# An Improved Genetic Map for *Castanea mollissima*/*Castanea dentata* and Its Relationship to the Genetic Map of *Castanea sativa*

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**Abstract**

**We have added 275 AFLP and 24 SSR markers and the 5SrDNA locus to a previously published genetic map based on a hybrid cross between *Castanea mollissima* and *C. dentata*. The SSR markers, 5SrDNA locus, and one isozyme locus also permitted us to correlate the linkage groups in the published genetic map of *C. sativa* with those in our *C. mollissima*/*C. dentata* map. Correlating the map of European chestnut with that of American and Chinese chestnut will allow greater progress in mapping important physiological and adaptive traits across species boundaries.**

## INTRODUCTION

Comparative genetic mapping between species and genera is a tool that is proving to be extremely useful to the study of genetic mechanisms of biological control. Blocks of genes on a particular chromosomal segment are often conserved across wide species boundaries. Knowing the location of these conserved genomic regions permits the cross-referencing of studies among species, even those that are rather distantly related (Bennetzen and Freeling, 1997; Trachtulec and Forejt, 2001; Dominguez et al., 2003; Bourque et al., 2004). The primary goal of this study was to create a comparative genetic map among three species of chestnut: *Castanea dentata*, *C. mollissima*, and *C. sativa*. Each of these three species has 12 chromosome pairs (Jaynes, 1962), and hybrids among all of them can be created quite easily (Jaynes, 1979). A genetic map based on a hybrid between *C. dentata* and *C. mollissima* was published in 1997 (Kubisiak et al., 1997), while the genetic map of *C. sativa* was published in 2001 (Casasoli et al., 2001). Each of these published maps described 12 linkage groups, which were thought to correspond to the 12 chromosome pairs. The 1997 paper labeled the linkage groups (LGs) A through L, while the 2001 paper labeled the groups 1 through 12. Our question was simple. Which among the twelve LGs in the *C. mollissima*/*C. dentata* map corresponded to LG1 in the map of *C. sativa*, which corresponded to LG2, etc. To answer this question we were able to make use of several microsatellite (Simple-Sequence-Repeat = SSR) markers that had been developed in the laboratories of Roberto Botta, Karen Russell, Josef Glossl and Herta Steinkellner. SSR markers are often conserved across species boundaries, are

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usually co-dominant, and are amenable to automated methods of scoring, making them very valuable for comparative mapping (Belaj et al., 2003).

A secondary goal of the study was to correct and improve upon the genetic map of *C. mollissima/C. dentata* that had been published (Kubisiak et al., 1997). Subsequent mapping of a backcross population originating from the same $F_1$ parents used in the 1997 study had suggested that LGs B and E might belong together on the same chromosome and that one linkage group was missing from the published map (Hebard and Kubisiak, person. commun.). To resolve this problem, our initial effort was to add several hundred AFLP markers to the *C. mollissima/C. dentata* map, taking advantage of remnant DNA of the mapping population that had been preserved (Clark et al., 2001). However, the AFLP markers were distributed in a similar fashion to the previously mapped RAPD markers and had a similar degree of linkage distortion. So it was critical to correlate the linkage groups described in the 1997 *C. mollissima/C. dentata* genetic map with those found in the 2001 genetic map of *C. sativa* (Casasoli et al., 2001).

## MATERIALS AND METHODS

### Plant Material

In 1990, an interspecific $F_2$ mapping population of 193 trees was generated by crossing two $F_1$ trees at the Connecticut Agricultural Experiment Station in New Haven, CT, USA. The Chinese cultivar 'Mahogany' was the female parent of both $F_1$ trees. Two different American chestnut trees from Roxbury, CT, were used as males to create the $F_1$ trees. The cross to create the $F_2$ population was made in one direction only, using R4T52 as female.

| $F_1$ Tree R4T52 | X | $F_1$ Tree R4T31 |
|---|---|---|
| (*C. mollissima* 'Mahogany' x *C. dentata*) | | (*C. mollissima* 'Mahogany' x *C. dentata*) |

$F_2$ trees planted: 193
$F_2$ trees rated for resistance to *Cryphonectria parasitica*: 185
$F_2$ trees analyzed with RAPDs, RFLPs, and isozymes: 102
$F_2$ trees analyzed with AFLPs: 89*
$F_2$ trees analyzed with SSRs: 87*
*The 89 and 87 trees included many of the original 102 plus others of the total set of 185 trees, depending on which DNA samples remained.

### Marker Analyses

DNA had been isolated from leaf tissue as described in Kubisiak et al. (1997). We used remnant DNA from the original study. SSR analyses were done at the Southern Institute of Forest Genetics in Saucier, Mississippi, USA, and AFLP analyses at North Carolina State University, Raleigh, NC, USA. Isozyme analyses were done at the CNR in Porano, Italy, using proteins extracted from dormant buds (Villani et al., 1991).

### Mapping Software

JoinMap ver. 3.0 (VanOoijen and Voorrips, 2001) was used for assigning markers to linkage groups using the CP population type (heterozygous parents, four possible alleles).

## RESULTS

### Addition of AFLP Markers

Catherine Clark in the Forest Biotechnology Laboratory at North Carolina State University added 275 AFLP markers to the *C. mollissima/C. dentata* map, making a total of 516 markers. Of these, 112 were distorted and not included in the linkage analysis. The AFLP markers were similar in distortion to the other marker types. Percent distorted for

each marker type were AFLPs 22%, RAPDs 20%, RFLPs 31%, and isozymes 25%. Although the total marker number was nearly doubled by the addition of AFLPs, the total genetic map distance increased by only 21 cM compared to the 1997 genetic map. The AFLP markers were distributed randomly throughout the genome, not clustered, as they are in the genetic maps of some other species.

**Addition of SSR Markers and Correlation with the Map of European Chestnut**

With the advice and assistance of Manuela Casasoli, Fiorella Villani, Teresa Barreneche, and Antoine Kremer, Tom Kubisiak at the Southern Institute of Forest Genetics added 24 SSR markers, the 5S ribosomal DNA locus and one isozyme marker (GOT) to the map. These markers were chosen because they were distributed across the European chestnut map (Barreneche et al., 2004). Dr. Kubisiak was not told which European chestnut linkage groups were involved until he had assigned the loci to the *C. mollissima/C. dentata* map. In this way we were able to correlate 11 of the 12 linkage groups between the *C. mollissima/C. dentata* genetic map with the linkage groups in the map of *C. sativa*, as shown in Table 1.

## DISCUSSION

**Advantages for QTL Analysis**

It is expected that many traits identified by Quantitative Trait Locus (QTL) analysis will have common loci governing them in all the chestnut species. The correlation of the European, American and Chinese chestnut maps will permit identification of these regions. Several QTLs have already been mapped in European chestnut (Casasoli et al., 2004).

**Identification of Contaminants and Reanalysis of the Chinese/American Genetic Map**

The SSR loci are so highly polymorphic that they revealed contaminants in the original Chinese/American chestnut $F_2$ mapping population. Of the 102 trees analyzed for the published map (Kubisiak et al., 1997), 18 proved to be the result of contaminating pollen. When the contaminant trees were removed from the mapping analysis, the results showed that there were only two loci governing resistance to *Cryphonectria parasitica* rather than three. The locus on LG G disappeared, leaving only the loci on LGs B and F.

**No Common Loci Found between LGs B and 11**

It is a reasonable conjecture that LG B on the Chinese/American chestnut map corresponds to LG11 on the European chestnut map. However, we have not yet mapped any markers that are common to the two linkage groups. Approximately 50 Expressed Sequence Tags (ESTs) have now been mapped on the European population, and it is our hope that some of these will allow these linkage groups to be correlated.

**Segregation Distortion Complicates Mapping of the Chinese/American $F_2$ Population**

The high degree of segregation distortion in the *C. mollissima/C. dentata* mapping population creates the possibility of pseudo-linkages. Several regions of the genetic map are distorted in the direction of the Chinese parent. Loci that are distorted in a similar manner are correlated, giving the appearance of genetic linkage for loci that may in fact be on different chromosomes. This has caused us problems in mapping LGs B and E in particular. For this reason it is very important for us to find common markers between the European genetic map and our LG B. We are also creating new intraspecific mapping populations within *C. dentata* and within *C. mollissima* in hopes of avoiding the segregation distortion we found in the interspecific mapping population. Nevertheless, the interspecific $F_2$ was the best population for measuring variation among trees for resistance to chestnut blight.

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**Tables**

Table 1. Related linkage groups of American, Chinese, and European chestnut.

| Chinese/American Chestnut Linkage Groups | European Chestnut Linkage Groups | Common Loci Mapped as of November, 2004 |
|---|---|---|
| A | 1 | QpZAG36,QrZAG07 |
| B | 11? | None |
| C | 8 | CsCAT01,CsCAT05,CsCAT15,CsCAT41 |
| D | 10 | CsCAT02,QpZAG58Sb,QrZAG11,QrZAG96 |
| E | 4 | EMCs38, 5SrDNA |
| F | 7 | EMCs4 |
| G | 3 | QrZAG04(b) |
| H | 6 | CsCAT07, CsCAT08, QrZAG20 |
| I | 5 | EMCs14, QpZAG09 |
| J | 12 | CsCAT03,GOT |
| K | 2 | CsCAT14, CsCAT17 |
| L | 9 | EMCs15,QpZAG15,QrZAG31 |