evaluated in breeding programs (Bradshaw and Stettler 1995). Carbon isotope discrimination ( $\Delta$ ) is a parameter related to the isotopic fractionation of carbon stable isotopes during the photosynthetic process (for review see Farquhar et al. 1989; Brugnoli and Farquhar 2000). Plant material is always enriched in  $^{13}\text{C}$  with respect to the isotopic composition ( $\delta^{13}\text{C}$ ) of atmospheric  $\text{CO}_2$ . This is particularly evident in  $\text{C}_3$  plants where the fractionation effect mostly occurs during  $\text{CO}_2$  diffusion from outside the leaf to the carboxylation sites into the chloroplasts, and during the carboxylation by ribulose 1,5-bisphosphate (RuBP) carboxylase. Due to its relationships with the diffusional path of photosynthetic gas exchange (for both  $\text{CO}_2$  and water vapor in reverse directions) and with the photosynthetic substrate demand ( $\text{CO}_2$  fixation by RuBP carboxylation activity),  $\Delta$  has been theoretically predicted and

empirically demonstrated to be inversely related to plant water-use efficiency (roughly the ratio of carbon gain to water losses; for deeper insights see Farquhar et al. 1989; Brugnoli and Farquhar 2000). Despite the complexity of this trait, significant heritabilities and low genotype  $\times$  environment interactions have been found for  $\Delta$  in crop species (Hall et al. 1994) encouraging the use of this parameter for breeding purposes.

### 5.5.2 Strategies and Methods Used for QTL Detection

In forest trees, QTLs for several traits have been detected, clearly showing the usefulness of this approach to dissect genomic regions controlling complex traits (Sewell and Neale 2000). With few exceptions (Brown et al. 2003; Jermstad et al. 2003), the size of segregating populations used in these studies is often small (150 to 200 individuals). Among factors influencing QTL detection power, small sample sizes and low trait heritability were shown to cause an overestimation of QTL effects and the underestimation of QTL number and to hamper detection of QTLs with low effects (Beavis 1995). For these reasons, a single QTL detection experiment does not give an exhaustive idea of the genetic architecture of a quantitative trait. One possible strategy to overcome these difficulties is to detect QTLs several times across different environments and developmental stages. In this way, environmental and temporal stability of QTLs can be verified and a more complete picture of genetic architecture of the complex trait can be drawn. Moreover, comparative QTL mapping between phylogenetically related species offers an important tool to validate QTLs from the evolutionary point of view. In oak and chestnut, a QTL-detection strategy based on multiple experiments across different environments and years has been performed to give an idea, as much as possible, of the complete genetic architecture of adaptive traits in both species. Afterwards, comparative QTL mapping for the three adaptive traits studied was carried out between the two species in order to identify genomic regions conserved through evolution controlling these traits.

In oak, QTL detection was done in both the French (3P\*A4) and Dutch (A1\*A2) *Q. robur* mapping pedigrees. In the French studies, phenotypic assessments were done over successive years using a clonal test

planted with the vegetative copies of the full-sibs belonging to the pedigree. The phenotypic data obtained so far all originate from the two plantations installed in the southwest of France. The assessments first addressed the same three major adaptive traits as for chestnut: phenology, growth, and carbon isotope discrimination (Scotti-Saintagne et al. 2004; Brendel et al. 2007). In addition, leaf morphology characters (Saintagne et al. 2004) and the ability for vegetative reproduction by cutting propagation (Scotti-Saintagne et al. 2005) were assessed. The focus on leaf morphological traits is related to their use in species discrimination as shown by previous analyses (Kremer et al. 2002). The Dutch study focused on QTL detection for morphological and growth characters in one specific full-sib cross that was grown for two successive years in a nursery (Bakker 2001).

In European chestnut (*C. sativa*), bud flush, growth, and carbon isotope discrimination measurements were performed for 3 years: 2000, 2001, and 2002, corresponding to the growing seasons 2, 3, and 4 since seed germination. Bud set timing was scored only in 2002. During the three years, plants were grown in central Italy (Istituto di Biologia Agroambientale e Forestale, CNR, Porano, central Italy, 42° 43' latitude, 500 m elevation) as previously reported. Details about phenotypic measurements are reported in Casasoli et al. (2004) and in Table 5.

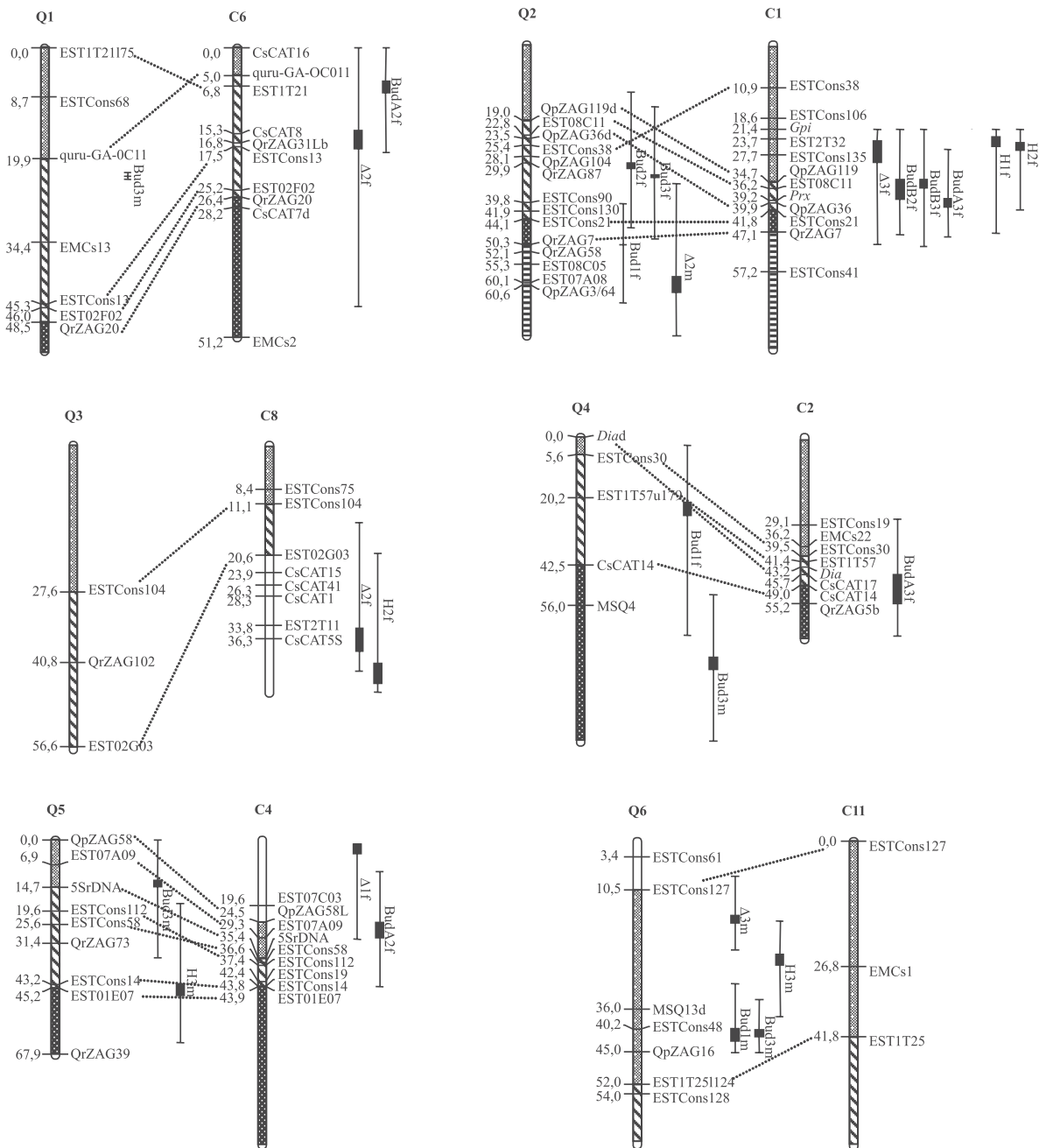
In American (*C. dentata*) and Asian (*C. mollissima*) chestnut, the blight resistance response of F<sub>2</sub> progeny was assessed by using the agar-disk cork-borer method (Griffin et al. 1983). During the growing season, each F<sub>2</sub> individual was inoculated with two different strains of *Cryphonectria parasitica*. Canker evaluations were made over two successive months. The mean canker sizes in each month for each isolate were used as relative measures of resistance. The degree of association between marker loci and blight resistance trait was investigated using successively single-locus or nonsimultaneous analysis of variance (ANOVA) models and multiple marker or simultaneous analysis of variance (ANOVA) models (Kubisiak et al. 1997). In European beech (*F. sylvatica*), leaf traits (size and shape) were assessed over 2 years, whereas growth and carbon isotope discrimination measurements were done only one year.

The MultiQTL software (Britvin et al. 2001, <http://esti.haifa.ac.il/~poptheor>) was used for QTL detection both in oak and chestnut in the French

Table 5. QTL data in oak, chestnut, and beech

Trait	Species (reference)	Pedigree	Number of offsprings phenotyped	Number of field plantations where the trait was assessed	Growing season(s) when the trait was assessed	Heritability or repeatability	Number of QTLs detected <sup>(f)</sup>	Range of variation of PEV (minimum – maximum)
Height growth	<i>Quercus robur</i> <sup>(a)</sup>	3P × A4	207	1	4 <sup>a</sup>	0.14–0.23 <sup>c</sup>	5	9.5–18.7
	<i>Quercus robur</i> <sup>(b)</sup>	A1 × A2	101	1	2	–	1	31.2
	<i>Castanea sativa</i> <sup>(c)</sup>	Bursa × Hopa	135–153	1	3	–	6	7.0–17.0
	<i>Fagus sylvatica</i> <sup>(d)</sup>	44 × 45	118	1	1	–	0	–
	<i>Quercus robur</i> <sup>(a)</sup>	3P × A4	174–278	3	8, 4, 5	0.15–0.52 <sup>c</sup>	12	3.1–10.7
Bud burst	<i>Quercus robur</i> <sup>(b)</sup>	A1 × A2	101	1	1	–	2	Trait not normally distributed.
Delta	<i>Castanea sativa</i> <sup>(c)</sup>	Bursa × Hopa	150–174	1	3	–	9	Nominal scale of 1–5
	<i>Fagus sylvatica</i> <sup>(d)</sup>	44 × 45	124	1	1	–	1	6.3–12.2
	<i>Quercus robur</i> <sup>(e)</sup>	3P × A4	121–207	2	4, 5, 5	0.32–0.80 <sup>c</sup>	5	27.3
	<i>Castanea sativa</i> <sup>(c)</sup>	Bursa × Hopa	152–155	1	3	–	7	4.4–34.4
	<i>Fagus sylvatica</i> <sup>(d)</sup>	44 × 45	102	1	1	–	0	5.7–13.2
Bud set	<i>Castanea sativa</i> <sup>(c)</sup>	Bursa × Hopa	151	1	1	–	3	–
Diameter growth	<i>Quercus robur</i> <sup>(b)</sup>	A1 × A2	101	1	2	–	–	8.9–17.1
	<i>Castanea sativa</i> <sup>(c)</sup>	Bursa × Hopa	136–153	1	3	–	4	No significant QTL detected
	<i>Castanea sativa</i> <sup>(c)</sup>	Bursa × Hopa	136–153	1	3	–	4	5.9–10.3

<sup>(a)</sup> Scotti-Saintagne et al. (2004)<sup>(b)</sup> Bakker (2001)<sup>(c)</sup> Casasoli et al. (2004)<sup>(d)</sup> Scalfi et al. (2004)<sup>(e)</sup> Brendel et al. (2007)<sup>(f)</sup> QTL detected at  $p < 0.05$  at the genome level



**Fig. 3.** Comparative QTL mapping between *Q. robur* and *C. sativa* (from Casasoli et al. 2006). Homeologous LGs between *Q. robur* (Q) and *C. sativa* (C) are named and ordered as in Figs. 1 and 2. Orthologous markers are linked by dotted lines. Common intervals between the two genomes identified by orthologous markers are filled with corresponding backgrounds in both oak and chestnut LGs. The figure was drawn using MapChart software (Voorrips 2002). Each QTL is represented on the right of the LG by its confidence interval (95% confidence intervals, black line) and the most probable position (Casasoli et al. 2006). QTLs were detected for three different phenotypic traits on the male (m) and female map (f): bud burst (Bud), total height (H), and carbon isotope discrimination ( $\Delta$ ). The phenotypic traits were observed over three seasons (indicated by subscripts 1 to 3). In oak, the date of bud burst was assessed as the date when the apical bud flushed. In chestnut, bud burst was assessed in two different ways: A = date of first observed unfolded leaf of a tree; B = date when 70% of buds showed an unfolded leaf BudA2f: QTL for bud burst assessed with method A during season 2 in female map. H2m: QTL for total height measured at season 2 and located on male map.  $\Delta$ 3f: QTL for carbon isotope discrimination assessed during season 3 and located on female map

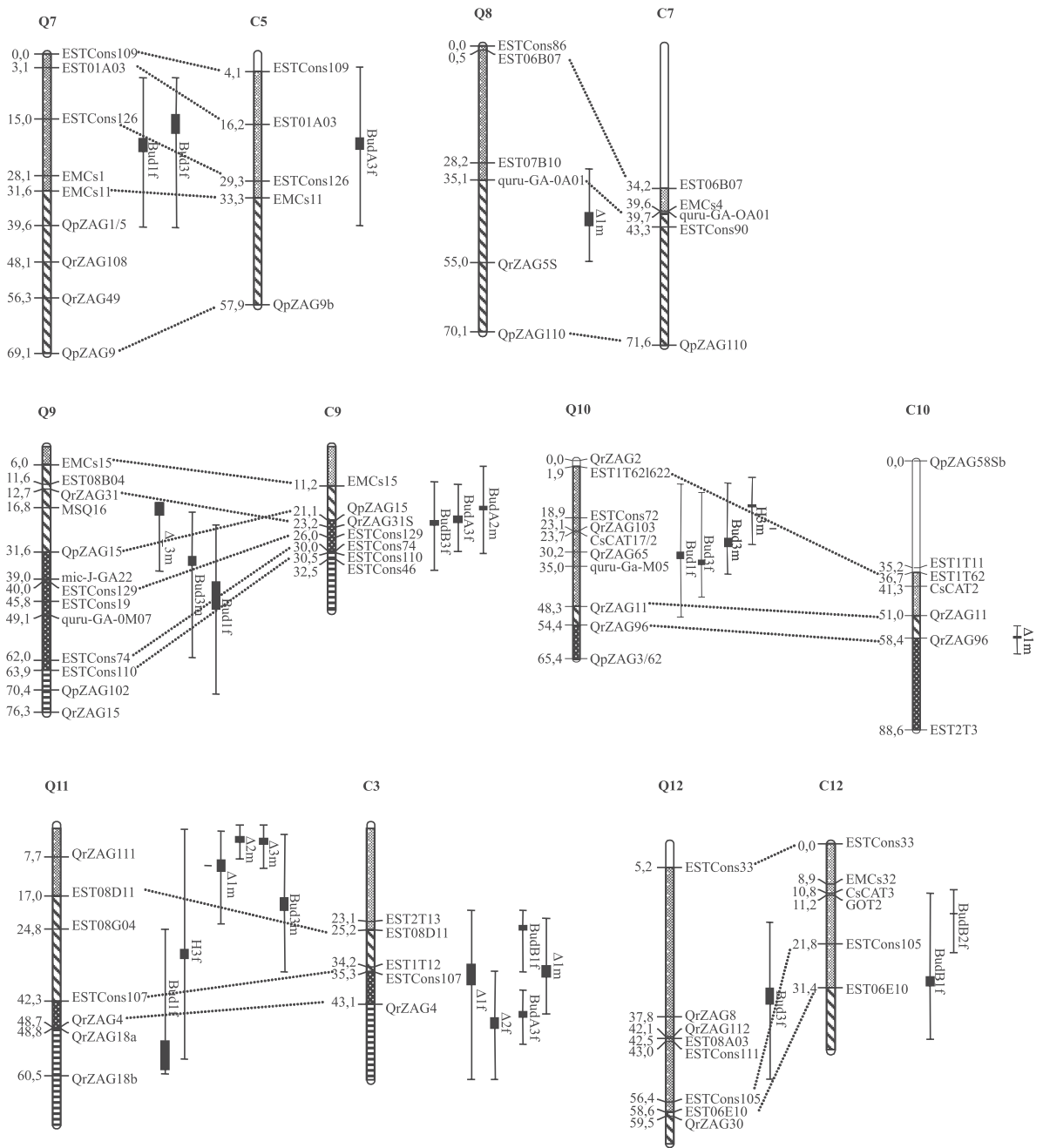


Fig. 3. (continued)

studies. This software was chosen for several reasons. First, the composite interval mapping was available (CIM, Jansen and Stam 1994; Zeng 1994); second, QTL significance thresholds could be computed by permutation (Churchill and Derge 1994); and finally, confidence intervals for QTL position could be estimated by bootstrap (Visscher et al. 1996). The same statistical analysis

was performed in oak and chestnut; the details are reported in Casasoli et al. (2004) and Scotti-Saintagne et al. (2004). The Dutch study used MapQTL 4.0 (<http://www.kyazma.nl/index.php/mc.MapQTL>) for QTL detection. For beech, QTL Cartographer 1.12 (Basten et al. 1994, 2002) and MultiQTL (Britvin et al. 2001) softwares were used to detect QTLs (Scalfi et al. 2004)



### 5.5.3 Number and Distribution of QTLs and Their Effects

The results of QTL detection are extremely heterogeneous across pedigrees and species. The survey of the results has been limited to traits that were assessed in at least two species (Table 5). Heterogeneity among the results is most likely related to the reduced size of the mapping pedigree. As already mentioned, with on average less than 200 sibs per pedigree, the contribution of a given QTL to the phenotypic variance of the trait is usually overestimated and exhibits a large sample variance (Beavis 1995). However, because the sampling efforts were similar and the phenotypic assessments were the same, a comparative analysis of QTL detection could be done between *Q. robur* and *C. sativa*.

The alignment of the 12 *Q. robur* and *C. sativa* LGs gives rise to a logical framework defined by common orthologous markers for comparing QTL location between the two species. Figure 3 shows the alignment of the 12 LGs, the common genomic regions identified by orthologous markers, and QTLs compared in oak and chestnut. Details about the definition of common genomic intervals and corresponding unique QTLs between the two species (i.e., more individual QTLs detected several times in the same genomic region in a single species) are reported in Casasoli et al. (2006). A total number of 34 common intervals were identified between the oak and chestnut genetic linkage maps thanks to the orthologous markers. Following the previously described criteria to declare unique QTLs, 13 and 10 unique QTLs were identified for timing of bud burst, 5 and 7 unique QTLs were identified for carbon isotope discrimination, and, finally, 5 and 6 unique QTLs for height growth were identified in oak and chestnut, respectively (Fig. 3). Among these unique QTLs, nine controlling timing of bud burst and two controlling height growth were collocated between the two species. No QTL involved in carbon isotope discrimination was collocated in the oak and chestnut map. Following Lin et al. (1995), the probability of obtaining these collocations by chance is  $p = 0.0002$  in the case of timing of bud burst and  $p = 0.20$  in the case of height growth. When QTL number and effects were compared for the three traits between the two species, a similar genetic architecture was observed for adaptive traits in oak and chestnut (Casasoli et al. 2006). From this simple comparison it was clear that adaptive traits are controlled by more loci of low and moderate

than large effect in both species. Timing of bud flush was the trait showing the higher number of detected and stable QTLs. Despite this similar genetic architecture, most of the QTLs for bud flush were conserved, whereas only a few QTLs were conserved for height growth, and none for carbon isotope discrimination. The different conservation of QTLs may be explained taking into account differences for the three adaptive traits investigated in trait heritability values, QTL stability across experiments, and QTL-by-environment interactions. The striking conservation of QTLs for bud flush is very interesting from an evolutionary point of view. Although correspondence of QTLs does not imply correspondence of genes underlying the QTLs, as already reported in other species (Doust et al. 2004), these findings showed that loci controlling bud flush have remained highly polymorphic in both species. This high polymorphism of loci controlling bud flush, despite strong natural selection acting on this adaptive trait, may be explained with selection pressures able to maintain diversity over long evolutionary times (balancing, disruptive, or frequency-dependent selection) as discussed in Casasoli et al. (2006).

## 5.6 Conclusion

Mapping experiments in Fagaceae were hampered by various biological constraints that have limited research activities in this field. First, for most species, it was not possible to find adequate  $F_2$  pedigrees that would allow us to screen the genome for QTLs of interest. This is somehow compensated by the high level of within-population diversity, which would allow segregation for QTLs of interest in  $F_1$  pedigrees as well. Second, controlled crosses to obtain mapping  $F_1$  pedigrees has been challenging in these species, and alternatives based on open-pollinated progeny screening using parentage analysis were implemented. Third, the development of mapping activities was restrained by the limited genomic resources available (genetic markers, ESTs) within this group of species. Despite these limitations, important progress has been made in the recent years as a result of international cooperation. Maps have been developed for each economically important genus (*Quercus*, *Castanea*, and *Fagus*), and the ongoing activities in comparative mapping suggest that there

is a strong macrosyteny between phylogenetically close genera (*Quercus* and *Castanea*). For some traits, e.g., bud burst, there is even a strong conservation of the QTL position between the two genera. Extension of comparative mapping to *Fagus* might be more problematic as illustrated by difficulties described in this review. However, comparative mapping should be much easier with *Lithocarpus* and *Castanopsis*, as these genera are close to *Quercus* and *Castanea*. Furthermore, the genome of the Fagaceae is of small enough size (e.g., only 3.5 to 6 times larger than *Arabidopsis*) to make comparative genomics easily applicable to this family. These expectations should enhance research activities in genetics within a large group of ecologically and economically important species growing throughout the northern hemisphere.

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