AN ABSTRACT OF THE THESIS OF

Brooke C. Peterschmidt for the degree of Master of Science in Horticulture presented on February 26, 2013

Title: DNA Markers and Characterization of Novel Sources of Eastern Filbert Blight Resistance in European Hazelnut (*Corylus avellana* L.)

Abstract approved:_____
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European hazelnut is a significant crop in the Pacific Northwest, and the US ranks 4th internationally for hazelnut production. Production in the Pacific Northwest is threatened, however, by the disease eastern filbert blight (EFB) caused by the fungus *Anisogramma anomala* (Peck) E. Müller. To meet the challenges faced by the hazelnut industry in Oregon and Washington, the breeding program at Oregon State University has focused on developing DNA marker technology and producing EFB resistant cultivars. This study focused on developing new microsatellite markers from hazelnut transcriptome sequences and on disease resistance from three accessions ('Culpla,' 'Crvenje,' and OSU 495.072) which showed no disease symptoms following a series of inoculations.

DNA markers have been useful in hazelnut breeding for marker-assisted selection, construction of genetic linkage maps, cultivar fingerprinting, and phylogeny studies. Previously developed markers include AFLP, RAPD, ISSR, and microsatellite (SSR) markers developed from enriched libraries and ISSR fragments. This study utilized the transcriptome sequence from 'Jefferson' hazelnut to mine for microsatellites, align with the genomic sequence, design primers, screen for polymorphism, and characterize and map polymorphic markers. A total of 1432 microsatellites were mined from the transcriptome sequence, and the most frequently found motifs were AG (35.8%), AT

(13.3%), and AAG (12.7%), and 382 primer pairs were designed. Screening showed that 119 markers were polymorphic, and these were characterized on sets of 50 and 14 accessions. Fifty-three markers that segregated in the mapping population or in three alternate populations were mapped and assigned to linkage groups. A dendrogram showed that accessions clustered mostly according to geographic origin. These results confirm the high level of diversity present in hazelnut, and the markers developed in this study will be useful for further genetics studies in hazelnut.

The three EFB resistant parents 'Culpla,' 'Crvenje,' and OSU 495.072 were subjected to two inoculation treatments: greenhouse inoculations and exposure under an inoculation structure. The accessions remained free of disease after both treatments. Progeny segregating for resistance were produced. The progeny were inoculated either in the greenhouse or under the structure, and disease response recorded for each individual. DNA was extracted from seedlings, and sets of 32 seedlings from each resistant parent were screened with previously mapped markers using PCR and capillary electrophoresis. All three resistance sources were correlated with marker A614, allowing the resistance loci to be assigned to linkage group (LG) 6. The progeny were then screened with all known microsatellite markers on LG 6, and linkage maps constructed of the marker loci and resistance loci. Markers KG821, LG628, and LG696 are especially close to the resistance loci and will be useful for marker-assisted selection. Although these resistance loci are located in the same region of LG 6 as the 'Gasaway' resistance gene, they are different from 'Gasaway,' and markers linked to resistance will be useful for introgressing and pyramiding resistance in new cultivars.

DNA Markers and Characterization of Novel Sources of Eastern Filbert Blight Resistance in European Hazelnut (*Corylus avellana* L.)

by
Brooke C. Peterschmidt

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented February 26, 2013
Commencement June 2013

Master of Science thesis of Brooke C. Peterschmidt presented on February 26, 2013.

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Brooke C. Peterschmidt, Author

ACKNOWLEDGEMENTS

I wish to thank my advisor Dr. Shawn Mehlenbacher for the leadership, mentoring, and advice shared during my time as a graduate student. His guidance made it possible to complete my degree. I also wish to thank my committee members, Dr. Nahla Bassil, Dr. Shaun Townsend, and Dr. Thomas Wolpert, who have been so gracious in offering their support and advice.

I wish to thank Dr. Vidyasagar Sathuvalli, who shared his wealth of knowledge and laboratory experience with me and made my research more successful. His encouragement throughout my research was invaluable. Thank you, David Smith, for teaching me so much about the field aspects of my research. I thank my colleagues in the department: Rebecca McCluskey, Gehendra Bhattarai, Megan Mathey, Renee Harkins, Christina Hagerty, Joseph Rothleutner, Oscar Vargas, Ryan King, Hannah King, Jeb Galloway, and Colton Ives. I am especially thankful to my wonderful friends for all the fun times we shared during these years: Allan Flemming, Nadine Hanhan, Colin Harthcock, Linden Rogers, Toby Polocoser, Matthew McIntire, Kyle Janssen, Sharon Smythe, Kim Ogren, Katie Engberg, Louise Doolittle, Malia Doolittle, Anna Sanner, Ariel Wasson, and Kathryn Brown.

I wish to say how grateful I am to my family and how deeply I love you all. Without you, this would not have been possible. Thank you, Mom and Dad, for your love and support and for teaching me since I was young. I thank my brothers Tyler, Jordan, Aaron, Drew, Evan, and Espen for the closeness and camaraderie we share. I give a warm thanks to all my extended family for the encouragement offered through the years. Finally, I thank my fiancé Gabe for loving, supporting, and cheering me to the finish line.

CONTRIBUTION OF AUTHORS

Dr. Shawn Mehlenbacher was the primary designer of the experiments (Chapters 2 and 3). Dr. Mehlenbacher also provided the equipment, facilities, and funding for this research. David Smith made controlled crosses in the field, propagated progeny from seed, grafted all the plant materials in the greenhouse for disease evaluation and was involved in field data collection. The plant materials were obtained from OSU hazelnut breeding program. Development of microsatellite markers, characterization of microsatellite markers, disease segregation analysis, primer screening, map construction, and writing the manuscript was performed by Brooke Peterschmidt.

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CHAPTER 1

INTRODUCTION

Introduction to European Hazelnut

Hazelnut (*Corylus*) is a genus of the birch (Betulaceae) family that contains 8 shrub species and 5 tree species that are found in the Northern hemisphere. The trees grow wild in many regions of the world, through Europe, Asia, and North America. The hazelnut fruit is a kernel enclosed in a hard, brown shell that grows either partially or fully covered by a leafy husk. The kernels of all *Corylus* species are edible, but the European hazelnut (*Corylus avellana* L.) is most suited to cultivation and crop production due to the size, quality, and quantity of the kernels. European hazelnut is an economically significant crop in a few areas with mild, temperate climates, but the wild range of European hazelnut is quite extensive. *Corylus avellana* trees are found throughout Europe, including the British Isles, Scandinavia, Russia, the Ural and Caucausus Mountains, Turkey, and Spain (Hummer, 2009). It is the European hazelnut that has the longest history of human cultivation and that is grown commercially in several regions around the world.

European hazelnuts are grown in orchards as multi-stemmed trees or pruned to a single trunk, and suckers are pruned out or burned with herbicide to maintain the desired form. Existing cultivars are suited to Mediterranean climates with mild winters and warm summers, since this provides the ideal environment for flowering and nut production.

Hazelnuts begin bearing fruit in their 3rd to 4th year and are fully mature by the 7th to 10th year. The trees have a small genome of 0.48 pg/1C and are diploid (2n = 2x = 22), monoecious, and dichogamous. Flowers bloom in the winter. Pollen is shed by catkins, and wind is responsible for transferring the pollen to female flowers. The trees express sporophytic incompatibility controlled by the S-locus, preventing self-pollination (Thompson, 1979a) and resulting in forced outcrossing. Fertilization of the ovule and nut set are completed between mid-May and early June, five months following pollination (Thompson, 1979b; Beyhan & Marangoz, 2007), and nuts are fully mature in September or October (Germain, 1994). Nuts may be harvested from the branches by hand labor, as is done in Turkey; or as is practiced in the Pacific Northwest of the US, the mature nuts are allowed to fall to the flat, highly manicured orchard floor and picked up by machine.

Worldwide commercial production of hazelnuts has grown steadily over the past decades. There are over 603,000 Ha of harvested land in hazelnut production worldwide, with more than 11,000 Ha in the United States (FAOSTAT, 2012). The greatest production of hazelnuts occurs in Turkey (74% of world production), Italy (15%), Spain (3%), Azerbaijan (2%), and the United States (2%) (USDA, 2004). United States annual production grew from less than 11,000 metric tons in 1961 to more than 25,000 metric tons in 2010 (FAOSTAT, 2012). Ninety-nine percent of US hazelnuts are produced in the temperate climate of Oregon's Willamette Valley (Mehlenbacher & Olsen, 1997).

Hazelnuts are sold in-shell or as kernels. The kernels are valued for their excellent flavor, high oil content, and good suitability for use in confections and chocolates (USDA, 2002). Hazelnut consumption has many nutritional benefits, as they are a good source of dietary fiber, Vitamin E, magnesium, B vitamins, and healthy monounsaturated

fats (oleic acid and alpha-linolenic acid) (USDA, 2012). Hazelnuts also contain high levels of proanthocyanidins, a category of phenolic compounds that are potent antioxidants when consumed in the diet (Gu et al., 2004), and hazelnuts contain a higher concentration of α–tocopherol than other nuts, adding significantly to their antioxidant profile. Hazelnuts rank quite high in total antioxidants compared to other foods such as blueberries, almonds, and apples (Wu et al., 2004). With all these beneficial vitamins and compounds, hazelnuts have been suggested to provide anti-cancer properties and reduce risk of coronary heart disease (Richardson, 1997), making hazelnuts an excellent choice for culinary uses.

In addition to the value of the kernels, hazelnuts have potential as biodiesel feedstock (Xu & Hanna, 2010; Moser, 2012). The oil derived from hazelnut has been evaluated and appears to be a satisfactory alternative to the common soybean or rapeseed derived feedstock, and in fact it is considered superior to soybean oil for biodiesel production (Xu et al., 2007).

Because hazelnuts are so valuable for their flavorful kernels as well as their potential for biodiesel production, there is increased interest in producing hazelnuts across the US. However, existing hazelnut production is largely limited to the Willamette Valley of Oregon. European hazelnuts were first brought to the USA in the late 1800's from Europe by a nurseryman named Felix Gillet (1835-1908), and he is credited with initiating the commercial hazelnut industry in the USA (Mehlenbacher and Miller, 1989). The first large commercial hazelnut orchard was started in 1900 by George Dorris in Springfield, Oregon (Hummer, 2001). Since then, commercial hazelnut production has thrived in the warm summers and mild winters provided by the Willamette Valley.

Currently, over 29,000 acres of hazelnuts are grown in Oregon (ODA, 2012), comprising a significant portion of total agricultural production in the state. The trees are generally grown in large orchards as single-trunk trees, and harvested by machine.

A breeding program for hazelnut has existed at Oregon State University since 1969. The program strives to develop new, improved cultivars to support the hazelnut industry in Oregon. The program focuses on developing high-yielding varieties with round nuts, thin shells, high kernel quality, early maturity, suitability for machine harvest, and disease resistance. The program uses many tools to evaluate the seedlings, including field trials and disease inoculations. With the advent of molecular technology, DNA markers have been developed for hazelnut and used in the breeding program.

DNA-Based Markers

Molecular markers are genomic-based tools that provide information about the genetic characteristics of an organism, and their use has become widespread throughout many disciplines, including plant breeding. Some of these markers are species- or genus-specific and are useful for determining genotype of an individual, cultivar identification, aiding in selection in breeding populations, determining genetic relatedness, map-based cloning of desired genes, construction of genetic linkage maps (Nam et al., 1989), and assisting in plant variety protection (Xu & Crouch, 2008). Markers may be developed from specific gene regions or random regions in the genome. Markers that show the presence or absence of specific alleles at a locus are useful for selection when those alleles are ‘linked’ to a trait of interest (Collard & Mackill, 2008). Markers linked to genes of interest, allow the breeder to select plants carrying a desirable allele, a procedure called marker-assisted selection (MAS). Or, if the trait of interest is under the control of

many genes or quantitative trait loci (QTL), genetic marker data can be used to identify the number and locations of loci that affect the trait and the potential of an individual plant.

Selecting for disease resistance (R) is an area that has benefited from the use of markers when markers linked to the R gene are available. Using molecular markers to screen parents and seedlings in a breeding population allows the breeder to determine which individuals carry a disease resistance gene. If more than one gene is desired and closely linked markers are available, each individual can be screened, and those carrying the desired gene(s) are kept, while the individuals lacking the resistance gene(s) are discarded. This process of marker-assisted selection is becoming widely used. It can greatly speed the process of selection, since a breeder will not be required to wait for phenotypic traits to become apparent. This is especially useful in crops with long juvenile periods, crops in which the pathogen has a long latent period, situations in which scoring the phenotype directly is time-consuming, difficult, and expensive, or to select for resistance in the absence of a pathogen. Using markers can also significantly reduce costs by allowing a breeder to more quickly cull individuals and reduce the numbers of plants planted and evaluated in the field.

There are several types of markers designed for research in plant genetics. Restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite or simple sequence repeat (SSR), inter-simple sequence repeat (ISSR), and single nucleotide polymorphism (SNP) are common forms of markers. In hazelnut breeding, RAPD and microsatellite markers have been used for MAS. Five hundred seventy RAPD markers

(Pomper et al., 1998; Mehlenbacher et al., 2004, 2006; Sathuvalli et al., 2011), 236 microsatellite markers (Bassil et al., 2005a, 2005b; Boccacci et al., 2005; Gurcan, 2009), and 13 ISSR markers (Gürcan et al., 2009) have been developed for use in hazelnut, allowing for construction of a genetic linkage map. Additionally, markers linked to eastern filbert blight resistance genes in 'Gasaway,' 'Ratoli,' OSU 408.040, and Georgian accession OSU 759.010 have been identified (Mehlenbacher et al., 1991; Sathuvalli et al., 2011a; 2011b; 2012).

RFLP was the first type of molecular marker developed, and the technique originated in the 1980's (Phillips & Vasil, 2001). They are created when a plant DNA sample is cut by a restriction enzyme that targets specific short sequences scattered through the genome. The resulting fragments of DNA are separated by gel electrophoresis, transferred to a membrane by Southern blotting, and fragments are hybridized with a DNA probe and visualized by autoradiography. Variations in fragment length are scored as different alleles and are useful for genetic analysis. This type of marker follows simple Mendelian segregation, and is codominant (Botstein et al., 1980). Over the years as molecular technology has continued to progress, these hybridization-based markers have largely been replaced with newer PCR-based techniques.

Random amplified polymorphic DNA (RAPD) markers are another PCR-based tool that targets random sites in the genome. They have been used for marker-assisted selection. These markers use an arbitrarily designed short primer sequence between 8 and 12, but most often 10, base pairs in length (Welsh & McClelland, 1990). To produce amplicons, the primer is required to anneal in opposite orientations on two complementary DNA strands that are sufficiently close together (250-2500 bp) to

produce a PCR product (Jones et al., 1997). RAPD primers anneal and amplify many locations in the genome. Polymorphism arises from annealing at different sites or in different orientations between individuals (Williams et al., 1990). PCR products are separated by size on agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. Polymorphism is detected generally as presence or absence of unique bands or occasionally as variation in the sizes of bands on the gel.

Advantages of RAPD markers are the ease and speed of development, low cost involved, and the requirement of only standard molecular laboratory equipment. RAPD markers are dominant, easy to score, and used often for genetic analysis. Drawbacks of RAPD markers have been the low rate of reproducibility between labs (Weeden et al., 1992; Jones et al., 1997), but standardized protocols, conversion to sequence characterized amplified regions (SCARs), and development of cleaved amplified polymorphic sequence (CAPS) markers have improved their usefulness (Paran & Michelmore, 1993; Konieczny & Ausubel, 1993).

In hazelnut, RAPD markers have been used for marker-assisted selection and for development of a genetic linkage map (Mehlenbacher et al., 2006). Markers linked to two important loci (EFB resistance and S-locus) have been identified. A total of 26 RAPD markers have been identified that are linked to the ‘Gasaway’ resistance gene, and UBC 152-800 and UBC 268-580 have been especially useful for selecting individuals carrying the ‘Gasaway’ gene (Davis & Mehlenbacher, 1997; Mehlenbacher et al., 2004). In addition, RAPD markers linked to specific incompatibility alleles in hazelnut have been identified (Pomper et al., 1998; Bassil & Azarenko, 2001).

Amplified fragment length polymorphism (AFLP) was first described by Vos et al. (1995). The technique uses two restriction enzymes to cut the genomic DNA into many fragments. Adapters are ligated to the fragments which are then amplified in two steps using the polymerase chain reaction (PCR). Separating the fragments by size uses acrylamide gel electrophoresis and is followed by staining with silver to visualize the fragments as bands. AFLP analysis is a dominant type marker. In hazelnut, the AFLP technique was used to identify markers linked to eastern filbert blight resistance genes in OSU 408.040 and 'Ratoli' (Chen et al., 2005; Chen, 2004; Sathuvalli et al., 2012).

Microsatellite, or simple sequence repeat (SSR) or short tandem repeat (STR), markers were described as early as 1993 (Morgante, 1993). These markers consist of tandemly repeated sequences 2-6 base pairs long. It is uncertain exactly how they form, but many suppose that the repeating sequence causes slippage of DNA polymerase during replication (Schlötterer & Tautz, 1992), resulting in additional or fewer sets of the repeated sequence. Unique alleles result from the different lengths of the microsatellite-containing region amplified by PCR. They are useful for analysis of genetic variation because they are multiallelic, codominant, highly reproducible, abundant throughout the genome, relatively easy to score, highly polymorphic, easily shared among labs, and useful in multiple populations (Zane et al., 2002). Studies have found an average density of microsatellites to be 1 per 6.0 kb among various crop plants (Varshney et al., 2002). Microsatellites have a high rate of transferability to related species and occasionally to related genera (Cavagnaro et al., 2011).

Microsatellite markers are often developed from enriched libraries, portions of the genome selected for their higher content of microsatellite loci (Sharapova et al., 2002;

Gürcan et al., 2010; Cavagnaro et al., 2011). The procedure involves using repeat-specific oligonucleotides to hybridize and amplify ssDNA from a genomic library, thereby creating dsDNA of only the fragments containing the desired repeat. Microsatellite-enriched libraries were especially useful before whole-genome sequencing became widespread, because they allowed for increased efficiency of microsatellite identification and characterization (Techen et al., 2010).

Microsatellites are PCR-based markers that are amplified using primer pairs designed from conserved sequences that flank the repeat region. Primer design requires either de novo sequencing of the repeat and surrounding region (Reiter, 2001) or existing sequence data from which to design the primers. With the increasing popularity and reduced cost of whole-genome sequencing, it is becoming commonplace to develop microsatellite markers directly from genome, transcriptome, or expressed sequence tag (EST) sequences (Table 1). With this approach, the microsatellite regions are mined using a program such as SSRIT (Kantety et al., 2002), WebSat (Kunkeaw et al., 2010), Perl scripts designed to extract SSR regions from sequence data (Sharopova et al., 2002), or other similar methods. Primers are designed from sequences flanking the tandem repeat to give a PCR product between 80 and 500 base pairs in length. Use of different lengths and fluorescent tags allows for efficient multiplexing of several markers when performing capillary electrophoresis to accurately score the size of each PCR product.

Table 1.1 Frequencies of most common polymorphic microsatellite motifs in EST and transcriptome derived microsatellites in plant species

Motif	***Nicotiana tabacum*[a]**	***Coffea* sp.[b]**	***Manihot esculenta*[c]**	***Capsicum* sp.[d]**	***Citrus* sp.[e]**
AAG/TTC	58.3%	26.0%	14.6%	17.0%	17.9%
AAT/TTA	31.2%	9.8%	14.6%	5.1%	16.2%
AAC/TTG	22.9%	4.9%	8.3%	8.9%	4.0%
AGC/TCG	0.0%	4.9%	12.0%	5.1%	16.1%
ACT/TGA	0.0%	18.7%	2.1%	8.0%	8.4%
ACC/TGG	2.1%	10.6%	4.2%	11.5%	4.5%
ATC/TAG	8.3%	4.9%	6.8%	7.3%	0.0%
ACG/TGC	0.0%	7.3%	0.0%	4.9%	11.1%
AGG/TCC	0.0%	8.1%	3.6%	7.1%	4.0%
CCG/GGC	0.0%	7.3%	3.1%	5.6%	3.0%
AAAT/TTTA	N/A*	0.8%	8.9%	1.9%	3.3%
AAAG/TTTC	N/A*	0.8%	8.3%	2.1%	2.6%
AAAC/TTTG	N/A*	0.0%	3.1%	0.7%	0.7%
AATT/TTAA	N/A*	0.0%	1.6%	1.1%	1.0%
AAAAG/TTTTC	N/A*	0.0%	N/A*	2.3%	0.9%

[a] Tong et al., 2012; [b] Aggarwal et al., 2007; [c] Sraphet et al., 2011; [d] Yi et al., 2006; [e] Chen et al., 2006

*N/A indicates microsatellites of this motif length were not developed.

Microsatellite markers have proven to be useful for fingerprinting, genetic mapping, marker-assisted selection, kinship studies, and cultivar identification. Many microsatellite marker loci have been developed for use in hazelnut (Bassil et al., 2005; Boccacci et al., 2005; Boccacci et al., 2006, Gurcan et al., 2010a; 2010b). Most of these have been added to the hazelnut linkage map (Mehlenbacher et al., 2006; Gürcan et al., 2010; Sathuvalli et al., 2012). Hazelnut microsatellite markers have also been shown to transfer to other *Corylus* species and genera in the Betulaceae (Gurcan et al., 2007).

Microsatellite markers are typically characterized by describing observed heterozygosity (H_o), expected heterozygosity (H_e), polymorphic information content (PIC), and frequency of null alleles. Observed heterozygosity is calculated as the number of heterozygous genotypes at a particular locus divided by the number of genotypes at that locus and gives a measure of the proportion of individuals with differing genotypes. Expected heterozygosity is considered to be the probability that two alleles chosen randomly from the population are different. This is calculated according to the formula $H_e = 1 - \sum p_i^2$, where p_i is the frequency of the i^{th} allele (Nei, 1973). The PIC value of a marker is the estimated probability that the parentage of an allele in an individual can be determined and is a measure of the marker's usefulness for linkage analysis. It is calculated by the formula:

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2\right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

Where p_i and p_j are the frequencies of the i^{th} and j^{th} alleles, respectively (Botstein et al., 1980). Null alleles are those that repeatedly fail to amplify with PCR and are not detected in individuals being genotyped, and the presence of null alleles can distort

estimates of heterozygosity and relatedness and interfere with parentage studies. The frequencies of null alleles (r) are often calculated according to a maximum likelihood estimate (Kalinowski et al., 2006) or as $r = H_e - H_o / 1 + H_e$ (Brookfield, 1996).

Inter-simple sequence repeat (ISSR) markers are semi-arbitrary markers generated by a single primer and amplification by PCR. The primers are composed of designated repeat motifs and anchored by two to four bases at the 3' or 5' end. These amplify complementary microsatellite regions of the template DNA and often amplify several fragments with one primer (Bornet and Branchard, 2001). The bands are scored based on product length, and polymorphism appears as variations in banding patterns. ISSR markers are plentiful in plant genomes and are useful for mapping, genotyping, and developing microsatellite markers, and the simplicity, speed, and low cost of development make them well-suited to these uses. ISSR marker have been applied for genomics studies in common bean (Marotti et al., 2006), perennial ryegrass (Ghariani et al., 2003), tomato (Tikunov et al., 2003), and hazelnuts (Ferreira et al., 2009; Gürcan et al., 2009), among others.

Single nucleotide polymorphism (SNP) markers are the newest development in molecular marker technology. SNPs arise from substitutions of one base in a DNA sequence, and generally chosen are loci with two alleles and conserved flanking sequences. They do not include insertions or deletions. These markers are used for marker assisted selection, cultivar identification, and diversity evaluations. There are several methods that can be used to identify SNPs, including alignment of sequences from a "diversity panel" of accessions (Gupta et al., 2001; Elshire et al., 2011). With availability of next-generation sequencing platforms such as Illumina, detection of SNPs

can be automated for efficiency and high-throughput. To increase efficiency of useful marker detection by increasing coverage of SNP-containing regions, specific restriction enzymes may be used to cleave and target non-repetitive regions and improve the ease of sequence alignment and. Many SNP detection techniques use this 'reduced representation' approach that is particularly useful in large genomes. Assays for genotyping SNP markers vary, and they may not require gel assays as with most previously developed markers (Gupta et al., 2001).

SNP markers have rapidly gained in popularity due to their abundance throughout the genome. In both mammals and plants, SNPs are more abundant than other forms of markers. These markers are functionally bi-allelic, because although a single SNP can theoretically have four alleles, only those with two alleles are actually chosen (Brookes, 1999; Gupta et al., 2001). The National Center for Biotechnology Information (NCBI) and National Human Genome Research Institute (NHGRI) have worked to create a public SNP database 'dbSNP' that is useful for researchers for archiving and annotating SNPs. In hazelnut, recent sequencing of the 'Jefferson' genome and transcriptome has allowed some preliminary SNP analysis to be done. The RNA-sequence data contains approximately one SNP every 193 bases (Bryant et al., 2009). In the genomic sequence of hazelnut, SNPs are even more abundant, possibly as many as one SNP in 50 base pairs (T. Mockler and E. Rowley, pers. comm.).

The cost of using SNP markers has been relatively high until recently. When this technology was first developed, each SNP locus required a mapped probe to be sequenced, PCR primers developed, and PCR to amplify the target fragment. Each fragment was then sequenced and characterized (Edwards & Mogg, 2001) to create

usable SNP markers. Initial SNP technology involved two predominant methods for amplifying and scoring SNP alleles. Illumina's Golden Gate assay allows for multiplexing up to 1536 SNPs in a single reaction during the PCR phase, followed by hybridizing to an array matrix or bead chip for imaging and genotyping. The second common approach is Illumina's Infinium assay. This involves whole-genome amplification, with fragmented amplicons hybridized to oligonucleotide bead sites on a chip, with hundreds of thousands of sites per chip. Fluorescent tagged bases complementary to the SNP allele are imaged to genotype each SNP. Once a SNP array or chip has been developed for a species, the tool is able to be used and shared among researchers for that species (Ganal et al., 2012.).

The development of SNP arrays and chips entails significant cost, but recent improvements in technology, namely next-generation sequencing platforms, have decreased the expense of identifying SNP markers by orders of magnitude (Deschamps et al., 2012). A newer approach is to genotype by sequencing (GBS), rather than developing expensive SNP chips (Elshire et al., 2011). This approach involves using a methylation-sensitive restriction enzyme and sequencing with a next-generation sequencing platform such as Illumina. This method allows for identification of SNPs, genotyping individuals at each SNP locus, and generating useful data for use for mapping, population studies, germplasm characterization, and breeding. GBS has been used in many crops to identify SNPs, and between 2,000 and 10,000 SNPs are routinely discovered in many species (Faria et al., 2012; Grabowski et al., 2012; Rauh et al., 2012). SNPs identified through GBS have also been useful for mapping, including difficult polyploid species (Byers et al., 2012).

Genetic Linkage Map Construction

DNA markers are useful for creating genetic linkage maps. Building a linkage map requires a segregating population. Linkage maps can be used to study gene-trait associations, identify QTL, identify markers linked to genes, and screen individuals for phenotypic traits based on genetic composition. A linkage map is constructed based on analysis of molecular markers and the frequencies of recombination between the markers on the chromosomes. For conifers, geneticists may use haploid megagametophytes to study linkage in gametes (Tulsieram et al., 1992; Nelson et al., 1993). In many field crops that tolerate inbreeding (i.e. self-pollinated crops), two inbred lines are crossed to create a population of recombinant inbred lines (Song et al., 2004; Grattapaglia & Sederoff, 1994). However, in many vegetatively propagated crops that are highly heterozygous and intolerant of inbreeding, this method is not feasible. More commonly, geneticists use a two-way or double pseudo-testcross approach in seedling populations created by crossing two highly heterozygous individuals (Grattapaglia and Sederoff, 1994; Celton et al., 2009). Many linkage maps for perennial crops and woody species have been developed using this approach, including eucalyptus (Grattapaglia & Sederoff, 1994), apple (Celton et al., 2009), poplar (Cervera et al., 2001), birch (Jiang et al., 2011), chestnut (Casasoli et al., 2001), oak (Barreneche et al., 1998), olive (Aabidine et al., 2010), and hazelnut (Mehlenbacher et al., 2006). The benefit of this approach is that the mapping population can be maintained clonally for future use.

The hazelnut (*Corylus avellana* L.) linkage map (Mehlenbacher et al., 2006) was generated using a segregating population from a controlled cross between OSU 252.146 and OSU 414.062. RAPD and SSR markers segregating in the population and the two-

way pseudo-testcross approach were used. Two linkage maps were created, one for the markers segregating from the female parent and one for markers segregating from the male parent, and 11 linkage groups were mapped for each parent. The female map included 249 RAPD and 20 SSR markers, spanning 661 cM, and the male map included 271 RAPD and 28 SSR markers, spanning a distance of 812 cM. Additional SSR markers were subsequently mapped, with a total of 180 SSRs mapped to date (Gürcan et al., 2010; Gürcan & Mehlenbacher, 2010; Sathuvalli et al., 2012).

When constructing a linkage map, details such as numbers of markers used, population size, and frequency of errors and missing values are considerations which play an important role in the process. Ideally, the data should have no missing values, no genotyping errors, and all markers segregate according to expected Mendelian ratios. It has been shown, however, that missing or erroneous data significantly affects the order of loci from maximum likelihood criteria calculations, and increased distance between loci exacerbates the problem (Hackett & Broadfoot, 2003). The loci are successfully ordered, but map lengths are inflated when errors and missing data are present. However, Hackett and Broadfoot (2003) also illustrated that segregation distortion at marker loci has little effect on the linkage maps. When constructing linkage maps, population size influences the results. Smaller populations are more susceptible to negative effects such as increased distance between loci when errors are present, but larger populations are less prone to such effects (Hackett & Broadfoot, 2003).

Eastern Filbert Blight of Hazelnut

Resistance to eastern filbert blight (EFB) is an important objective of the OSU hazelnut breeding program. The pathogen causing the disease is the pyrenomycete

Anisogramma anomala (Peck) E. Müller. This fungus is endemic to the eastern United States and infects only *Corylus*. It occasionally produces small cankers on indigenous American hazelnut (*Corylus americana*), but the disease is much more severe on European hazelnut. Infection results in severe stem cankers, yield reduction, and girdling and death of branches. EFB will cause death of the entire plant within five to fifteen years if left untreated (Pinkerton et al., 1993).

The fungus has a two-year life cycle, which has been well-documented (Pinkerton et al., 1992, 1995, 1998, 2001; Johnson et al., 1996). The cycle begins with ascospore release from early fall to late spring during periods of prolonged branch wetness (Gottwald & Cameron, 1979). The spores are disseminated by wind or water, and host tissue is susceptible to infection during spring and early summer, just after budbreak, when the fungus is able to penetrate the host tissue (Pscheidt, 2010). Most invading hyphae are halted by a hypersensitive response of the host, but a fraction of the number of spores that adhere to and attempt to invade the host are successful in infecting the host (Pinkerton et al., 1995). The hyphae spread through the vascular tissue of the host during the first year of infection, colonize the xylem, cambium, and phloem, overwinter in the host, and produce sunken cankers and stromata from phloem tissue 12-16 months after the initial infection (Gottwald & Cameron, 1979; Stone et al., 1992). Perithecia containing ascospores mature during the fall, producing and releasing spores that serve as inoculum to other host plants.

EFB was limited to the eastern US until the first sighting of it in 1968 by a grower in western Washington (Davison & Davidson, 1973). Since that time, it has been spreading through the Willamette Valley at a rate of two to three kilometers per year

(Pinkerton et al., 2001). EFB symptoms were first detected on trees near Corvallis, OR on September 4, 2004, posing a risk to conservation of the hazelnut germplasm collection at the USDA-ARS National Clonal Germplasm Repository and susceptible selections and cultivars in the OSU hazelnut breeding program. Control measures involve regular scouting for signs of disease, pruning out infected branches 30 cm below the canker, and fungicide application beginning at budbreak and again at two-week intervals throughout the spring (Johnson et al., 1994; Pscheidt, 2006). These fungicide applications are expensive for growers and have significant environmental impacts, so it is desirable to find alternate methods for managing EFB in hazelnut orchards.

While cultural practices can reduce the impact of EFB on hazelnuts in the Pacific Northwest, disease pressure remains much higher in the eastern and mid-west regions of the US where the pathogen is native, severely limiting the ability of farmers to grow European hazelnuts in those areas (Capik & Molnar, 2012). Because of the costs and labor involved with controlling the disease with cultural practices and fungicides, it is desirable to find alternate ways to impede disease spread (Mehlenbacher & Smith, 2004; Mehlenbacher et al., 2007, 2008). Genetic host resistance is considered the most viable and economical strategy for managing EFB (Mehlenbacher, 1994).

Genetic Host Resistance

Genetic host resistance has been used in many crops to prevent infection from pathogens, and it is the preferred method of managing disease in many crops (Sama et al., 2012; McDonald & Linde, 2002). Resistance genes are generally divided into two groups: qualitative, conferring complete resistance (i.e. immunity) to the pathogen, or quantitative, providing a reduction in the amount or severity of the disease. More than 50

resistance genes have been sequenced for different pathogens (Hulbert et al., 2001; Dilbirligi & Gill, 2004). Most of these genes fit the gene-for-gene model first described by Flor (1956), in which the host has a resistance gene (R gene) conferring resistance and the pathogen has a corresponding avirulence gene (Avr gene) that allows the host with the R gene to detect and defend against the invader with a hypersensitive response. Many sequenced resistance genes have nucleotide binding site (NBS) and leucine rich repeat (LRR) sequences. Other resistance genes contain protein kinase domains. In contrast to these studied model pathosystems, hazelnut is a highly heterozygous, perennial tree, and *A. anomala* is a canker disease with a latent period of 12-16 months. In this hazelnut-EFB pathosystem, a gene-for-gene relationship has not been demonstrated, and typical hypersensitive reactions are not observed.

A wide array of hazelnut germplasm has been screened for resistance to EFB, and sources conferring both qualitative and quantitative resistance have been found. In the early 1900's, resistant *C. americana* were hybridized with *C. avellana* in an attempt to develop a commercial quality variety with resistance conferred by the *C. americana* parent. These efforts met with limited success, due to limited knowledge about EFB and the narrow genetic spectrum of hazelnut parents that were used (Thompson et al., 1996). EFB resistance in *C. avellana* was first discovered in 'Gasaway' pollinizers in a heavily infected 'DuChilly' orchard in Washington state (Cameron, 1976). Other resistant cultivars and selections in *C. avellana* include 'Ratoli' from Spain, 'Zimmerman' from Oregon, OSU 408.040 from Minnesota, OSU 759.010 from the Republic of Georgia, COR 157 from Finland (to be confirmed), OSU 495.072 from Russian, 'Culpla' from Spain, 'Crvenje' and 'Uebov' from Serbia, Moscow #2 from Moscow, Russia, H3R13-40

and H3R4-23 and H3R4-30 from Krasnodar, Russia, and H3R14-26 and H3R12-58 and H3R12-62 and H3R7-7 and H3R10-88 from Crimea, Ukraine (Sathuvalli et al., 2010; Capik & Molnar, 2012).

Breeding for Disease Resistance

Selections with qualitative resistance controlled by single, dominant genes are useful for breeding. Resistance in 'Gasaway' and 'Ratoli' is conferred by dominant alleles at different loci (Mehlenbacher et al., 1991; Chen et al., 2007; Sathuvalli et al., 2011). The ‘Gasaway’ gene has been successfully introgressed into the breeding program to confer EFB resistance. Cultivar releases containing the ‘Gasaway’ resistance gene include ‘Jefferson,’ ‘Santiam,’ ‘Yamhill,’ ‘Dorris,’ ‘Wepster,’ and the pollinizers ‘Eta,’ ‘Theta,’ ‘York,’ and ‘Felix.’ The goal of the OSU hazelnut breeding program is to produce new cultivars resistant to EFB in addition to desired agronomic characteristics such as high yield and high-quality kernels.

Little is known about genetic diversity in the pathogen, *A. anomala*. It is unknown how many strains or pathotypes exist in nature, and thus it is uncertain if existing sources of EFB resistance would be robust in the presence of all variants of the pathogen. Isolates may already exist that are able to overcome the available resistance genes, and new isolates could arise from mutation or recombination. Many hazelnut accessions and several cultivars have been identified as completely resistant to EFB over many years of screening in the Pacific Northwest. ‘Gasaway,’ for example, has been used extensively in the OSU hazelnut breeding program because of its single gene conferring complete resistance. However, after ‘Gasaway’ and one of its offspring ‘VR20-11’ containing the resistance gene were screened for EFB resistance in New Jersey, EFB

cankers and stromata were observed (Molnar et al., 2010). Another recent study of EFB resistance in numerous *Corylus* selections was performed in New Jersey to assess the robustness of host resistance in a different environment (Capik & Molnar, 2012). They discovered that many genotypes resistant to EFB in the Pacific Northwest actually showed disease symptoms in New Jersey, perhaps as a result of higher disease pressure or different strains of the pathogen. Additionally, small cankers have been observed in Oregon on 'Jefferson' and some seedlings containing the 'Gasaway' gene (S.A. Mehlenbacher, pers. comm.). Cankers observed on resistant trees are often larger in New Jersey than in Oregon, but the resistance still limits the size and number of cankers that develop. Many resistance sources, including OSU 408.040, OSU 495.072, 'Ratoli' and others, show no signs of disease in New Jersey (Capik and Molnar, 2012). The host-pathogen interaction between *C. avellana* and *A. anomala* is a complex one, and the resistance sources appear to have varying degrees of efficacy under different disease pressures.

The phenomenon of a 'breakdown' in disease resistance is not uncommon in crop plants. In many host-pathogen interactions, mutations in the pathogen allow it to overcome the resistance mechanism of the host. Relying on single, dominant resistance genes leaves the host especially vulnerable to new pathogenic strains or an increase in disease pressure, as has been seen with European hazelnut in New Jersey (Capik & Molnar, 2012). Therefore, it is advantageous to have varied genetic sources of resistance and the ability to combine multiple resistance genes in the host plant. This concept of 'pyramiding' disease resistance is intended to supply multiple means of defense against a

pathogen, so that a new strain or mutant will be less likely to overcome the host resistance if there is more than one mode of resistance present.

As the genetics of host-pathogen interactions has been studied and observed, genetic host resistance often "breaks down" over time. This has occurred numerous times in cereals with rust (*Puccinia* sp.) (Samborski, 1985; Hulbert et al., 1991; Kolmer, 1992) and powdery mildew (*Blumeria graminis*) pathogens (Brown et al., 1993; Wolfe & McDermott, 1994). The failure of resistance genes in these cases was due to genetic mutations in the pathogen population. Once a mutant pathogen strain has arisen, it is better able to survive and reproduce on the host plant than the less virulent strains, allowing it to more rapidly reproduce and become widespread (McDonald & Linde, 2002).

With the observed EFB cankers on hazelnut accessions with an R gene under the environmental conditions of *A. anomala* in New Jersey and the potential for a new race of the pathogen to arise in the Pacific Northwest, it is imperative for plant breeders to employ strategies for increasing the durability of disease resistance. Some general approaches to controlling disease include using quantitative sources of resistance to lower selection pressure on the pathogen, using multiple and unique resistance genes, mixing crop varieties to include resistant and non-resistant genotypes, alternating different forms and levels of resistance across years, employing management strategies to limit the sexual reproduction of the pathogen, removal of alternate hosts, and 'pyramiding' multiple R genes (McDonald & Linde, 2002). The concept of pyramiding resistance genes has become popular among plant breeders. This method combines multiple major resistance genes in one genotype with the goal that mutations in the pathogen would be

less likely to simultaneously overcome several different resistance genes. McDonald and Linde (2002) have identified the pyramid gene approach to be most advantageous for use with pathogens showing low genetic diversity. These two traits are characteristic of *A. anomala* (Cai et al., 2011a, 2011b), which has not been observed to reproduce sexually and which is readily transferred across locations. With these attributes in mind, hazelnut breeders should continue pursuing the use of resistance genes and pyramiding multiple genes for improving the durability of host resistance to eastern filbert blight.

Pyramiding is an approach to plant breeding that has been used in many programs to combine multiple resistance sources and increase the durability of resistance against pathogens. Pyramiding has been used to combine quantitative resistance as well as single, dominant R genes in a single plant. The approach has been used in apple (*Malus* sp.) to combine multiple scab (caused by *Venturia inaequalis*) resistance genes for increased durability of resistance (Kellerhals et al., 2009). Breeders pyramiding multiple quantitative genes have found an increased level and a broader spectrum of resistance with multiple genes than with just the individual genes alone, as seen in the case of bacterial blight (caused by *Xanthomonas oryzae*) resistance in rice (*Oryza* sp.) (Huang et al., 1997) and stripe rust (caused by *Puccinia striiformis*) resistance in barley (*Hordeum vulgare*) (Richardson et al., 2006). Other instances of pyramiding quantitative genes have failed to show even an additive effect of the combined genes in the case of resistance to root knot nematode (*Meloidogyne* sp.) in potato (*Solanum tuberosum*) (Tan et al., 2009).

In the case of breeding and selecting for dominant gene resistance, the ideal scenario would be for introgression of several unique R genes with different resistance mechanisms to be combined into a single cultivar. This would be greatly beneficial to

breeders and growers, because multiple qualitative R genes would be less likely to break down in the face of a new pathogen strain than a single R gene. This strategy has been used successfully in apple and soybean, to combine multiple R genes for the same pathogen and for different strains of a pathogen (Kellerhals et al., 2009; Maroof et al., 2008). With hazelnuts, this approach is feasible, especially since a wide variety of germplasm has been screened and multiple sources of qualitative resistance have been discovered (Mehlenbacher et al., 1991; Coyne et al., 1998; Chen et al., 2007; Sathuvalli et al., 2010). With these resources available, hazelnut breeders have the opportunity to focus on introgressing individual R genes and then combining unique R genes for added durability of resistance.

When planning to breed resistant cultivars using single, dominant resistance genes, molecular markers are very useful to the plant breeder. In hazelnuts, molecular markers are a significant benefit to breeding and selecting for resistance. Using molecular markers can greatly speed the selection process, especially with resistance genes for *A. anomala*, where field inoculations typically take 16 months to show results (Davis & Mehlenbacher, 1997; Mehlenbacher et al., 2004; Chen et al., 2005; Sathuvalli et al., 2011, 2012). Additionally, as more resistance genes are discovered and linked markers are identified, the DNA markers will make it possible to pyramid unique resistance genes in a single cultivar with the goal of producing more durable resistance to *A. anomala*.

Research Objectives

Hazelnut is an important commercial crop in Oregon's Willamette Valley and other places worldwide. The nuts grown in Oregon are premium quality and are sold on the in-shell and kernel markets. Additionally, demand for hazelnuts is likely to increase

as the kernel market continues to increase and alternate uses for hazelnut are pursued. As growers continue to plant new orchards and replace old orchards with newer cultivars, it is important for the OSU hazelnut breeding program to continue developing and releasing cultivars to meet the needs of hazelnut growers. The program strives to develop new cultivars that have a desirable, round nut shape, medium to large nut size, high percent kernel, good blanching, excellent flavor, few defects, early maturity, precocity, free-husking nuts, high yield, and resistance to eastern filbert blight. Several cultivars with the 'Gasaway' gene for resistance have been released, improving the outlook for growers in the Willamette Valley. However, since all of these cultivars share the same single, dominant resistance gene, there is concern that a mutation or recombination in the pathogen or the introduction of a new strain of the pathogen would result in a breakdown of the resistance. Therefore, it is ideal to pyramid several different resistance genes to reduce the risk of resistance breaking down (Werner et al., 2005). This can be accomplished most efficiently using DNA markers.

DNA markers have proven to be useful tools for breeders. Markers allow researchers to perform kinship studies, find markers linked to important genes, discover quantitative trait loci, and build genetic linkage maps. Of particular use to breeders is the potential to use markers for marker-assisted selection. RAPD, AFLP, and microsatellite markers have already been developed for hazelnut, and a genetic linkage map has been created using a full-sib population from the cross OSU 252.146 and OSU 414.062. EFB resistance sources 'Gasaway,' 'Ratoli,' OSU 759.010, and OSU 408.040 have been studied and markers linked to resistance genes identified, and these markers have been useful to the breeding program.

The current research has two aims. The first is to develop, characterize, and map new microsatellite markers from ‘Jefferson’ transcriptome data. The second is to study three unique sources of EFB resistance (‘Culpla,’ ‘Crvenje,’ and OSU 495.072), including segregation for resistance in seedling populations and the mapping of the resistance loci. Markers linked to resistance can be used for marker-assisted selection.

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Chapter 2

DEVELOPMENT AND MAPPING OF MICROSATELLITE MARKERS FROM HAZELNUT TRANSCRIPTOME SEQUENCES

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Abstract

Microsatellite markers are useful in genetics and plant breeding for marker-assisted selection, cultivar fingerprinting, kinship studies, and cultivar identification. They have previously been developed for European hazelnut (*Corylus avellana* L.) from enriched libraries, ISSR fragments, and BAC sequences. This study utilized the 'Jefferson' transcriptome sequence to develop useful microsatellite markers for hazelnut. Microsatellites were mined from the transcriptome sequence and aligned with the corresponding genomic sequence using a BLASTN search. Primers were designed from the genomic sequence. A total of 1432 microsatellites were identified, and the most frequently found motifs were AG (35.8%), AT (13.3%), and AAG (12.7%). Motifs of three base pairs or longer and at least five repeats were chosen for further pursuit, and 382 primer pairs were designed. Primers were screened for polymorphism with a set of 24 *C. avellana* accessions, and those that were polymorphic were characterized with sets of 50 and 14 accessions. One hundred nineteen polymorphic microsatellite markers were identified and characterized, with AAG, AGC, and ACT being the most common motifs. Fifty-three of these loci were placed on the existing linkage map constructed with the mapping population OSU 252.146 x OSU 414.062, and 24 loci were assigned to linkage groups using alternate segregating populations. A dendrogram constructed from the fingerprints at 116 new marker loci showed clusters of accessions from similar geographic origins and confirming the tremendous amount of genetic diversity present within *C. avellana*. These markers will be useful for fingerprinting, marker-assisted selection, and genetic studies in hazelnut.

Introduction

Microsatellite or simple sequence repeat (SSR) markers have been used widely for cultivar fingerprinting, genetic mapping, marker-assisted selection (MAS), kinship studies, and cultivar identification. These markers consist of tandemly repeated sequences 2-6 base pairs long. It is uncertain exactly how they form, but many suppose that the repeating sequence causes slippage of DNA polymerase during replication (Schlötterer & Tautz, 1992), resulting in additional or fewer sets of the repeated sequence. Unique alleles result from differences in the lengths of the microsatellite-containing region when amplified by PCR. They are useful for analysis of genetic variation because they are multiallelic, codominant, highly reproducible, abundant throughout the genome, relatively easy to score, highly polymorphic, easily shared among labs, and useful in multiple populations (Zane et al., 2002).

Microsatellites have been developed through several methods. Before the advent of widespread genome sequencing, identification of microsatellite loci often relied on developing libraries enriched for microsatellites from hybridization with sequence-specific oligonucleotides (Sharapova et al., 2002; Gürcan et al., 2010; Cavagnaro et al., 2011). The plant DNA in recombinant plasmids in these libraries would then be sequenced, repeat regions identified, and primers designed from the flanking regions. Since the advent of next-generation technology allowing for fast and low-cost genome and transcriptome sequencing, microsatellites can be mined directly from sequence data, greatly increasing the speed and ease of marker development.

Many microsatellite markers have been developed for hazelnut from enriched libraries (Bassil et al., 2005, 2006; Boccacci et al., 2005; Gürcan et al., 2010a), ISSR fragments (Gürcan et al., 2010b), and BAC sequences (Sathuvalli et al., 2012) and these

have been useful for generating a linkage map for hazelnut (Mehlenbacher et al., 2006; Gürcan et al., 2010a; Sathuvalli et al., 2011, 2012). Microsatellite markers are especially useful for mapping, since they act as anchor loci and are polymorphic in multiple populations. In hazelnut, microsatellites allowed mapping of eastern filbert blight resistance from OSU 408.040 (Sathuvalli et al., 2012). Sequencing of the 'Jefferson' hazelnut genome and transcriptome (Rowley et al., 2012) has generated much sequence data which can be mined for microsatellite loci. The goal of this project is to develop new polymorphic microsatellite loci from 'Jefferson' transcriptome sequences, characterize them, and assign them to linkage groups.

Materials and Methods

Plant Material and DNA Extraction

Three sets of genotypes were used for screening and characterizing the microsatellite loci (Appendices A, B, C). Initial screening of primers for polymorphism on agarose gels involved a set of 24 diverse *Corylus avellana* selections from a wide range of geographic locations (Appendix A). These individuals were chosen to increase the likelihood of identifying polymorphic microsatellites. For characterization of polymorphic microsatellite loci, an additional 24 accessions plus the two parents of our mapping population were used. The 50 accessions (Appendix B) were a diverse set of accessions characterized by Gökirmak et al. (2008) and Gürcan and Mehlenbacher (2010a) and chosen to represent the genetic diversity in the *Corylus avellana* germplasm collection. One hundred forty-four seedlings of the F_1 mapping population OSU 252.146 X OSU 414.062 were used to place the microsatellite loci on the hazelnut linkage map

(Mehlenbacher et al., 2006). The maternal parent OSU 252.146 is susceptible to eastern filbert blight, caused by *Anisogramma anomala* (Peck) E. Müller. The paternal parent OSU 414.062 is heterozygous at the disease resistance locus. An additional set of 14 parents were amplified to identify progenies not segregating in the mapping population that could be used for linkage group assignment (Appendix C). We used three populations of 32 full-sib seedlings to assign the markers to linkage groups. These populations, 01035 (OSU 713.068 x OSU 495.072), 05024 (OSU 675.028 x 'Culpla'), and 06027 (OSU 675.028 x 'Crvenje') segregate for resistance to eastern filbert blight from three different sources. Their pedigrees are shown in Figures 2.1, 2.2, and 2.3.

Leaves from all accessions were collected from trees in the field collections at the USDA-ARS National Clonal Germplasm Repository (NCGR) and Oregon State University in Corvallis, Oregon. Total DNA was extracted from 2-4 fresh, young leaves as described by Lunde et al. (2000) with no RNAse treatment. DNA was quantified by a BioTek Synergy2 microplate reader paired with Gen5 data analysis software (BioTek Instruments, Winooski, VT) and diluted with TE buffer to a concentration of 20 ng/µl.

Microsatellite Identification and Marker Development

Microsatellite-containing regions were identified *in silico* from the 'Jefferson' hazelnut transcriptome sequences supplied by Rowley et al. (2012) and included sequences derived from leaves, catkins, bark, and whole seedlings (Appendix D). Sequences containing microsatellite regions were identified using Gramene SSRIT (Temnykh et al., 2001) and Evopipes.net "findSSR" (Kane and Rieseberg, 2007; Barker et al., 2010). From the microsatellite regions identified, those containing motifs of three to six base pairs with at least five repeats were selected, and 17 di-repeat motifs were also

selected. Transcriptome sequences containing microsatellites were truncated to 400 base pairs, with the microsatellite region located in the middle. The sequences were aligned with the corresponding hazelnut genomic sequences with a BLASTN search of the 'Jefferson' reference genome sequence (http://corylus.cgrb.oregonstate.edu:8080/) (Sathuvalli and Mehlenbacher, 2011). The microsatellite-containing genomic sequences were aligned using CodonCode Aligner (CodonCode Corporation, Centerville, MA), and sequences from previously identified microsatellite sequences (Gürcan et al., 2010a, 2010b) were included in the alignment. Sequences containing microsatellites that had been previously identified were eliminated. Primers were designed from the genomic sequences using the Primer3 program (http://frodo.wi.mit.edu/). Design criteria specified a primer length of 18 to 27 base pairs, 60 °C annealing temperature, and 20-80% GC content. Non-fluorescent forward and reverse primers were ordered from Eurofins MWG Operon (Huntsville, AL).

Initial Screening of Markers

Polymerase chain reactions were performed with each of the primer pairs on DNA from the set of 24 accessions (Appendix A) to amplify the microsatellite region. The PCR mix was a total of 10µl per reaction and contained 0.3 µM each of the forward and reverse primer, 1x Biolase NH_4 reaction buffer, 2 mM $MgCl_2$, 200 µM each of dATP, dTTP, dGTP, and dCTP, 20 ng template DNA, and 0.25 units of Biolase DNA polymerase (Bioline USA Inc., Boston, MA). Ninety-six reactions were run simultaneously on GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) and MyCycler (Bio-Rad, Hercules, CA) thermal cyclers. The PCR program was: denaturation at 94 °C for 5 minutes followed by 40 cycles of 94 °C for 40 seconds, 60 °C

for 40 seconds, 72 °C for 40 seconds; and 72 °C for 7 minutes of extension, ending with an infinite hold at 4 °C. The PCR products were separated by electrophoresis on 3% w/v agarose (ISC Bioexpress, Kaysville, UT) gels with TBE buffer that ran at 90 V for 3.5 hours. The gels were then stained with ethidium bromide (Sigma-Aldrich Co., St. Louis, MO) and imaged under UV light using a BioDoc-It® Imaging System (UVP, Upland, CA). Polymorphic SSRs were indicated by size differences among the 24 genotypes.

Characterization and Mapping of Polymorphic Markers

Primers showing polymorphism on agarose gels were pursued further with fluorescent labeling. The forward primer of each primer pair was labeled with FAM, NED, or HEX with consideration for efficient multiplexing. NED-labeled forward primers were synthesized by Applied Biosystems (Foster City, CA), and FAM- and HEX-labeled primers were made by Integrated DNA Technologies (Coralville, IA). PCR reactions were performed as described earlier, except that 64 accessions (50 accessions plus 14 parents; Appendices B and C) were amplified, and the fluorescent tagged forward primer was used in place of the non-fluorescent forward primer. The PCR products from each reaction were multiplexed, with six to twelve different products in each multiplex set. Two µl of each product were combined in 150 µl water, and a 1 µl aliquot was submitted to the CGRB Core Lab facility at Oregon State University for genotyping with an ABI 3730 DNA Analyzer (Life Technologies, Carlsbad, CA). The capillary electrophoresis fragment size data was analyzed with ABI Gene Mapper® software (Life Technologies, Carlsbad, CA). The length of the amplified fragments was recorded for each primer pair for each genotype in the set. For data points that could not be scored

with confidence, PCR and capillary electrophoresis were repeated and the fragment size scored, confirmed, and recorded.

Marker loci polymorphic in the mapping population (OSU 252.146 x OSU 414.062) were placed on the genetic linkage map of Mehlenbacher et al. (2006). A two-way pseudo testcross analysis in Join Map 4.0 (Kayzma, Wageningen, Netherlands) was used to construct the map. Markers not segregating in the mapping population were assigned to linkage groups using one of the three additional segregating populations. New markers were added to the existing genetic linkage map for the OSU 252.146 x OSU 414.062 mapping population, which had been constructed using JoinMap 4.0 (van Ooijen and Voorrips, 2001) and the two-way pseudo testcross approach.

Data Analysis

Marker Characterization

For each marker, observed heterozygosity (H_o), expected heterozygosity (H_e), and polymorphism information content (PIC) were calculated using PowerMarker (Liu and Muse, 2005), and the frequency of null alleles (r) was calculated using Cervus (Field Genetics Ltd., London, UK). The calculated values are influenced by the number of unique alleles for each marker and the relative frequencies of the alleles in the panel of 50 accessions (48 accessions plus 2 parents). Observed heterozygosity was calculated as the number of heterozygous genotypes at a particular locus divided by the number of genotypes at that locus. Expected heterozygosity is the probability that two alleles chosen randomly from the population are different, calculated according to the formula $H_e = 1 - \sum p_i^2$, where p_i is the frequency of the *i*th allele (Nei, 1973). The PIC value of a marker is the estimated probability that the parentage of an allele in an individual can be

determined and is a measure of the marker's usefulness for linkage analysis (Botstein et al., 1980). It is calculated by the formula:

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2\right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

where p_i and p_j are the frequencies of the i^{th} and j^{th} alleles, respectively (Botstein et al., 1980). The frequency of null alleles (r) was calculated according to Kalinowski et al. (2006, 2007). A dendrogram was constructed using PowerMarker (Liu and Muse, 2005) and Mega5 (Tamura et al., 2011).

Correlation Analysis in Alternate Populations

For markers segregating in any of the three alternate segregating populations, a PROC CORR function was performed with SAS 9.2 (SAS Institute Inc., Cary, NC), and correlation ($|r| > 0.50$) with previously mapped markers was used to assign each locus to a linkage group.

Results

One thousand four hundred thirty-two microsatellites were identified in the transcriptome data. Of these, 429 microsatellite regions were mined from the bark transcriptome data, 206 from catkin, 748 from leaf, and 49 from seed. Repeat motifs of three base pairs or longer plus 17 di-repeat regions were selected for primer design, and 731 di-repeat microsatellites were not pursued. A BLASTN search against the 'Jefferson' genomic sequence indicated that 75 (12.7%) microsatellites were duplicates of previously identified microsatellites and were not pursued further. Twenty (3.4%) microsatellite regions lacked sufficient flanking sequence for primer design in the genomic sequences,

and 117 (19.8%) microsatellite-containing sequences had no BLASTN match and thus could not have primer pairs designed. For the remaining 382 (64.6%) identified regions, primer pairs flanking the simple sequence repeat were designed. In the initial screening on agarose gels with 24 accessions, 149 loci (39%) were scored as polymorphic. For these a forward primer with a fluorescent tag was ordered: 50 FAM, 52 HEX, and 47 NED. Amplification of 50 accessions using 149 primer pairs confirmed that 119 (79.9%) were polymorphic, including 6 which amplified 3 or 4 bands rather than 1 or 2. Repeat motif lengths ranged from dinucleotide to hexanucleotide. Of the polymorphic loci, 4 (3.4%) are di-repeats, 99 (83.2%) are tri-repeats, 3 (2.5%) are tetra-repeat, 2 (1.7%) are penta-repeats, and 2 (1.7%) are hexa-repeats. The most common repeat motifs are AAG (31.9%), AGC (18.6%), and ACT (13.4%) (Table 2.1).

The polymorphic microsatellite loci were characterized in 50 accessions by calculating the number of alleles, H_e, H_o, PIC, and frequency of null alleles (Table 2.2). Alleles per locus ranged from 2 to 14, with an average of 4.3. The expected heterozygosity ranged from a low of 0.02 to a high of 0.86 with a mean of 0.42. Observed heterozygosity ranged from 0 to 0.86 with a mean of 0.40. Two loci monomorphic in the set of 50 for screening (BR210 and BR240) (Appendix B) were polymorphic in the other set of 16 parents (Appendix C). The PIC values ranged from 0.02 to 0.84 with a mean of 0.38. The most informative markers, those with a high PIC value, are BR259, BR379, and BR483, with PIC values of 0.84, 0.84, and 0.79, respectively. The least informative markers are BR210 and BR398, both with PIC values of 0.02. The frequency of null alleles ranged from -0.18 to 0.79. BR240, BR340, BR374, and BR433 had the highest

frequencies of null alleles. No correlation between length of microsatellite in the transcriptome sequence and number of alleles was observed ($|r| = 0.26$, $n = 111$ loci).

Of the 61 loci segregating in the mapping population, 53 (36.8%) were placed on the maps of Mehlenbacher et al. (2006) and Gürcan et al. (2010a) (Figures 2.4 to 2.9). An additional 24 loci (16.7%) were assigned to linkage groups using one of the three alternate populations (Table 2.3). The remaining 42 loci (29.2%) could not be assigned to a linkage group. Of these, 13 were polymorphic in the mapping and segregating populations, including BR227 and BR277 that had multiple bands, but were unlinked to any other markers, and 29 failed to segregate in any of the four populations used for mapping and linkage group assignment.

Discussion

Developing microsatellite markers from transcriptome sequences has several advantages. Since the transcriptome is the expressed portion of the genome, any markers developed from it should be in functional regions. This increases the likelihood of developing useful markers which are closely linked to functional genes.

Microsatellite markers are co-dominant, highly reproducible, and have substantial cross-species transferability. Gürcan and Mehlenbacher (2010) showed that microsatellites from *C. avellana* amplified over 80% across *Corylus* species. A high rate of transferability has been reported in other plant genera. In *Brassica*, microsatellite markers show between 42% and 100% transferability (Ramchiary et al., 2011). Microsatellites mined from EST sequences of *Capsicum annuum* showed 100% transferability to other *Capsicum* species (Shirasawa et al., 2013), and markers developed

for *Solanum lycopersicon* showed an 85% amplification rate in *S. pennellii* (Shirasawa et al., 2010). Microsatellites have been the marker of choice for breeders because of their suitability for genetic mapping, genotyping, and marker-assisted selection. Of the 113 polymorphic microsatellites developed in this study, tri-nucleotide repeats were the most abundant. Tri-repeat and larger motifs were selected in preference over di-nucleotide repeats, since significant numbers of di-repeat microsatellites had been developed previously (Bassil et al., 2005; Gürcan et al., 2010a, 2010b).

Di-nucleotide repeats were most prevalent in the transcriptome, although tri- and larger motifs were selected for marker development (Table 2.2). Fifty-two percent of the mined repeats were di-nucleotide repeats, 45% tri-repeats, 1% tetra-repeats, 0.3% penta-repeats, and 1% hexa-repeats (Table 2.4). The most common motifs found in hazelnut were AG (35.8%), AT (13.3%), and AAG (12.7%). AG repeats were most common in microsatellites mined from EST sequences of *Coffea* and *Citrus* (Chen et al., 2006; Aggarwal et al., 2007). AT repeats were common, especially in *Nicotiana tabacum* and *Coffea* species (Aggarwal et al., 2007; Tong et al., 2012), and AAG repeats were found frequently in *Nicotiana tabacum* and *Capsicum* species as well (Yi et al., 2006; Tong et al., 2012). Almost no CG repeats and very few AC repeats were found, which is similar to findings in other species (Yi et al., 2006; Tong et al., 2012).

Of the new polymorphic microsatellite markers developed in this study from hazelnut transcriptome sequences, the most common repeat motifs are AAG (31.9%), AGC (18.6%), and ACT (13.4%) (Table 2.1). Tri-repeats have been found to be the most abundant microsatellite in transcriptome-derived microsatellites of rice, wheat, barley, soybean, and chickpea (La Rota et al., 2005; Hisano et al., 2007; Garg et al., 2011).

Metzgar et al. (2000) suggested that the reason tri-repeats are the most common microsatellites in coding regions is that non-triplet changes in repeat length causes frameshift mutations that alter gene expression. This would indicate that tri-repeats are selected over other motifs since they are less likely to cause detrimental mutations. For repeats other than tri-motifs, it is possible that they are located in 5' or 3' UTRs instead of coding regions, where mutations in length of the microsatellite would not cause detrimental effects to expression of the gene.

The mining of hazelnut transcriptome sequences is an efficient method for identifying microsatellite-containing regions. Compared with older methods of microsatellite development involving enriched libraries and ISSR marker sequencing (Sharapova et al., 2002; Gürcan et al., 2010b; Cavagnaro et al., 2011), in silico mining of transcriptome sequences is a relatively inexpensive alternative (Tang et al., 2008). Although there is still time and cost involved in primer design, screening for polymorphism, and characterization of the loci, our approach identified a large number of polymorphic microsatellites. In addition, the 'Jefferson' genome sequence (T. Mockler, pers. comm.) and 'Jefferson' transcriptome sequence (Rowley et al., 2012) provide a wealth of information from which to develop DNA markers, and the 748 di-repeat microsatellites that were not pursued in this study for marker development could be pursued in future work.

The microsatellite markers developed in this study will be useful for further genetics research in *Corylus* species. Of particular interest are markers that have high PIC values and low frequency of null alleles, are easy to score, are suitable for multiplexing, and cover all the linkage groups. Twenty-seven of the identified markers appear to be

most useful for genetics research (Table 2.5). Three markers are located on linkage group (LG) 1, one on LG 2R/7S, two on LG 3, six on LG 4, three on LG 5, one on LG 7, two on LG 8, two on LG 9, two on LG10, and three on LG 11. No marker loci were mapped to LG 6, so none of the 27 markers of this set are located on LG 6.

Some markers have unique and rare alleles or are nearly monomorphic. Several loci have unique alleles that occurred only once in the 64 genotypes used to characterize them (Table 2.6). These and other unique alleles were confirmed by repeating the PCR and capillary electrophoresis. The following individuals were notable for having multiple unique alleles: OSU 495.072 (7 unique), 'Fusco Rubra' (6), 'B-3' (4), OSU 495.049 (4), OSU 759.010 (4), OSU 1185.126 (4), and OSU 1187.101 (4). The presence of unique alleles was expected based on previous results (Gürcan et al., 2010a) and the high level of genetic diversity in the set of hazelnut accessions used to characterize microsatellite markers (Boccacci and Botta, 2010). Because hazelnuts are wind-pollinated and self-incompatible, they have a high degree of heterozygosity, so it is reasonable that the cultivars studied here exhibit high levels of diversity.

Microsatellite loci were placed on the existing linkage map for hazelnut (Figures 2.4 to 2.9) or assigned to linkage groups by correlation analysis in segregating populations. The linkage map consists of separate maps for loci segregating in the female and male parents, and there was some inconsistency between the two with linkage groups 2 and 7. The female map had 11 distinct linkage groups, but the male map merged LG 2 and LG 7 into one linkage group. It is suspected that a reciprocal translocation exists in the male parent, which has caused linkage groups 2 and 7 to become linked. This phenomenon has been observed in an interspecific hybrid between peach (*Prunus*

persica) and almond (*Prunus dulcis*), where two previously mapped, distinct linkage groups merged to form a single linkage group in the progeny of the cross (Jáuregui et al., 2001). To confirm this hypothesis in hazelnut, cytogenetic studies of each parent of the mapping population could be performed to investigate meiotic abnormalities in the male parent, especially presence of ring and line patterns indicating translocation.

There were 13 markers that segregated in the mapping and alternate segregating populations that were unlinked to any previously mapped markers. This could be due to gaps in the genetic linkage maps, if there are portions of the genome that have yet to be mapped. The new microsatellite loci which were mapped appear to be fairly well-distributed throughout the genome. Our new loci were assigned to every linkage group, except LG 6 on which the 'Gasaway' gene for resistance to EFB is located (Mehlenbacher et al., 2006). These markers should be useful for fingerprinting and marker-assisted selection. Markers BR259, BR253, and BR427 are located on LG 5 and co-segregate with the S-locus (Figure 2.6). The S-locus controls pollen-stigma incompatibility (Thompson, 1979), and RAPD markers linked to the S-locus were previously identified (Pomper et al., 1998). The three microsatellite markers will be very useful for map-based cloning of the S-locus.

Fifty accessions were fingerprinted at 113 polymorphic microsatellite marker loci (Appendix E) and used to draw two dendrograms. The UPGMA dendrogram shows inferred relationships of the group (Figure 2.10). As in previously constructed dendrograms of hazelnut accessions (Ferreira et al., 2009; Boccacci and Botta, 2010; Gürcan et al., 2010a; Gürcan and Mehlenbacher, 2010), the accessions mostly cluster according to geographic origin. There are distinct groups of Russian, German, Turkish,

English and French, and Spanish and Italian clusters. The Spanish-Italian, Turkish, and German clusters are nearly always present in characterization studies such as this (Gürcan et al., 2010). However the groupings are not tight. For example, 'Gasaway' originally from Washington, USA groups with the French accessions, and 'Gem,' also from the USA, groups with the Spanish-Italian accessions. 'Fusco Rubra' from Germany groups with the Russian-Georgian accessions, and COR 187 from Finland and 'Barcelloner Zellernuss' from Spain both group with the English and French accessions. These observed discrepancies in clustering could be a result of gene flow between major geographical regions, or of human error in naming and recording the background information of the original accessions, or simply different loci in the studies giving different results. Regardless, the fingerprint data from these accessions and the groupings of the dendrograms are a demonstration of the tremendous genetic diversity within *C. avellana*.

The transcriptome-derived microsatellite markers developed in this study are highly polymorphic and will be useful for continuing genetic studies in hazelnut. These loci were developed from expressed regions of the genome and thus are likely to be located in or close to coding regions. Many of the markers were mapped and will be useful in marker-assisted selection and further studies of genetic diversity, cultivar fingerprinting, and other genetic studies.

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Table 2.1 Frequencies of repeats of polymorphic microsatellite loci developed from transcriptome sequences in *Corylus avellana*

Repeat motif	Count	Frequency
AG/TC	7	6.2%
AT/TA	1	1.0%
AAC/TTG	10	8.8%
AAG/TTC	26	23.0%
AAT/TTA	1	1.0%
ACC/TGG	15	13.3%
ACT/TGA	14	12.4%
AGC/TCG	18	15.9%
AGG/TCC	10	8.8%
CGC/GCG	4	3.5%
AGAT/TCTA	1	1.0%
ATTC/TAAG	1	1.0%
ATGG/TACC	1	1.0%
AAAAT/TTTTA	1	1.0%
ACCAT/TGGTA	1	1.0%
AACAGA/TTGTCT	1	1.0%
ACAACC/TGTTGG	1	1.0%

Table 2.2 Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR114b	$(TTC)_6$	340-356	FAM-F-GCAAAAGAGAAAGTGGCTGATT R-ACAGAAGCACCCTGTGAAAACT	5	0.422	0.4	0.404	0.008	NA
BR173	$(CAA)_5$	223-235	HEX-F-GACAAAGCTGAGGAGCCAAC R-GAAGGGCGTATATGCAGGAA	4	0.528	0.66	0.417	-0.116	10[a]
BR177	$(CAC)_7$	386-395	NED-F-ATCAGAGCCTTCACAAAGAACC R-ATGAACCCAGAAGAGGAATTGA	2	0.058	0.06	0.057	-0.007	NA
BR182	$(ACC)_5$	227-230	HEX-F-TTCTACCGTTTTCTCCGACATT R-AACAGCAGCAACAACTCTTTCA	2	0.273	0.286	0.236	0.029	NA
BR190	$(AGC)_5$	287-293	HEX-F-GGCATAGACTGACACCAATTCA R-AAGACAATCCCAAATCATGTCC	4	0.325	0.26	0.3	0.136	5S
BR193	$(TCC)_5$	339-342	HEX-F-GGACGATGTTCCCTGTGATATT R-ACACCCATTTGCTCTTCATTCT	2	0.455	0.54	0.352	-0.085	NA
BR199	$(TCC)_5$	297-309	FAM-F-CTCTACATCTTCTGCTTGGCCT R-TGGGTCTGGCTCTAACTCTAGC	5	0.247	0.229	0.239	0.141	10
BR202	$(GGC)_5$	180-201	NED-F-CCCATGCAATCCCTACTCAT R-GTCCAAATGATCCCATCTGC	6	0.487	0.48	0.451	0.007	11[a]

*Linkage group assigned based on segregation in mapping population or one of three alternate populations: a) 01035 b) 05024 c) 06027

Table 2.2 (Cont.) Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR205	$(GCT)_5$	169-172	FAM-F-CCTTCGTCTTGGTCTGCTATTT R-CTTTGATGGACTTGATAAGGGC	3	0.384	0.3	0.324	0.115	11S
BR210	$(TTC)_6$	238-241	NED-F-GAAGGTGGGTTGGAGACAGA R-CAATGGTGAGCAATTTGGTG	2	0.0	0.0	0.0	ND	4[c]
BR211	$(ATC)_8$	137-160	FAM-F-CCAATTTCCTGTGCTGGTTT R-CGTGTAGCCAATCCTCTCGT	6	0.478	0.52	0.422	-0.049	11S, 11R
BR215	$(CGC)_5$	120-129	HEX-F-TGAAATCTTCACCTCTTAAAAGATCC R-GGAATCTGAGCTGCCAAGTC	6	0.686	0.694	0.623	0.01	7S, 7R
BR216	$(ACC)_6$	118-139	HEX-F-AGGGGTGTTGGAGGACTTTT R-GAATCATTTTGGCCTTTGGA	6	0.556	0.48	0.458	0.079	4[c]
BR227a	$(TTC)_{10}$	284-305	HEX-F-CTACACACCTTCTTTTGGAGGC R-GTCATCTCTTGCCTGTCTTCCT	7	0.647	0.571	0.588	0.068	NA
BR227b	$(CTT)_8$	321-324	HEX-F-CTACACACCTTCTTTTGGAGGC R-GTCATCTCTTGCCTGTCTTCCT	2	0.19	0.213	0.172	0.155	NA
BR229	$(CGA)_5$	297-306	HEX-F-ATGTCGAACTCTTTCACACCCT R-TCCCTCAACACCTCTCTCTCTC	4	0.637	0.66	0.578	-0.022	1S
BR230	$(CAC)_5$	368-371	FAM-F-ATGGAGGAGGAGGAGAGAGAAT R-AGTCAGATTTCCACCGAGTACA	2	0.403	0.28	0.322	0.18	7S, 2R

*Linkage group assigned based on segregation in mapping population or one of three alternate populations: a) 01035 b) 05024 c) 06027

Table 2.2 (Cont.) Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR231	$(ACC)_5$	128-134	HEX-F-CATGAACGGAAAATCGGAGT R-CCCGAAAAACGACTTCATCT	3	0.304	0.32	0.273	-0.036	2S, 2R
BR233	$(ACC)_6$	109-115	HEX-F-CCATAGGGTGCACTTGACCT R-TTCTAGGCCCTCATTTGGTG	3	0.059	0.06	0.058	-0.007	NA
BR238	$(GATG)_6$	266-274	HEX-F-ATATCCACATAGGCCAGCAAAC R-ATGACCGAGGAAGAAGCATTAG	6	0.619	0.46	0.549	0.158	7S, 2R
BR240	$(GCA)_5$	229-241	FAM-F-GGTGGTGCTGCTGCTAGTG R-CTCTTTGTGCATCGTAATTGGA	4	0.115	0	0.113	0.789	9[a]
BR242	$(TTC)_6$	284-287	HEX-F-TGGATTTTCAGGCTTTAGAGGA R-ACATTTAGGTGGCTTTGGAGAA	3	0.059	0.06	0.058	-0.007	NA
BR245	$(TCA)_5$	279-285	NED-F-GCACAAGTGTAAGCTATGCTCG R-AACTCAGGATCTACCAACCGAA	5	0.548	0.58	0.487	-0.045	NA
BR246	$(AGG)_5$	175-183	HEX-F-ACCATATTCATTCCGGTCAATC R-ACCCACCAAGCAAAAGTAGAAA	2	0.164	0.14	0.15	0.078	9[a]
BR249	$(AACAGA)_5$	283-303	HEX-F-CGTGAGTGATTGAGTTGATGGT R-CAGATGAAGAAATCTCCTTGGC	7	0.58	0.52	0.501	0.061	10S
BR253	$(CCAACA)_5$	324-342	NED-F-GGTCTTAACTTTCAAGCATGGG R-GTTCATCACCTCCTACCTCGAC	9	0.632	0.7	0.582	-0.057	5S, 5R

*Linkage group assigned based on segregation in mapping population or one of three alternate populations: a) 01035 b) 05024 c) 06027

Table 2.2 (Cont.) Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR255	$(CTG)_5$	219-221	FAM-F-GACCTTGTTGTGTTCTTTGTCG R-TAATGGGCTCACTTCTTGGATT	2	0.077	0.04	0.074	0.284	2
BR257	$(TCG)_5$	359-371	NED-F-TGCTCGAAGAGGAATGACTACA R-ACTTTAACCCTAACCCTGGCTC	3	0.508	0.62	0.432	-0.104	9[b]
BR259	$(TCA)_{10}$	224-251	NED-F-GAAGGATGAATGGAAGTTGGAG R-AAGATCGGCTTCGAGAATATCA	9	0.857	0.86	0.841	-0.002	5S
BR261	$(TCT)_6$	142-148	NED-F-AGCCACCGTAGAAGACCAAA R-AATCCCAAGCTCATCGTCAG	3	0.325	0.32	0.299	0.01	9S, 9R
BR262	$(CAA)_6$	93-108	FAM-F-TGGGCTATGGGAGTTGGTAG R-CTCCGCTCTCAGCCTCAATA	3	0.096	0.1	0.094	-0.016	NA
BR264	$(AGA)_8$	116-128	NED-F-GGAAGACGCAGCAGAGAATC R-GTTTGCCACGACATTTTCCT	4	0.491	0.52	0.389	-0.028	8S
BR267	$(GCG)_5$	123-129	HEX-F-TGAAATCTTCACCTCTTAAAAGATCC R-GGAATCTGAGCTGCCAAGTC	4	0.675	0.66	0.607	0.013	8S, 8R
BR270	$(CTG)_6$	87-99	FAM-F-AGCACCTCCTCTGCTTCCTA R-TTCCTCCTCTGCTCCAAATG	5	0.572	0.54	0.48	0.033	1S
BR276	$(CTG)_6$	337-340	NED-F-GATTTCTGCTGTGGAGGGTATC R-TTCTGGGAGTATGCCTGGTACT	2	0.164	0.14	0.15	0.078	8[a]

*Linkage group assigned based on segregation in mapping population or one of three alternate populations: a) 01035 b) 05024 c) 06027

Table 2.2 (Cont.) Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR277	$(ACC)_5$	233-239	FAM-F-TATAGAGGAGAAAGTCCGCCAC R-TGTGGTAAAGAAGAGCGACAGA	5	0.279	0.22	0.258	0.134	NA
BR277b	$(ACC)_5$	357-359	FAM-F-TATAGAGGAGAAAGTCCGCCAC R-TGTGGTAAAGAAGAGCGACAGA	2	0.404	0.438	0.323	0.018	NA
BR279	$(ACC)_6$	125-131	NED-F-GGTAGCGGAAATCTCTGTCATC R-GAGTCGCAGTCCTGTTAGGTTT	3	0.423	0.38	0.349	0.045	10^c
BR284	$(CAG)_5$	386-395	HEX-F-CAACAGATCCCAGGTTAAAAGG R-TATGTTTCGGGACTTGGACTTC	4	0.551	0.62	0.492	-0.074	1S, 1R
BR288	$(TGC)_5$	366-369	FAM-F-ATTGTCAGGCTCTTCTATTGGC R-TTTCATCTCTGAACCACTTCCC	2	0.215	0.204	0.192	0.095	NA
BR292	$(CCA)_7$	320-323	HEX-F-TAATTCCCACCAGACCCATAAC R-TTGGCAGACTAACCTTTTCTCA	4	0.302	0.204	0.281	0.248	1R
BR294	$(CTT)_5$	308-311	NED-F-GGGACGACGGATACTCTTGTAA R-GCATCAAGGTGTTATGTTTGGA	2	0.113	0.12	0.106	-0.022	5S, 5R
BR302	$(CTT)_6$	121-127	HEX-F-CTTCCAGGACGACCCTCATA R-AACCTCTGTGGGATCTCTCG	3	0.078	0.08	0.076	-0.011	1^a
BR307	$(GAG)_7$	84-90	FAM-F-TGTGAAGGTATCCACCACGA R-ATCATCCACGTCATCATCCA	2	0.48	0.6	0.365	-0.111	NA

*Linkage group assigned based on segregation in mapping population or one of three alternate populations: a) 01035 b) 05024 c) 06027

Table 2.2 (Cont.) Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR311	$(GAA)_6$	111-117	FAM-F-GACAAAGCAGCCCAAGTAGC R-CTTCTCCCAACAGGCTTCTG	3	0.149	0.16	0.14	-0.033	NA
BR315	$(CAG)_6$	133-136	FAM-F-CTTCGGGCAGATATCCACAT R-ATCTGCAAATGAACCCGTCT	2	0.495	0.5	0.373	-0.005	9S, 9R
BR316	$(GAG)_5$	128-133	NED-F-TCAGCAATACCAGGATGCAA R-CCCAGGAAGTAAGCCAACAA	3	0.165	0.14	0.155	0.08	NA
BR322	$(ACT)_7$	99-108	FAM-F-TCTCTTCCTTGCCACCTCAG R-AAGATGGGGTTCGAGGAGAC	5	0.612	0.58	0.562	0.02	8S
BR325	$(GAG)_5$	147-156	HEX-F-CCAGAATTGGAGGGACAGTG R-CGGTTTTCCATCATCATCCT	5	0.629	0.58	0.571	0.039	8S, 8R
BR327	$(CCA)_7$	228-231	HEX-F-CCACGCTTCTTCAGTTCCTC R-CATTGTCCAGCGTCTGATCT	3	0.229	0.14	0.207	0.238	NA
BR331	$(AGA)_5$	126-132	HEX-F-CGAATTTCCAAAGGGAAACA R-GGATCGAAAAAGCCATTGAA	3	0.394	0.38	0.331	0.026	3S
BR332	$(GGT)_6$	345-351	HEX-F-CATAGGGTGGAGCAGAAGATG R-TGAACAACATCATAAAGCTGGC	4	0.115	0.12	0.113	-0.021	NA
BR339	$(GTG)_8$	125-131	NED-F-GGTAGCGGAAATCTCTGTCATC R-GAGTCGCAGTCCTGTTAGGTTT	3	0.423	0.38	0.349	0.045	10[c]

*Linkage group assigned based on segregation in mapping population or one of three alternate populations: a) 01035 b) 05024 c) 06027

Table 2.2 (Cont.) Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR340	$(TCT)_5$	370-391	HEX-F-CCAGCCGATTCCAATTCTATTT R-ACCATTCTCGACCTGTTCTCC	6	0.604	0.163	0.556	0.58	1R
BR341	$(TCT)_8$	104-116	FAM-F-CACCTACACCACCCCTAAGC R-GAGAGGCTGGAGAAGGATCA	5	0.582	0.58	0.538	0.015	1S
BR343	$(TGC)_6$	386-395	NED-F-CAACAGATCCCAGGTTAAAAGG R-TATGTTTCGGGACTTGGACTTC	4	0.551	0.62	0.492	-0.074	1S, 1R
BR344	$(TTG)_5$	121-136	NED-F-CTACTTCGAGGATGTCGTTGC R-CGGAAATGTTGACGATGATG	5	0.609	0.26	0.548	0.408	NA
BR345	$(GAT)_5$	112-115	FAM-F-TGCTTCAGATGACGGAAATG R-TGGTACCTTTTTCGTTTCTTGG	2	0.226	0.22	0.201	0.014	NA
BR347	$(CAT)_5$	386-401	NED-F-CCAGTTGAAGAACCTGTAAGGG R-TAAACACACCATGCCAGATAGG	9	0.516	0.46	0.495	0.051	1R
BR349	$(GCA)_5$	228-240	FAM-F-GGTGGTGCTGCTGCTAGTG R-CTCTTTGTGCATCGTAATTGGA	4	0.269	0.1	0.256	0.448	9[a]
BR352	$(GAA)_5$	109-118	HEX-F-AGAAAGCAAGATGGCAGACC R-CGTTGGCTTACCTGGATGAC	5	0.573	0.74	0.482	-0.149	10R
BR355	$(TCA)_8$	198-204	FAM-F-GGAAGTGGTTGTTGTGATTGTG R-TTCTGTGCCATCTAGTCACGTT	4	0.169	0.18	0.164	-0.038	NA

*Linkage group assigned based on segregation in mapping population or one of three alternate populations: a) 01035 b) 05024 c) 06027

Table 2.2 (Cont.) Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR357	$(TGT)_5$	109-116	NED-F-GCCCAAGCTTTCTCACTTCA R-GCAAGGCTTTTAGAACCAACA	4	0.647	0.54	0.573	0.094	4S
BR358	$(TTC)_8$	116-128	FAM-F-GGAAGACGCAGCAGAGAATC R-GTTTGCCACGACATTTTCCT	4	0.491	0.52	0.389	-0.028	8S
BR359	$(TCT)_5$	384-399	HEX-F-TACCTAACACAACAGCCACCAC R-TCAGAATGGTAATTGCACCTTG	13	0.695	0.64	0.675	0.037	4S, 4R
BR361	$(TGC)_7$	368-377	NED-F-GCTATCTTGCTTGCTTCCTTGT R-ATCCCCTTCCAAAACTAACCAT	3	0.426	0.52	0.343	-0.103	1S
BR362	$(CCT)_6$	201-204	FAM-F-GATGTGATGGTCAAAAGCTCAA R-AAGAAGAGCAGCGATCTCAAGT	2	0.18	0.08	0.164	0.379	4[c]
BR371	$(TGT)_6$	270-282	NED-F-TATTGAAATGGGGAGAGGAGTG R-AGGGGATCTTCTAGGATTTTCG	4	0.533	0.46	0.45	0.076	11[a]
BR374	$(GGA)_7$	218-251	FAM-F-GCAACCCCCATGGATATAAA R-TGGACATTGTTGGTGGAGAA	10	0.786	0.18	0.757	0.633	NA
BR375	$(GAA)_7$	256-265	NED-F-GGACAGTGAGGGAGAAACAACT R-GGATACCTGGATTTGACGAGAG	6	0.705	0.8	0.651	-0.068	9S, 9R
BR379	$(GAA)_7$	112-151	HEX-F-AACCCCGAGAAACAGAGGAT R-GCGTCTGCTCATCGTATTGA	14	0.853	0.84	0.837	-0.003	7S

Table 2.2 (Cont.) Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR381	$(TTC)_8$	124-140	HEX-F-TCGGAAACCAAACAGAGTGA R-CTGACGCACAAACCTGAAGA	4	0.41	0.429	0.341	0.001	NA
BR387	$(CAA)_5$	365-380	NED-F-AACAGCAACAACAACAACTGCT R-GAGGATGAGAAGTCGAGGAACT	8	0.701	0.54	0.667	0.138	NA
BR389	$(AAG)_5$	320-329	FAM-F-GGTAAAGAGCATCACTCTGCAA R-CTCAACCAAGCCAATTAAGCTC	2	0.245	0.286	0.215	-0.019	NA
BR392	$(GCA)_6$	215-221	HEX-F-TCTGTTGCTGTTGTTGTTGTTG R-CTCAATCGCAGTCTCTCATCAC	2	0.226	0.22	0.201	0.014	NA
BR396	$(ATC)_6$	139-148	NED-F-TTTGGGTGAATCTTCATCAGC R-CCAGTGCATCACAGCAGTTT	3	0.114	0.12	0.111	-0.022	NA
BR397	$(TCT)_9$	238-253	HEX-F-AAGAGTTGTGGAAGAGGCAGAG R-TACTTGAAACCACGAGACGAGA	4	0.705	0.76	0.655	-0.031	NA
BR398	$(AGAT)_5$	164-172	NED-F-GATAGCCAGGAGGAACAGAGAA R-AGGGCAGTGTCAGAGAAGAAAG	2	0.02	0.02	0.02	-0.001	NA
BR402	$(GGC)_6$	128-140	NED-F-GGGTGGAAACTTGACACCAG R-GTGAGCTGCTCCATCATCAA	5	0.674	0.66	0.62	0.006	4R
BR406	$(ACC)_6$	212-218	FAM-F-TAGGACTCGTCCCTGTAGGC R-TTCTAGGCCCTCATTTGGTG	3	0.039	0.04	0.039	-0.003	NA

*Linkage group assigned based on segregation in mapping population or one of three alternate populations: a) 01035 b) 05024 c) 06027

Table 2.2 (Cont.) Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR410	$(ACT)_5$	322-325	FAM-F-ACACAAACTGGATGTATGCCAA R-TGGAAAAGACAACACTGGAATG	2	0.484	0.38	0.367	0.12	9S, 9R
BR411	$(AGC)_5$	118-133	HEX-F-CCGGATGGTTTCAGGTACAG R-TCCAGAGAAAGACGGAGAGC	5	0.485	0.42	0.447	0.086	3[c]
BR413	$(TTG)_8$	227-245	NED-F-AAACCTCAAACAACATGGAACC R-CCTCTTCTTCTGCTTGCTCTTC	5	0.375	0.34	0.354	0.075	4S
BR414	$(AAT)_6$	112-134	HEX-F-ATCGCATCACGGAAGAGAAG R-TGACGAGAACCTAGGGATCTATTT	10	0.674	0.78	0.635	-0.1	9S, 9R
BR415	$(GAA)_7$	248-260	NED-F-GATTGGAAGAAGGCAAAGAATG R-TAAAACCTTGATGGGTCGTCTT	6	0.621	0.56	0.573	0.045	5S, 5R
BR418	$(AAAAT)_6$	122-136	NED-F-GAACTAAATGGCCCAAGCAA R-TCCATTGCCATACAGCTCAA	3	0.057	0.02	0.058	0.372	NA
BR420	$(TGC)_5$	88-100	FAM-F-GACGTTCGATCCAGAAGAGC R-TGATGGGTTTGACCCTTTGT	6	0.583	0.54	0.522	0.046	NA
BR423	$(GAA)_6$	103-115	HEX-F-ACAAACCAAAGGGAGTGTGG R-CAAGCTTTCCATCATCGTCA	4	0.692	0.74	0.63	-0.036	1S, 1R
BR425	$(CTC)_6$	265-283	FAM-F-GGGACCACTTGCACTTGAAT R-TGCTGCAACTTTCCCTTGTA	5	0.318	0.3	0.284	0.059	2[b]

*Linkage group assigned based on segregation in mapping population or one of three alternate populations: a) 01035 b) 05024 c) 06027

Table 2.2 (Cont.) Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR427	$(CCA)_5$	304-319	FAM-F-CAGGAGCAGAAGGAGAAGATGT R-CTCTGGTAGTGATTCGGGTTCT	5	0.539	0.48	0.432	0.06	5S
BR430	$(TCC)_6$	260-266	HEX-F-AGGAAGCAAGGACAACATCACT R-CAACAAGACTGGAAACAACCAA	3	0.114	0.12	0.109	-0.022	2[a]
BR433	$(TACCA)_5$	134-150	NED-F-GCCAATCCAGAGGAGATAAGG R-TCACATCTTGAAAACGGAGAG	6	0.547	0.163	0.514	0.555	NA
BR437	$(TGC)_5$	145-151	NED-F-GCTCATCGTAGCAAATTACGC R-GGCGCAATTAACGTATGGAA	4	0.355	0.2	0.329	0.286	NA
BR438	$(TCA)_8$	191-199	FAM-F-ATCTCTGCCCCTCTCTCTCTCT R-AACTAACACCGTTGCTGATCCT	4	0.539	0.66	0.479	-0.11	11S
BR442	$(GAT)_5$	172-225	NED-F-CTGCCCTACTTCCCTTTTCTTT R-ATCATAGACCCCACCAAGTCCT	3	0.509	0.46	0.389	0.051	2S, 2R
BR444	$(TCT)_5$	103-106	FAM-F-CAGAGCAGCGAAGGAAAAAG R-CTTGCTCAGTCTTCACCATCC	3	0.492	0.7	0.38	-0.178	NA
BR446	$(CAA)_5$	153-162	FAM-F-GATTGATGCTGATGGTGCTG R-TACGCCCTCAAATCAAGACC	4	0.668	0.84	0.601	-0.114	11S, 11R
BR451	$(CAA)_5$	126-137	HEX-F-ACACCCTTCACCAAAACCAC R-GCTTCATCCCAGCAGAGAAC	2	0.058	0.02	0.057	0.371	NA

*Linkage group assigned based on segregation in mapping population or one of three alternate populations: a) 01035 b) 05024 c) 06027

Table 2.2 (Cont.) Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR456	$(TCA)_7$	133-142	FAM-F-CCTCTGTCCAACGGTTGTTT R-TGGTCACCTAGGGCATGTTT	2	0.343	0.36	0.284	-0.024	3[c]
BR464	$(ATC)_7$	269-296	FAM-F-GTGCAAACAGTCGCTATCATCT R-CGAGGACCCATAAGAGAACATC	5	0.531	0.52	0.464	0.018	3S
BR467	$(ATTC)_5$	141-154	NED-F-GCATTAAGAAGGCGTCTTGG R-ATTCCCCCACCATTCAAAAC	3	0.202	0.22	0.192	-0.051	1S
BR468	$(TGA)_8$	361-376	NED-F-GGAGATTCCCTCATCTTTCTCA R-AGACTGAAGTGCCCAAAGTACC	3	0.077	0.08	0.076	-0.011	1[c]
BR470	$(CAA)_5$	334-340	HEX-F-AAACTCAAGCATCCAATCTGGT R-CCTAAACTCCCAAAAGGGTTTC	2	0.113	0.12	0.106	-0.022	NA
BR474	$(TTC)_6$	122-125	HEX-F-ACCAGAACCTCCATTACCACAC R-AAAAGAAGGAGAAGACGAAGGG	2	0.412	0.46	0.327	-0.055	3S
BR475	$(TCT)_7$	237-243	FAM-F-TCACAAACAAACCCCAGACA R-CACATGCTTCAACACCTCGT	3	0.441	0.5	0.352	-0.057	7S, 2R
BR478	$(GA)_8$	203-207	FAM-F-TCCATGGGCATATATGGATCT R-GAAGCCTGTGGTGAAGAAGG	4	0.456	0.4	0.416	0.078	8S, 8R
BR480	$(TC)_{11}$	132-128	NED-F-TGGTGTTGCTGATGGGACTA R-ACATGAGGTGCCCAATTCTC	9	0.737	0.64	0.701	0.074	4S

*Linkage group assigned based on segregation in mapping population or one of three alternate populations: a) 01035 b) 05024 c) 06027

Table 2.2 (Cont.) Primer sequences and characteristics of 113 new microsatellite loci from transcriptome sequences of European hazelnut (*Corylus avellana* L.)

Locus	Repeat motifs	Target size	Primer 5' – 3'	n	He	Ho	PIC	r	LG*
BR482	$(AG)_9$	282-304	FAM-F-GGTGAAGCTGTGACTGTTGAAG R-AGCAGCCAAACCAAAACTCTTA	6	0.555	0.56	0.516	-0.001	4S, 4R
BR483	$(AG)_{12}$	282-310	NED-F-TTACCACCACTTTTCAACACCA R-GGTACATCAAAGAAGGGAGCAC	9	0.815	0.76	0.792	0.033	11S, 4R
BR484	$(AT)_8$	363-375	HEX-F-CAAAGCCACCAGATTCACTTACT R-GTCCGTGGAAGGAGTATTCAAG	5	0.531	0.48	0.491	0.047	4[b]
BR485	$(AG)_9$	127-135	HEX-F-CGGAAAGTGGACAGTGGATT R-ATCCGCAAAACCAAAACAAA	5	0.723	0.74	0.677	-0.017	3S, 3R
BR487	$(AG)_9$	369-381	NED-F-TCTCGAAATCCTTATCCGTAGC R-CAATATGAAACCAAAGCGACAC	6	0.719	0.54	0.673	0.145	4S
BR488	$(AG)_{13}$	258-266	NED-F-GAAAGGAAAGTGAGAATGGGAA R-TATTGATAACCCGGATCGAAAG	5	0.416	0.44	0.391	-0.046	NA

*Linkage group assigned based on segregation in mapping population or one of three alternate populations: a) 01035 b) 05024 c) 06027

Table 2.3 Polymorphic microsatellite loci assigned to linkage groups using alternate segregating populations

Marker	Population used for LG assignment	Linkage Group	Marker, correlation coefficient, and P-value
BR173	01035	10	A640 of LG10 (0.514, 0.0036)
BR199	97035	10	B664 of LG10 (-0.671, <0.0001)
BR202	01035	11	B657 of LG11 (0.544, 0.0019)
BR210	06027	4	B738 of LG4 (0.535, 0.0033), BR335 of LG4 (0.86, <0.0001)
BR216	06027	4	BR335 of LG4 (-0.79, <0.0001), BR210 (0.92, <0.0001)
BR240	01035	9	SMN_E of LG9 (0.659, <0.0001), BR349 of LG9 (0.914, <0.0001)
BR246	01035	9	B732 of LG9 (0.6, 0.0007)
BR255	01033	2	B789 of LG2 (0.732, <0.0001), K76_1_26 of LG2 (0.933, <0.0001)
BR257	05024	9	B795 of LG9 (0.709, <0.0001)
BR276	01035	8	B665 of LG8 (-0.53, 0.0027)
BR279	06027	10	BL021 of LG10 (-0.818, <0.0001)
BR335	06027	4	B701 of LG4 (-0.57, 0.0009)
BR339	06027	10	BL021 of LG10 (-0.81, <0.0001)
BR349	01035	9	SMN_E of LG9 (0.736, <0.0001), BR240 of LG9 (0.914, <0.0001)
BR362	06027	4	B634 of LG4 (0.53, 0.0026), B774 (0.93, <0.0001)
BR371	01035	11	SMN_N of LG11 (-0.881, <0.0001)
BR411	06027	3	B619_162 of LG3 (0.795, <0.0001), B662_225 of LG3 (0.535, 0.003)
BR425	05024	2	B751 of LG2 (0.559, 0.002)
BR430	01035	2	B751 of LG2 (-0.736, <0.0001)
BR456	06027	3	B619 of LG3 (-0.933)
BR468	06027	1	B029 of LG1 (-0.519, 0.0039), B737 (-0.8, <0.0001)
BR484	05024	4	B634 of LG4 (0.555, 0.0018)

Table 2.4 Comparison of microsatellite (di-, tri-, tetra-, penta-, and hexa-repeats) motifs mined from transcriptome and EST sequences among 6 plant genera and proportions found polymorphic

	Corylus avellana transcriptome	*Nicotiana tabacum*[a] EST	*Coffea sp.*[b] EST	*Manihot esculenta*[c] EST	*Capsicum sp.*[d] EST	*Citrus sp.*[e] EST
Number microsatellite sequences mined	1432	85,716	425	NA	1201	3278
Number primer pairs designed	382	3604	224	1500	812	100
Markers polymorphic (%)	26.68%	6.10%	75.00%	13.80%	29.20%	87.00%
Repeat motifs						
AT/TA	13.3%	46.8%	15.3%	3.9%	3.2%	10.4%
AC/TG	3.1%	3.8%	13.1%	41.0%	2.3%	13.8%
AG/TC	35.8%	13.4%	32.5%	28.1%	12.4%	17.2%
CG/GC	0.1%	0.0%	0.6%	0.0%	0.0%	0.0%
AAG/TTC	12.7%	11.3%	0.1%	3.9%	14.0%	9.5%
AAT/TTA	1.5%	13.9%	3.8%	3.9%	4.2%	8.6%
AAC/TTG	3.7%	5.7%	1.9%	2.2%	7.3%	2.1%
AGC/TCG	7.0%	0.9%	1.9%	3.2%	0.0%	8.5%
ACT/TGA	6.9%	2.1%	1.9%	0.0%	6.6%	1.9%
ACC/TGG	4.4%	1.1%	4.1%	1.1%	9.4%	2.4%
ATC/TAG	1.6%	0.0%	4.4%	2.4%	6.0%	2.6%
ACG/TGC	1.5%	0.0%	2.8%	0.0%	8.2%	5.9%
AGG/TCC	4.6%	0.0%	3.1%	1.0%	5.8%	2.1%
CCG/GGC	1.3%	0.9%	0.9%	0.8%	4.6%	1.6%

Table 2.4 (cont.) Comparison of microsatellite (di-, tri-, tetra-, penta-, and hexa-repeats) motifs mined from transcriptome and EST sequences among 6 plant genera and proportions found polymorphic

AAAT/TTTA	0.1%	0.0%	0.3%	2.4%	1.6%	1.8%
AAAG/TTTC	0.4%	0.0%	0.3%	2.2%	1.7%	1.4%
AAAC/TTTG	0.0%	0.0%	0.0%	0.8%	0.6%	0.4%
AACC/TTGG	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%
AACG/TTGC	0.0%	0.0%	0.0%	0.1%	0.2%	0.0%
AACT/TTGA	0.1%	0.0%	0.0%	0.0%	0.3%	0.1%
AAGT/TTCA	0.1%	0.0%	0.3%	0.3%	0.3%	0.5%
AATT/TTAA	0.0%	0.0%	0.0%	0.4%	0.9%	0.5%
AAGG/TTCC	0.0%	0.0%	0.0%	0.0%	0.8%	0.0%
ACAT/TGTA	0.0%	0.0%	0.3%	0.0%	0.4%	0.5%
ACAG/TGTC	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
ACCT/TGGA	0.1%	0.0%	0.3%	0.1%	0.0%	0.0%
ACCG/TGGC	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
ACGC/TGCG	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%
ACTC/TGAG	0.0%	0.0%	0.0%	0.0%	0.1%	0.2%
AGAT/TCTA	0.1%	0.0%	0.0%	0.0%	0.0%	0.2%
AAAAT/TTTTA	0.1%	0.0%	0.0%	0.0%	0.7%	0.0%
AACCT/TTGGA	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
ACCAT/TGGTA	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
AAAAAG/TTTTTC	0.1%	0.0%	0.0%	0.0%	0.0%	0.2%
AAAAAT/TTTTTA	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
AAACAG/TTTGTC	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
AACACC/TTGTGT	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
AACAGA/TTGTCT	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
AAGAGG/TTCTCC	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 2.4 (cont.) Comparison of microsatellite (di-, tri-, tetra-, penta-, and hexa-repeats) motifs mined from transcriptome and EST sequences among 6 plant genera and proportions found polymorphic

AAGATG/TTCTAC	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%
ACCCGC/TGGGCG	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
AGAGGT/TCTCCA	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
AGCAGG/TCGTCC	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
AGATGG/TCTACC	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%

[a] Tong et al., 2012; [b] Aggarwal et al., 2007; [c] Sraphet et al., 2011; [d] Yi et al., 2006; [e] Chen et al., 2006

Table 2.5 Microsatellite loci recommended for future genetics research in hazelnut (*C. avellana*).

Locus	Repeat motifs	Target size	n	He	Ho	PIC	r	LG
BR173	$(CAA)_5$	223-235	4	0.528	0.66	0.417	-0.116	10
BR229	$(CGA)_5$	297-306	4	0.637	0.66	0.578	-0.022	1S
BR238	$(GATG)_6$	266-274	6	0.619	0.46	0.549	0.158	7S, 2R
BR249	$(AACAGA)_5$	283-303	7	0.580	0.52	0.501	0.061	10S
BR259	$(TCA)_{10}$	224-251	9	0.857	0.86	0.841	-0.002	5S
BR270	$(CTG)_6$	87-99	5	0.572	0.54	0.480	0.033	1S
BR322	$(ACT)_7$	99-108	5	0.612	0.58	0.562	0.020	8S
BR325	$(GAG)_5$	147-156	5	0.629	0.58	0.571	0.039	8S, 8R
BR341	$(TCT)_8$	104-116	5	0.582	0.58	0.538	0.015	1S
BR357	$(TGT)_5$	109-116	4	0.647	0.54	0.573	0.094	4S
BR359	$(TCT)_5$	384-399	13	0.695	0.64	0.675	0.037	4S, 4R
BR375	$(GAA)_7$	256-265	6	0.705	0.80	0.651	-0.068	9S, 9R
BR379	$(GAA)_7$	112-151	14	0.853	0.84	0.837	-0.003	7S
BR387	$(CAA)_5$	365-380	8	0.701	0.54	0.667	0.138	NA
BR397	$(TCT)_9$	238-253	4	0.705	0.76	0.655	-0.031	NA
BR402	$(GGC)_6$	128-140	5	0.674	0.66	0.620	0.006	4R
BR414	$(AAT)_6$	112-134	10	0.674	0.78	0.635	-0.100	9S, 9R
BR415	$(GAA)_7$	248-260	6	0.621	0.56	0.573	0.045	5S, 5R
BR427	$(CCA)_5$	304-319	5	0.539	0.48	0.432	0.060	5S
BR438	$(TCA)_8$	191-199	4	0.539	0.66	0.479	-0.110	11S
BR446	$(CAA)_5$	153-162	4	0.668	0.84	0.601	-0.114	11S, 11R
BR464	$(ATC)_7$	269-296	5	0.531	0.52	0.464	0.018	3S
BR480	$(TC)_{11}$	132-128	9	0.737	0.64	0.701	0.074	4S
BR482	$(AG)_9$	282-304	6	0.555	0.56	0.516	-0.001	4S, 4R
BR483	$(AG)_{12}$	282-310	9	0.815	0.76	0.792	0.033	11S, 4R
BR485	$(AG)_9$	127-135	5	0.723	0.74	0.677	-0.017	3S, 3R
BR487	$(AG)_9$	369-381	6	0.719	0.54	0.673	0.145	4S

Table 2.6 Unique alleles found in 66 accessions at 113 polymorphic microsatellite marker loci

Locus	Allele	Accession	Locus	Allele	Accession
BR114b	340	OSU 1187.101	BR325	159	Albania 55
BR169	172	Tombul Ghiaghli	BR332	348	Fusco Rubra
BR169	196	Sant Jaume	BR340	379	Aurea
BR169	201	Palaz	BR341	110	Fusco Rubra
BR169	213	Cutleaf	BR344	133	Artellet
BR169	218	Tonda Romana	BR358	116	OSU 759.010
BR169	229	Tonda Gentile d. Langhe	BR358	125	Pendula
BR169	231	Imperiale de Trebizonde	BR371	282	OSU 495.072
BR169	235	Barcelona	BR379	149	OSU 651.011
BR169	247	Tombul Ghiaghli	BR379	153	Gasaway
BR169	251	B-3	BR387	368	Albania 55
BR169	259	Des Anglais	BR398	164	OSU 495.049
BR169	266	Du Chilly	BR398	172	OSU 1187.101
BR169	271	Du Chilly	BR402	140	OSU 681.078
BR173	220	OSU 495.049	BR406	218	OSU 681.078
BR202	180	Tonda Romana	BR406	206	Fusco Rubra
BR210	241	Crvenje	BR406	215	Artellet
BR211	132	Fusco Rubra	BR414	127	Albania 55
BR211	160	OSU 495.072	BR414	151	Crvenje
B216	139	OSU 1187.101	BR418	130	Culpla
BR216	153	Palaz	BR418	136	OSU 556.027
BR216	159	B-3	BR420	97	Aurea
BR227	293	OSU 495.049	BR425	265	B-3
BR231	125	OSU 675.028	BR425	274	IannusaRacinante
BR240	229	OSU 1187.101	BR427	319	OSU 408.040
BR240	238	OSU 759.010	BR428	128	Culpla
BR242	281	Fusco Rubra	BR430	260	OSU 26.072
BR246	183	OSU 495.072	BR442	176	Fusco Rubra
BR249	308	OSU 495.049	BR451	126	OSU 495.072
BR264	116	OSU 759.010	BR456	137	Crvenje
BR264	125	Pendula	BR464	281	OSU 495.072
BR264	128	OSU 495.072	BR475	243	OSU 26.072
BR270	93	OSU 26.072	BR478	209	B-3
BR270	99	OSU 759.010	BR479	91	OSU 1185.126
BR277	239	Pendula	BR479	104	OSU 1185.126
BR303	268	OSU 252.146	BR479	118	Daviana
BR307	90	OSU 495.072	BR482	304	OSU 1185.126
BR311	117	Mortarella	BR487	377	OSU 1185.126
BR316	128	Culpla			

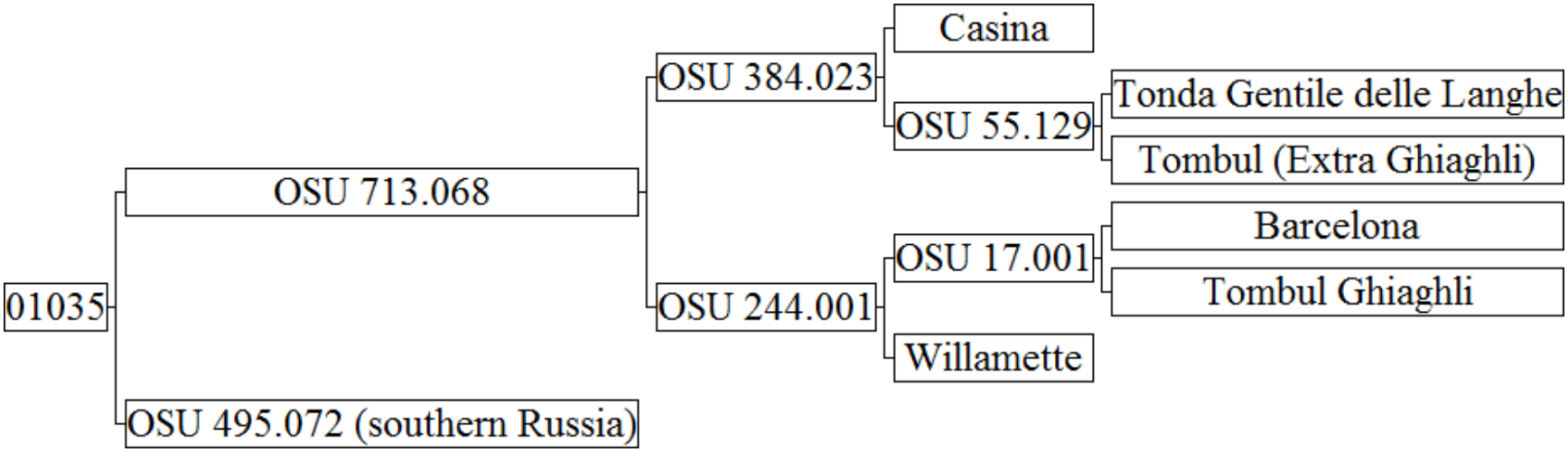

Figure 2.1 Pedigree of progeny 01035 segregating for eastern filbert blight resistance and useful for assigning loci to linkage groups.

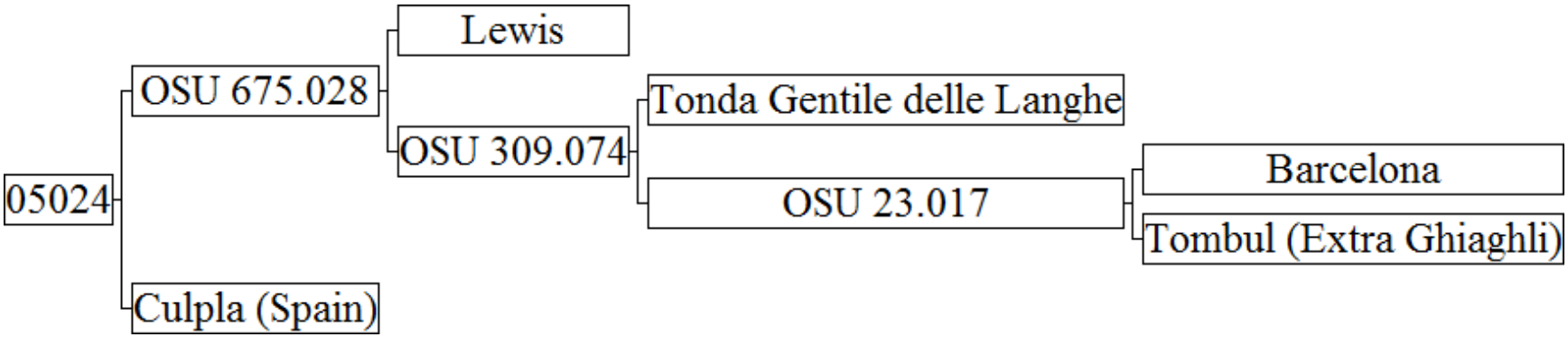

Figure 2.2 Pedigree of progeny 05024 segregating for eastern filbert blight resistance and useful for assigning loci to linkage groups.

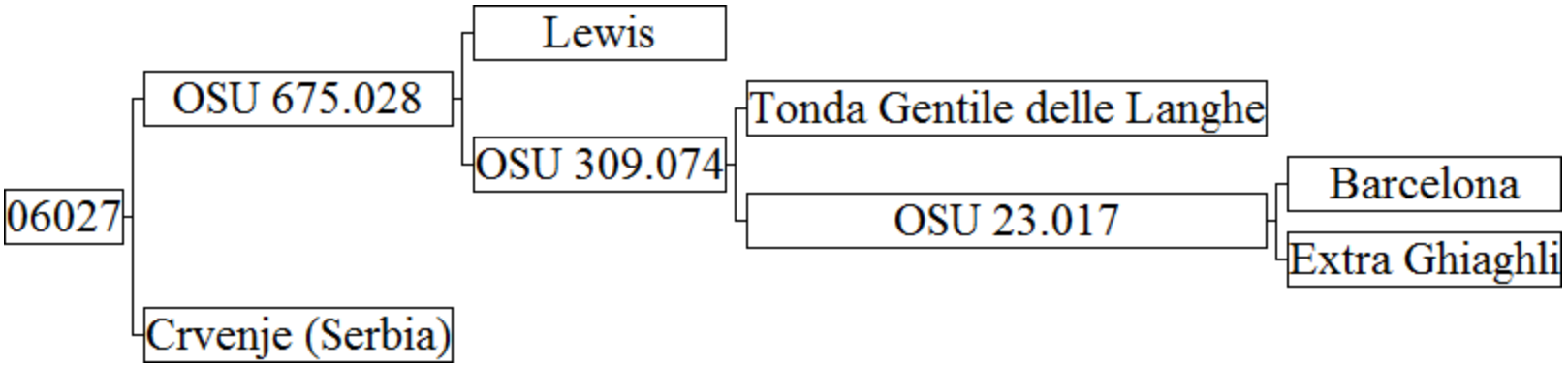

Figure 2.3 Pedigree of progeny 06027 segregating for eastern filbert blight resistance and useful for assigning loci to linkage groups.

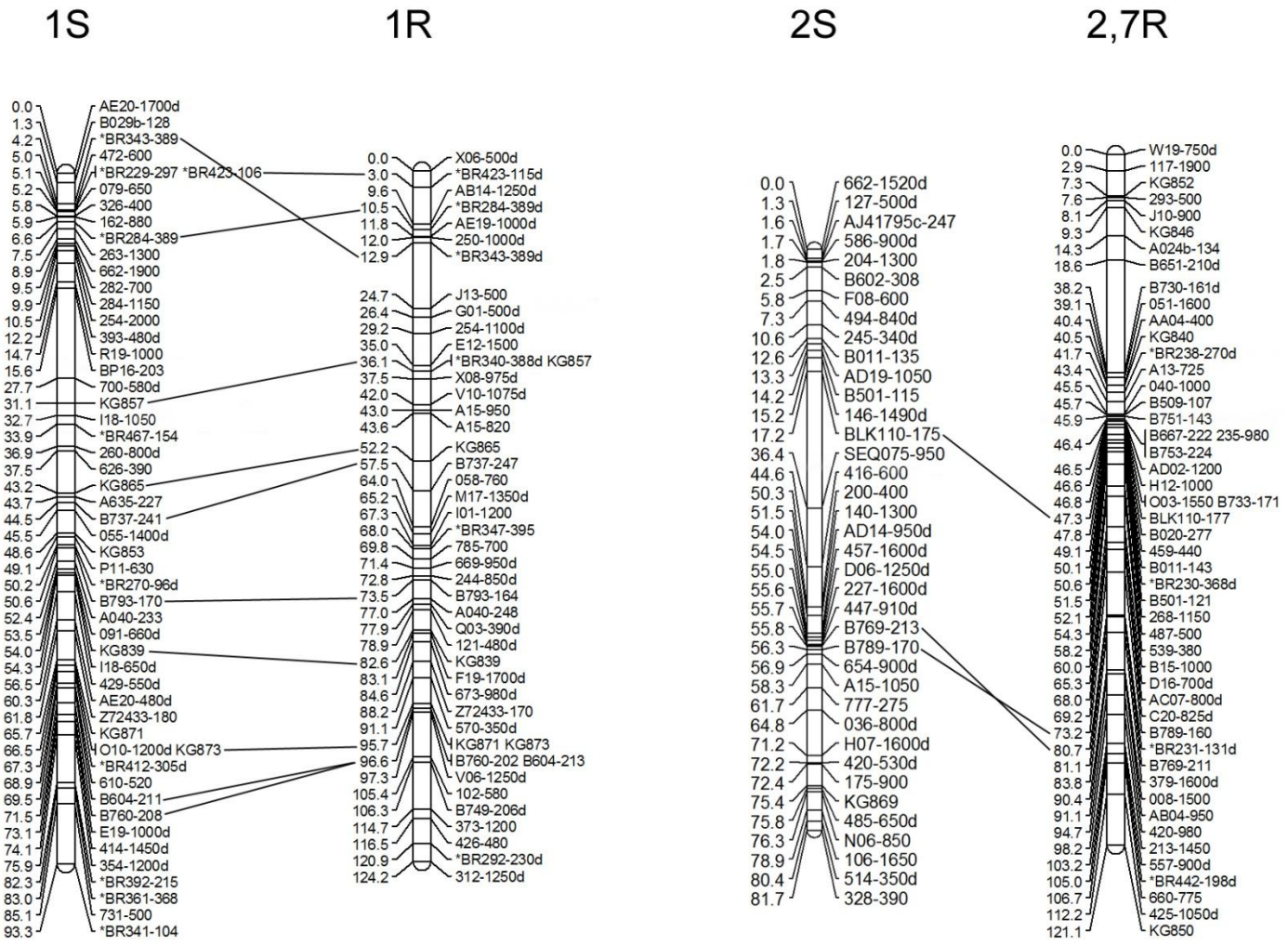

Figure 2.4 Linkage groups 1 & 2 of susceptible parent OSU 252.146 and resistant parent OSU 414.062

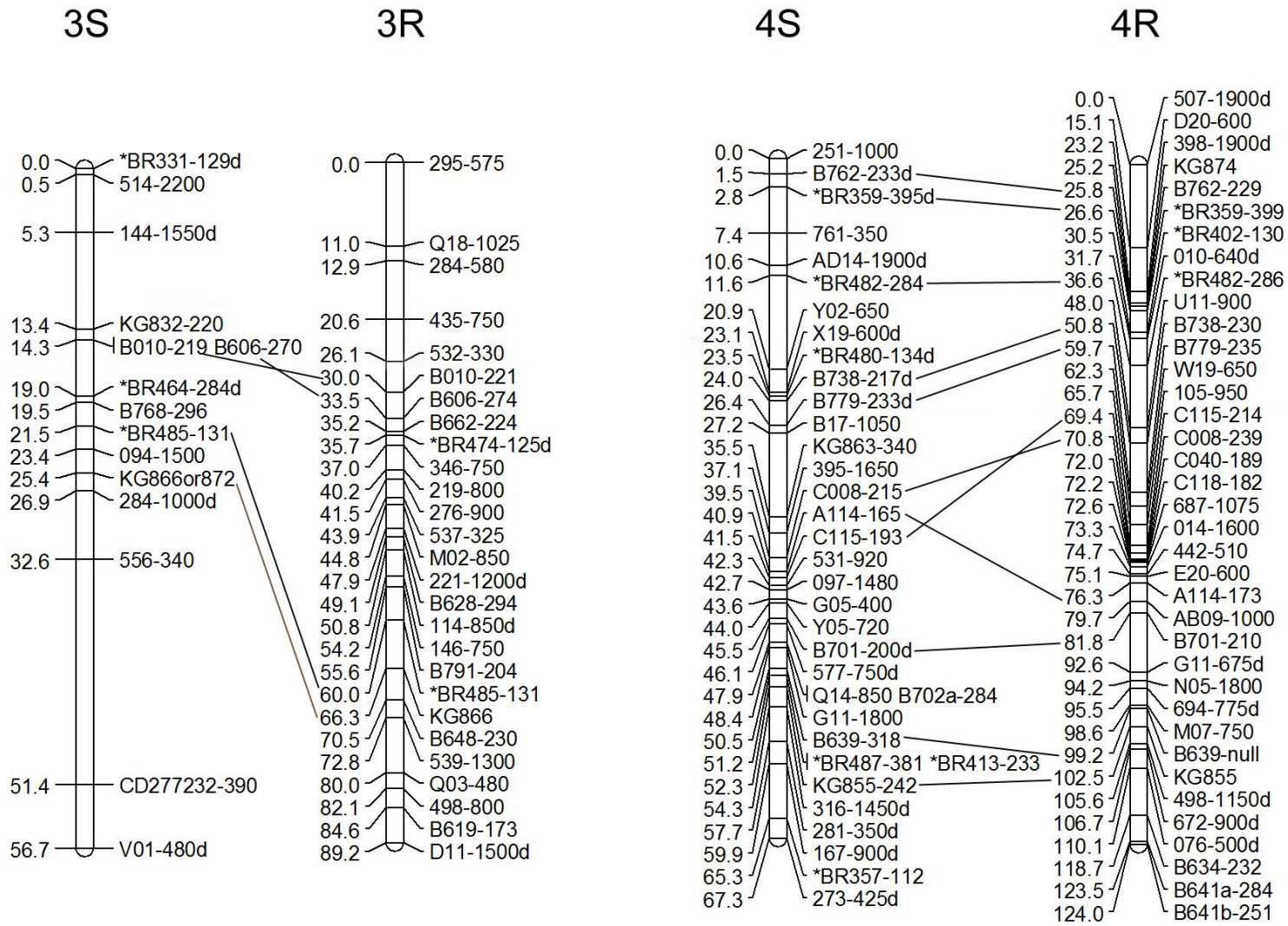

Figure 2.5 Linkage groups 3 & 4 of susceptible parent OSU 252.146 and resistant parent OSU 414.062

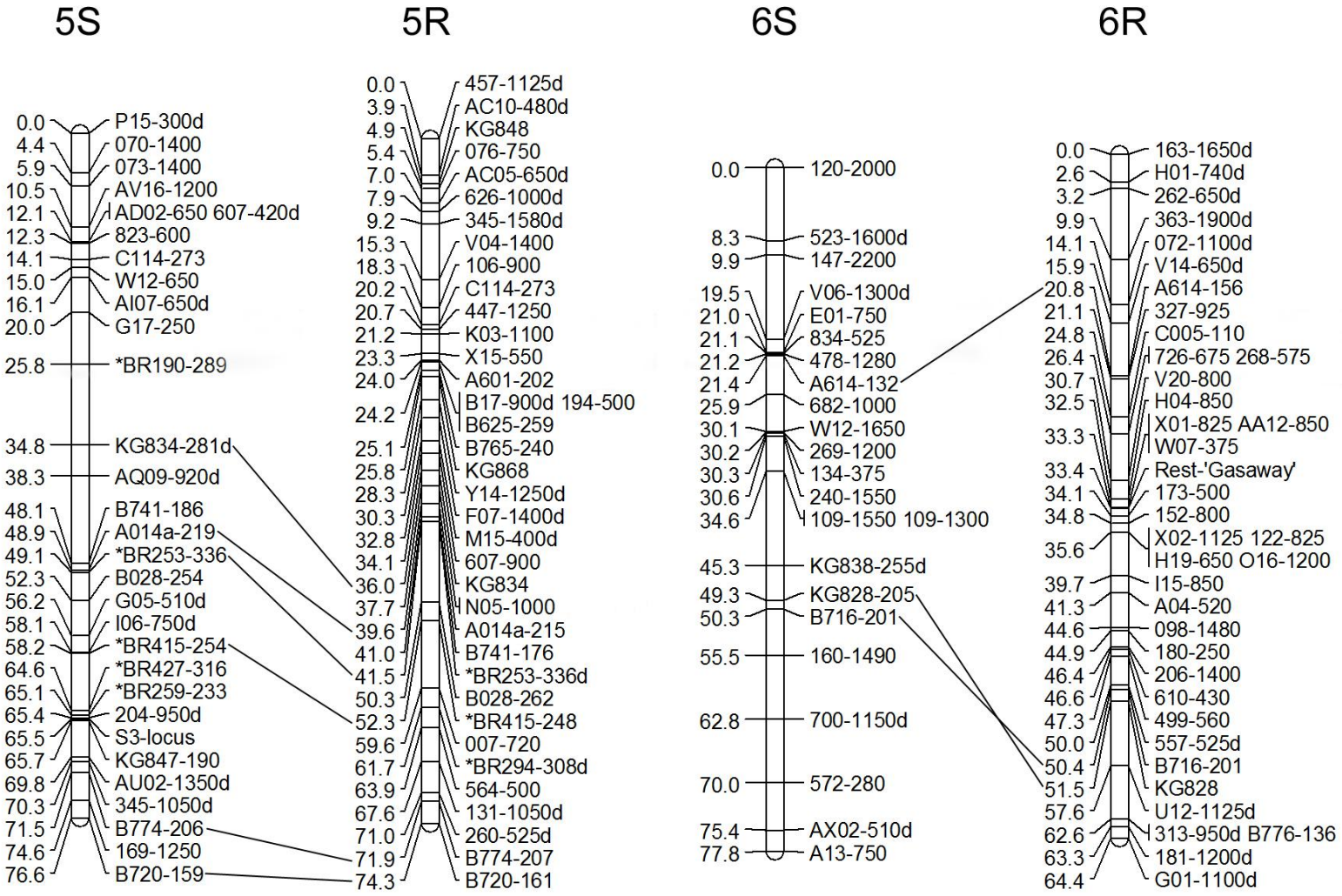

Figure 2.6 Linkage groups 5 & 6 of susceptible parent OSU 252.146 and resistant parent OSU 414.062

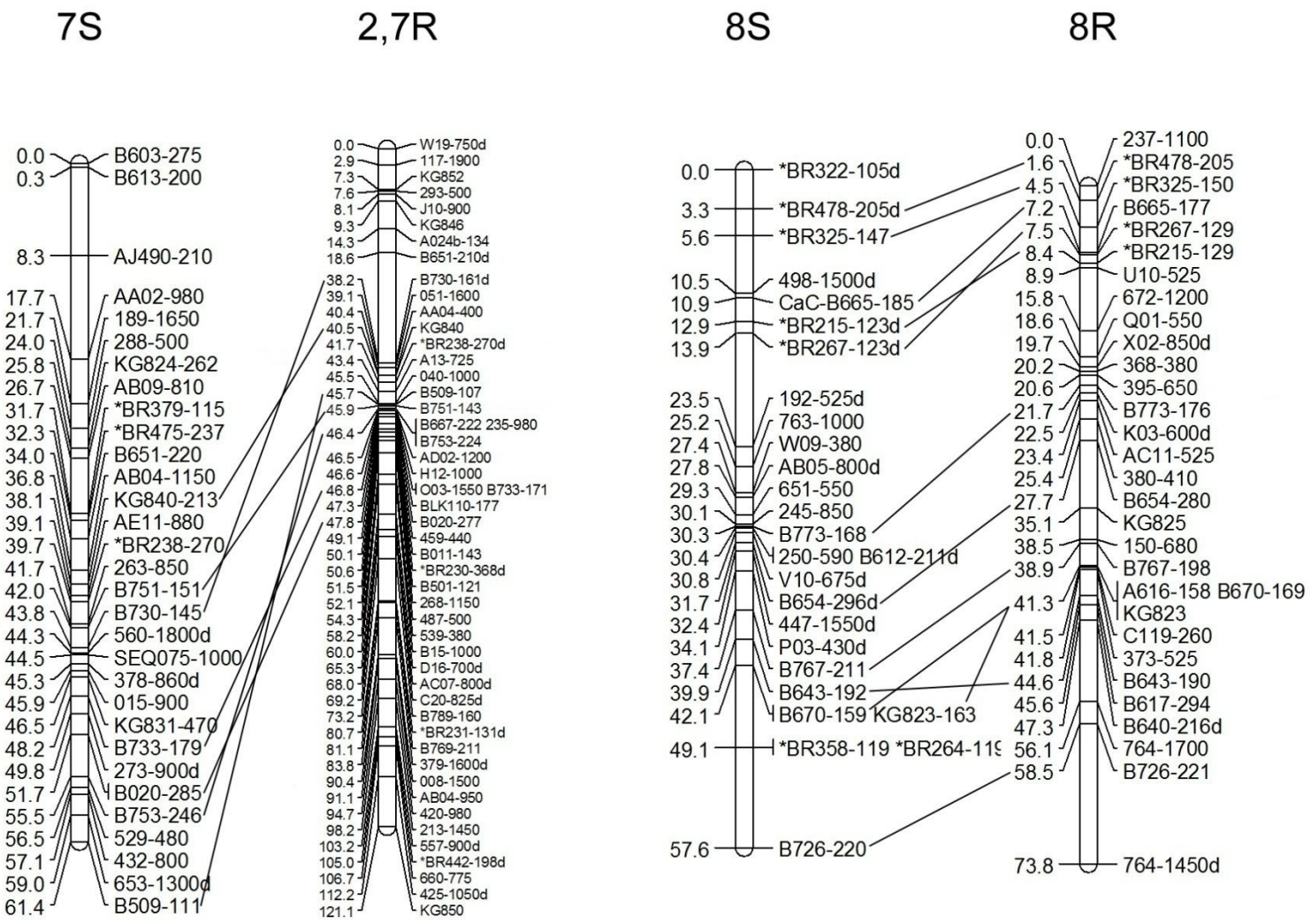

Figure 2.7 Linkage groups 7 & 8 of susceptible parent OSU 252.146 and resistant parent OSU 414.062

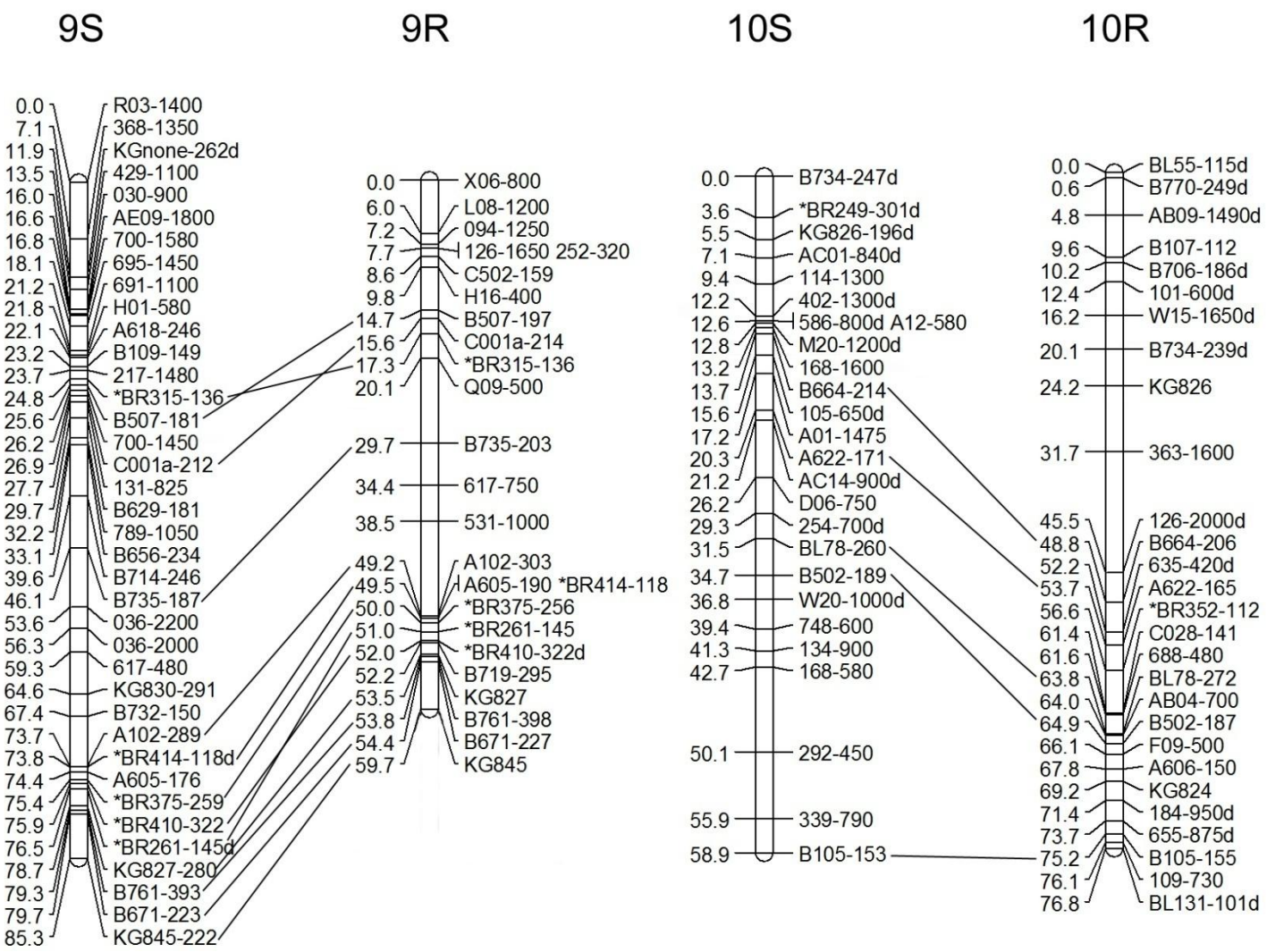

Figure 2.8 Linkage groups 9 & 10 of susceptible parent OSU 252.146 and resistant parent OSU 414.062

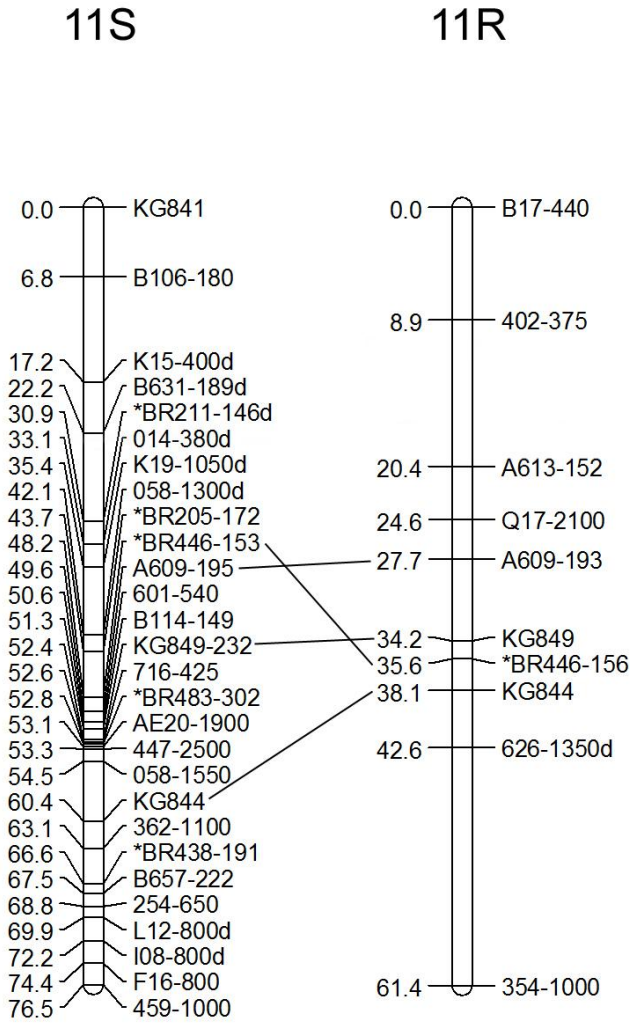

Figure 2.9 Linkage groups 11 of susceptible parent OSU 252.146 and resistant parent OSU 414.062

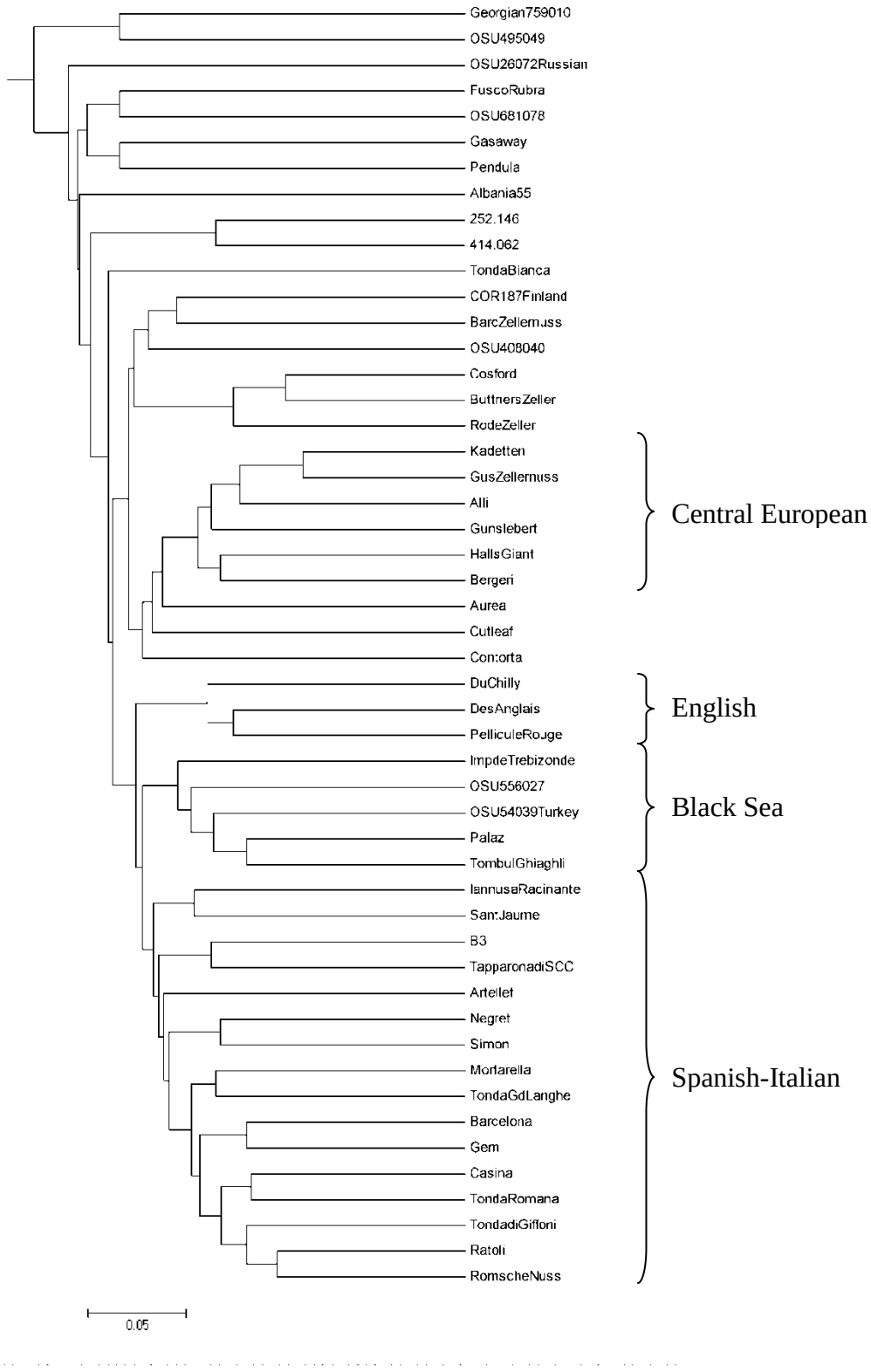

Figure 2.10 UPGMA dendrogram of 50 accessions fingerprinted with 113 microsatellite loci

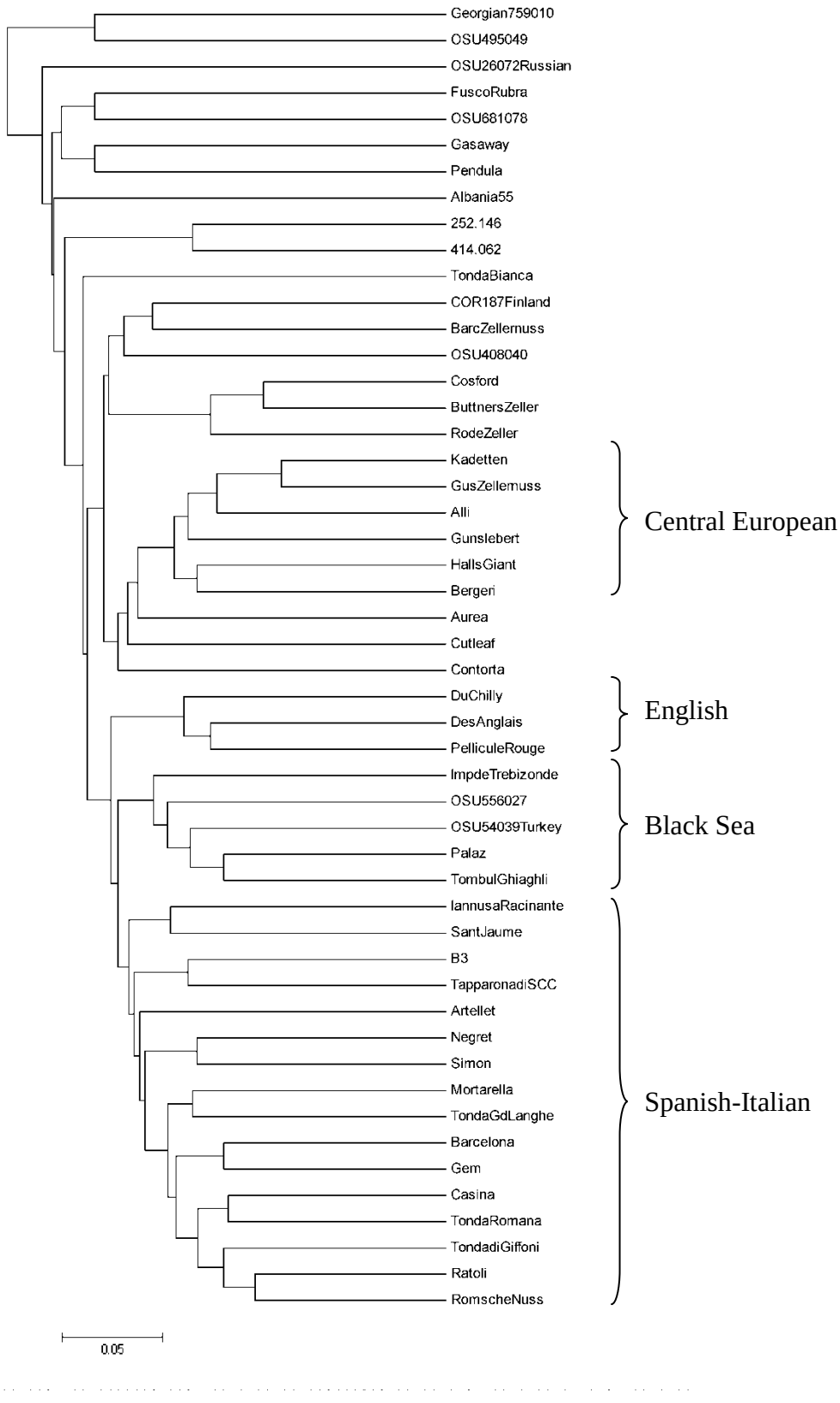

Figure 2.10 (cont.) NJ dendrogram of 50 accessions fingerprinted with 113 microsatellite loci

Chapter 3

NOVEL SOURCES OF EASTERN FILBERT BLIGHT RESISTANCE IN HAZELNUT ACCESSIONS ‘CULPLA,’ ‘CRVENJE,’ AND OSU 495.072

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Abstract

European hazelnut (*Corylus avellana* L.) is a significant crop in Oregon, where 99% of US hazelnuts are produced. Eastern filbert blight (EFB) caused by *Anisogramma anomala* (Peck) E. Müller is a significant disease that infects the trees, reduces yield, and causes premature death. Managing the disease through cultural methods and fungicide applications is laborious and expensive, and genetic host resistance is considered the most viable option for controlling EFB in hazelnuts. Genetic resistance from 'Gasaway' has been successfully introgressed into breeding lines, and resistant cultivars developed. This study investigated the resistance sources 'Culpla,' 'Crvenje,' and OSU 495.072, comparing the resistance with 'Gasaway,' observing segregation of resistance in progeny and mapping the resistance loci. RAPD markers linked to resistance from 'Gasaway' were absent in all three accessions, indicating that the resistance is different. In progeny populations, segregation did not exactly fit a 1:1 ratio for all progenies as expected for single, dominant resistance genes, suggesting that chromosomal abnormalities during meiosis may be affecting the segregation of chromatids. Microsatellite marker A614, previously mapped to linkage group (LG) 6, co-segregated with resistance in progeny of 'Culpla,' 'Crvenje,' and OSU 495.072, allowing the resistance loci to be assigned to LG 6. Maps were constructed for each resistant parent with microsatellite markers from LG 6, and each resistance locus was mapped to the same region, which indicates that these three resistance genes may be the same gene or different resistance genes that are located in the same region. Markers LG628, LG610, and LG696 will be very useful for breeding to introgress resistance from 'Culpla,' 'Crvenje,' and OSU 495.072.

Introduction

European hazelnut (*Corylus avellana* L.) is an important crop in Oregon's Willamette Valley, where the trees thrive in the mild, Mediterranean climate. The value of hazelnuts produced in Oregon ranks second to blueberries of all fruit and nut production in Oregon and is the 12th most valuable commodity produced in the state (Oregon Department of Agriculture, 2012). Ninety-nine percent of all commercial hazelnut production in the US is in Oregon (Mehlenbacher and Olsen, 1997). The trees are valued for their high quality kernels that have excellent flavor, high oil content, and are ideally suited for use in confections and chocolates (USDA, 2002). Hazelnuts also contain anti-cancer properties and reduce the risk of coronary heart disease in consumers (Richardson, 1997). Hazelnuts have been grown commercially in Oregon since the early 1900's, and the acreage in hazelnut production has grown through the decades to over 29,000 currently (Oregon Department of Agriculture, 2012).

The pathogen *Anisogramma anomala* (Peck) E. Müller causes the disease eastern filbert blight (EFB) on susceptible trees of *C. avellana*. This fungus is endemic to the eastern United States and infects only *Corylus* species. Many native *C. americana* selections are resistant to, or tolerant of, the pathogen (Capik and Molnar, 2012a), but infections are much more severe on European hazelnut (Pinkerton et al., 1993). The lifecycle of *A. anomala* is well documented (Gottwald and Cameron, 1979; Johnson et al., 1996; Pinkerton et al., 1992, 1998a, 1998b; Stone et al., 1992). The fungus has a two-year life cycle, which begins with ascospore release from perithecia in the early fall to late spring during prolonged wet conditions. The spores are disseminated by wind or water, and host tissue is susceptible to infection during spring and early summer. The disease is difficult to prevent, and control measures involve scouting and pruning out

diseased limbs and multiple, expensive fungicide applications during the spring. Infection results in stem cankers, girdling and death of branches, reduced yield, and premature tree death.

Genetic host resistance has been used in many crops to impede disease development. Scab resistance genes have been used in apple (Kellerhals et al., 2009). In lettuce, more than 25 genes conferring resistance to 7 different diseases, including downy mildew (*Bremia lactucae*), lettuce mosaic virus (*Potyvirus* sp.), root aphid (*Pemphigus bursarius*), and others, have been identified and used in breeding (McHale et al., 2009). By developing cultivars resistant to disease, there is significant economic savings to the growers in terms of reduced pesticide costs and the potential for increased yield. Because of the cost and difficulty of managing the spread of *A. anomala*, genetic host resistance is considered the most viable option for managing EFB in hazelnuts (Mehlenbacher, 1994).

Selections of *C. avellana* have been found which exhibit qualitative and quantitative resistance to EFB (Mehlenbacher et al., 1991; Chen et al., 2007; Sathuvalli et al., 2010; Capik and Molnar 2012). The cultivar ‘Gasaway’ was the first *C. avellana* selection observed to express complete resistance to EFB (Cameron, 1976), and this selection was found to have a single, dominant gene conferring resistance (Mehlenbacher et al., 1991). The hazelnut breeding program has been successful in introgressing the ‘Gasaway’ resistance gene into several cultivars that have been released for commercial production (Mehlenbacher et al., 2007, 2009, 2011, 2012). Additional accessions with dominant gene resistance have been identified and are of interest for use in the breeding program (Lunde et al., 2006; Sathuvalli et al., 2011, 2012).

Genetic host resistance from ‘Gasaway’ has been a tremendous benefit to growers in Oregon and Washington, and cultivars with resistance conferred by the ‘Gasaway’ gene has been a popular solution among growers for managing eastern filbert blight, since these cultivars do not require the intense management practices that susceptible cultivars require. However resistance genes are vulnerable to “breakdown” over time as new isolates of the pathogen arise or are introduced (McDonald and Linde, 2002). Accessions expressing complete resistance in Oregon have been screened for resistance in New Jersey, where *A. anomala* is native and disease pressure is higher, and EFB cankers and stromata were observed on some trees, including ‘Gasaway’ (Molnar et al., 2010; Capik and Molnar, 2012). In Oregon, small cankers have been observed on ‘Jefferson’ and some seedlings containing the ‘Gasaway’ gene (S.A. Mehlenbacher, pers. comm.), indicating that some selections containing the ‘Gasaway’ gene occasionally develop cankers under high disease pressure.

In light of the potential breakdown of resistance to EFB, it is desirable to identify and introgress new, diverse sources of disease resistance. Ideally, multiple resistance genes could be combined in a single selection. This concept of “pyramiding” disease resistance is intended to reduce the likelihood of mutation in the pathogen enabling it to overcome resistance mechanisms. Pyramiding of major resistance genes has been used successfully in several crops, including scab resistance in apple (Kellerhals et al., 2009) and bacterial blight resistance in rice (Huang et al., 1997). In barley, each quantitative trait locus (QTL), which confers a degree of disease resistance, has been pyramided in a single isogenic line with additive amounts of disease resistance from each QTL (Richardson et al., 2006).

To increase the speed and efficiency of introgressing single, dominant resistance genes in hazelnut, DNA markers are very useful. Markers allow the breeder to screen selections for presence/absence of genes of interest, cull unwanted individuals, and bypass the 16 month latent period of *A. anomala* to show disease symptoms. Many types of markers have been investigated in hazelnut, including RAPD, AFLP, and microsatellite (SSR) markers (Davis and Mehlenbacher, 1997; Mehlenbacher et al., 2004; Chen et al., 2005; Sathuvalli et al., 2011, 2012), and RAPD and SSR markers have been used in marker-assisted selection (MAS) (Davis and Mehlenbacher, 1997; Mehlenbacher et al, 2004; Sathuvalli et al, 2012).

This study investigated EFB resistance from these new sources: 'Culpla,' 'Crvenje,' and OSU 495.072. In addition to studying the inheritance of the resistance, we identified DNA markers linked to the resistance, and mapped the location of the resistance genes.

Materials and Methods

Plant Materials

The three hazelnut accessions used in this study are 'Culpla,' 'Crvenje,' and OSU 495.072. 'Culpla' is a Spanish cultivar received as scions from IRTA Mas Bove. It is believed to have originated in Tarragona, and molecular studies have shown it to be closely related to the cultivars 'Tonda Gentile della Langhe' from Piemonte, Italy and 'Sant Pere' from Spain (Boccacci and Botta, 2010). 'Culpla' was screened for resistance to eastern filbert blight by Chen et al. (2007), and it showed no signs of infection following greenhouse inoculation. In 2005, 'Culpla' was crossed with susceptible parents

OSU 675.028 and OSU 612.015 (Table 3.1, Figure 3.1), generating 117 and 92 seedlings, respectively.

The second accession used in this study is ‘Crvenje.’ It was received as scions from the Fruit Research Institute in Čačak, Serbia. ‘Crvenje’ was screened for resistance to EFB in greenhouse inoculations and found to be completely resistant (Sathuvalli et al., 2010). In 2006, ‘Crvenje’ was crossed with susceptible parents OSU 675.028 and OSU 679.114 (Table 3.1, Figure 3.1), generating 239 and 224 seedlings, respectively.

The third EFB resistant accession studied is OSU 495.072. This tree was selected from a group of seedlings grown from seeds sent in 1989 from the N.I. All-Russian Scientific Research Institute of Plant Industry in St. Petersburg, Russia. It is believed that the seeds were collected from southern Russia (Mehlenbacher, pers. comm.). OSU 495.072 has been screened for resistance to EFB in Oregon and New Jersey and has remained free of disease symptoms in both environments (Gökirmak et al., 2008; Molnar et al., 2010; Sathuvalli et al., 2010; Capik and Molnar, 2012). In 2001 and 2002, OSU 495.072 was crossed with susceptible parent OSU 713.068 and OSU 651.011 (Table 3.1, Figure 3.1), generating 112 and 14 seedlings, respectively. In 2009, two resistant selections from the first set of crosses were used as parents to generate four additional seedling populations (Fig 3.1). OSU 1136.051 is a seedling from a cross of OSU 713.068 x OSU 495.072, and OSU 1154.027 is from a cross of OSU 495.072 x OSU 651.011.

Structure Inoculations

Potted trees of progenies 05023, 05024, 06027, 06028, 09029, 09030, 09031, and 09032 were placed under the inoculation structure at the OSU Smith Horticulture

Research Farm from March to June during the years 2007, 2008, and 2011. Potted trees of susceptible varieties ‘Ennis’ and ‘Tonda di Giffoni’ were placed under the inoculation structure with the seedlings as positive controls. The structure for inoculation was composed of a wood frame supporting wire mesh platforms held over the trees, as described by Pinkerton et al. (1993). Hazelnut wood bearing cankers with stromata from *A. anomala* was collected from the OSU Smith Horticulture Research Farm and placed on the mesh above the plants. After trees from the progenies listed were exposed under the structure, they were transplanted to nursery rows.

Structure inoculated trees were scored for disease phenotype 18 months after exposure. Seedlings were observed for the presence of cankers and stromata. Disease severity was scored on a scale of 0 to 5, with 0 being absence of disease symptoms and 5 being very severe disease symptoms. For clonal selections, approximately 12 potted trees of each were exposed, and total numbers of cankers per tree were recorded along with the length of each canker, the sum of canker length per tree, the sum of canker length per selection, and the square root of the sum canker length per selection.

Greenhouse Inoculations

Scions were collected from the seedling populations in the winters of 2008, 2010, and 2011. Scions were stored at -1°C until they were grafted in April and May. Three replicates of each seedling were grafted onto rooted layers, potted in 5L pots containing a potting medium composed of equal volumes fine bark, pumice, and peat and 9g of Sierra 3-4 month release fertilizer. The trees were grown in the greenhouse under 24°C days and 18°C nights until the plants were growing vigorously and had developed 4 to 5 nodes.

The inoculation chamber was constructed of a frame of polyvinyl chloride tubing (1.27cm diameter) and covered with 4mm polythene sheeting on the walls, with the roof remaining open. The chamber spanned three greenhouse benches each measuring 2.44m x 0.88m. A misting system was placed above the chamber. Three misters per bench (7.57L/hr) spaced 0.3m apart were mounted 0.9m above each bench top. The misters were programmed with an automated misting unit (Model No. DE 8 PR2; Davis Engineering, Canoga Park, CA) to run for 10 seconds every 30 minutes during the daytime hours (8:00 to 19:00) and 10 seconds every hour during the night (19:00 to 8:00).

Diseased wood with cankers bearing mature stromata were collected from the OSU Smith Horticulture Research Farm in December annually and stored in polyethylene bags at -20°C until the following summer when they were used for greenhouse inoculations. Stored inoculum was removed from the freezer and thawed at room temperature under high humidity. Perithecia containing ascospores were removed from the shoots using a spatula and ground in a mortar with a small amount of water to release the spores. Water was added to bring the spore suspension to 100 ml, the concentration was measured using a hemacytometer (Fisher Scientific, Hampton, NH), and the suspension diluted to a concentration of 1×10^6 spores/ml. Inoculations were performed once per day either in early morning (7:00) or late evening (20:00) at three day intervals for a total of two inoculations. The inoculations were conducted during the cool hours of the day to increase the success rate of the inoculation and reduce escapes. A spray bottle was used to apply the spore suspension to the one or two most vigorous shoot tips on each tree. Each shoot tip was sprayed until it was wet. Three days following the second inoculation, the trees were removed from the inoculation chamber and placed on benches

under the greenhouse conditions described previously. Seedlings were inoculated in the greenhouse at Oregon State University (OSU) in 2008, 2010, and 2011. Susceptible varieties ‘Ennis’ and ‘Tonda di Giffoni’ were included in the inoculation as positive checks. Trees were allowed to remain in the greenhouse for 3-6 months before being transplanted to a field at the OSU Smith Horticultural Research Farm in Corvallis, OR.

Greenhouse inoculated trees were scored for presence or absence of disease between October and November, approximately 14-16 months after inoculation. Genotypes exhibiting cankers and stromata were scored as susceptible, and genotypes were scored as resistant if all three replications of the genotype were free from infection. If at least one replicate of a genotype exhibited disease symptoms, the genotype was scored as susceptible.

Data Analysis

The observed segregation ratios of susceptible:resistant were compared with expected segregation ratios and a chi-square goodness-of-fit test (Tables 3.2, 3.3, and 3.4). A test of heterogeneity was also performed with progenies sharing the same resistance source, to determine if the data from the progenies could be pooled for analysis.

DNA Extraction

DNA was extracted from parents and seedlings inoculated in the greenhouse and from some seedlings inoculated under the structure (Table 3.2). Whole-genome DNA was extracted from all 3 resistant parents, the 4 susceptible parents, 112 seedlings from progeny 01035, 81 seedlings of 05023, 117 seedlings from cross 05024, 157 seedlings of

progeny 06027, and 68 seedlings of progeny 06028 (Table 3.2). DNA was extracted from fresh, young leaves collected during the spring. DNA extraction followed the protocol described in Lunde et al. (2000) with slight modifications and no RNAase treatment. DNA was quantified by a BioTek Synergy2 microplate reader paired with Gen5 data analysis software (BioTek Instruments, Winooski, VT) and diluted with Tris-EDTA buffer to a concentration of 20 ng/ml. DNA was not extracted from progenies 09029, 09030, 09031, and 09032 but is planned for spring 2013.

Screening with DNA Markers

The resistant parents 'Culpla,' 'Crvenje,' and OSU 495.072 were screened using polymerase chain reaction (PCR) and the RAPD primers UBC268 and UBC152 which genetic markers linked to 'Gasaway' resistance. PCR was performed as described by Mehlenbacher et al. (2004). PCR products were separated by capillary gel electrophoresis on 2% agarose gels, stained with ethidium bromide (Sigma-Aldrich Co., St. Louis, MO) and imaged under UV light using a BioDoc-It® Imaging System (UVP, Upland, CA). Presence of a band of 580 base pairs or 800 base pairs for markers $UBC268_{580}$ and $UBS152_{800}$, respectively, indicates presence of the 'Gasaway' gene.

Sets of 32 seedlings plus the two parents of progenies 01035, 05024, and 06027 were screened with microsatellite markers. A set of 24 microsatellite markers previously mapped was selected to screen for correlation with resistance (Mehlenbacher et al., 2006). The selected markers were distributed across the genome, with markers representing each linkage group. PCR was performed with each of the 24 primers. The PCR mix was a total of 10 µl per reaction and contained 0.3 µM each of the fluorescent-

labeled forward and non-fluorescent reverse primer, 1x Biolase NH_4 reaction buffer, 2 mM $MgCl_2$, 200 µM each of dATP, dTTP, dGTP, and dCTP, 20 ng template DNA, and 0.25 units of Biolase DNA polymerase (Bioline USA Inc., Boston, MA). Ninety-six reactions were run simultaneously on GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) and MyCycler (Bio-Rad, Hercules, CA) thermal cyclers. The PCR program was: denaturation at 94 °C for 5 minutes followed by 40 cycles of 94 °C for 40 seconds, 60 °C for 40 seconds, 72 °C for 40 seconds, followed by 72 °C for 7 minutes of extension and ending with an infinite hold at 4 °C. PCR products from each reaction were multiplexed, with six to twelve different primer products in each multiplex set. Two µl of each product were combined in 150µl water, and a 1µl aliquot of the mixture was submitted to the CGRB Core Lab facility at Oregon State University for genotyping with capillary electrophoresis using an ABI 3730 DNA Analyzer (Life Technologies, Carlsbad, CA). The fragment sizes were estimated with AB Gene Mapper® software (Life Technologies, Carlsbad, CA). The length of the amplified fragments was recorded for each primer pair for each genotype in the set.

Genotyping data was entered into a spreadsheet with disease response data for each of the three populations screened. Disease response was scored as 1 if the individual was resistant and 0 if the individual was susceptible. Markers were scored for each seedling for the presence (1) or absence (0) of that allele. A correlation coefficient was calculated in a spreadsheet for each marker and the corresponding disease response data. Coefficients with absolute values greater than 0.5 indicated the linkage group of the resistance gene and were investigated further.

Mapping Resistance Loci

When correlation coefficients indicated the linkage group, microsatellite markers previously mapped to that linkage group were used to amplify the parents of the seedling populations. For those markers that segregated in the progeny, 96 individuals of progenies 01035, 05024, and 06027 and 48 individuals of progenies 05024 and 06028 were genotyped as described above. Marker data and resistance data was scored as 'h' for resistance/allele present and 'a' for susceptibility/allele absent. Data was analyzed using Join Map 4.0 (Van Ooijen and Voorips, 2006). Using the BC1 function, maps were constructed from microsatellite markers located within 20 cM of the resistance and map distances shown in centimorgans (cM).

Results

In the greenhouse inoculation studies, resistant parents 'Culpla,' 'Crvenje,' and OSU 495.072 consistently showed no disease symptoms, while the susceptible parents OSU 675.028, OSU 612.015, and OSU 679.114 developed cankers. OSU 713.068 did not show disease symptoms when it was inoculated in 2005, but that year's inoculation was weak, and this accession is presumed to be susceptible (Mehlenbacher, pers. comm.). Each of the resistant parents 'Culpla,' 'Crvenje,' and OSU 495.072 were screened with RAPD markers $UBC268_{580}$ and $UBC152_{800}$ linked to 'Gawaway' resistance. The RAPD markers were absent for each of the resistant parents and present in each of the positive checks 'Gasaway,' 'Jefferson,' and OSU 414.062 containing the 'Gasaway' resistance genes (Figure 3.2). These results indicate that the resistance in 'Culpla,' 'Crvenje,' and OSU 495.072 is different than 'Gasaway.'

Disease response scores and segregation ratios were calculated for the nine progenies. Progeny 05024 from 'Culpla' segregates 1:1 as expected (Table 3.3). The

second ‘Culpla’ progeny 05023 does not fit a 1:1 segregation ratio and has a surplus of resistant seedlings. This progeny fits a 3:1 segregation ratio. The ‘Crvenje’ progenies 06027 and 06028 likewise do not fit the expected 1:1 segregation ratio (Table 3.4). Both of these populations have an excess of susceptible seedlings. The progeny 01035 from OSU 495.072 has more resistant seedlings than susceptible (Table 3.5), and it does not fit either a 1:1 or 3:1 segregation ratio. Of the four additional progenies containing resistance from OSU 495.072, three exhibit a 1:1 segregation, but progeny 09029 fits a 3:1 segregation.

Microsatellite markers correlated ($|r|>0.5$) with resistance were found for ‘Culpla,’ ‘Crvenje,’ and OSU 495.072. Marker A614 was found to correlate with resistance from all three resistant parents. This marker had correlation coefficients of 0.62 for ‘Culpla,’ 0.71 for ‘Crvenje,’ and 0.63 for OSU 495.072. A614 had previously been mapped to linkage group 6 (LG6) (Gürcan et al., 2010), indicating that all three resistance loci were on LG6.

Ninety-six seedlings of progenies 01035, 05024, and 06027 and 48 seedlings of progenies 05023 and 06028 were fingerprinted with segregating microsatellite markers from LG6, giving 5 maps. There are 16 additional seedlings with DNA extracted from progeny 01035, 21 additional seedlings with DNA of progeny 05024, and 61 additional seedlings with DNA of progeny 06027 that have disease phenotype scores but were not fingerprinted with LG6 microsatellite markers. Marker and disease phenotype segregation for the fingerprinted genotypes were fairly consistent, with some exceptions (Appendix G). In the ‘Culpla’ progeny 05024, 2 individuals scored as resistant had SSR markers indicating susceptibility, and 1 individual scored as susceptible showed DNA

marker data consistent with resistant phenotypes. The second ‘Culpla’ progeny 05023 similarly had 2 resistant trees and 1 susceptible tree that conflicted with marker data.

The first ‘Crvenje’ progeny 06027 had 4 resistant and 4 susceptible individuals conflicting with data from all markers they were screened with. The second ‘Crvenje’ progeny 06028 had 1 susceptible individual conflicting with DNA marker data. The seedling population of OSU 495.072 had 1 resistant and 2 susceptible seedlings conflicting with all markers they were screened with.

Resistance loci were mapped on LG6 for ‘Culpla,’ ‘Crvenje,’ and OSU 495.072. Linkage maps were constructed (for the three populations) of 96 individuals in each of three progenies and aligned with the existing linkage map constructed in a population segregating for resistance from ‘Gasaway.’ Several markers are closely linked with each resistance locus. The ‘Culpla’ resistance locus in both populations is closely linked to several markers, including LG628, LG637, LG639, LG682, LG687, LG688, and LG696. In the ‘Crvenje’ populations, markers LG628, LG639, LG648, LG675, and LG696 are linked to resistance. SSR markers LG648, LG 668, LG687, and LG696 are closely linked to OSU 495.072 resistance.

Discussion

Absence of RAPD markers indicates that these three resistance sources do not carry the ‘Gasaway’ gene, indicating that the resistance source is novel (Figure 3.2). The progenies of ‘Culpla,’ ‘Crvenje,’ and OSU 495.072 inherited varying proportions of complete resistance. If the resistant parent is heterozygous at a single locus, a 1:1 segregation (resistant:susceptible) is expected in the progeny. ‘Culpla’ progeny 05024 is the only one of the five progenies to show the expected 1:1 segregation (Table 3.3). The

second ‘Culpla’ progeny 05023 does not fit the 1:1 ratio, but instead has exactly a 3:1 ratio of resistant to susceptible seedlings. The ‘Crvenje’ seedling populations 06026 and 06028 show a different trend (Table 3.4). They also do not fit the 1:1 model, however these populations have an excess of susceptible individuals, about 60% susceptible and 40% resistant. OSU 495.072 progeny 01035 does not fit the 1:1 ratio and has approximately 60% resistant seedlings and 40% susceptible seedlings (Table 3.5). Of the 4 progenies with resistance from OSU 495.072 two generations back, three exhibit 1:1 segregation, while progeny 09029 fits a 3:1 ratio.

Each of these three parents has exhibited complete resistance in multiple trials in Oregon and in New Jersey (Chen et al., 2007; Gökirmak et al., 2008; Molnar et al., 2010; Sathuvalli et al., 2010; Capik and Molnar, 2012). It seemed reasonable to expect that resistance is controlled by a single, dominant gene as observed in other resistant selections of *C. avellana* (Mehlenbacher et al., 1991; Sathuvalli et al., 2011a, 2011b, 2012.). Differences were observed between the segregation ratios of the two progenies of ‘Culpla,’ and only one fit the expected 1:1 model. The progeny of the other resistant populations show skewed proportions of resistant and susceptible seedlings. It is suspected that chromosomal abnormalities are causing the skewed segregation ratios. Chromosomal abnormalities have been observed (Salesses, 1973; Salesses and Bonnet, 1988); some common cultivars form quadrivalents or trivalents at meiosis. If any of the parents used in this study express heterozygous pairing of linkage group 6 during meiosis, it could explain the differing ratios of disease segregation. If abnormalities such as this occur in the pollen parent, there may be lower pollen fertility (Salesses and Bonnet, 1998), reducing the chance of transmitting the trait to the progeny. However, if

the abnormality occurs in the female parent, female gametes are more tolerant of chromosomal abnormalities, potentially increasing the chance that heterozygous pairing would be transmitted to the offspring. There are 5 progenies (01035, 05023, 06027, 06028, and 09029) that do not exhibit the expected 1:1 ratio of resistant to susceptible, and two of these progenies have resistance contributed by the female parent. It is likely that heterozygous pairing of LG6 during meiosis and/or abnormalities contributed by female gametes are responsible for the skewed segregation.

There were some mismatches between observed disease phenotype and expectation based on marker data. In the cases where seedlings from the inoculation trials were scored as resistant but marker data indicates susceptibility, the likely explanation is that the individuals escaped infection from *A. anomala* by chance but actually do have susceptible phenotypes. The opposite scenario, when inoculated seedlings are scored as susceptible but marker data indicate that the individual should have a resistant phenotype, is more difficult to understand. It is possible for the resistance gene to be present and for the tree to sometimes develop some small cankers (Mehlenbacher, pers. comm.). Another possibility is that the wrong DNA may have been used or DNA collected from the wrong seedling, or human error may have been made in the scoring of phenotypes in the field. This occurrence of a mismatch between phenotypes and marker scores (ie: resistant seedlings with small cankers) has been observed in segregating populations of ‘Zimmerman’ hazelnut (Lunde et al., 2006), and in both that study and this, the number of mismatched disease phenotypes with marker data is small.

The resistance loci of ‘Culpla,’ ‘Crvenje,’ and OSU 495.072 all mapped to the same region of LG6 that the ‘Gasaway’ and OSU 408.040 resistance loci map to, but it is

uncertain if these three resistant parents have different or the same resistance genes. There has been some hypothesizing that resistance genes cluster in the genome. Resistance gene clusters have been observed in lettuce (McHale et al., 2009), perennial ryegrass (Dracatos et al., 2009), and *Arabidopsis* (Field et al., 2011). There have been increasing numbers of plants found to have clusters of genes related to defense mechanisms. Even genes coding for peroxidase, a secondary metabolite involved in broad plant defense, have been observed in clusters in barley (González et al., 2010). The resistance gene clusters are thought to have arisen by duplication, genome organization, and neofunctionalization (Field et al., 2011). In hazelnut, the observed resistance loci mapping to the same region of LG6, if they are all distinct resistance genes, could have arisen by any one of these suggested routes. In order to determine if these three resistance loci are the same gene, map-based cloning paired with real time PCR to monitor gene expression following inoculation or a candidate gene approach to mapping resistance gene analogs would be necessary. Three candidate genes (Contig4_g19, Contig5_g4, and Contig4_g25) have been suggested for EFB resistance from 'Gasaway' (Sathuvalli, 2011), so these could be targeted for further investigation.

Several microsatellite markers were found to be closely linked to the resistance loci in all 5 populations. Markers LG648, LG668, LG687, and LG696 were all located either 1.8 or 2.1 centimorgans from the resistance loci, and all these markers co-segregated almost completely with resistance, with the few mismatches between phenotype and marker data mentioned previously. In the 'Culpla' populations, markers LG628, LG637, and LG682 also mapped within 1.1 cM of the resistance, and these markers co-segregated with resistance. The two 'Crvenje' populations also had some of

the same markers closely linked to resistance: LG648, LG675, and LG696. Each of these markers listed are useful for marker-assisted-selection (MAS). To date, 'Gasaway' has been used extensively as a source of EFB resistance, and several resistant cultivars containing the 'Gasaway' gene have been released. However, with the potential for breakdown of 'Gasaway' resistance, other sources of resistance are desirable. With the aid of the linked markers previously mentioned, resistance from 'Culpla,' 'Crvenje,' and OSU 495.072 can be used to select resistant seedlings quickly, and new cultivars containing these new sources of resistance developed after one or two backcross generations.

An added benefit of identifying markers linked to disease resistance from multiple sources is that it opens up the possibility of pyramiding resistance genes. MAS could be used to combine two resistance genes in the same individual, which would hopefully increase the robustness of resistance to *A. anomala*. This approach has been used successfully in apple (Kellerhalls et al., 2009), barley (Richardson et al., 2006), and rice (Huang et al., 1997). With markers linked to resistance in 'Gasaway' (Mehlenbacher et al., 2004), 'Ratoli' (Sathuvalli et al., 2011a), OSU 759.010 (Sathuvalli et al., 2011b), and OSU 408.040 (Sathuvalli et al., 2012), and now 'Culpla,' 'Crvenje,' and OSU 495.072, there is great potential to pyramid these resistance genes in hazelnut.

'Culpla,' 'Crvenje,' and OSU 495.072 offer promising sources of resistance, and their agronomic traits will also impact how desirable they are as parents in breeding. 'Culpla' has small, round nuts borne in clusters of 3 to 5 with hirtsute, slit husks, and the tree has a small canopy size. 'Crvenje' has small, slightly oblong nuts and long, fringed husks, 1 to 2 nuts per cluster, and a tree with moderate to large canopy size. OSU

495.072 has very small, slightly long and pointed nuts that are borne in clusters of 2 to 4 in long, slit husks, and a tree with moderate canopy size. While these trees have some less desirable traits, such as small nut size, it is feasible to introgress resistance with a modified backcross approach to combine disease resistance in a selection with desirable agronomic traits. This approach has been successful with ‘Gasaway,’ with very small nut size and very low nut yield, and several cultivars containing the ‘Gasaway’ resistance gene have been released (Mehlenbacher et al., 2007, 2009, 2011, 2012). The markers found in this study linked to resistance in ‘Culpla,’ ‘Crvenje,’ and OSU 495.072 will be useful for introgression of the resistance genes from these selections.

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Table 3.1 Progeny of controlled crosses used in study eastern filbert blight resistance.

Progeny	Maternal parent	Paternal parent	No. seedlings	Inoculation Method	
				Structure	Greenhouse
01035	OSU 713.068	OSU 495.072	112	0	112
02020	OSU 495.072	OSU 651.011	14	0	14
05023	OSU 612.015	‘Culpla’	92	32	60
05024	OSU 675.028	‘Culpla’	117	37	80
06027	OSU 675.028	‘Crvenje’	239	123	116
06028	‘Crvenje’	OSU 679.114	224	115	109
09029	OSU 1136.051	OSU 1031.015	60	60	0
09030	OSU 1136.051	OSU 1041.069	69	69	0
09031	OSU 1154.027	OSU 1029.039	52	52	0
09032	OSU 1154.027	OSU 1039.051	23	23	0

Table 3.2 Number of seedlings inoculated and DNA extraction performed of progenies in this study

Progeny	Maternal parent	Paternal parent	No. seedlings with disease scores	No. seedlings with DNA	No. seedlings with both
01035	OSU 713.068	OSU 495.072	112	112	112
05023	OSU 612.015	'Culpla'	92	81	81
05024	OSU 675.028	'Culpla'	117	117	117
06027	OSU 675.028	'Crvenje'	239	157	157
06028	'Crvenje'	OSU 679.114	224	68	68
09029	OSU 1136.051	OSU 1031.015	60	0	0
09030	OSU 1136.051	OSU 1041.069	69	0	0
09031	OSU 1154.027	OSU 1029.039	52	0	0
09032	OSU 1154.027	OSU 1039.051	23	0	0

Table 3.3 Segregation for resistance to eastern filbert blight in progenies of 'Culpla'

Progeny	Parents	Plants (no.)		Expected Ratio	χ^2	
		Resistant	Susceptible		Value	*p*
05024	OSU 675.028 x 'Culpla'	60	57	1:1	0.077	0.782
05023	OSU 612.015 x 'Culpla'	69	23	1:1	23	1.62E-06
05023	OSU 612.015 x 'Culpla'	69	23	3:1	0	1
	Total	129	80	1:1	23.08	0.78

Table 3.4 Segregation for resistance to eastern filbert blight in progenies of ‘Crvenje’

Progeny	Parents	Plants (no.)		Expected Ratio	χ^2	
		Resistant	Susceptible		Value	*p*
06027	OSU 675.028 x 'Crvenje'	103	134	1:1	4.055	0.044
06028	Crvenje' x OSU 679.114	40	60	1:1	4	0.046
Pooled data		143	194	1:1	7.718	0.006
Heterogeneity χ^2 (degrees of freedom = 1)					0.34	0.56

Table 3.5 Segregation for resistance to eastern filbert blight in progenies of OSU 495.072

Progeny	Parents	Plants (no.)		Expected Ratio	χ^2	
		Resistant	Susceptible		Value	*p*
01035	OSU 713.068 x OSU495.072	58	38	1:1	4.17	0.041
09029	OSU 1136.051 x OSU 1031.015	41	19	1:1	8.067	0.005
09029	OSU 1136.051 x OSU 1031.015	41	19	3:1	1.422	0.233
09030	OSU 1136.051 x OSU 1041.069	31	38	1:1	0.710	0.399
09031	OSU 1154.027 x OSU 1029.039	22	30	1:1	1.231	0.267
09032	OSU 1154.027 x OSU 1039.051	13	10	1:1	0.391	0.532
	Total	206	154	1:1	14.57	1.477

Figure 3.1 Pedigrees of progeny segregating for resistance to eastern filbert blight from 'Culpla,' 'Crvenje,' and OSU 495.072.

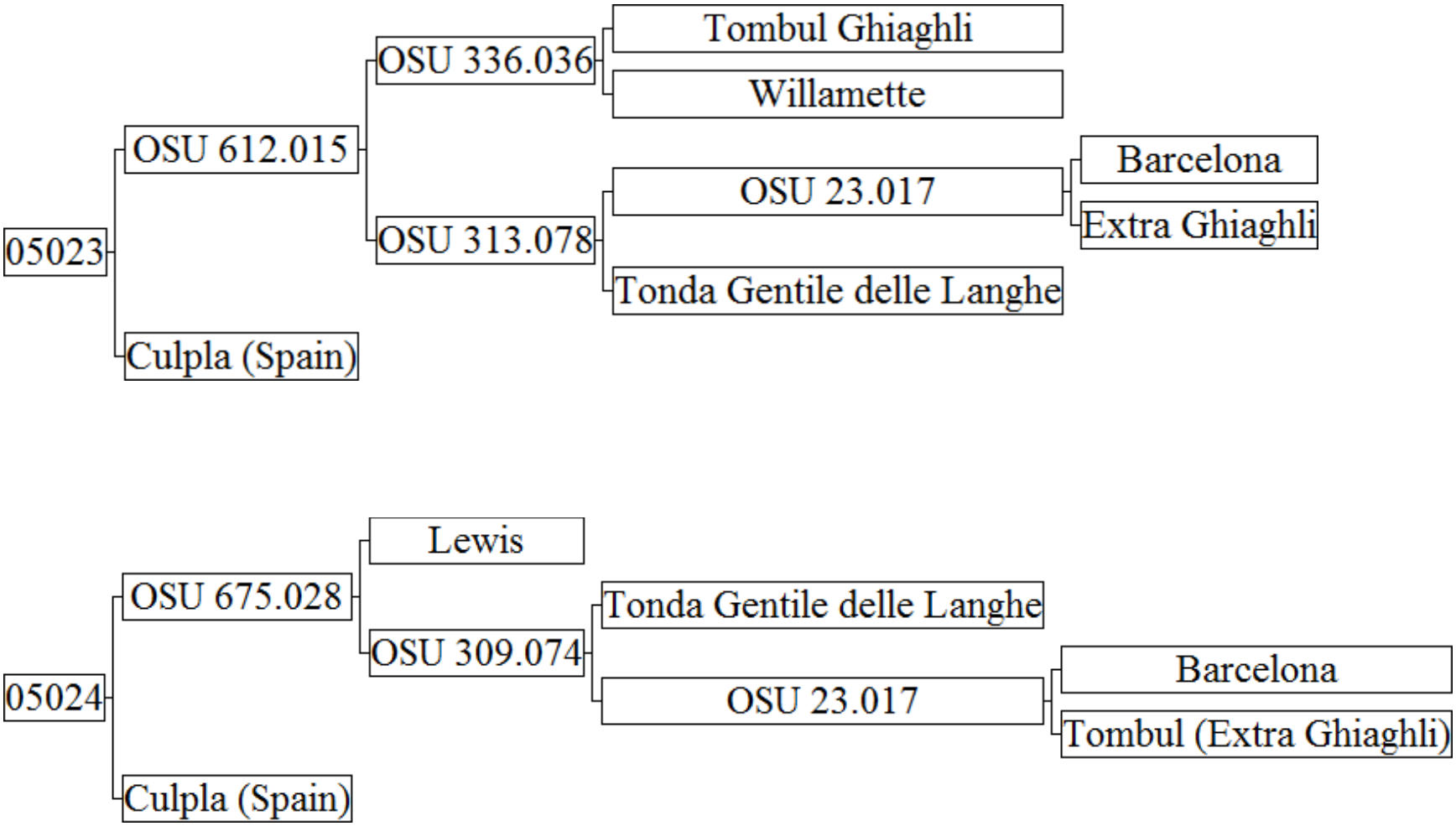


Figure 3.1 (cont.) Pedigrees of progeny segregating for resistance to eastern filbert blight from 'Culpla,' 'Crvenje,' and OSU 495.072.

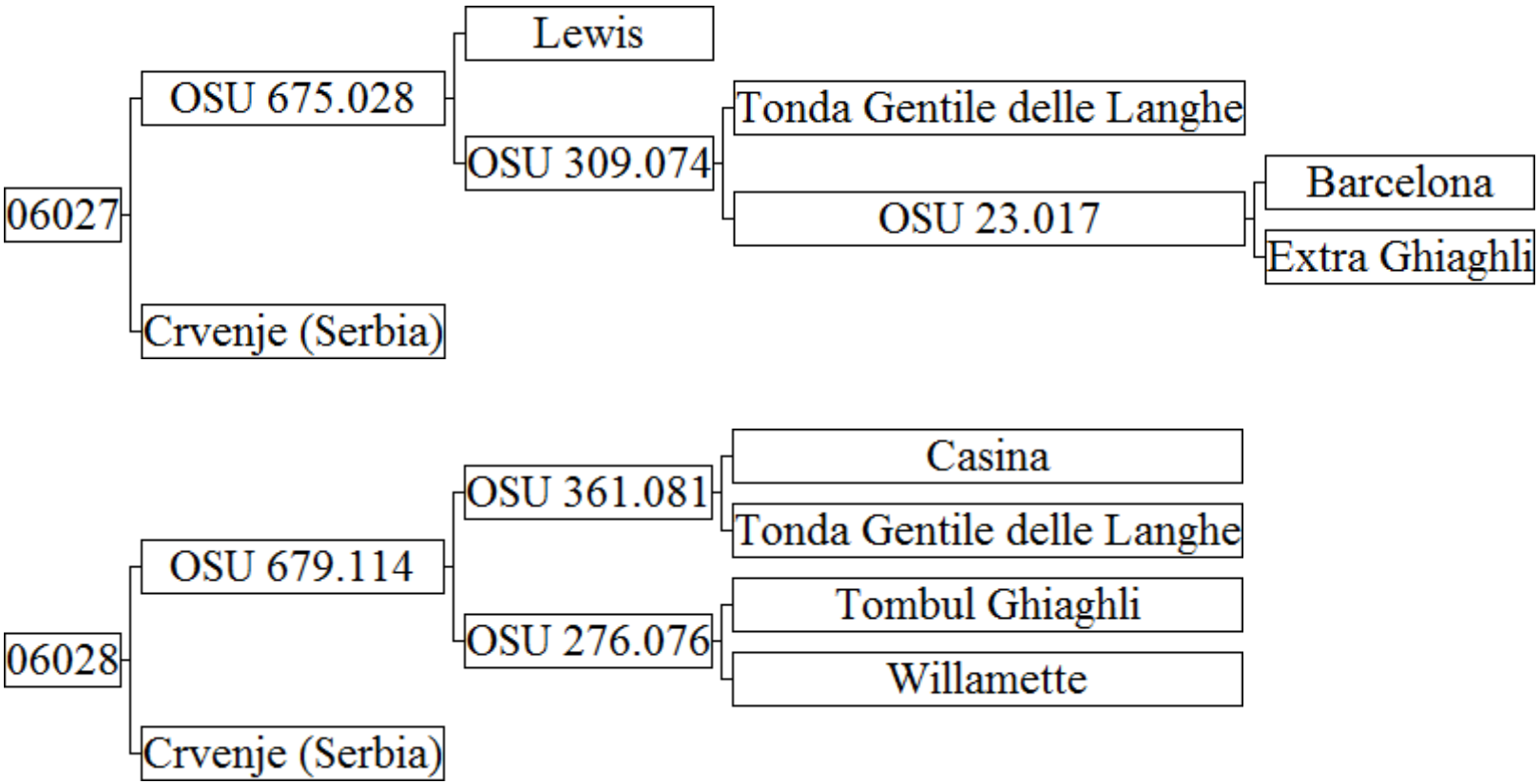


Figure 3.1 (cont.) Pedigrees of progeny segregating for resistance to eastern filbert blight from 'Culpla,' 'Crvenje,' and OSU 495.072.

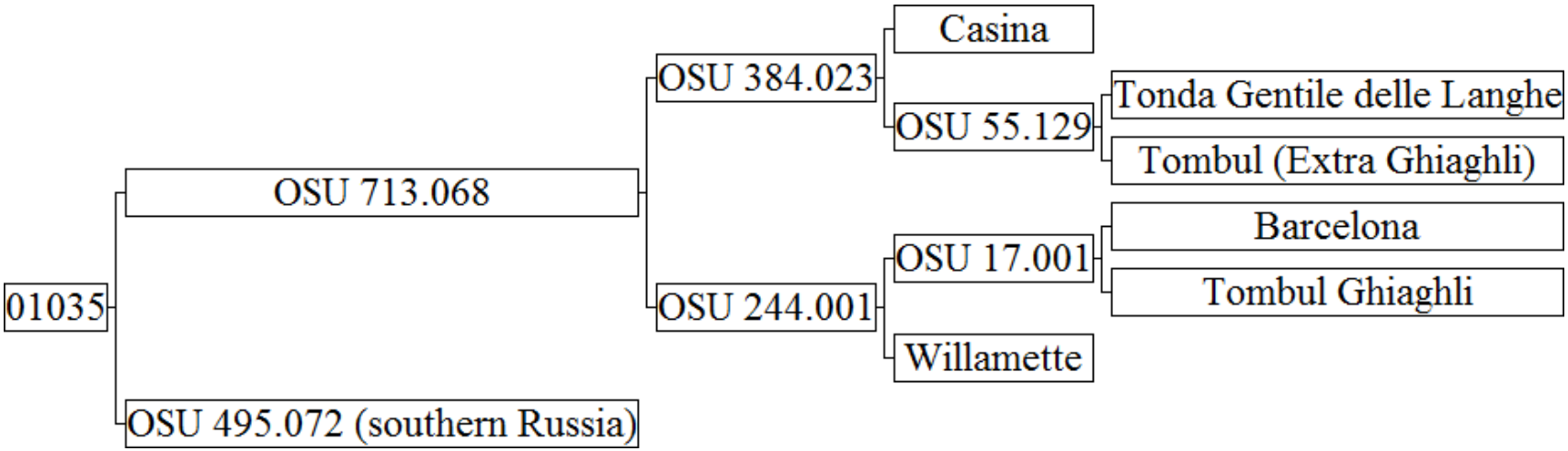


Figure 3.1 (cont.) Pedigrees of progeny segregating for resistance to eastern filbert blight from 'Culpla,' 'Crvenje,' and OSU 495.072.

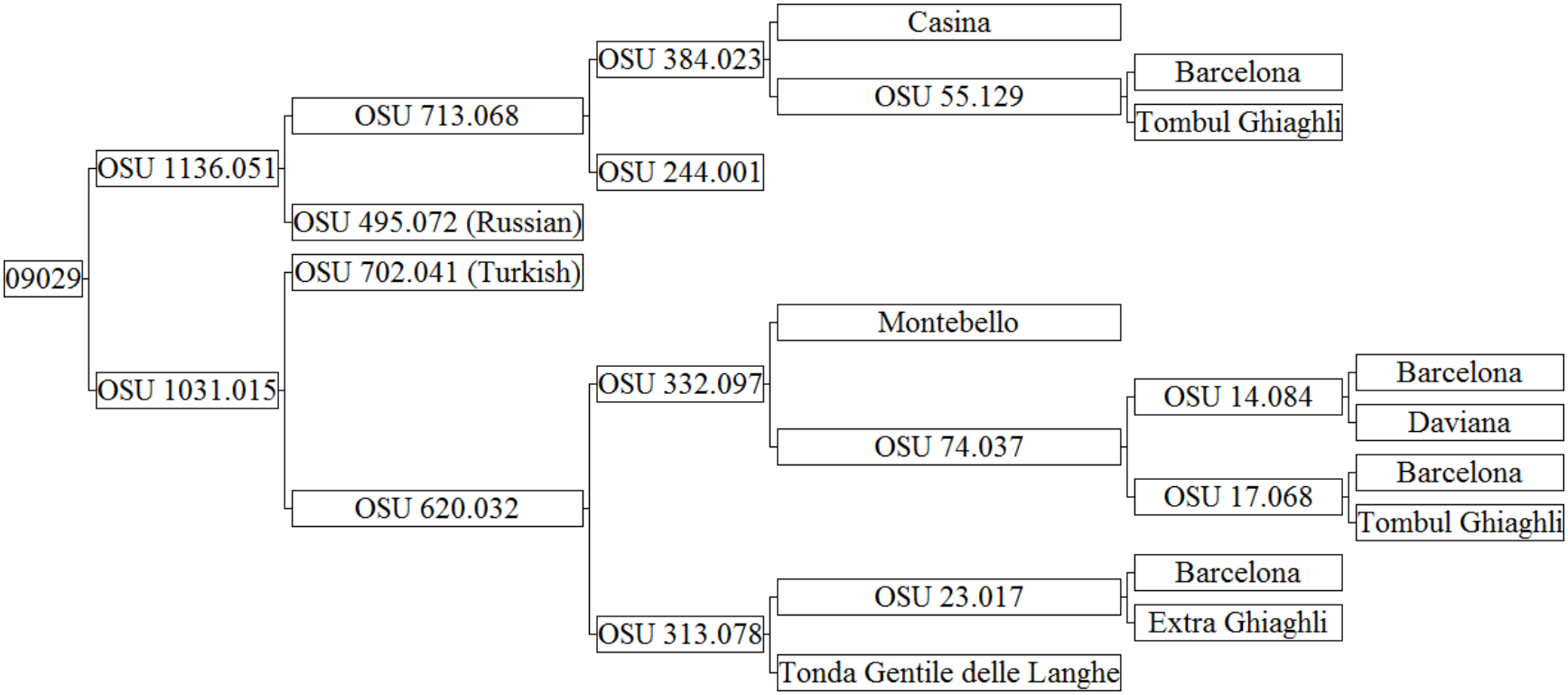


Figure 3.1 (cont.) Pedigrees of progeny segregating for resistance to eastern filbert blight from 'Culpla,' 'Crvenje,' and OSU 495.072.

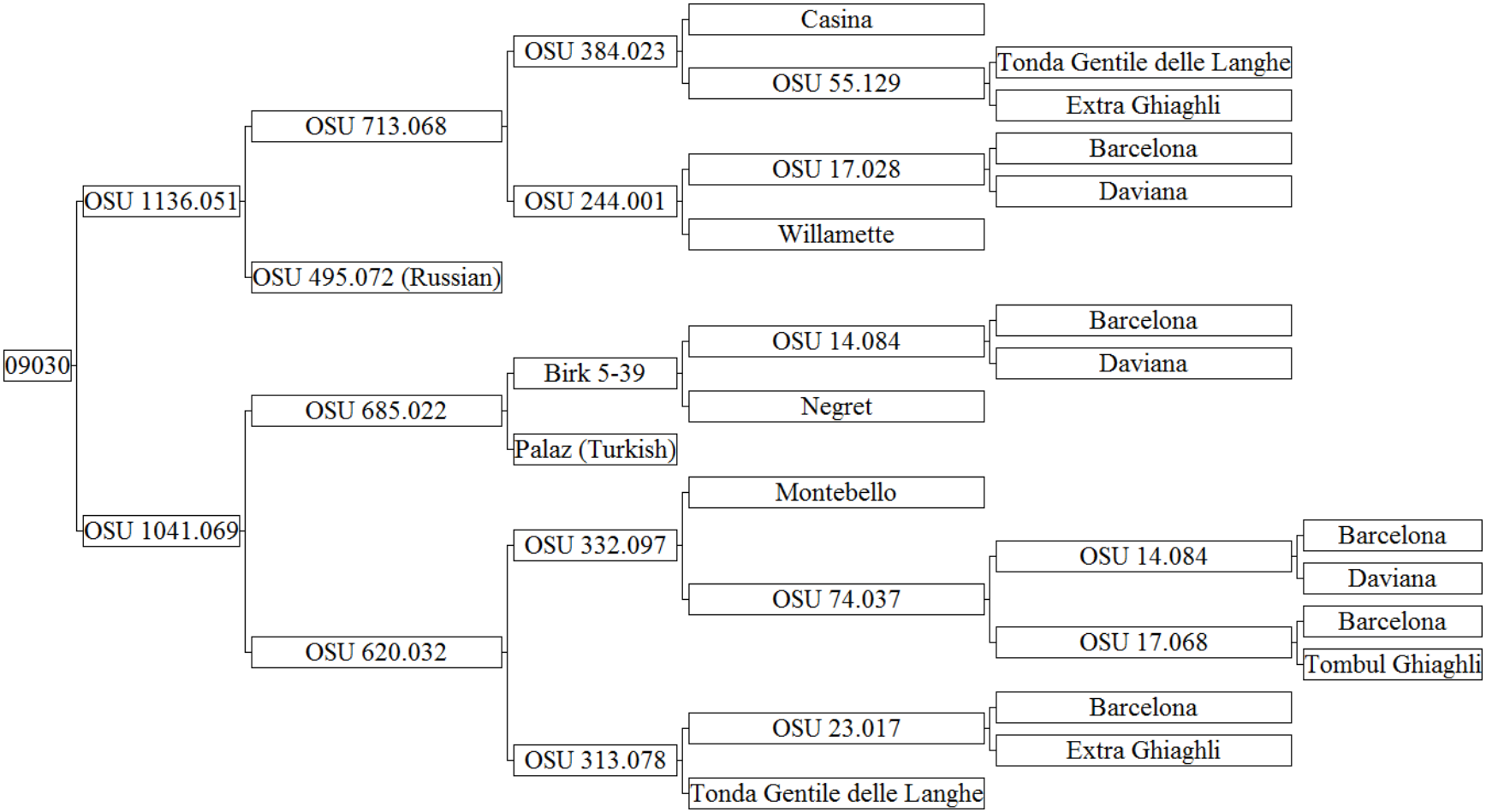


Figure 3.1 (cont.) Pedigrees of progeny segregating for resistance to eastern filbert blight from 'Culpla,' 'Crvenje,' and OSU 495.072.

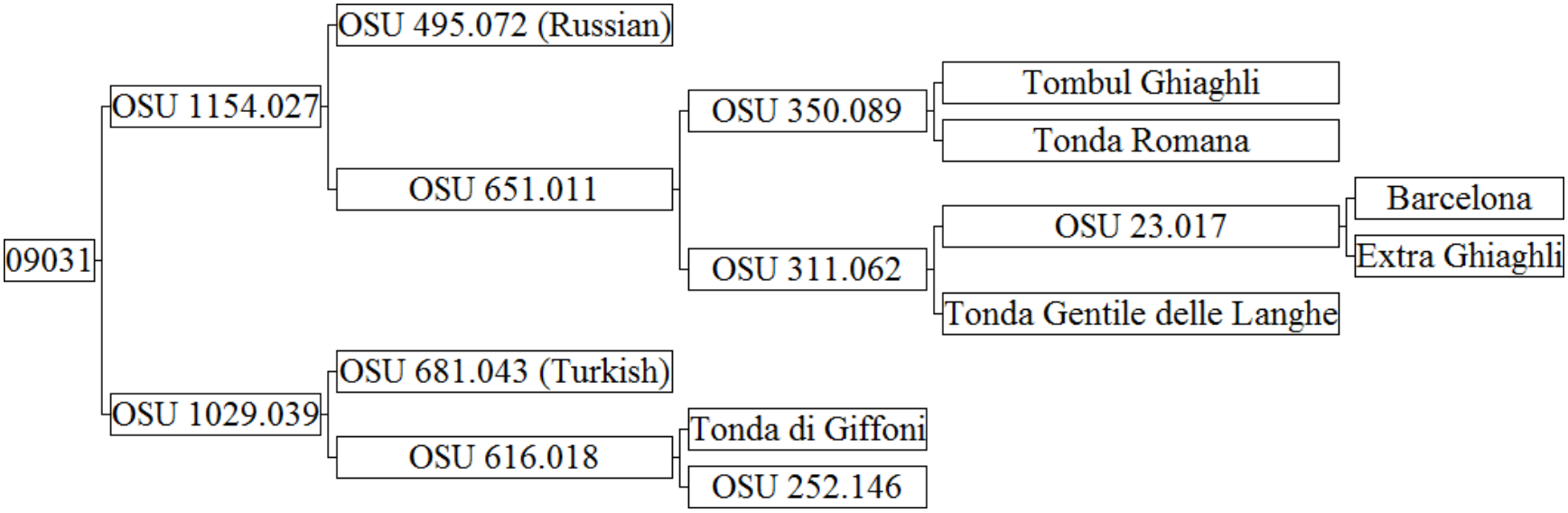


Figure 3.1 (cont.) Pedigrees of progeny segregating for resistance to eastern filbert blight from 'Culpla,' 'Crvenje,' and OSU 495.072.

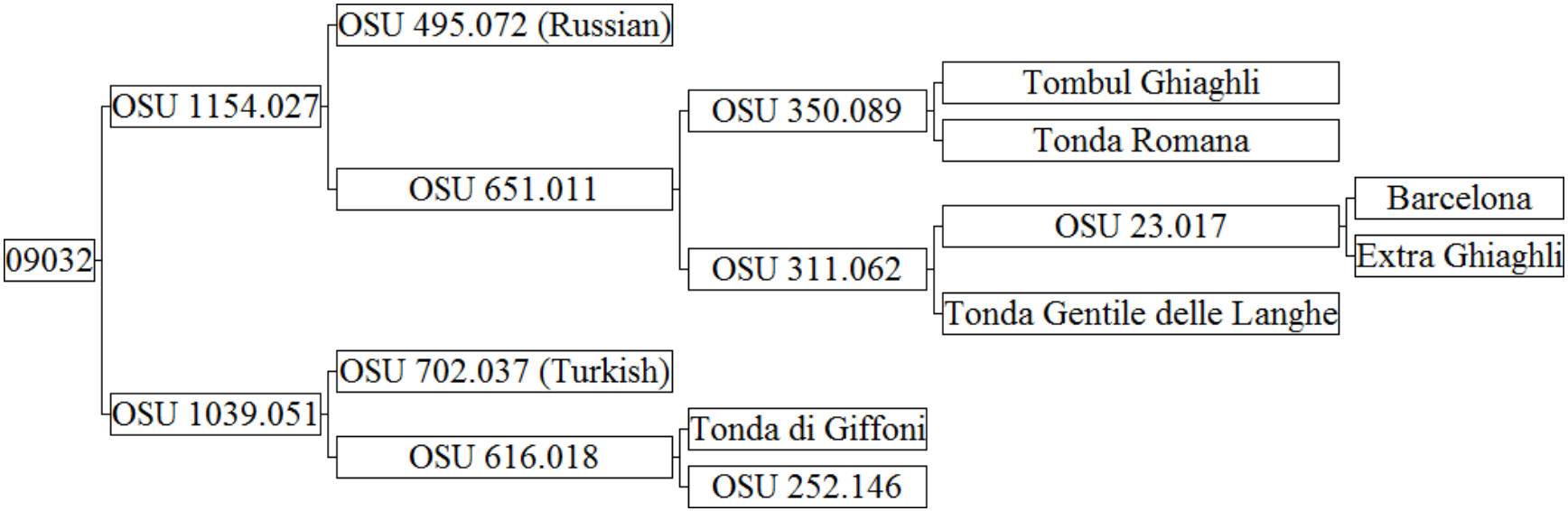


Figure 3.2 Robust RAPD markers UBS268$_{580}$ and UBC152$_{800}$ for ‘Gawaway’ resistance are absent in ‘Culpla,’ ‘Crvenje,’ and OSU 495.072.

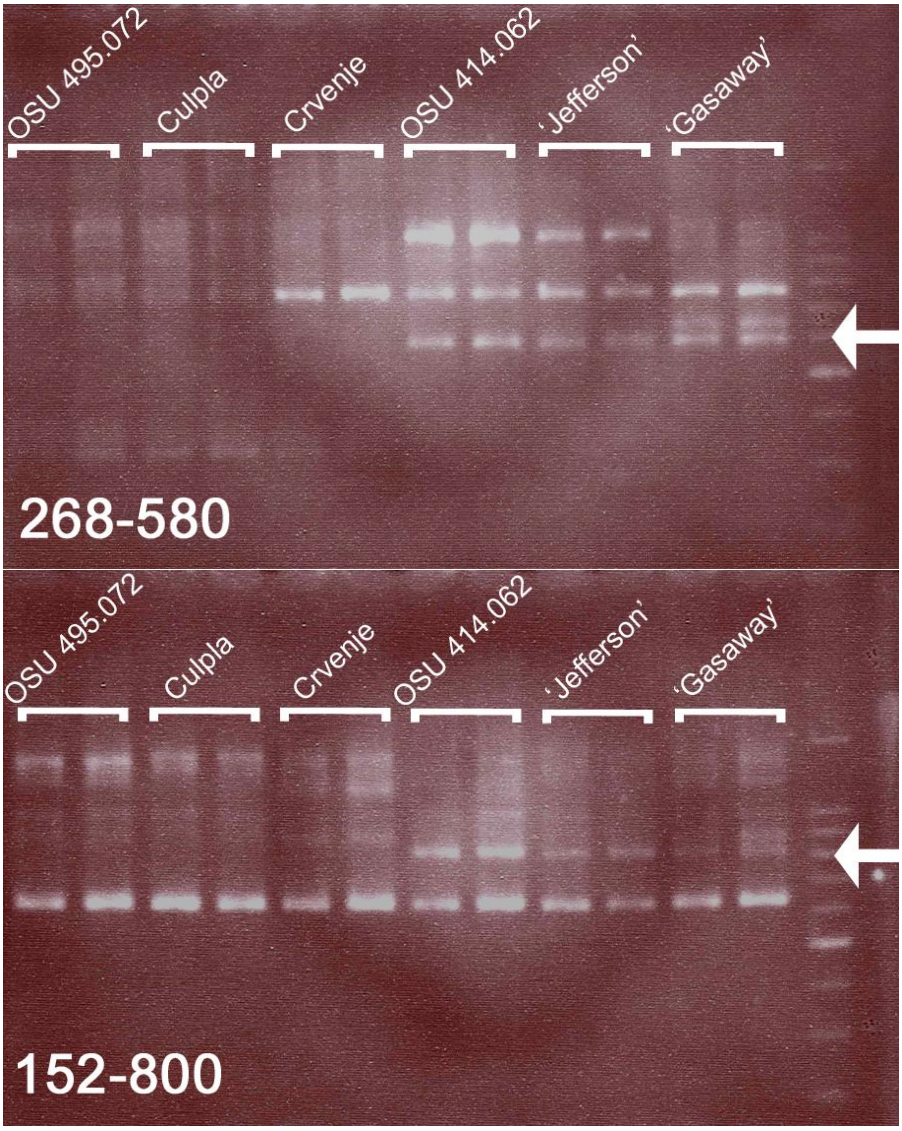
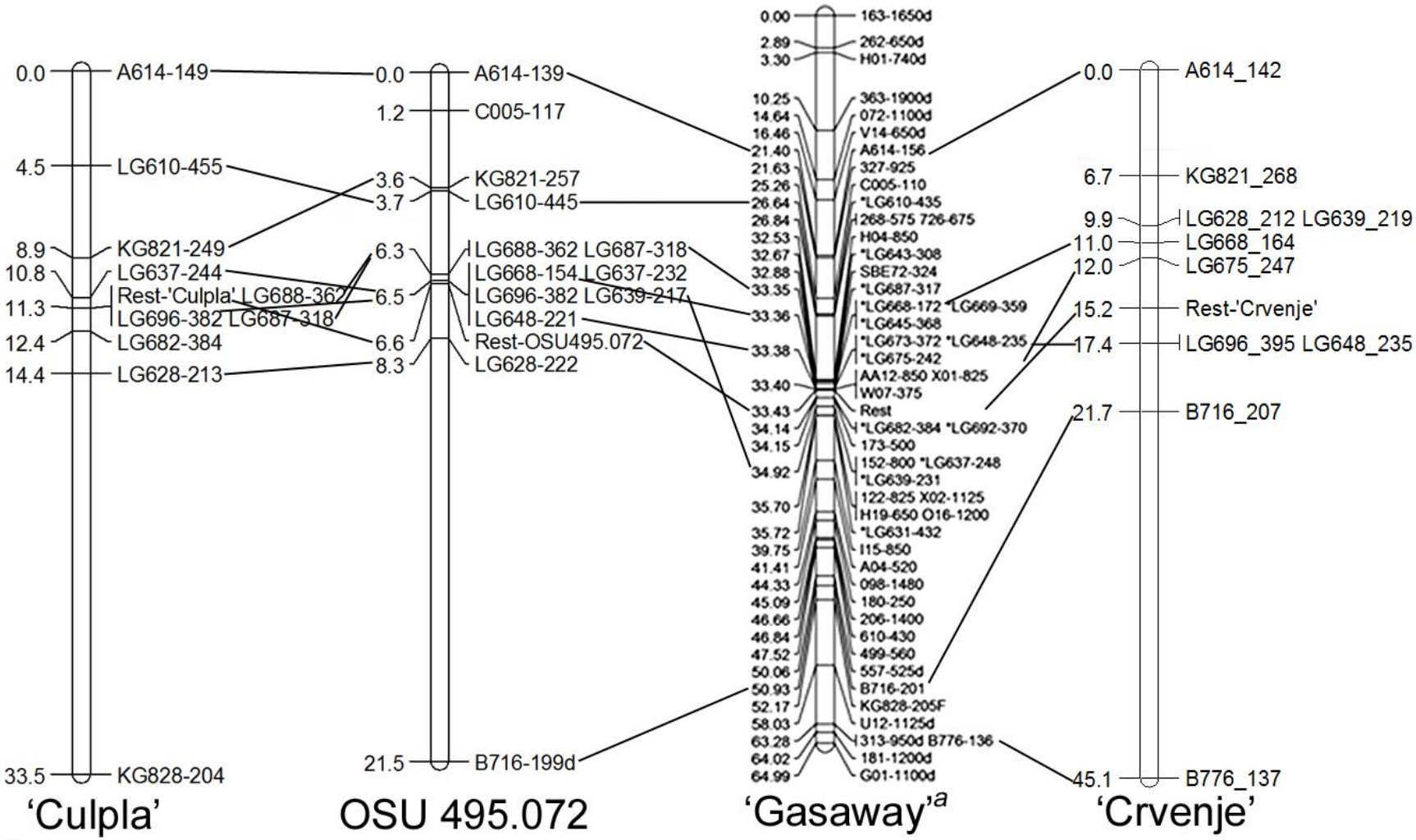


Figure 3.3 Linkage group 6 of populations segregating for eastern filbert blight resistance and the locations of resistance loci



[a] Sathuvalli et al., 2013 (in press)

Figure 3.4 Linkage maps of two half-sib ‘Culpla’ progenies segregating for eastern filbert blight resistance and showing the location of the resistance locus.

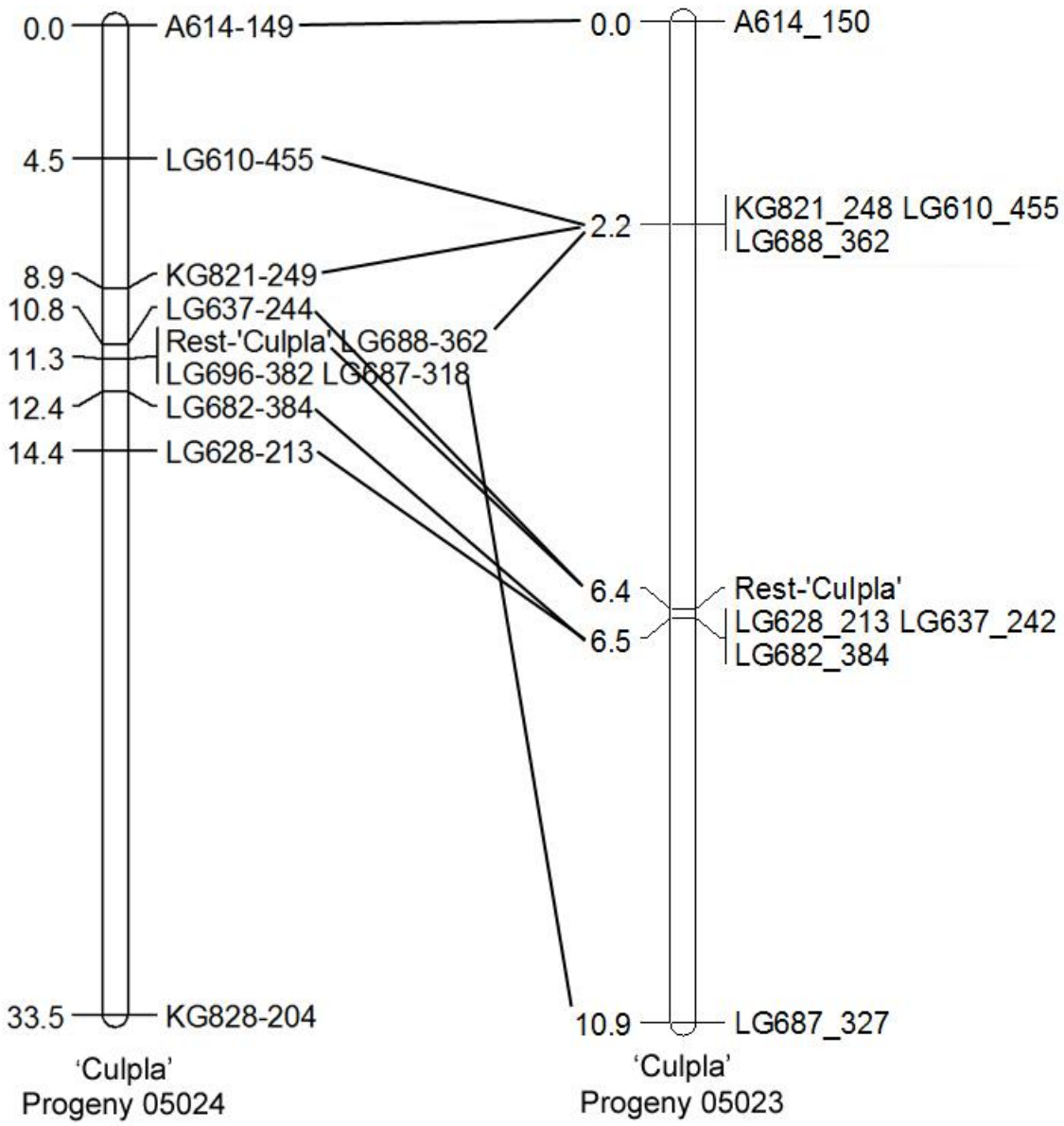
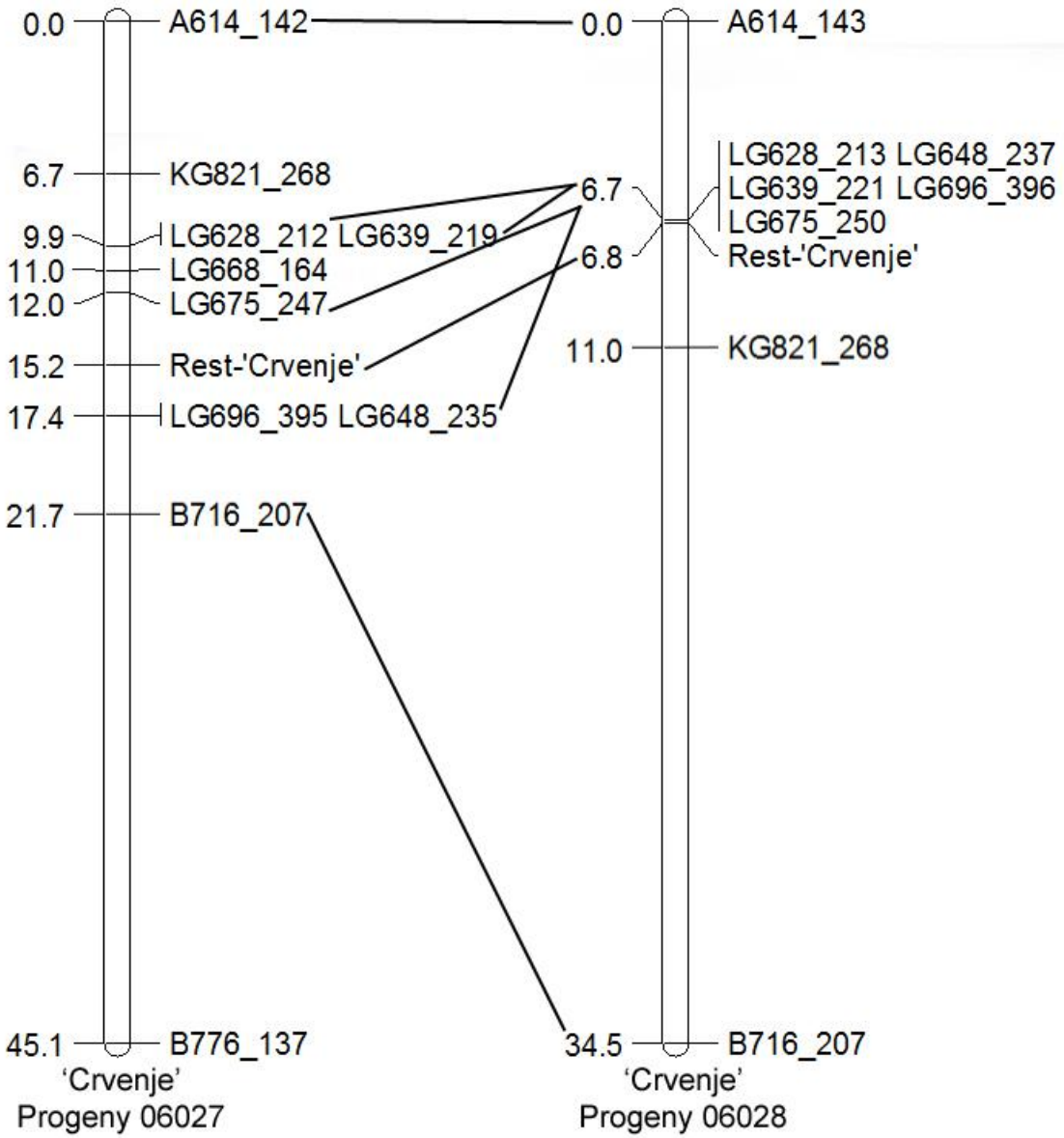


Figure 3.5 Linkage maps of two half-sib ‘Crvenje’ progenies segregating for eastern filbert blight resistance and showing the location of the resistance locus.



Chapter 4

CONCLUSION

European hazelnut is an economically significant crop in Oregon's Willamette Valley. The state produces more than 25,000 metric tons annually, and the US ranks 4th internationally for hazelnut production. The hazelnut breeding program at Oregon State University is focused on improving the hazelnut industry in Oregon by releasing superior cultivars for commercial production, with particular focus on developing DNA marker technology and producing cultivars resistant to eastern filbert blight (EFB) caused by *Anisogramma anomala*. The purpose of this study was to develop new microsatellite markers from hazelnut transcriptome sequence and to investigate and map the location of three sources of EFB resistance.

Microsatellite markers have been used in hazelnut and other crops for marker-assisted selection, genetics studies, and cultivar fingerprinting. Previous work with hazelnut has developed AFLP, RAPD, microsatellite (SSR), and ISSR markers for hazelnut, and a linkage map for hazelnut was constructed from RAPD and microsatellite markers. Previously developed microsatellites were generated from enriched libraries and ISSR fragments. This study utilized the transcriptome sequence of hazelnut to mine for microsatellite loci. A total of 1432 microsatellites of di- to hexa-repeat motifs with at least five repeats were mined. Tri- through hexa-repeats were selected for primer design, and 382 primer pairs were designed. One hundred nineteen of the microsatellite loci were found to be polymorphic when screened on a set of 24 diverse accessions. These markers

were characterized with sets of 50 and 14 accessions, and observed heterozygosity, expected heterozygosity, frequency of null alleles, and polymorphic information content calculated.

Markers segregating in the mapping population for hazelnut were mapped and added to the existing linkage map. Additional markers were assigned to linkage groups based on segregation in alternate populations. The marker loci were well distributed throughout the genome, and since they were developed from transcriptome data, they should be located in or close to functional genes. Dendrograms constructed from the fingerprint data of the markers tended to cluster accessions by their geographical region, with a few exceptions. The dendrograms illustrate the huge range of genetic diversity present in hazelnut, which results from high heterozygosity in individuals and forced outcrossing in populations. These new markers showed high levels of polymorphism and should be useful for marker-assisted selection in hazelnut and related genera.

The three EFB resistant accessions ‘Culpla,’ ‘Crvenje,’ and OSU 495.072 were studied to observe segregation of resistance in the progeny and to map the resistance loci. These parents have displayed complete resistance in all inoculation trials, and RAPD markers suggest that the resistance source is different than ‘Gasaway’ resistance. Nine populations containing resistance from one of the three resistant parents were generated, and the progeny were inoculated either in the greenhouse or under an outdoor structure. In some populations, resistance segregated 1:1, suggesting that the resistance is conferred by a single, dominant gene. In other populations, the resistance fit a 3:1 ratio or fell somewhere between 2:1 and 3:1. We suspect that resistance in each of the three parents is

controlled by a single, dominant gene, but that chromosomal abnormalities is responsible for the skewed ratios of segregation observed in the progeny.

Resistance loci from ‘Culpla,’ ‘Crvenje,’ and OSU 495.072 were assigned to linkage group 6. The resistance loci were mapped using microsatellite markers, and all of the resistance loci cluster in the same region, very close to the ‘Gasaway’ resistance gene. We know that these three resistance genes are different than ‘Gasaway,’ but it is uncertain if these three are identical to each other or unique. Resistance genes have been observed to cluster in the same functional region of the genome in other species, so it is not surprising that these three loci are located very close to the ‘Gasaway’ locus. The markers KG821 and LG628 flank the resistance loci in ‘Culpla’ and OSU 495.072 populations and will be useful for marker-assisted selection to select for these resistance genes. In ‘Crvenje,’ the markers KG821 and LG696 flank the resistance locus and will be useful for marker-assisted selection. These markers linked to the resistance loci would hopefully allow for pyramiding of multiple resistance genes in a single cultivar.

The new microsatellite markers developed in this study and the information about the three novel sources of EFB resistance in hazelnut will be useful for future hazelnut breeding. The markers have expanded the resources available for molecular marker technology in hazelnut, and they will especially be useful for marker-assisted selection and cultivar fingerprinting. The three resistant accessions can be utilized in the breeding program to introgress novel sources of EFB resistance into the breeding lines. These will be valuable for increasing robust resistance to *A. anomala* and for developing new, disease resistant cultivars.

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Appendices

Appendix A. 24 accessions used for screening new microsatellite markers mined from transcriptome data for polymorphism.

Accession	Designation[z]	Origin[y]	Source[x]
Albania 55	PI 617207	Albania	seed from Cajup, Albania
Aurea	PI 557050	France	Morton Arboretum, Lisle, IL, USA
Barcelona	PI 557156	Spain	Faversham, England, UK
Bergeri	PI 557114	Belgium-Luttich	ISF Rome, Italy
Casina	PI 557033	Spain-Asturias	Q.B. Zielinski (Asturias, Spain)
Cosford	PI 557039	England-Reading	NYAES, Geneva, NY
Cutleaf	PI 557306	England	Arnold Arboretum, Boston, MA
DuChilly	PI 557099	England	OSU Entomology Farm, OR, USA
Finland COR 187	PI 557080	Finland	seed from Lappa, Finland
Fusco Rubra	PI 557047	Germany	Morton Arboretum, Lisle, IL, USA
Gasaway	PI 557042	USA-Washington	orchard in Washington, USA
Hall's Giant	PI 557027	Germany/France	OSU Entomology Farm
Imperiale de Trebizonde	PI 271105	Turkey	Q.B. Zielinski (France)
Negret	PI 270340	Spain-Tarragona	Q.B. Zielinski (Spain)
OSU 408.040	PI 617266	Univ. Minnesota	Horticulture research farm seeds
OSU 495.072	PI 557421	Russia-Southern	seeds from VIR, southern Russia
OSU 681.078	PI 634203	Russia-Moscow	J. Henkin collected seeds, Russia
Palaz	PI 304632	Turkey-Ordu	Q.B. Zielinski (Greece)
Pendula	PI 557048	France	Arnold Arboretum, Boston, MA, USA
Ratoli	PI 557167	Spain-Tarragona	IRTA Mas Bove, Reus, Spain
Rode Zeller	PI 271280	Netherlands	Q.B. Zielinski (Netherlands)
Tombul Ghiaghli	PI 304634	Turkey	Q.B. Zielinski (Greece)
Tonda di Giffoni	PI 296207	Italy-Campania	Q.B. Zielinski (Italy)
Tonda Gentile della Langhe	PI 557075	Italy-Piemonte	Univ. di Torino, Italy

[z] Accessions are designated by Plant Inventory (PI) number, Corvallis *Corylus* accession number, or tree location

[y] Origin indicates the country (and province) where the accession was selected

[x] Source indicates the institution, location, or person who sent the accession. Most accessions were received as scions. A few were collected as seeds and individual trees selected from the resulting seedlings.

Appendix B. Origins and sources of 50 accessions used for characterization of 119 microsatellite loci mined from transcriptome data.

Accession	Designation[z]	Origin[y]	Source[x]
Albania55	PI 617207	Albania	seed from Cajup, Albania
Alli	R72.02	Estonia	Polli, Estonia
Artellet	PI 557108	Spain-Tarragona	IRTA Mas Bove, Reus, Spain
Aurea	PI 557050	France	Morton Arboretum, Lisle, IL, USA
B-3	PI 557122	Macedonia	Skopje, Macedonia
Barcelona	PI 557037	Spain-Tarragona	Oregon Nursery, USA
Barcelloner Zellernuss	PI 557156	Spain	Faversham, England, UK
Bergeri	PI 557114	Belgium-Luttich	ISF Rome, Italy
Buttner's Zellernuss	PI 557094	Germany-Landsberg	Faversham, England, UK
Casina	PI 557033	Spain-Asturias	Q.B. Zielinski (Asturias, Spain)
Contorta	PI 557049	England	Arnold Arboretum, Boston, MA, USA
Cosford	PI 557039	England-Reading	NYAES, Geneva, NY
Cutleaf	PI 557306	England	Arnold Arboretum, Boston, MA
DesAnglais	PI 557423	unknown	INRA, Bordeaux, France
DuChilly	PI 557099	England	OSU Entomology Farm, OR, USA
Finland COR 187	PI 557080	Finland	seed from Lappa, Finland
Fusco Rubra	PI 557047	Germany	Morton Arboretum, Lisle, IL, USA
Gasaway	PI 557042	USA-Washington	orchard in Washington, USA
Gem	PI 557029	USA-Washington	orchard in Oregon, USA
Gunslebert	PI 557191	Germany-Gunsleben	INRA Bordeaux, France
Gustav's Zellernuss	PI 557085	Germany-Landsberg	Faversham, England, UK
Hall's Giant	PI 557027	Germany/France	OSU Entomology Farm
Iannusa	PI 557183	Italy-Sicily	Univ. di Torino, Italy
Imperiale de Trebizonde	PI 271105	Turkey	Q.B. Zielinski (France)
Kadetten Zellernuss	PI 557090	Germany	Faversham, England, UK
Mortarella	PI 339723	Italy-Campania	Q.B. Zielinski (Italy)
Negret	PI 270340	Spain-Tarragona	Q.B. Zielinski (Spain)

Appendix B (cont.). Origins and sources of 50 accessions used for characterization of 119 microsatellite loci mined from transcriptome data.

OSU 26.072	PI 323961	Russia-North Caucasus	H. Brooks collected seeds, Russia
OSU 54.039	PI 557060	Turkey-Giresun/Ordu	M.M. Thompson collected seeds, Turkey
OSU 252.146	--	OSU	Parent of mapping population
OSU 408.040	PI 617266	Univ. Minnesota	Horticulture research farm seeds
OSU 414.062	--	OSU	Parent of mapping population
OSU 495.049	PI 557421	Russia-Southern	seeds from VIR, southern Russia
OSU 556.027	PI 617269	Turkey-Istanbul	Istanbul market seeds, Turkey
OSU 681.078	PI 634203	Russia-Moscow	J. Henkin collected seeds, Russia
OSU 759.010	759.016	Georgia	L. Lazareishivili, Tbilisi, Georgia
Palaz	PI 304632	Turkey-Ordu	Q.B. Zielinski (Greece)
Pellicule Rouge	PI 271110	France	Q.B. Zielinski (France)
Pendula	PI 557048	France	Arnold Arboretum, Boston, MA, USA
Ratoli	PI 557167	Spain-Tarragona	IRTA Mas Bove, Reus, Spain
Römsche Nuss	PI 557171	unknown	Hermansverk, Norway
Rode Zeller	PI 271280	Netherlands	Q.B. Zielinski (Netherlands)
Sant Jaume	PI 557103	Spain-Tarragona	IRTA Mas Bove, Reus, Spain
Simon	PI 557166	Spain-Tarragona	IRTA Mas Bove, Reus, Spain
Tapparona di SCC[w]	PI 617239	Italy-Liguria	Univ. di Torino, Italy
Tombul Ghiaghli	PI 304634	Turkey	Q.B. Zielinski (Greece)
Tonda Bianca	PI 296206	Italy-Campania	Q.B. Zielinski (Italy)
Tonda di Giffoni	PI 296207	Italy-Campania	Q.B. Zielinski (Italy)
Tonda Gentile delle Langhe	PI 557075	Italy-Piemonte	Univ. di Torino, Italy
Tonda Romana	PI 557025	Italy-Lazio	ISF Rome, Italy

[z] Accessions are designated by Plant Inventory (PI) number, Corvallis *Corylus* accession number, or tree location

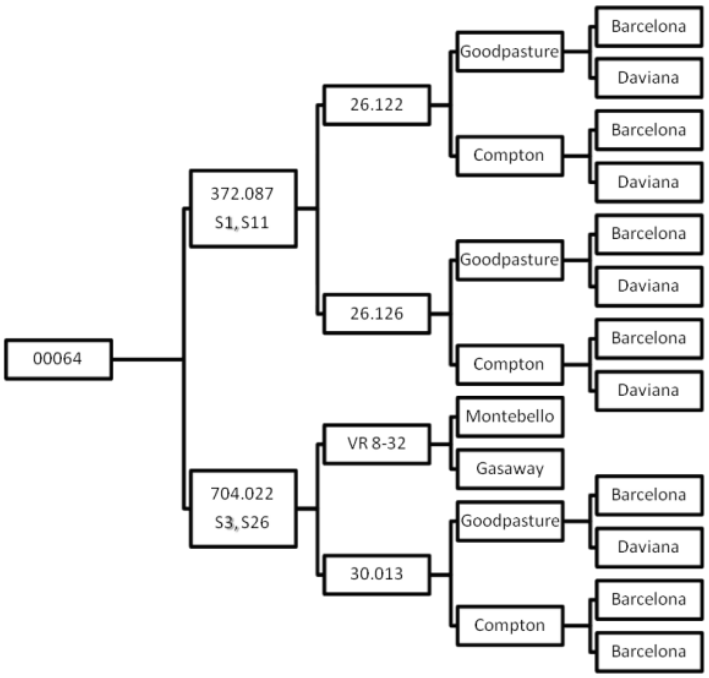
[y] Origin indicates the country (and province) where the accession was selected

[x] Source indicates the institution, location, or person who sent the accession. Most accessions were received as scions. A few were collected as seeds and individual trees selected from the resulting seedlings.

[w] Tapparona di San Colombano Cortemoli

Appendix C. Origins and sources of 16 parents used for characterization of 119 microsatellite loci mined from transcriptome data.

Accession	Designation[z]	Origin[y]	Source[x]
Barcelona	PI 557156	Spain	Faversham, England, UK
Culpla	COR 255.002	Spain	Scions from IRTA Mas Bove, Spain
OSU 612.015	--	OSU	Parent of progeny 05023
OSU 675.028	--	OSU	Parent of progeny 05024, 06027
OSU 495.072	PI 557421	Russia	Seeds from VIR, southern Russia
OSU 651.011	--	OSU	Parent of progeny 02020
OSU 713.068	--	OSU	Parent of progeny 01035
Crvenje		Serbia	Scions from Fruit Research Institute, Čačak, Serbia
OSU 679.114	--	OSU	Parent of progeny 06028
OSU 252.146	--	OSU	Parent of mapping population
OSU 414.062	--	OSU	Parent of mapping population
OSU 372.087	--	OSU	(S-locus, yellow styles)
OSU 704.022	--	OSU	(S-locus, red styles)
OSU 1187.101	--	Russia	Seed from market, Holmkij, Russia
OSU 1185.126	--	Crimea	Seed from roadside vendor
Daviana	PI 557040	England	England, UK, Richard Webb



Pedigree of progeny 00064 from the cross of OSU 372.087 x OSU 704.022 for studying segregation of style color in hazelnut.

Appendix D In silico development of SSR markers from hazelnut transcriptome sequences

Step 1. SSR regions were mined from .fasta files of assembled hazelnut transcriptome sequences for seedlings, catkins, leaf, and bark. SSR regions were identified using two programs: Gramene SSRIT (http://www.gramene.org/db/markers/ssrtool) and Evopipes.net "find SSR" (http://evopipes.net/findssr.html).

Gramene SSRIT:

When using Gramene SSRIT, the maximum motif length allowed was hexamer and the minimum number of repeats allowed was 5. Between 4 and 10 contigs from the sequence data were pasted into the Gramene SSRIT sequence box at a time. The information for each SSR region identified along with the entire contig it was located in and the SSR region in bold were listed in a document as follows:

scaf175|size1149-1 tcttca 7 321 362 1149

>SCAF175|SIZE1149,(TCTTCA)7,267,TTCGTCTTCGTCTTCATCTTCA,22,59.993,TTGGATAAGTGGCTAGAAGGGA,22,60.089,320,293,538

>scaf175|size1149
ATCCAAAAAAAAAGGAAACTCTCTTGCTCCACGCACTTCCTTGTTGCT
TGATTTTTCTACTAAATCTGTTTACACCTAGGCCTTAATCTTCTCCTCGC
AGTTCCGATTGCTTGATTTCAGACCGTAAAAAGGCATCTGTTAACCCA
AAGGGGCTATCCACTTTATATTTTTCTTCCCAAGCAAGCGATATGTCCA
ATCTGTTCTCCCCGGGCATGTGAGAAATATCAACTACAATGCAGCGAA
CATCAGTATATGCATCAGTATATACATGAGTAAATACTTCAGTATATA
CTTCGTCTTCGTCTTCATCTTCATCTTCT**TCTTCATCTTCATCTTCATC**
TTCATCTTCATCTTCATCTTCATTTTCATCTCCATCTCCATCTCCATCT
CCATCTCCATCTCCATCTCCATCTCCATCTCCATCTCCATCTTCATCGG
ATTTCATACGACGAGCCGTTTGTAAGATGCAGAACCTCTTGTTCTCTAA
ATGCACAAGACAAGGAACTGGTGAACTGGAAATTCCGAGATGCTTAA
GACTCCCTTCTAGCCACTTATCCAAGTCTAAATTATAGGCAATCAAGA
ATGCTTCCTCCTCCATCCCATCCTCTACCCAAGTGGTCTCTACCCAAGT
GGTCCAGTATAAAGTATTATGTGCAGTTATCGTCCTTTGACAACTCCCG
ACAGGGAACATAGAGTGTATCTTGCGCTCAGGAGGGGCAAGCACTTC
CCAAGATCGATGCTGCACATTATATGTATAAAACATTGCAGAATAGCG
TGTATTCGGAACATGGAAAGCCACAAGAATCCTGCTTGGATTCTCAAG
AGCTGCAGAAAGGATTATAGAACTCGAATCATATGGAGGATCCGGTA
AGGCTTCCCATTTTCCATAATCGGGATCAAAAACCTCACCCCACTTGA
CGGTAGGCGACGTATGCTGTAGGCGACTAGAAAAAAAATAAATCTTC
CCGTCTAGAGCAAAACTTAGCGGTTTGTATTTGGGAGAAATCATCGGA
GAAACACGCTTTAAGGAGTAGGGGCCACGGCTGGTAAAGTCTAATTTG
CAGACTTCAGATAGAGAGGTTGGAATCGTCGCGTAGACGGGTTTCGGC
CCACAACCAAAAGCGTAGACGTGGGAACGTAGGGCTGCAA

Evopipes.net "find SSR":

When using Evopipes.net "find SSR," the .fasta file containing the sequence data was uploaded to the online job launch site. The program generated a list of SSR regions, and those with repeat motifs of 3 base pairs or longer and a total repeat length of at least 15 base pairs were selected for primer design. The entire contig containing each SSR region and the SSR characteristics were listed in a document with the SSR region in bold as follows:

scaf552|size760-1 aga 5 350 364 760

>SCAF552|SIZE760,(AGA)5,356,AGAACATAGTCGCGTACAAGCA,22,59.969,CTACCACTTTTGGTGGCTCTTC,22,60.165,349,95,429

>scaf552|size760
TGCATCAGGCTTTTCTGTGTAGGCTCCCCCCCCTCCATTGGGGAATTTT
CATGTCAGCACTTTGGTTTGAACTTTGATGAAGATGATGATTCCCAGA
ACATAGTCGCGTACAAGCAAAGAAAACAAGGCAGGATTTTTGAGACC
TTTTCCTAAGTCCGAAGCTGCCTCTCTGTCTTGTTTTATTCTCTTGCTTT
TAACGGCTCTCCTCCTTTTTTCTAGCAGCATACTAGGCGGTTGAAAGCA
ATATCCAACGCTCCCAGCTCTTCCCACTTTAATAGACAACCAATTGAA
TCATCATAAACACGAAGAAAAAGAATAGCGGGCCAATACCCAATTGC
CATCTTTCCAT**AGAAGAAGAAGAAGA**AATGGACAAATAACCGGTAGA
AGAACTCGCCGGCGTGGAGGGTTGGGTACCGGAAGTCCCAGGAGAAG
AGCCACCAAAAGTGGTAGCATTGCTAGAACCGTCGTTCATGGCAATCC
CAAGCGAGTACGCGGGCGTCCCATCGGGCTTAAACAAGCCGTAGTTCC
TCTCCGAAGTAGGACCAGGCTTCATGTTCTCGTTGAACAAAGCGAAGA
CGTAGATGTTCAGCTCCGAGCTGGGCCTCATGGGCGTGCCCTTCTTCTT
CGGCTCCGTCATCAGCTTAATGAGATTCCCATTGTACTTCTTGGCGTTC
TCCGGGGAGGCGCCGGCCTCGTCCCCGTCCCCTCTCGACGGCCACCCC
GTCTCCGATAGGTGCACCGGCAGCTTCTTGTACCCCAA

Step 2. In a spreadsheet, each scaffold containing an SSR was listed, along with the SSR region and a truncated 400 base pair region from each contig, with the SSR located in the middle. Each unique SSR was given a letter/number designation (ie: BR111).

Step 3. The truncated 400 base pair contig was used to search the hazelnut genome sequence using BLASTN to find the corresponding genomic sequence (http://corylus.cgrb.oregonstate.edu:8080/). A document was made, containing the original SSR and RNA contig information, blast results, and genomic contig as follows, with the SSR in bold:

RNA contig (shortened to 400 bp)

Leaf
>209
NNNNNNNNNNNNNAGCTATACATACCAGCATGCACTGGCTTAACACCTGA
GGGAGTCATTAGAGCAGTCAAGACATTATCCGAACGTCATCACAGCTTAAAG
AGCCTGAGGATAAATGGTATCTACAACATAAAGAAAGAAAACCTACAAACA
ATTCTCTCTTATCTGCAAATGAACCTGTCT**CAGCAGCAGCAGCAG**AGAAAGC

AGCAACCTACTACCCTCTATCATCACAGGAACTTCCCAATTTTCAGAAATGAA
GATAGTGATCCCATGCTTGATGTGGATATCTGCCCGAAGTGCAACGACGTAA
GAGCGGTTTATGAGTGTGTAAGAGAAACCTGCAAAAGGAAGAGGGAGCGGC
TATCAACCGTTTGTAGAGGCTGCAACCTTTGCATTCC

Corylus blast results

Query= 209
(399 letters)

Sequences producing significant alignments:	Score (bits)	E Value
VM_c16190	726	0.0
VM_c85872	38	0.27
VM_c642	38	0.27

>VM_c16190
Length = 2137

Score = 726 bits (366), Expect = 0.0
Identities = 383/388 (98%), Gaps = 3/388 (0%)
Strand = Plus / Minus

```
Query: 15   agctatacataccagcatgcactggcttaacacctgagggagtcattagagcagtcaaga 74
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1945 agctatacataccagcatgcactggcttaacacctgagggagtcattagagcagtcaaga 1886


Query: 75   cattatccgaacgtcatcacagcttaaagagcctgaggataaatggtatctacaacataa 134
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1885 cattatccgaacgtcatcacagcttaaagagcctgaggataaatggtatctacaacataa 1826


Query: 135  agaaagaaaacctacaaacaattctctcttatctgcaaatgaacctgtctcagcagcagc 194
            |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct: 1825 agaaagaaaacctacaaacaattctctcttatctgcaaatgaacccgtctcagcagcagc 1766


Query: 195  agcag---agaaagcagcaacctactaccctctatcatcacaggaacttcccaattttca 251
            |||||   ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1765 agcagcagagaaagcagcaacctactaccctctatcatcacaggaacttcccaattttca 1706


Query: 252  gaaatgaagatagtgatcccatgcttgatgtggatatctgcccgaagtgcaacgacgtaa 311
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1705 gaaatgaagatagtgatcccatgcttgatgtggatatctgcccgaagtgcaacgacgtaa 1646


Query: 312  gagcggtttatgagtgtgtaagagaaacctgcaaaaggaagagggagcggctatcaaccg 371
            |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct: 1645 gagcggtttatgagtgtgtaagagaaacctgcaagaggaagagggagcggctatcaaccg 1586


Query: 372  tttgtagaggctgcaacctttgcattcc 399
            ||||||||||||||||||||||||||||
Sbjct: 1585 tttgtagaggctgcaacctttgcattcc 1558
```

```
>VM_c85872
          Length = 616

 Score = 38.2 bits (19), Expect = 0.27
 Identities = 25/27 (92%)
 Strand = Plus / Minus


Query: 323 gagtgtgtaagagaaacctgcaaaagg 349
           |||||| |||| ||||||||||||||
Sbjct: 75  gagtgtttaagtgaaacctgcaaaagg 49



>VM_c642
          Length = 7072

 Score = 38.2 bits (19), Expect = 0.27
 Identities = 22/23 (95%)
 Strand = Plus / Minus


Query: 188  cagcagcagcagagaaagcagca 210
           ||||||||||||||| |||||||
Sbjct: 2264 cagcagcagcagagagagcagca 2242
```

Step 4. The top two BLASTN hits for each SSR were added to the spreadsheet from Step 2. The genomic contig containing the SSR was also added to the spreadsheet as well as a separate text document for just the genomic contigs, with the SSR name added to the contig name.

Step 5. A search for duplicate contigs was performed using CodonCode Aligner. The text document of genomic sequences created in Step 4 was used for this search. Any duplicate contigs were removed from the master list so as to not design more than one primer pair for each genomic SSR region.

Step 6. Primers were designed for each SSR using the genomic sequence contigs. The Primer3 program was used (http://frodo.wi.mit.edu/). Design criteria specified a primer size of 18 to 27 base pairs, 60 °C annealing temperature, and 20-80% primer GC content. If Primer3 was unable to design primers for the specified sequence, WebSat (http://wsmartins.net/websat/) was used to design primers. Forward and reverse primer sequences along with each respective annealing temperature and expected product size were recorded on the spreadsheet from Steps 2 & 4.

Step 7. Primers were ordered from Integrated DNA Technologies (Coralville, IA).

Step 8. Initial screening of primers utilized a set of 24 highly diverse *Corylus avellana* selections (Appendix A) from a wide range of geographic locations. These individuals were chosen based on their high level of diversity in order to increase the likelihood of indentifying polymorphic SSRs. The PCR mix was a total of 10µl per reaction and contained 0.3 µM each of the forward and reverse primer, 1x Biolase NH_4 reaction

buffer, 2 mM $MgCl_2$, 200 µM each of dATP, dTTP, dGTP, and dCTP, 20 ng template DNA, and 0.25 units of Biolase DNA polymerase (Bioline USA Inc., Boston, MA). The PCR program ran according to the following protocol: denaturation at 94 °C for 5 minutes followed by 40 cycles of 94 °C for 40 seconds, 60 °C for 40 seconds, 72 °C for 40 seconds; then 72 °C for 7 minutes of extension, ending with an infinite hold at 4 °C. The PCR products were evaluated with agarose gel electrophoresis. The PCR products were run on 3% agarose gels at 90 V for 3.5 hours. The finished gels were stained with ethidium bromide and imaged under UV light using a BioDoc-It® Imaging System (UVP, Upland, CA). Polymorphic SSRs were identified by examining the gel images and looking for PCR products that varied in size among the 24 genotypes.

Step 9. Primers showing polymorphism were selected for fluorescent labeling. The forward primer of each primer pair was labeled with FAM, NED, or HEX tag (company info). A set of 24 + 48 genotypes were used for characterizing each of the SSRs. PCR reactions were performed as described above, except that the fluorescent tagged forward primer was used in place of the non-fluorescent forward primer. Products were multiplexed post-PCR, with six to twelve different primer products in each multiplex set. 2 µl of each product were combined in 150 µl water and submitted to the CGRB Core Lab facility at Oregon State University for genotyping with an AB 3730 DNA Analyzer (Life Technologies). The genotyping data was analyzed with AB Gene Mapper® software (Life Technologies). The length of the amplified region was recorded for each SSR region on each of the genotypes in the set.

Step 10. SSRs with polymorphism in the parents of the mapping population (OSU 252.146 and OSU 414.062) were mapped using the segregating seedling population and markers on the existing genetic linkage map constructed by Mehlenbacher et al. (2006).

Appendix E. SSR profiles of 50 accessions at 113 loci.

Cultivar	BR114b	BR173	BR177	BR182	BR190	BR193	BR199
OSU 495.049	353/353	220/223	395/395	227/230	287/287	339/342	303/303
Albania 55	347/350	223/226	395/395	227/227	287/287	342/342	300/303
Fusco Rubra	347/347	226/226	395/395	227/227	287/290	339/342	303/303
Finland COR 187	347/347	223/226	395/395	227/227	287/287	339/342	303/303
Pendula	347/347	226/226	395/395	227/227	287/287	339/342	303/303
Hall's Giant	347/347	223/226	395/395	227/227	287/287	339/342	303/303
Gasaway	345/347	226/226	395/395	230/230	287/287	339/342	297/297
Rode Zeller	347/350	223/226	395/395	227/227	287/287	342/342	303/303
Cosford	350/350	223/226	386/395	227/227	287/287	339/342	303/303
DuChilly	347/347	223/226	395/395	227/230	287/287	342/342	303/303
Palaz	347/356	223/226	395/395	227/227	287/287	342/342	303/303
Pellicule Rouge	347/347	223/223	395/395	227/227	287/287	342/342	303/303
Imperiale de Trebizon.	347/356	226/226	395/395	227/227	287/287	342/342	303/303
Tombul Ghiaghli	347/353	223/223	395/395	NA/NA	287/287	342/342	303/303
Tonda Bianca	345/347	223/226	395/395	227/230	287/287	342/342	300/303
Negret	345/347	223/223	395/395	227/227	286/289	339/342	303/309
Tonda Gentile delle L.	347/347	223/226	395/395	227/227	287/287	342/342	303/303
Tonda Romana	347/350	223/226	395/395	227/227	287/290	339/342	303/303
Romische Nuss	347/347	223/226	395/395	227/227	287/290	342/342	303/303
Casina	347/347	223/226	395/395	227/227	290/290	339/342	303/303
Ratoli	347/347	223/226	395/395	227/227	287/290	339/342	303/303
Mortarella	347/347	223/226	395/395	227/230	287/287	339/342	303/303
Tonda di Giffoni	347/347	223/223	395/395	227/230	287/287	342/342	300/303
Barcelona	347/347	223/226	395/395	227/227	287/290	342/342	303/303
Cutleaf	345/347	223/223	395/395	227/230	287/287	339/339	300/303
OSU 681.078	347/347	226/226	395/395	227/227	287/287	339/339	303/309
Barcelloner Zellernuss	345/347	223/226	395/395	227/227	287/287	339/342	303/303
Aurea	347/347	223/223	395/395	227/230	287/287	339/342	303/303
OSU 408.040	347/347	226/226	395/395	227/227	287/287	339/342	303/309
Des Anglais	345/347	226/226	395/395	227/227	287/287	339/342	297/303
OSU 26.072	347/353	223/223	395/395	227/227	287/287	339/342	303/309
Bergeri	347/347	223/226	395/395	227/230	287/287	339/342	303/303
Alli	347/347	223/226	395/395	227/230	287/287	339/339	NA/NA
Kadetten Zellernuss	347/347	223/226	395/395	227/230	287/287	339/342	303/303
OSU 759.010	353/356	223/226	395/395	227/227	287/290	342/342	303/312
Contorta	347/350	223/226	395/395	227/230	287/287	339/339	303/303
OSU 556.027	347/347	223/226	395/395	227/230	287/290	339/342	303/303
B3	347/347	223/226	386/395	227/227	287/287	342/342	303/303
OSU 54.039	347/353	223/226	395/395	227/227	287/287	339/342	303/303
Gunslebert	347/347	223/226	395/395	227/227	287/290	339/342	303/303
Sant Jaume	347/347	223/223	395/395	227/227	287/287	339/342	NA/NA
Iannusa Racinante	347/356	223/228	395/395	227/227	287/287	339/342	303/303
Gem	347/347	223/228	395/395	227/227	287/290	342/342	303/303
Artellet	347/356	223/226	395/395	227/227	287/290	342/342	303/309
Simon	345/347	223/226	395/395	227/227	287/290	339/342	303/303
Gustav's Zellernuss	347/347	223/226	395/395	227/230	287/287	339/342	303/303
Buttner's Zellernuss	347/350	226/226	395/395	227/227	287/287	339/342	303/303
Tapparona di SCC	347/347	223/226	386/395	227/227	287/287	342/342	303/303
OSU 252.146	347/347	223/223	395/395	227/227	286/289	342/342	303/303
OSU 414.062	347/347	223/226	395/395	227/230	286/286	342/342	303/303

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR202	BR205	BR210	BR211	BR215	BR216	BR227
OSU 495.049	192/192	172/172	238/238	143/143	123/123	130/130	293/296
Albania 55	192/192	169/172	238/238	147/147	126/129	133/133	296/305
Fusco Rubra	192/195	169/169	238/238	132/143	120/123	130/133	296/302
Finland COR 187	192/195	169/172	238/238	143/143	120/126	130/130	296/296
Pendula	192/192	169/169	238/241	143/146	126/129	130/133	296/302
Hall's Giant	192/192	169/169	238/238	143/143	126/126	130/133	284/296
Gasaway	186/192	169/169	238/238	137/146	126/129	133/146	296/305
Rode Zeller	192/192	169/169	238/238	137/146	123/126	133/133	296/296
Cosford	192/192	169/169	238/238	143/146	123/123	124/133	296/296
DuChilly	186/186	169/169	238/238	143/143	126/129	133/133	296/296
Palaz	192/192	169/172	238/238	143/146	129/129	130/153	296/299
Pellicule Rouge	186/192	169/169	238/238	143/146	129/129	133/133	296/296
Imperiale de Trebizonde	192/192	172/172	238/238	143/149	123/129	133/133	299/299
Tombul Ghiaghli	192/192	169/172	238/238	143/146	123/129	130/133	296/299
Tonda Bianca	192/198	169/172	238/238	143/143	129/129	133/133	296/299
Negret	192/195	169/169	238/238	143/146	123/129	130/133	296/296
Tonda Gentile delle Langhe	192/192	169/169	238/238	143/146	123/126	130/133	296/299
Tonda Romana	180/192	169/169	238/238	143/143	123/129	133/133	296/299
Romische Nuss	192/192	169/172	238/238	143/146	123/126	130/133	284/299
Casina	192/195	169/169	238/238	143/146	123/123	133/133	299/299
Ratoli	192/192	169/172	238/238	143/143	123/123	130/133	296/299
Mortarella	192/195	169/169	238/238	143/146	123/126	130/130	296/296
Tonda di Giffoni	192/192	169/169	238/238	143/143	123/129	133/133	299/299
Barcelona	192/192	169/172	238/238	143/143	123/126	130/133	299/299
Cutleaf	192/192	169/169	238/238	143/143	123/129	130/133	299/299
OSU 681.078	192/195	169/169	238/238	137/143	126/129	124/133	305/305
Barcelloner Zellernuss	192/195	169/172	238/238	143/146	123/129	130/130	296/302
Aurea	195/198	169/169	238/238	143/146	129/129	130/130	296/296
OSU 408.040	192/192	169/169	238/238	143/146	126/126	130/130	284/284
Des Anglais	186/198	169/169	238/238	143/146	129/129	130/133	284/299
OSU 26.072	192/195	169/169	238/238	143/146	126/129	130/130	NA/NA
Bergeri	192/192	169/169	238/238	143/143	126/126	130/133	280/296
Alli	192/192	169/169	238/238	143/146	127/135	130/133	296/299
Kadetten Zellernuss	192/195	169/169	238/238	143/143	126/129	130/130	296/296
OSU 759.010	192/192	169/172	238/238	143/146	123/129	130/130	296/302
Contorta	186/192	169/169	238/238	143/146	129/129	130/130	296/299
OSU 556.027	192/192	169/172	238/238	143/143	129/129	130/133	284/296
B3	186/195	169/169	238/238	137/143	123/129	133/159?	296/299
OSU 54.039	192/195	172/172	238/238	143/146	129/129	130/130	296/296
Gunslebert	192/192	169/172	238/238	143/143	123/126	130/133	299/299
Sant Jaume	192/195	169/169	238/238	143/143	NA/NA	130/130	296/299
Iannusa Racinante	192/198	169/169	238/238	143/143	123/126	133/133	296/299
Gem	186/192	169/172	238/238	143/143	123/126	133/133	299/299
Artellet	195/195	169/172	238/238	143/143	123/129	130/133	296/299
Simon	192/195	169/169	238/238	143/143	126/129	130/130	296/299
Gustav's Zellernuss	192/195	169/169	238/238	143/143	126/129	130/133	296/299
Buttner's Zellernuss	192/192	169/169	238/238	143/143	123/129	124/133	284/302
Tapparona di SCC	183/192	172/172	238/238	143/143	126/129	130/133	296/296
OSU 252.146	192/192	169/172	238/238	143/146	123/126	130/130	299/299
OSU 414.062	192/192	170/170	238/238	146/146	126/129	130/130	296/296

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR229	BR230	BR231	BR233	BR238	BR240
OSU 495.049	297/303	368/368	131/134	109/109	266/266	241/241
Albania 55	300/300	368/368	128/131	109/109	266/270	235/235
Fusco Rubra	300/303	368/368	131/131	109/115	266/266	235/235
Finland COR 187	300/303	371/371	131/131	109/109	266/270	235/235
Pendula	297/297	368/368	131/131	109/109	270/270	235/235
Hall's Giant	300/300	368/368	131/131	109/109	266/266	235/235
Gasaway	297/303	371/371	131/131	109/109	270/270	235/235
Rode Zeller	303/303	368/371	131/131	109/109	266/266	235/235
Cosford	300/303	368/371	131/131	109/109	266/274	235/235
DuChilly	303/306	371/371	131/131	109/109	266/266	235/235
Palaz	297/303	368/371	131/134	109/109	266/270	235/235
Pellicule Rouge	303/306	368/371	131/131	109/109	266/266	235/235
Imperiale de Trebizonde	303/303	368/371	131/134	109/109	266/270	235/235
Tombul Ghiaghli	303/303	368/371	131/134	109/109	266/270	235/235
Tonda Bianca	297/300	368/368	131/134	109/109	270/270	235/235
Negret	297/303	368/368	131/131	109/109	266/270	235/235
Tonda Gentile delle Langhe	303/303	368/368	131/131	109/109	270/270	235/235
Tonda Romana	303/303	368/368	128/131	109/109	266/270	235/235
Romische Nuss	300/303	368/368	131/131	109/109	270/270	235/235
Casina	303/303	371/371	131/131	109/109	266/270	235/235
Ratoli	303/303	371/371	131/131	109/109	270/270	235/235
Mortarella	303/303	368/368	131/134	109/109	270/270	235/235
Tonda di Giffoni	300/303	368/368	131/134	109/109	270/270	235/235
Barcelona	297/303	368/368	131/134	109/109	269/274	235/235
Cutleaf	297/297	368/368	131/131	109/109	266/266	235/235
OSU 681.078	297/303	368/368	134/134	109/115	266/266	235/235
Barcelloner Zellernuss	297/303	368/368	131/131	109/109	270/274	235/235
Aurea	297/300	368/368	131/131	109/109	266/270	235/235
OSU 408.040	300/300	368/371	131/134	109/109	274/274	235/235
Des Anglais	300/306	368/368	131/131	109/109	266/274	235/235
OSU 26.072	303/303	368/368	131/131	109/109	266/266	235/235
Bergeri	300/303	368/371	131/131	109/109	266/266	235/235
Alli	300/303	368/368	131/131	109/109	266/274	235/235
Kadetten Zellernuss	297/303	368/368	131/131	109/109	266/266	235/235
OSU 759.010	303/303	368/371	128/131	109/109	266/270	238/238
Contorta	297/303	368/368	131/131	109/109	266/266	235/235
OSU 556.027	297/303	368/368	131/131	109/109	266/266	235/235
B3	300/306	371/371	131/134	109/109	266/266	235/235
OSU 54.039	297/300	371/371	131/134	109/109	266/226	235/235
Gunslebert	300/303	368/368	131/131	109/109	266/270	235/235
Sant Jaume	300/303	368/371	131/131	109/109	269/274	235/235
Iannusa Racinante	297/303	368/368	131/131	109/109	270/270	235/235
Gem	297/303	368/371	131/131	109/109	266/270	235/235
Artellet	300/303	368/368	131/131	109/112	270/270	235/235
Simon	297/303	368/368	131/131	109/109	269/273	235/235
Gustav's Zellernuss	297/300	368/368	131/131	109/109	266/266	235/235
Buttner's Zellernuss	303/303	368/371	131/131	109/109	266/266	235/235
Tapparona di SCC	297/303	368/368	131/131	109/109	266/270	235/235
OSU 252.146	297/303	368/371	131/134	109/109	270/274	235/235
OSU 414.062	303/303	368/371	131/134	109/109	270/274	232/232

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR242	BR245	BR246	BR249	BR253	BR255
OSU 495.049	284/284	279/285	177/177	301/308	336/336	221/221
Albania 55	284/287	279/279	177/177	294/301	336/336	219/219
Fusco Rubra	281/284	279/279	177/177	286/301	330/336	219/219
Finland COR 187	284/284	276/279	177/177	301/303	318/336	219/219
Pendula	284/284	276/276	177/180	301/301	336/345	219/219
Hall's Giant	284/284	279/279	177/180	303/303	336/345	219/219
Gasaway	284/284	279/279	177/180	301/303	324/336	219/219
Rode Zeller	284/284	279/285	177/180	301/301	329/342	219/219
Cosford	284/284	279/279	177/177	301/301	336/342	219/219
DuChilly	284/284	279/285	177/177	283/303	324/336	219/219
Palaz	284/284	279/285	177/177	301/301	336/342	219/219
Pellicule Rouge	284/284	285/285	177/177	301/303	324/336	219/219
Imperiale de Trebizonde	284/284	282/285	177/177	301/301	336/342	219/219
Tombul Ghiaghli	284/284	279/285	177/180	301/301	336/342	219/219
Tonda Bianca	284/284	279/279	177/177	301/301	336/336	219/219
Negret	284/284	279/285	177/177	301/303	324/342	219/219
Tonda Gentile delle Langhe	284/287	279/285	177/177	301/301	342/342	219/219
Tonda Romana	284/284	279/285	177/177	301/303	324/336	219/219
Romische Nuss	284/284	279/285	177/177	301/301	324/336	219/219
Casina	284/284	279/285	177/177	301/303	324/342	219/219
Ratoli	284/284	279/285	177/177	301/301	324/342	219/219
Mortarella	284/284	279/285	177/177	301/301	318/342	219/219
Tonda di Giffoni	284/284	279/285	177/177	301/301	336/342	219/219
Barcelona	284/284	279/285	177/177	301/303	336/342	219/219
Cutleaf	284/284	279/279	177/177	301/303	336/336	219/219
OSU 681.078	284/284	279/282	177/177	283/303	336/366	219/219
Barcelloner Zellernuss	284/284	276/279	177/177	294/301	336/336	219/219
Aurea	284/284	279/279	177/177	301/303	336/336	219/219
OSU 408.040	284/284	276/276	177/177	283/303	318/336	219/219
Des Anglais	284/284	279/285	177/177	301/301	324/336	219/219
OSU 26.072	284/284	276/279	177/177	289/303	324/336	219/219
Bergeri	284/284	279/279	177/180	303/303	336/336	219/219
Alli	284/284	276/279	177/177	301/303	336/336	219/219
Kadetten Zellernuss	284/284	279/279	177/177	303/303	336/336	219/219
OSU 759.010	284/284	279/285	177/177	301/301	336/336	219/221
Contorta	284/284	279/285	177/177	303/303	333/336	219/221
OSU 556.027	284/284	282/285	177/177	301/308	336/342	219/219
B3	284/284	285/285	177/177	301/303	336/342	219/219
OSU 54.039	284/284	279/279	180/180	301/301	336/342	219/219
Gunslebert	284/284	279/279	177/177	301/301	336/342	219/219
Sant Jaume	284/284	279/285	177/177	301/303	336/336	219/219
Iannusa Racinante	284/284	279/285	177/177	301/303	336/342	219/219
Gem	284/284	279/279	177/177	283/303	336/342	219/219
Artellet	284/284	270/279	177/177	301/303	324/342	219/219
Simon	284/284	279/285	177/177	301/303	342/342	219/219
Gustav's Zellernuss	284/284	279/279	177/180	303/303	336/336	219/219
Buttner's Zellernuss	284/284	279/279	177/177	303/303	342/342	219/219
Tapparona di SCC	284/284	279/285	177/177	303/303	336/342	219/219
OSU 252.146	284/284	279/279	177/177	301/303	336/342	219/219
OSU 414.062	284/284	279/279	177/177	303/303	336/342	219/219

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR257	BR259	BR261	BR262	BR264	BR267
OSU 495.049	368/371	233/248	145/145	102/102	119/122	123/123
Albania 55	362/368	224/239	142/148	102/102	119/119	129/129
Fusco Rubra	362/368	230/239	142/145	93/102	122/122	120/123
Finland COR 187	362/362	227/245	145/145	102/102	119/122	120/126
Pendula	368/368	230/230	145/145	102/108	119/125	126/129
Hall's Giant	362/368	227/233	145/145	102/102	119/119	126/126
Gasaway	362/368	233/233	145/148	102/102	119/122	126/129
Rode Zeller	362/368	230/239	145/145	102/102	119/122	123/126
Cosford	362/368	224/239	145/145	102/102	119/122	123/123
DuChilly	362/371	224/236	145/145	102/102	119/122	126/129
Palaz	362/368	224/233	145/145	102/102	119/119	129/129
Pellicule Rouge	368/371	233/236	145/148	102/102	122/122	129/129
Imperiale de Trebizonde	362/368	224/236	145/145	102/102	119/122	123/129
Tombul Ghiaghli	368/368	233/248	145/145	102/102	122/122	123/129
Tonda Bianca	362/368	233/242	145/145	102/102	119/119	123/126
Negret	368/368	233/236	145/145	93/102	119/122	123/129
Tonda Gentile delle Langhe	362/368	224/239	145/145	102/108	119/119	123/126
Tonda Romana	368/368	233/236	145/145	102/102	119/119	123/129
Romische Nuss	368/371	236/242	145/145	102/102	119/122	123/126
Casina	368/368	236/245	145/145	102/102	119/119	123/123
Ratoli	362/368	224/236	145/145	102/102	119/119	123/123
Mortarella	368/368	224/239	145/148	102/102	119/122	123/126
Tonda di Giffoni	362/368	224/233	145/145	102/102	119/122	123/129
Barcelona	368/368	224/242	145/148	102/102	119/119	123/126
Cutleaf	362/368	230/239	145/145	93/102	122/122	123/129
OSU 681.078	362/362	224/239	145/145	102/102	122/122	126/129
Barcelloner Zellernuss	362/368	236/245	142/145	102/102	119/122	123/129
Aurea	368/368	227/239	145/145	102/102	119/119	129/129
OSU 408.040	362/368	233/233	145/145	102/102	119/122	126/126
Des Anglais	362/368	224/239	145/148	102/102	119/122	129/129
OSU 26.072	362/368	224/236	145/145	102/102	119/119	123/129
Bergeri	362/368	236/239	142/145	102/102	119/122	126/126
Alli	362/368	227/242	142/145	102/102	119/119	126/129
Kadetten Zellernuss	368/368	242/245	145/145	102/102	119/119	126/129
OSU 759.010	368/368	233/248	145/145	102/102	116/122	123/129
Contorta	368/371	233/236	145/148	102/102	119/122	129/129
OSU 556.027	368/368	224/248	145/145	102/102	119/122	129/129
B3	368/371	224/242	145/145	102/102	119/122	123/129
OSU 54.039	362/371	239/239	145/145	102/102	119/122	129/129
Gunslebert	362/368	233/233	145/145	102/102	119/119	123/126
Sant Jaume	362/368	239/242	142/142	102/102	119/122	126/126
Iannusa Racinante	368/368	227/242	145/148	102/102	119/119	123/126
Gem	362/368	224/224	145/148	102/102	119/119	123/126
Artellet	362/368	236/239	145/148	102/102	119/119	123/129
Simon	368/368	233/239	145/145	102/102	119/122	126/129
Gustav's Zellernuss	368/368	227/242	145/145	102/102	119/119	126/129
Buttner's Zellernuss	362/368	224/239	145/145	102/102	119/122	123/129
Tapparona di SCC	368/368	224/242	145/145	102/102	119/122	123/123
OSU 252.146	368/368	233/248	145/148	102/102	119/122	123/126
OSU 414.062	368/368	242/242	145/148	102/102	122/122	126/129

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR270	BR276	BR277	BR279	BR284	BR288	BR292
OSU 495.049	96/96	337/337	237/237	125/125	392/395	366/366	320/320
Albania 55	96/96	337/337	235/235	125/128	395/395	366/366	320/320
Fusco Rubra	87/90	337/337	235/235	125/128	395/395	366/366	320/320
Finland COR 187	90/96	337/337	235/235	125/128	395/395	366/369	320/320
Pendula	87/90	337/337	237/239	125/125	395/395	369/369	320/323
Hall's Giant	96/96	337/337	235/235	125/128	386/395	366/366	320/320
Gasaway	90/90	337/337	235/235	128/128	395/395	366/369	320/323
Rode Zeller	96/96	340/340	235/235	125/128	395/395	366/366	320/320
Cosford	90/96	337/337	235/235	125/128	395/395	366/366	320/320
DuChilly	90/96	337/340	235/235	125/128	389/395	366/366	320/320
Palaz	90/96	337/337	235/237	125/125	392/395	366/366	320/320
Pellicule Rouge	90/96	337/337	235/237	125/125	389/395	366/366	320/320
Imperiale de Trebizonde	90/96	337/337	235/237	125/125	389/395	366/366	320/320
Tombul Ghiaghli	96/96	337/337	235/235	125/125	389/389	366/366	320/320
Tonda Bianca	90/96	337/337	235/235	128/128	386/395	366/369	320/320
Negret	90/96	337/337	233/235	125/125	389/395	366/366	221/221
Tonda Gentile delle Langhe	96/96	337/337	235/235	125/125	389/395	366/366	320/320
Tonda Romana	90/90	337/337	235/235	125/125	389/395	366/366	320/320
Romische Nuss	90/96	337/337	235/237	125/128	386/389	366/366	320/320
Casina	96/96	337/337	235/235	125/125	389/395	366/369	320/320
Ratoli	90/90	337/337	235/235	125/128	389/389	366/366	320/320
Mortarella	87/96	337/337	235/235	125/128	389/395	366/366	320/320
Tonda di Giffoni	90/96	337/337	235/235	125/128	389/395	366/369	320/320
Barcelona	90/90	337/337	235/235	125/125	389/389	366/366	320/320
Cutleaf	90/96	337/337	235/235	125/125	386/395	366/366	320/320
OSU 681.078	87/90	337/340	235/235	125/128	389/395	366/366	320/323
Barcelloner Zellernuss	90/90	337/337	235/235	125/125	395/395	366/366	320/320
Aurea	96/96	337/337	235/235	128/128	395/395	366/366	320/320
OSU 408.040	90/96	337/340	235/235	125/125	395/395	366/366	320/320
Des Anglais	90/90	337/340	235/237	125/128	389/395	366/366	320/320
OSU 26.072	93/96	337/337	235/237	125/125	392/395	366/366	320/320
Bergeri	90/90	337/337	235/235	125/125	386/395	366/366	320/323
Alli	90/90	337/337	235/235	125/125	386/395	366/369	320/323
Kadetten Zellernuss	87/96	337/337	235/235	125/125	386/395	366/369	323/323
OSU 759.010	96/99	337/337	237/237	131/131	386/395	366/366	NA/NA
Contorta	90/96	337/340	235/235	125/128	395/395	366/366	320/323
OSU 556.027	90/96	337/337	235/237	125/128	389/395	366/366	320/320
B3	90/96	337/337	235/235	125/125	389/395	366/369	320/320
OSU 54.039	90/96	337/337	235/235	125/125	392/395	366/366	320/320
Gunslebert	96/96	337/337	235/235	125/128	395/395	366/366	320/323
Sant Jaume	90/90	337/337	235/235	125/128	389/395	NA/NA	320/320
Iannusa Racinante	96/96	337/337	235/235	125/128	386/389	366/366	320/320
Gem	90/90	337/340	235/235	125/128	389/395	366/366	320/320
Artellet	90/90	337/337	235/235	128/128	395/395	366/369	320/320
Simon	90/96	337/337	233/235	125/125	395/395	366/366	320/320
Gustav's Zellernuss	87/96	337/337	235/235	125/125	395/395	366/369	320/323
Buttner's Zellernuss	90/90	337/340	235/235	125/125	395/395	366/366	320/320
Tapparona di SCC	90/96	337/337	235/235	125/125	389/395	366/366	320/323
OSU 252.146	90/96	337/337	235/235	125/125	389/395	366/366	215/221
OSU 414.062	90/90	337/337	235/357	125/125	389/395	366/366	221/221

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR294	BR302	BR307	BR311	BR315	BR316
OSU 495.049	308/308	121/124	87/87	114/114	133/133	133/133
Albania 55	308/308	124/127	87/87	114/114	133/136	133/133
Fusco Rubra	308/308	124/124	87/87	114/114	133/133	133/136
Finland COR 187	308/308	124/124	87/87	114/114	133/133	133/133
Pendula	308/308	124/124	87/87	111/114	133/136	133/133
Hall's Giant	308/308	124/124	87/87	114/114	133/133	133/133
Gasaway	308/308	124/124	87/87	114/114	133/133	133/133
Rode Zeller	308/308	124/124	84/87	114/114	133/136	133/133
Cosford	308/308	124/124	84/87	114/114	133/136	133/133
DuChilly	308/308	124/124	84/87	111/114	133/136	133/133
Palaz	308/308	124/124	84/87	114/114	136/136	133/133
Pellicule Rouge	308/308	124/124	84/87	114/114	136/136	130/133
Imperiale de Trebizonde	308/308	124/124	87/87	114/114	133/133	133/133
Tombul Ghiaghli	308/308	124/124	84/87	114/114	136/136	133/133
Tonda Bianca	308/311	124/124	84/87	114/114	133/136	133/133
Negret	308/308	124/124	84/87	114/114	133/136	133/133
Tonda Gentile delle Langhe	308/308	124/124	84/84	114/114	133/133	133/133
Tonda Romana	308/308	124/124	84/87	114/114	133/136	133/133
Romische Nuss	308/308	124/124	84/87	114/114	133/136	133/133
Casina	308/308	124/124	84/87	114/114	133/133	133/133
Ratoli	308/308	124/124	84/87	114/114	133/133	133/133
Mortarella	308/308	124/124	84/87	114/117	133/136	130/133
Tonda di Giffoni	308/308	124/124	84/87	114/114	133/136	133/133
Barcelona	308/311	124/124	84/87	114/114	133/136	133/133
Cutleaf	308/308	124/124	84/84	114/114	133/133	133/133
OSU 681.078	308/308	124/124	84/84	114/114	133/136	133/133
Barcelloner Zellernuss	308/308	124/124	87/87	111/114	133/133	130/130
Aurea	308/308	124/124	84/87	114/114	133/133	133/133
OSU 408.040	308/308	124/127	84/87	114/114	133/133	133/133
Des Anglais	308/308	124/124	84/87	114/114	133/136	130/133
OSU 26.072	308/308	124/124	84/84	114/114	136/136	133/133
Bergeri	308/308	124/124	87/87	114/114	136/136	130/133
Alli	308/308	124/124	84/87	114/114	133/136	133/133
Kadetten Zellernuss	308/308	124/124	84/87	114/114	136/136	133/133
OSU 759.010	308/308	124/124	84/87	111/114	133/133	133/133
Contorta	308/308	124/124	87/87	111/114	133/136	133/133
OSU 556.027	308/308	124/124	84/87	114/114	136/136	133/133
B3	308/308	124/124	84/87	114/114	133/136	133/133
OSU 54.039	308/308	124/124	84/87	114/114	133/133	133/133
Gunslebert	308/308	124/124	84/87	114/114	133/136	130/133
Sant Jaume	308/311	124/124	87/87	114/114	133/136	133/133
Iannusa Racinante	308/311	121/124	87/87	114/114	133/136	133/133
Gem	308/308	124/124	84/87	111/114	136/136	133/133
Artellet	308/308	124/124	84/87	114/114	133/136	133/133
Simon	308/308	124/124	84/87	114/114	133/136	133/133
Gustav's Zellernuss	308/308	124/124	84/87	114/114	133/136	133/133
Buttner's Zellernuss	308/308	124/124	87/87	111/114	133/136	133/133
Tapparona di SCC	308/308	124/124	84/87	114/114	136/136	130/133
OSU 252.146	308/311	124/124	84/84	114/114	133/136	133/133
OSU 414.062	308/311	124/124	87/87	114/114	136/136	133/133

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR322	BR325	BR327	BR331	BR332	BR335
OSU 495.049	102/102	147/150	228/231	126/129	345/345	143/143
Albania 55	99/99	150/159	228/228	126/126	345/345	143/147
Fusco Rubra	102/102	150/156	228/288	129/132	345/348	143/147
Finland COR 187	99/102	150/156	228/228	126/126	345/345	143/147
Pendula	99/105	150/150	228/231	126/126	345/345	143/147/151
Hall's Giant	99/102	141/156	228/228	126/126	345/345	143/147/157
Gasaway	96/99	147/156	228/228	126/132	345/345	143/147
Rode Zeller	105/108	150/150	228/228	129/129	345/345	143/147
Cosford	102/105	150/150	228/228	126/129	345/345	143/147
DuChilly	102/102	150/156	228/228	126/126	345/345	143/157
Palaz	102/108	147/150	228/231	126/129	345/345	143/147/157
Pellicule Rouge	108/108	150/156	228/228	126/129	345/345	143/157
Imperiale d.Trebizon.	105/108	150/150	228/228	126/129	345/345	143/154
Tombul Ghiaghli	102/105	150/156	228/231	126/129	345/345	143/147/157
Tonda Bianca	102/102	150/150	228/228	126/126	345/345	143/143
Negret	102/105	150/156	228/228	126/129	345/345	147/147
Tonda Gentile delleL.	102/105	150/150	228/228	126/129	345/345	143/147
Tonda Romana	102/105	147/150	228/228	129/129	345/345	143/147/157
Romische Nuss	102/105	147/150	228/228	126/129	345/345	143/147/157
Casina	102/105	150/156	228/228	126/129	345/345	143/147/157
Ratoli	102/105	141/150	231/231	126/126	345/345	143/147/157
Mortarella	102/105	150/150	228/228	126/126	345/357	143/147
Tonda di Giffoni	102/102	150/150	228/228	126/129	345/345	143/143/157?
Barcelona	102/105	150/156	228/228	126/126	345/351	143/147/157
Cutleaf	102/102	156/156	228/228	126/126	345/345	143/147
OSU 681.078	99/102	150/150	228/228	126/126	345/351	143/147
BarcellonerZellernuss	105/105	141/150	228/228	126/126	345/345	143/147
Aurea	102/105	150/156	228/228	126/126	345/345	143/147/151
OSU 408.040	99/102	147/150	228/228	126/126	345/345	143/147/151
Des Anglais	102/102	141/156	228/228	126/126	345/345	143/149/157
OSU 26.072	102/102	141/147	228/228	126/129	345/345	143/147
Bergeri	102/102	147/156	228/228	126/126	345/345	143/147/157
Alli	99/102	156/156	228/228	126/126	345/345	147/157
Kadetten Zellernuss	99/99	156/156	228/228	126/126	345/345	143/147/157
OSU 759.010	102/102	147/150	228/231	126/129	345/345	143/147/149
Contorta	99/102	150/156	228/228	126/126	345/345	143/147/149
OSU 556.027	102/102	156/156	228/228	129/129	345/345	143/147
B3	102/102	150/156	231/231	126/126	345/345	143/147/157
OSU 54.039	102/108	150/150	231/231	126/126	345/345	143/147
Gunslebert	99/105	150/156	228/228	126/126	345/345	143/147
Sant Jaume	102/102	156/156	228/228	126/126	345/345	147/147
Iannusa Racinante	102/102	147/147	228/228	126/126	345/345	143/147
Gem	102/105	150/150	228/228	126/126	345/351	143/157
Artellet	102/105	150/150	228/228	126/129	345/345	143/149
Simon	102/102	150/150	228/228	126/126	345/345	143/147
Gustav's Zellernuss	99/99	156/156	228/228	126/126	345/345	143/147/157
Buttner's Zellernuss	102/105	147/150	228/228	126/129	345/345	143/147/157
Tapparona di SCC	102/105	150/150	228/231	126/129	345/345	143/147/157
OSU 252.146	102/105	147/150	228/228	126/129	345/345	147/147
OSU 414.062	102/102	147/150	228/228	126/126	345/357	147/147

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR339	BR340	BR341	BR343	BR344	BR345	BR347
OSU 495.049	125/125	382/385	104/113	392/395	127/127	112/112	386/395
Albania 55	125/128	NA/NA	107/107	395/395	124/124	112/115	389/408
Fusco Rubra	125/128	388/388	107/110	395/395	124/124	112/115	386/398
Finland COR 187	125/128	370/370	104/107	395/395	127/136	112/112	389/392
Pendula	125/125	385/385	107/107	395/395	136/136	112/112	389/389
Hall's Giant	125/128	388/388	107/107	386/395	124/136	112/115	389/398
Gasaway	128/128	388/388	107/113	395/395	130/136	112/112	401/401
Rode Zeller	125/128	382/385	107/107	395/395	124/124	112/112	389/395
Cosford	125/128	385/385	107/107	395/395	124/127	112/112	389/395
DuChilly	125/128	385/385	107/107	389/395	124/124	112/112	389/392
Palaz	125/125	382/382	107/107	392/395	124/127	112/115	389/389
Pellicule Rouge	125/125	382/382	107/107	389/395	124/124	112/112	389/389
Imperiale de Trebizonde	125/125	385/385	107/116	389/395	124/124	112/112	395/395
Tombul Ghiaghli	125/125	385/385	107/107	389/389	127/127	112/112	389/395
Tonda Bianca	128/128	385/385	104/116	386/395	127/127	115/115	389/401
Negret	125/125	382/382	104/107	389/395	124/127	112/112	389/389
Tonda Gentile delle Langhe	125/125	382/385	107/107	389/395	127/127	112/112	389/389
Tonda Romana	125/125	385/385	104/104	389/395	127/127	112/112	389/389
Romische Nuss	125/128	385/385	104/116	386/389	127/127	112/112	389/389
Casina	125/125	385/385	107/107	389/395	127/127	112/115	389/389
Ratoli	125/128	385/385	104/113	389/389	127/127	112/112	389/389
Mortarella	125/128	385/385	104/107	389/395	127/127	112/112	389/401
Tonda di Giffoni	125/128	382/382	104/116	389/395	127/127	112/115	389/389
Barcelona	125/125	382/385	104/107	389/389	127/127	112/112	389/389
Cutleaf	125/125	385/388	104/116	386/395	127/136	112/112	389/395
OSU 681.078	125/128	388/388	107/116	389/395	127/127	112/112	401/401
Barcelloner Zellernuss	125/125	388/388	107/107	395/395	127/127	112/112	389/389
Aurea	128/128	379/385	107/113	395/395	130/130	112/115	389/392
OSU 408.040	125/125	370/370	107/113	395/395	124/124	112/115	389/389
Des Anglais	125/128	388/388	107/116	389/395	124/124	112/112	389/389
OSU 26.072	125/125	382/388	104/107	392/395	127/127	112/115	389/389
Bergeri	125/125	388/388	107/107	386/395	127/136	112/112	386/392
Alli	125/125	388/388	107/113	386/395	124/127	112/112	389/389
Kadetten Zellernuss	125/125	385/385	107/107	386/395	127/136	112/112	389/389
OSU 759.010	131/131	382/382	107/113	386/395	124/124	112/112	386/389
Contorta	125/128	382/382	107/116	395/395	136/136	112/112	389/396
OSU 556.027	125/128	385/385	107/113	389/395	127/127	112/112	389/401
B3	125/125	385/385	104/104	389/395	127/127	112/112	389/389
OSU 54.039	125/125	385/385	104/113	392/395	127/127	112/112	389/395
Gunslebert	125/128	385/385	107/107	395/395	127/136	112/115	389/389
Sant Jaume	125/128	385/385	107/113	389/395	124/127	112/112	389/389
Iannusa Racinante	125/128	385/385	107/116	386/389	127/127	112/112	389/407
Gem	125/128	385/385	104/107	389/395	124/124	112/112	389/392
Artellet	128/128	385/385	104/107	395/395	133/136	112/112	389/395
Simon	125/125	385/385	104/107	395/395	127/127	112/112	389/389
Gustav's Zellernuss	125/125	385/385	107/107	395/395	136/136	112/112	389/389
Buttner's Zellernuss	125/125	385/385	107/107	395/395	124/124	112/112	389/389
Tapparona di SCC	125/125	385/385	107/107	389/395	127/127	112/112	389/395
OSU 252.146	125/125	385/385	104/107	389/395	127/127	112/115	389/389
OSU 414.062	125/125	385/388	107/107	389/395	127/127	112/112	389/395

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR349	BR352	BR355	BR357	BR358	BR359
OSU 495.049	240/240	100/100	198/198	109/109	119/122	390/399
Albania 55	234/234	100/109	198/198	112/112	119/119	384/384
Fusco Rubra	234/234	118/118	198/201	109/112	122/122	384/384
Finland COR 187	234/234	109/109	198/198	109/112	119/122	384/395
Pendula	234/234	109/112	198/198	112/116	119/125	384/384
Hall's Giant	234/234	109/112	198/198	112/112	119/119	389/389
Gasaway	234/234	109/109	198/198	109/109	119/122	384/384
Rode Zeller	234/234	109/112	198/201	112/112	119/122	384/389
Cosford	234/234	109/112	198/201	112/116	119/122	384/389
DuChilly	234/237	109/112	198/198	112/116	119/122	389/389
Palaz	234/234	109/112	198/198	109/116	119/119	384/384
Pellicule Rouge	234/234	112/112	198/198	109/116	122/122	389/393
Imperiale de Trebizonde	234/234	112/112	198/198	109/116	119/122	389/393
Tombul Ghiaghli	234/234	112/112	198/198	109/116	122/122	384/394
Tonda Bianca	234/234	109/109	198/198	109/109	119/119	383/391
Negret	235/235	109/112	198/198	109/109	119/122	385/394
Tonda Gentile delle Langhe	234/234	109/112	198/198	109/109	119/119	384/384
Tonda Romana	234/234	109/112	198/204	109/116	119/119	384/390
Romische Nuss	234/237	109/112	198/198	109/116	119/122	383/384
Casina	234/237	109/112	198/198	112/116	119/119	393/393
Ratoli	234/237	109/112	198/198	109/112	119/119	384/393
Mortarella	234/234	109/112	198/198	109/109	119/122	383/384
Tonda di Giffoni	234/234	109/112	195/198	109/116	119/122	384/393
Barcelona	234/234	109/112	198/198	109/112	119/119	384/397
Cutleaf	234/234	109/112	198/198	109/116	122/122	384/384
OSU 681.078	234/234	100/109	198/198	109/109	122/122	383/384
Barcelloner Zellernuss	234/234	109/109	198/204	112/112	119/122	383/384
Aurea	234/234	109/112	198/198	112/112	119/119	383/383
OSU 408.040	234/234	109/112	198/198	116/116	119/122	383/384
Des Anglais	234/234	109/112	198/198	109/116	119/122	384/390
OSU 26.072	234/234	103/112	198/198	109/109	119/119	383/396
Bergeri	234/234	109/112	198/198	112/116	119/122	384/389
Alli	234/234	109/109	198/198	112/112	119/119	383/384
Kadetten Zellernuss	234/234	109/112	198/198	112/112	119/119	384/384
OSU 759.010	237/237	103/112	198/201	109/109	116/122	384/399
Contorta	234/237	109/112	198/198	112/112	119/122	384/384
OSU 556.027	234/234	109/112	198/198	109/112	119/122	384/384
B3	234/234	109/112	198/198	109/112	119/122	384/384
OSU 54.039	234/234	112/112	198/198	109/109	119/122	384/386
Gunslebert	234/234	109/112	195/198	109/112	119/119	384/390
Sant Jaume	234/234	109/112	198/198	112/112	119/122	384/384
Iannusa Racinante	234/234	109/112	195/198	109/116	119/119	384/384
Gem	234/234	109/109	198/198	109/116	119/119	384/389
Artellet	234/234	109/112	198/198	112/112	119/119	384/397
Simon	234/234	109/112	198/198	109/116	119/122	393/397
Gustav's Zellernuss	234/234	109/112	198/198	112/112	119/119	384/390
Buttner's Zellernuss	234/234	109/112	198/198	112/116	119/122	383/389
Tapparona di SCC	234/234	109/112	198/198	112/116	119/122	384/384
OSU 252.146	235/235	109/109	198/198	109/112	119/122	384/395
OSU 414.062	235/235	109/112	198/198	109/109	122/122	384/399

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR361	BR362	BR371	BR374	BR375	BR379
OSU 495.049	377/377	201/204	273/273	239/239	253/253	115/133
Albania 55	368/377	204/204	270/270	216/223	250/253	130/130
Fusco Rubra	377/377	204/204	270/270	225/225	253/256	112/127
Finland COR 187	377/377	204/204	270/270	239/239	253/253	133/136
Pendula	377/377	204/204	270/279	225/225	253/259	112/112
Hall's Giant	368/377	204/204	273/273	225/225	253/259	115/133
Gasaway	368/377	201/201	270/270	225/225	256/259	153/153
Rode Zeller	368/377	204/204	270/273	225/225	253/259	112/112
Cosford	377/377	204/204	270/273	225/225	253/259	112/115
DuChilly	368/377	204/204	270/273	239/239	253/256	112/150
Palaz	368/377	204/204	273/273	235/235	256/268	133/161
Pellicule Rouge	368/377	204/204	270/273	216/216	256/265	145/150
Imperiale de Trebizonde	377/377	204/204	273/279	235/235	256/268	115/161
Tombul Ghiaghli	368/377	204/204	270/273	225/225	253/256	133/150
Tonda Bianca	368/368	204/204	270/270	223/225	256/256	115/150
Negret	377/377	201/204	270/273	236/242	253/256	130/133
Tonda Gentile delle Langhe	377/377	201/201	270/270	235/245	253/256	127/133
Tonda Romana	368/377	204/204	270/270	216/216	256/259	115/115
Romische Nuss	368/377	204/204	270/270	235/235	253/256	115/133
Casina	377/377	204/204	270/270	235/235	256/256	112/133
Ratoli	368/377	204/204	270/273	235/235	253/256	133/150
Mortarella	368/377	201/201	273/273	235/235	253/259	133/150
Tonda di Giffoni	368/377	204/204	270/270	235/235	256/256	133/150
Barcelona	368/377	204/204	270/270	235/235	256/259	133/150
Cutleaf	368/377	204/204	270/273	225/225	256/256	130/146
OSU 681.078	368/377	204/204	270/273	225/225	253/259	115/133
Barcelloner Zellernuss	377/377	204/204	270/270	239/239	253/259	136/136
Aurea	377/385	204/204	270/270	225/225	256/259	133/146
OSU 408.040	377/377	204/204	270/270	216/225	256/259	112/146
Des Anglais	368/377	204/204	270/273	216/216	253/265	150/164
OSU 26.072	377/377	204/204	270/273	241/241	253/253	127/143
Bergeri	377/377	204/204	270/276	225/225	253/256	133/150
Alli	368/377	204/204	270/276	216/225	253/256	133/146
Kadetten Zellernuss	368/377	204/204	270/276	216/216	250/253	133/146
OSU 759.010	377/377	204/204	273/273	235/235	253/265	115/127
Contorta	368/377	204/204	270/270	241/241	253/256	127/150
OSU 556.027	368/377	204/204	273/273	235/235	256/259	130/150
B3	368/377	204/204	270/270	216/216	253/256	115/150
OSU 54.039	368/377	204/204	270/273	225/225	265/265	127/150
Gunslebert	377/377	204/204	270/276	223/225	253/256	115/146
Sant Jaume	377/377	204/204	270/270	235/235	253/259	133/150
Iannusa Racinante	377/377	204/204	270/270	235/235	253/259	133/150
Gem	368/368	204/204	270/273	216/223	256/259	133/150
Artellet	377/377	204/204	270/270	235/235	256/259	127/130
Simon	377/377	201/204	270/273	235/235	256/256	133/150
Gustav's Zellernuss	368/377	204/204	273/276	225/225	253/259	115/146
Buttner's Zellernuss	377/377	204/204	270/273	241/241	253/253	112/112
Tapparona di SCC	377/377	201/204	270/273	235/243	253/256	129/133
OSU 252.146	368/377	204/204	273/273	236/236	256/259	115/133
OSU 414.062	377/377	204/204	273/273	236/236	256/259	133/133

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR381	BR387	BR389	BR392	BR396	BR397
OSU 495.049	127/130	374/377	320/320	221/221	145/145	250/250
Albania 55	127/130	368/368	320/329	215/221	139/145	238/247
Fusco Rubra	NA/NA	377/377	320/329	221/221	145/145	238/238
Finland COR 187	127/130	371/377	320/320	221/221	145/145	250/250
Pendula	127/127	371/377	320/320	221/221	145/145	247/250
Hall's Giant	127/127	377/377	320/320	215/221	145/145	238/247
Gasaway	127/127	377/377	320/329	221/221	139/145	247/250
Rode Zeller	127/127	374/374	320/320	221/221	145/145	250/250
Cosford	127/127	365/365	320/329	221/221	145/145	247/250
DuChilly	127/130	374/377	320/320	221/221	145/145	250/250
Palaz	127/127	371/377	320/320	221/221	145/148	250/253
Pellicule Rouge	127/130	371/377	320/320	221/221	145/145	250/253
Imperiale de Trebizonde	127/130	365/374	320/329	221/221	145/148	247/253
Tombul Ghiaghli	127/127	371/377	320/320	221/221	145/145	250/250
Tonda Bianca	127/127	374/377	320/329	215/215	145/145	238/250
Negret	127/130	365/372	320/320	221/221	145/145	238/253
Tonda Gentile delle Langhe	127/127	371/377	320/320	221/221	145/145	238/253
Tonda Romana	127/130	377/377	320/320	221/221	145/145	247/253
Romische Nuss	127/130	377/377	320/320	215/221	145/145	250/253
Casina	127/130	365/365	320/320	221/221	145/145	250/253
Ratoli	130/130	371/377	320/320	215/221	145/145	250/253
Mortarella	127/130	371/371	320/320	221/221	145/145	238/253
Tonda di Giffoni	127/130	377/377	320/329	215/221	145/145	238/253
Barcelona	127/130	374/380	320/320	215/221	145/145	238/253
Cutleaf	127/127	365/365	320/320	221/221	145/145	238/250
OSU 681.078	127/140	371/377	320/320	221/221	145/145	250/250
Barcelloner Zellernuss	127/127	365/377	320/320	221/221	145/145	250/250
Aurea	127/127	377/377	320/320	221/221	145/145	238/247
OSU 408.040	127/127	377/377	320/320	221/221	145/145	250/250
Des Anglais	130/130	377/377	320/320	215/221	145/145	250/253
OSU 26.072	124/127	374/374	320/320	215/221	145/145	247/247
Bergeri	127/127	377/377	NA/NA	221/221	145/145	238/250
Alli	127/130	377/377	320/320	221/221	145/145	238/250
Kadetten Zellernuss	127/127	377/377	320/329	221/221	145/145	238/250
OSU 759.010	127/130	365/371	320/320	221/221	145/145	250/250
Contorta	127/127	365/374	320/329	221/221	145/145	247/250
OSU 556.027	127/127	371/377	320/320	221/221	145/145	238/250
B3	127/130	377/377	320/320	221/221	139/145	250/253
OSU 54.039	127/127	371/374	320/329	221/221	145/145	250/250
Gunslebert	127/127	374/377	320/329	221/221	145/145	238/253
Sant Jaume	130/130	374/377	320/320	221/221	145/145	250/253
Iannusa Racinante	127/130	374/377	320/329	221/221	145/145	238/253
Gem	127/127	380/380	320/320	215/221	145/145	250/253
Artellet	127/130	374/377	320/320	221/221	145/145	238/253
Simon	127/127	371/377	320/320	221/221	145/145	238/253
Gustav's Zellernuss	127/127	374/377	320/329	221/221	145/145	238/250
Buttner's Zellernuss	127/127	365/377	320/329	215/221	145/145	238/250
Tapparona di SCC	127/130	377/377	320/320	221/221	139/145	247/253
OSU 252.146	127/127	372/372	320/320	215/221	145/145	250/253
OSU 414.062	127/127	374/374	320/320	221/221	145/145	247/253

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR398	BR402	BR406	BR406	BR410	BR411	BR412
OSU 495.049	164/168	130/134	212/212	212/212	325/325	124/124	281/290/296/302
Albania 55	168/168	134/134	212/212	212/212	322/322	124/124	287/293/296/302
Fusco Rubra	168/168	128/134	212/212	212/212	322/322	124/124	275/284/290/299
Finland COR 187	168/168	128/128	212/212	212/212	322/325	124/130	290/299
Pendula	168/168	128/134	212/212	212/212	322/325	124/124	290/299
Hall's Giant	168/168	128/129	212/212	212/212	322/322	124/124	290/302
Gasaway	168/168	128/134	212/212	212/212	322/322	124/130	299/302
Rode Zeller	168/168	128/129	212/212	212/212	322/325	124/124	272/281/290/299
Cosford	168/168	130/134	212/212	212/212	322/322	124/124	290/299
DuChilly	168/168	130/130	212/212	212/212	322/325	121/124	272/281/290/299
Palaz	168/168	130/134	212/212	212/212	325/325	124/124	272/290
Pellicule Rouge	168/168	130/134	212/212	212/212	325/325	121/124	272/281/290/299
Imperiale d. Trebizon.	168/168	134/134	212/212	212/212	322/325	121/124	272/275/281/284
Tombul Ghiaghli	168/168	134/134	212/212	212/212	325/325	124/124	272/290
Tonda Bianca	168/168	128/128	212/212	212/212	322/325	124/133	296/299/302
Negret	168/168	134/134	212/212	212/212	325/325	124/124	290/299
Tonda Gentiledelle L.	168/168	130/134	212/212	212/212	322/322	124/124	290/302
Tonda Romana	168/168	128/129	212/212	212/212	325/325	121/124	281/284/296/299
Romische Nuss	168/168	130/134	212/212	212/212	325/325	124/130	278/287/290/299
Casina	168/168	134/134	212/212	212/212	325/325	121/121	287/290
Ratoli	168/168	128/134	212/212	212/212	322/325	124/124	299/299
Mortarella	168/168	128/129	212/212	212/212	322/322	121/130	290/299
Tonda di Giffoni	168/168	128/134	212/212	212/212	322/325	124/133	296/299
Barcelona	168/168	130/134	212/212	212/212	322/325	121/130	278/287/290/299
Cutleaf	168/168	134/134	212/212	212/212	322/322	121/124	299/299
OSU 681.078	168/168	134/140	212/218	212/218	322/322	121/124	278/287/290/299
BarcellonerZellernuss	168/168	128/134	212/212	212/212	322/322	121/124	299/299
Aurea	168/168	128/134	212/212	212/212	322/322	121/124	290/293/299/303
OSU 408.040	168/168	128/134	212/212	212/212	322/325	121/121	278/287/290/299
Des Anglais	168/168	128/134	212/212	212/212	322/325	121/124	272/281/290/299
OSU 26.072	168/168	134/134	212/212	212/212	322/325	124/124	287/290/299/303
Bergeri	168/168	128/129	212/212	212/212	322/322	124/124	290/299
Alli	168/168	128/134	212/212	212/212	322/325	124/124	278/287/290/299
Kadetten Zellernuss	168/168	128/129	212/212	212/212	322/322	124/124	278/290/302
OSU 759.010	168/168	130/134	212/212	212/212	322/325	124/124	281/290
Contorta	168/168	128/134	212/212	212/212	325/325	124/124	299/299
OSU 556.027	168/168	130/134	212/212	212/212	322/325	124/124	290/299
B3	168/168	128/129	212/212	212/212	322/322	124/124	299/299
OSU 54.039	168/168	134/134	212/212	212/212	325/325	124/124	272/290
Gunslebert	168/168	128/128	212/212	212/212	322/325	124/133	290/302
Sant Jaume	168/168	130/134	212/212	212/212	322/322	121/130	299/299
Iannusa Racinante	168/168	128/128	212/212	212/212	322/322	124/127	290/299
Gem	168/168	130/130	212/212	212/212	322/325	121/130	278/287/290/299
Artellet	168/168	130/134	212/215	212/215	322/322	124/124	296/296
Simon	168/168	134/134	212/212	212/212	325/325	121/124	290/299
Gustav's Zellernuss	168/168	130/130	212/212	212/212	322/322	124/124	278/290/302
Buttner's Zellernuss	168/168	130/134	212/212	212/212	322/322	124/124	290/299
Tapparona di SCC	168/168	130/134	212/212	212/212	322/322	124/124	278/287/290/299
OSU 252.146	168/168	134/134	212/212	212/212	322/325	124/124	278/287/296/305
OSU 414.062	168/168	128/134	212/212	212/212	322/325	127/127	296/299

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR413	BR414	BR415	BR418	BR420	BR423
OSU 495.049	242/242	118/121	248/257	122/122	91/94	112/115
Albania 55	242/242	127/137	248/254	122/122	94/100	106/112
Fusco Rubra	242/242	121/134	254/254	122/122	85/94	103/106
Finland COR 187	242/242	121/121	248/254	122/122	85/91	103/106
Pendula	233/242	121/134	239/254	122/122	94/94	103/106
Hall's Giant	242/242	121/121	248/257	122/122	94/94	106/115
Gasaway	239/242	121/131	251/254	122/122	91/94	103/106
Rode Zeller	230/233	121/134	254/257	122/122	91/100	103/115
Cosford	233/242	121/121	257/257	122/122	91/94	112/115
DuChilly	242/242	118/131	248/248	122/122	94/94	106/115
Palaz	242/242	118/118	248/251	122/122	91/94	112/115
Pellicule Rouge	242/242	118/121	248/248	122/122	91/94	112/115
Imperiale de Trebizonde	242/242	118/134	248/248	122/122	91/91	112/115
Tombul Ghiaghli	242/242	118/121	248/251	122/122	91/94	115/115
Tonda Bianca	242/242	121/121	248/254	122/122	94/94	112/115
Negret	242/245	118/121	248/248	122/122	88/91	106/115
Tonda Gentile delle Langhe	242/242	118/121	248/254	122/122	94/94	112/115
Tonda Romana	239/242	118/121	248/248	122/122	88/94	112/115
Romische Nuss	242/242	118/121	248/248	122/122	94/94	115/115
Casina	242/242	118/121	248/254	122/122	94/94	106/115
Ratoli	242/242	118/121	248/248	122/122	94/94	115/115
Mortarella	242/245	121/137	248/248	122/122	91/94	106/115
Tonda di Giffoni	242/242	118/121	248/248	122/122	91/94	115/115
Barcelona	242/245	118/121	248/254	122/122	91/94	106/115
Cutleaf	242/242	121/134	254/254	122/122	94/94	112/112
OSU 681.078	242/242	109/121	248/248	122/122	94/100	106/106
Barcelloner Zellernuss	233/242	109/121	248/260	122/122	91/100	112/115
Aurea	233/233	109/112	239/254	122/122	94/97	106/112
OSU 408.040	242/242	118/121	239/239	122/122	100/100	112/112
Des Anglais	242/242	118/121	248/254	122/122	94/100	106/115
OSU 26.072	230/233	112/121	248/254	122/122	85/91	115/115
Bergeri	242/242	121/137	248/257	122/122	94/94	106/115
Alli	242/242	121/121	248/254	122/122	91/94	106/112
Kadetten Zellernuss	242/242	121/121	248/248	122/122	94/94	112/112
OSU 759.010	242/242	112/121	248/251	122/122	91/94	112/112
Contorta	233/242	118/121	248/248	122/122	91/94	112/112
OSU 556.027	233/242	118/121	248/254	122/136	91/91	112/115
B3	242/242	124/124	248/254	122/122	91/100	106/115
OSU 54.039	242/242	118/121	248/248	122/122	91/94	106/115
Gunslebert	242/242	118/121	248/248	122/122	94/94	112/115
Sant Jaume	230/245	121/121	254/254	122/122	91/91	112/115
Iannusa Racinante	242/242	121/121	251/254	122/122	94/94	112/115
Gem	242/242	118/121	248/248	122/122	94/94	106/106
Artellet	242/245	118/121	248/257	122/122	91/94	106/106
Simon	242/242	112/118	248/248	122/122	91/91	106/112
Gustav's Zellernuss	242/242	119/121	248/257	122/122	94/94	112/115
Buttner's Zellernuss	233/242	119/121	257/257	122/122	91/94	106/112
Tapparona di SCC	242/245	124/124	248/254	122/122	94/94	106/115
OSU 252.146	233/242	118/121	248/254	122/122	94/94	106/112
OSU 414.062	242/242	118/121	248/254	126/126	94/94	112/115

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR425	BR427	BR430	BR433	BR437	BR438	BR442
OSU 495.049	277/277	310/310	263/266	134/134	145/148	195/197	200/200
Albania 55	277/277	316/316	263/263	144/144	148/151	191/193	200/225
Fusco Rubra	277/277	316/316	263/263	134/134	148/148	193/195	198/198
Finland COR 187	277/277	310/316	263/263	139/139	148/148	193/193	198/198
Pendula	277/277	316/316	263/263	139/139	148/148	191/193	198/198
Hall's Giant	277/277	310/316	263/263	134/139	151/151	191/193	200/200
Gasaway	277/277	316/316	263/263	139/139	145/148	193/195	198/198
Rode Zeller	277/277	316/316	263/263	150/150	148/148	193/193	200/200
Cosford	277/277	316/316	263/263	144/144	151/151	191/193	200/200
DuChilly	277/283	310/316	263/263	149/149	151/151	193/195	200/200
Palaz	277/277	310/310	263/263	139/144	148/148	193/195	198/200
Pellicule Rouge	277/283	310/310	263/266	NA/NA	148/151	193/193	200/200
Imperiale de Trebizon.	277/277	310/310	263/263	139/144	148/148	191/193	198/200
Tombul Ghiaghli	277/283	310/310	263/263	144/144	148/148	193/195	198/200
Tonda Bianca	277/277	310/316	263/263	144/144	148/148	191/193	198/198
Negret	268/277	310/316	263/263	101/144	148/148	193/193	200/200
Tonda Gentile delle L.	277/277	310/310	263/263	144/144	148/148	193/193	198/198
Tonda Romana	277/283	310/316	263/263	144/144	148/148	191/193	198/198
Romische Nuss	277/283	310/316	263/263	144/149	148/148	191/193	198/200
Casina	277/283	310/316	263/263	144/144	148/148	193/193	198/200
Ratoli	277/283	310/310	263/263	144/144	148/148	191/193	198/198
Mortarella	277/277	310/316	263/263	144/144	148/148	193/193	198/200
Tonda di Giffoni	277/283	310/316	263/263	144/144	148/148	191/193	200/200
Barcelona	277/283	310/310	263/263	144/149	148/148	191/193	198/198
Cutleaf	277/277	316/316	263/263	139/144	145/148	193/193	198/198
OSU 681.078	277/277	304/316	263/263	144/144	148/148	191/191	200/200
BarcellonerZellernuss	277/277	304/310	263/263	139/139	148/148	191/193	198/198
Aurea	277/277	316/316	263/263	144/144	148/148	193/193	198/200
OSU 408.040	277/277	316/319	263/263	144/144	148/148	193/193	200/200
Des Anglais	277/283	310/310	263/263	144/144	145/151	193/193	198/200
OSU 26.072	277/277	310/316	260/263	139/139	145/145	193/197	198/200
Bergeri	277/283	310/316	263/263	144/144	148/148	191/193	198/198
Alli	277/277	316/316	263/263	144/144	148/148	193/193	198/200
Kadetten Zellernuss	277/277	316/316	263/263	144/144	148/148	193/193	198/200
OSU 759.010	277/277	310/313	263/266	149/149	148/148	193/197	198/198
Contorta	277/277	310/310	263/263	144/144	148/148	193/193	198/198
OSU 556.027	277/277	310/310	263/266	144/144	145/148	191/195	200/200
B3	265/283	310/316	263/263	149/149	148/148	191/195	198/200
OSU 54.039	277/277	310/310	263/263	144/144	145/148	193/195	198/200
Gunslebert	277/277	310/316	263/266	144/144	148/148	191/193	200/200
Sant Jaume	277/277	310/316	263/263	144/144	148/148	193/193	198/200
Iannusa Racinante	274/283	310/316	263/263	144/144	142/148	193/193	198/200
Gem	283/283	310/316	263/263	144/149	148/151	191/193	198/200
Artellet	277/277	310/316	263/263	144/144	148/148	191/193	198/200
Simon	277/277	316/316	263/263	144/144	148/148	191/193	198/200
Gustav's Zellernuss	277/277	316/316	263/263	144/144	148/148	191/193	198/198
Buttner's Zellernuss	277/277	316/316	263/263	139/139	151/151	191/193	198/200
Tapparona di SCC	277/283	310/316	263/263	144/144	148/148	191/193	198/200
OSU 252.146	277/277	310/316	263/263	150/150	148/148	191/193	198/200
OSU 414.062	277/277	310/310	263/263	144/144	148/148	191/193	198/200

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR444	BR446	BR451	BR456	BR464	BR467
OSU 495.049	106/106	156/162	137/137	139/139	284/296	150/150
Albania 55	103/103	153/162	134/134	139/139	278/284	150/150
Fusco Rubra	103/106	153/153	134/134	139/139	272/278	150/150
Finland COR 187	103/103	153/162	134/134	139/139	278/278	150/150
Pendula	103/106	153/153	134/134	139/139	278/290	150/150
Hall's Giant	103/106	156/162	134/134	139/139	278/278	150/150
Gasaway	103/103	153/153	134/134	139/142	278/278	150/150
Rode Zeller	103/106	156/162	134/134	139/142	284/284	150/150
Cosford	103/106	156/162	134/134	139/142	284/284	150/150
DuChilly	103/106	156/162	134/134	139/139	278/284	141/150
Palaz	103/106	156/162	134/134	139/139	278/278	150/154
Pellicule Rouge	103/106	156/162	134/134	139/139	284/284	141/150
Imperiale de Trebizonde	103/106	156/162	134/137	139/139	278/284	150/150
Tombul Ghiaghli	103/106	153/162	134/134	139/139	278/284	150/150
Tonda Bianca	103/106	153/162	134/134	139/142	278/284	150/150
Negret	103/106	153/156	134/134	139/142	278/278	150/154
Tonda Gentile delle Langhe	103/106	153/156	134/134	139/142	278/284	150/150
Tonda Romana	103/106	153/156	134/134	139/142	278/284	150/150
Romische Nuss	103/106	156/159	134/134	139/139	278/278	150/150
Casina	103/106	153/156	134/134	139/142	278/284	150/150
Ratoli	103/106	156/156	134/134	139/139	278/278	150/150
Mortarella	106/106	153/156	134/134	139/139	278/284	150/150
Tonda di Giffoni	103/106	156/162	134/134	139/139	278/278	150/150
Barcelona	103/106	153/156	134/134	139/139	278/284	150/150
Cutleaf	103/103	153/162	134/134	139/139	278/278	141/150
OSU 681.078	103/106	153/162	134/134	139/139	278/290	150/150
Barcelloner Zellernuss	103/103	153/162	134/134	139/142	284/290	150/150
Aurea	103/103	153/153	134/134	139/142	278/278	141/150
OSU 408.040	103/106	153/159	134/134	139/139	284/290	150/150
Des Anglais	103/106	153/156	134/134	139/139	278/284	141/150
OSU 26.072	103/106	156/162	134/134	139/142	284/284	150/150
Bergeri	103/106	156/159	134/134	139/142	284/290	150/150
Alli	103/106	153/162	134/134	139/142	278/278	150/150
Kadetten Zellernuss	103/106	153/153	134/134	142/142	278/284	150/150
OSU 759.010	103/106	156/162	134/134	139/139	278/284	150/150
Contorta	103/106	153/162	134/134	142/142	278/278	150/150
OSU 556.027	103/103	153/162	134/134	139/139	278/278	150/150
B3	103/103	153/156	134/134	139/142	278/290	141/150
OSU 54.039	103/103	153/156	134/134	139/139	278/278	150/154
Gunslebert	103/103	153/153	134/134	139/142	278/278	150/150
Sant Jaume	103/106	153/156	134/134	139/139	278/278	150/150
Iannusa Racinante	103/106	153/156	134/134	139/139	278/278	150/150
Gem	103/106	153/156	134/134	139/139	278/278	150/150
Artellet	100/103	156/156	134/134	139/142	278/290	150/150
Simon	103/106	153/156	134/134	139/139	278/284	150/154
Gustav's Zellernuss	106/106	153/156	134/134	139/142	278/278	150/150
Buttner's Zellernuss	103/103	156/162	134/134	139/142	278/284	150/150
Tapparona di SCC	103/103	153/156	134/134	139/139	278/290	150/150
OSU 252.146	103/106	153/156	134/134	139/139	278/284	150/154
OSU 414.062	103/106	153/156	134/134	139/139	278/278	150/150

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR468	BR470	BR474	BR475	BR478	BR480
OSU 495.049	361/373	334/334	122/125	237/237	205/205	134/138
Albania 55	373/373	334/334	122/122	237/237	203/205	139/145
Fusco Rubra	373/373	334/334	122/122	237/240	205/205	137/139
Finland COR 187	373/373	334/334	125/125	237/237	203/207	137/137
Pendula	373/373	334/334	125/125	237/237	207/207	137/137
Hall's Giant	373/373	334/334	122/125	237/240	203/205	137/137
Gasaway	373/373	334/334	122/125	237/237	203/205	134/137
Rode Zeller	373/373	334/340	122/122	237/237	205/205	137/139
Cosford	373/373	334/340	122/122	237/237	205/207	137/137
DuChilly	373/373	334/340	125/125	237/237	205/205	132/137
Palaz	373/373	334/334	122/122	237/240	205/205	137/137
Pellicule Rouge	373/373	334/334	122/125	237/237	205/205	132/137
Imperiale de Trebizonde	373/373	334/334	122/125	237/237	205/205	132/137
Tombul Ghiaghli	373/373	334/334	122/122	237/240	205/205	134/138
Tonda Bianca	373/373	334/334	122/122	237/237	203/207	137/137
Negret	373/373	334/334	122/122	237/240	205/207	138/138
Tonda Gentile delle Langhe	373/376	334/334	122/122	237/240	205/207	132/132
Tonda Romana	373/373	334/334	122/122	237/240	205/205	132/137
Romische Nuss	373/373	334/334	122/122	237/240	205/205	132/132
Casina	373/373	334/334	122/122	237/240	205/205	132/132
Ratoli	373/373	334/334	122/122	237/240	205/205	132/138
Mortarella	373/373	334/334	122/125	237/240	205/205	132/132
Tonda di Giffoni	373/373	334/334	122/122	237/240	205/207	137/137
Barcelona	373/373	334/334	122/125	237/240	203/205	132/137
Cutleaf	373/373	334/334	122/125	237/240	205/205	137/137
OSU 681.078	373/373	334/334	122/122	237/240	205/205	134/137
Barcelloner Zellernuss	373/373	334/340	122/122	237/237	205/207	134/137
Aurea	373/373	334/334	122/122	237/240	203/207	132/148
OSU 408.040	373/373	334/334	122/122	237/237	205/205	134/137
Des Anglais	373/373	334/334	122/125	237/237	205/205	134/137
OSU 26.072	373/376	334/334	122/125	240/243	205/205	132/132
Bergeri	373/373	334/334	122/125	237/240	205/205	132/132
Alli	373/373	334/334	122/122	240/240	205/205	131/137
Kadetten Zellernuss	373/373	334/334	122/125	237/240	203/205	131/134
OSU 759.010	373/373	334/334	122/125	240/240	205/205	134/137
Contorta	373/373	334/334	122/125	237/237	205/205	134/134
OSU 556.027	373/373	334/334	122/125	237/237	205/205	132/138
B3	373/373	334/334	122/122	237/237	205/209	132/138
OSU 54.039	373/373	334/334	122/122	237/237	205/205	131/134
Gunslebert	373/373	334/334	122/125	237/237	203/205	134/137
Sant Jaume	373/373	334/334	122/122	237/240	203/205	131/137
Iannusa Racinante	373/376	334/334	122/125	237/240	207/207	132/137
Gem	373/373	334/340	122/125	237/240	205/205	132/137
Artellet	373/373	334/334	122/125	237/237	205/205	132/137
Simon	373/373	334/334	122/125	237/240	203/205	137/137
Gustav's Zellernuss	373/373	334/334	122/122	237/237	203/203	134/137
Buttner's Zellernuss	373/373	334/340	122/125	237/237	205/205	137/137
Tapparona di SCC	373/373	334/334	122/125	240/240	205/207	132/137
OSU 252.146	373/373	334/334	122/125	237/240	205/207	134/136
OSU 414.062	373/373	334/334	122/122	237/240	205/207	138/138

Appendix E (cont). SSR profiles of 50 accessions at 113 loci.

Cultivar	BR482	BR483	BR484	BR485	BR487	BR488
OSU 495.049	286/296	298/310	363/371	127/131	371/375	258/264
Albania 55	282/286	296/296	373/373	131/133	369/369	264/264
Fusco Rubra	286/286	288/296	373/375	127/133	371/371	264/268
Finland COR 187	286/286	288/296	367/367	133/133	371/375	264/264
Pendula	282/282	296/302	367/367	127/127	371/371	258/264
Hall's Giant	286/286	288/310	367/367	127/135	371/375	258/264
Gasaway	286/286	288/298	363/367	127/127	371/371	264/264
Rode Zeller	286/286	310/310	367/367	127/135	371/381	264/266
Cosford	284/286	288/310	363/367	127/135	371/381	264/266
DuChilly	286/286	296/310	363/367	127/127	369/373	264/264
Palaz	282/288	288/298	367/367	131/131	369/375	264/268
Pellicule Rouge	286/288	288/310	363/367	127/135	369/375	264/264
Imperiale de Trebizonde	286/288	282/298	367/367	131/135	375/375	264/268
Tombul Ghiaghli	282/288	288/298	367/367	127/131	369/369	264/264
Tonda Bianca	284/286	306/306	367/367	127/127	371/375	250/258
Negret	288/288	296/310	367/367	131/133	371/373	264/266
Tonda Gentile delle Langhe	286/286	288/310	363/367	131/133	371/371	264/264
Tonda Romana	286/290	296/310	363/367	131/135	369/371	264/264
Romische Nuss	286/286	282/292	363/367	131/133	369/375	264/264
Casina	286/288	288/310	367/375	127/135	369/371	264/264
Ratoli	286/288	282/282	367/375	127/131	369/369	264/264
Mortarella	286/286	292/310	363/367	133/135	369/375	264/264
Tonda di Giffoni	286/288	306/310	367/367	127/131	369/369	258/264
Barcelona	282/286	302/310	367/367	133/135	369/371	264/264
Cutleaf	282/286	296/296	363/367	127/135	371/371	264/264
OSU 681.078	286/286	296/306	367/375	127/127	371/371	264/264
Barcelloner Zellernuss	286/288	288/288	367/375	127/135	371/371	264/266
Aurea	286/286	296/296	375/375	127/135	375/375	264/264
OSU 408.040	286/286	302/310	367/375	127/135	371/375	264/264
Des Anglais	286/288	296/310	367/375	127/135	369/379	264/264
OSU 26.072	286/290	292/308	367/371	127/133	373/373	268/268
Bergeri	282/286	296/310	367/375	127/129	369/375	264/264
Alli	286/286	296/302	375/375	127/135	375/375	264/266
Kadetten Zellernuss	286/286	296/296	375/375	127/135	371/375	264/264
OSU 759.010	286/288	288/288	363/367	131/131	371/373	264/264
Contorta	286/286	296/296	373/375	129/129	371/379	264/266
OSU 556.027	286/288	288/298	367/367	127/131	371/371	264/266
B3	286/286	296/310	367/367	127/131	369/371	264/266
OSU 54.039	282/288	282/298	367/367	131/131	369/369	264/266
Gunslebert	284/286	296/296	367/375	127/135	375/375	258/264
Sant Jaume	286/286	292/310	367/367	131/133	371/371	264/266
Iannusa Racinante	286/286	306/310	367/367	131/135	375/375	264/264
Gem	286/286	302/310	367/367	127/133	369/369	264/264
Artellet	282/286	282/296	367/375	127/127	371/375	264/264
Simon	282/288	302/310	367/367	131/135	371/371	264/264
Gustav's Zellernuss	286/286	296/310	367/375	135/135	371/375	258/264
Buttner's Zellernuss	286/288	288/310	367/367	127/127	375/381	264/266
Tapparona di SCC	286/288	296/296	367/367	127/131	369/373	264/266
OSU 252.146	282/284	302/310	367/367	127/131	371/381	264/264
OSU 414.062	282/286	292/310	367/367	127/131	371/371	264/264

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR114b	BR169	BR173	BR177	BR182	BR190	BR193	BR199
Barcelona	347/347	186/186	223/223	395/395	NA/NA	286/289	342/342	303/303
Culpla	347/347	186/186	223/226	395/395	NA/NA	286/289	342/342	303/303
OSU 612.015	347/350	186/186	223/226	395/395	227/227	286/286	342/342	303/303
OSU 675.028	347/347	186/186	223/226	395/395	227/227	286/286	342/342	303/303
OSU 495.072	353/353	186/189	223/226	395/395	NA/NA	289/289	339/342	303/303
OSU 651.011	347/347	186/186	226/226	395/395	227/227	286/289	339/342	303/303
OSU 713.068	347/353	186/186	223/235	395/395	227/227	286/289	342/342	303/303
Crvenje	347/347	186/189	226/235	395/395	227/227	286/289	339/339	303/303
OSU 679.114	347/347	186/186	226/226	395/395	227/227	286/289	339/342	303/303
OSU 252.146	347/347	186/186	223/223	395/395	227/230	286/286	342/342	303/303
OSU 414.062	347/347	186/186	223/223	395/395	227/227	286/289	342/342	303/303
OSU 372.087	350/350	186/186	223/223	395/395	227/227	286/286	339/342	303/303
OSU 704.022	347/350	186/186	223/226	386/395	227/227	286/286	339/339	303/303
OSU 1187.101	340/350	186/186	223/226	395/395	227/227	289/289	339/342	303/303
OSU 1185.126	347/347	186/186	226/226	395/395	227/227	286/289	342/342	303/303
Daviana	347/350	186/186	223/223	386/395	227/227	287/287	339/339	303/303

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR202	BR205	BR209	BR210	BR211	BR215	BR216	BR227
Barcelona	192/192	169/172	366/366	238/238	143/143	123/126	130/133	299/299
Culpla	192/192	169/172	366/369	238/238	143/143	129/129	130/133	296/299
OSU 612.015	192/192	169/169	366/366	238/238	143/146	126/129	133/133	299/305
OSU 675.028	192/192	169/172	366/366	238/238	143/146	123/126	123/133	296/299
OSU 495.072	192/201	169/169	366/366	238/238	137/160	126/129	130/130	296/296
OSU 651.011	192/192	169/169	366/366	238/238	143/146	123/126	117/133	284/299
OSU 713.068	192/192	NA/NA	NA/NA	238/238	143/146	123/129	117/130	299/305
Crvenje	192/192	169/169	366/366	238/241	143/146	126/129	130/133	296/296
OSU 679.114	192/198	169/172	366/366	238/238	143/143	123/123	117/123	299/299
OSU 252.146	192/192	170/170	366/366	238/238	146/146	126/129	130/130	299/299
OSU 414.062	192/192	169/172	NA/NA	238/238	143/146	123/126	130/130	296/299
OSU 372.087	192/192	169/169	366/366	238/238	137/143	126/126	123/123	296/299
OSU 704.022	192/192	169/169	366/366	238/238	143/146	123/126	123/130	296/305
OSU 1187.101	192/192	169/169	366/369	238/238	146/146	123/123	130/139	296/299
OSU 1185.126	183/192	169/169	366/366	238/238	143/143	129/129	130/133	296/296
Daviana	192/192	169/169	366/366	238/238	143/146	123/129	123/133	296/296

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR229	BR230	BR231	BR233	BR238	BR240	BR242	BR245
Barcelona	297/303	368/368	131/134	109/109	270/274	235/235	284/284	279/285
Culpla	300/303	368/368	131/131	109/109	270/270	235/235	284/284	279/285
OSU 612.015	303/303	368/371	131/131	109/109	270/274	235/235	284/287	279/285
OSU 675.028	297/303	368/371	125/131	109/109	274/274	235/235	284/284	279/279
OSU 495.072	303/303	368/368	131/131	109/109	266/266	235/241	284/284	279/279
OSU 651.011	303/303	368/368	128/131	109/109	266/270	235/235	284/284	279/285
OSU 713.068	303/303	368/371	131/131	109/109	270/270	235/235	284/284	279/285
Crvenje	297/303	368/371	131/131	109/109	266/270	235/235	284/284	279/279
OSU 679.114	297/303	368/368	131/131	109/109	270/274	235/235	284/284	279/285
OSU 252.146	303/303	368/371	131/134	109/109	270/274	232/232	284/284	279/279
OSU 414.062	297/303	368/371	131/134	109/109	270/274	235/235	284/284	279/279
OSU 372.087	303/303	368/371	131/131	109/109	270/274	235/235	284/284	279/285
OSU 704.022	303/303	368/368	131/131	109/109	270/274	235/235	284/284	279/285
OSU 1187.101	300/300	368/368	131/134	109/109	266/270	229/229	284/284	285/285
OSU 1185.126	300/300	368/371	131/131	109/112	266/266	235/235	284/284	279/279
Daviana	303/303	368/371	131/131	109/109	266/274	235/235	284/284	279/285

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR246	BR249	BR253	BR255	BR257	BR259	BR261
Barcelona	177/177	301/303	336/342	219/219	368/368	224/242	145/148
Culpla	177/177	283/301	324/336	219/219	362/368	236/236	142/145
OSU 612.015	177/177	301/303	336/342	219/219	368/368	224/242	145/145
OSU 675.028	177/177	301/301	336/342	219/219	368/368	224/248	145/148
OSU 495.072	177/183	301/303	336/336	219/219	368/368	239/239	145/145
OSU 651.011	177/177	301/303	324/342	219/219	368/368	224/236	145/145
OSU 713.068	177/177	301/301	336/336	219/219	368/368	233/236	145/145
Crvenje	177/177	294/294	336/336	219/219	362/362	224/233	145/145
OSU 679.114	177/177	301/301	324/336	219/219	368/368	242/245	145/145
OSU 252.146	177/177	303/303	336/342	219/219	368/368	242/242	145/148
OSU 414.062	177/177	301/303	336/342	219/219	368/368	233/248	145/148
OSU 372.087	177/177	303/303	336/336	219/219	362/371	242/242	145/148
OSU 704.022	177/177	301/303	336/336	219/219	362/368	233/233	145/148
OSU 1187.101	177/177	301/301	336/336	219/220	368/371	224/239	145/145
OSU 1185.126	177/177	301/301	336/336	219/219	362/362	227/233	145/145
Daviana	177/177	301/301	336/336	219/219	362/371	224/230/233/239	145/148

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR262	BR264	BR267	BR270	BR276	BR277	BR279
Barcelona	102/102	119/119	123/126	90/90	337/337	235/235 & 357/359	125/125
Culpla	102/102	122/122	129/129	87/96	337/337	235/235 & 359/359	125/128
OSU 612.015	102/108	122/122	126/129	90/96	337/337	235/235 & 357/359	125/125
OSU 675.028	102/102	119/122	123/126	90/96	337/337	235/235 & 357/359	125/125
OSU 495.072	102/102	119/128	126/129	99/99	337/340	237/237 & 357/357	125/125
OSU 651.011	102/102	119/122	123/126	96/96	337/337	235/235 & 357/359	125/125
OSU 713.068	102/102	119/122	123/129	89/96	337/337	235/235 & 357/359	125/128
Crvenje	102/102	119/119	126/129	90/96	337/337	235/235 & 359/359	125/128
OSU 679.114	102/108	119/122	123/123	96/96	337/337	235/235 & 359/359	125/125
OSU 252.146	102/102	122/122	126/129	90/90	337/337	235/357	125/125
OSU 414.062	102/102	119/122	123/126	90/96	337/337	235/235	125/125
OSU 372.087	102/102	119/119	126/126	90/96	337/337	235/235 & 359/359	125/128
OSU 704.022	102/102	119/122	123/126	90/90	337/337	235/235 & 359/359	128/128
OSU 1187.101	102/102	119/122	123/123	96/99	337/337	235/237 & 357/359	125/131
OSU 1185.126	102/102	119/119	129/129	96/96	337/337	237/237 & 357/357	128/128
Daviana	102/102	122/122	123/129	90/96	337/337	235/237 & 357/359	125/128

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR284	BR288	BR292	BR294	BR302	BR303	BR307	BR311
Barcelona	389/389	366/366	221/221	308/311	124/124	271/271	84/87	114/114
Culpla	389/395	366/369	221/221	308/308	124/124	271/271	84/87	114/114
OSU 612.015	389/389	366/366	215/221	308/311	121/124	270/270	84/87	114/114
OSU 675.028	389/395	366/366	221/221	308/308	124/124	270/270	84/87	114/114
OSU 495.072	392/395	366/366	221/221	308/311	124/127	272/272	90/90	114/114
OSU 651.011	389/395	366/366	221/221	308/308	124/124	271/271	84/87	111/114
OSU 713.068	389/389	366/366	215/221	308/308	121/124	270/270	87/87	114/114
Crvenje	395/395	366/366	221/221	308/308	124/124	270/270	87/87	114/114
OSU 679.114	386/389	366/366	215/221	308/311	124/124	270/270	84/87	114/114
OSU 252.146	389/395	366/366	221/221	308/311	124/124	268/268	87/87	114/114
OSU 414.062	389/395	366/366	215/221	308/311	124/124	270/270	84/84	114/114
OSU 372.087	389/395	366/366	215/221	308/308	124/124	272/272	87/87	114/114
OSU 704.022	389/395	366/366	215/221	308/308	121/124	270/270	84/87	114/114
Holmskij	392/392	366/366	221/221	308/308	124/124	270/270	84/87	111/114
Crimea	386/386	366/366	215/221	308/308	124/124	270/272	84/87	114/114
Daviana	395/395	366/366	221/221	308/308	124/124	270/270	87/87	114/114

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR315	BR316	BR322	BR325	BR327	BR331	BR332	BR335
Barcelona	133/136	133/133	102/105	150/156	228/228	126/126	345/351	147/157
Culpla	133/133	128/133	102/102	150/150	228/228	126/129	345/345	147/157
OSU 612.015	133/133	133/133	102/102	150/156	228/231	126/126	345/345	147/157
OSU 675.028	133/136	133/133	102/105	150/156	228/228	126/129	345/345	147/147
OSU 495.072	133/136	133/133	102/102	147/150	228/228	126/126	345/345	147/147
OSU 651.011	133/133	133/133	102/105	150/156	228/228	129/129	345/345	147/147
OSU 713.068	133/133	133/133	102/105	150/150	228/231	126/126	345/345	147/147
Crvenje	133/136	133/133	99/102	150/150	228/228	126/129	345/345	147/151
OSU 679.114	133/133	133/133	102/105	150/156	228/228	129/129	345/345	147/147
OSU 252.146	136/136	133/133	102/102	147/150	228/228	126/126	345/357	147/147
OSU 414.062	133/136	133/133	102/105	147/150	228/228	126/129	345/345	147/147
OSU 372.087	133/136	133/133	102/102	147/147	228/228	126/126	345/345	147/147
OSU 704.022	133/136	133/133	102/102	147/150	228/228	126/129/132	345/345	147/147
Holmskij	133/133	133/133	102/108	147/150	228/231	129/129	345/345	147/147
Crimea	133/133	130/133	105/108	150/156	228/228	126/129	345/345	147/147
Daviana	136/136	133/133	102/105	150/150	228/228	126/129	345/345	143/147/157

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR339	BR340	BR341	BR343	BR344	BR345	BR347	BR349
Barcelona	125/125	385/385	104/107	389/389	127/127	112/112	389/389	235/235
Culpla	125/128	382/385	107/113	389/395	127/127	112/112	389/389	235/235
OSU 612.015	125/125	385/385	107/107	389/389	127/127	112/112	389/389	235/235
OSU 675.028	125/125	385/385	107/107	389/395	121/127	112/115	389/389	235/235
OSU 495.072	125/125	385/388	107/107	392/395	121/127	112/112	386/395	235/241
OSU 651.011	125/125	385/385	104/107	389/395	121/127	112/115	389/389	235/235
OSU 713.068	125/128	382/385	104/107	389/389	121/127	112/112	389/389	235/235
Crvenje	125/128	385/385	107/107	395/395	127/127	112/112	389/401	234/234
OSU 679.114	125/125	382/385	104/107	386/389	127/127	112/115	389/389	235/238
OSU 252.146	125/125	385/388	107/107	389/395	127/127	112/112	389/395	235/235
OSU 414.062	125/125	385/385	104/107	389/395	127/127	112/115	389/389	235/235
OSU 372.087	125/128	385/385	104/104	389/395	127/127	112/112	389/389	235/235
OSU 704.022	128/128	385/385	104/107	389/395	124/127	112/112	389/398	235/235
Holmskij	125/131	382/382	107/107	392/392	124/127	112/112	389/395	228/228
Crimea	128/128	382/385	104/116	386/386	127/136	112/115	386/395	235/235
Daviana	125/128	388/388	107/107	395/395	124/124	112/112	389/395	234/234

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR352	BR355	BR357	BR358	BR359	BR360	BR361	BR362
Barcelona	109/112	198/198	109/112	119/119	385/398	109/109	368/377	201/204
Culpla	109/112	198/198	112/116	122/122	394/394	109/109	377/377	204/204
OSU 612.015	112/112	198/198	109/116	122/122	385/395	109/109	368/377	201/204
OSU 675.028	109/109	198/198	109/116	119/122	385/398	109/109	377/377	201/204
OSU 495.072	109/118	198/198	112/116	119/128	385/396	103/109	368/377	204/204
OSU 651.011	109/112	198/204	109/116	119/122	385/385	109/109	368/377	201/204
OSU 713.068	109/112	198/198	109/112	119/122	385/395	103/109	368/377	204/204
Crvenje	109/109	198/198	116/116	119/119	394/399	109/109	368/377	201/201
OSU 679.114	109/118	198/198	112/116	119/122	385/395	109/109	368/377	204/204
OSU 252.146	109/112	198/198	109/109	122/122	384/399	109/109	377/377	204/204
OSU 414.062	109/109	198/198	109/112	119/122	384/395	109/109	368/377	204/204
OSU 372.087	109/112	198/198	116/116	119/119	385/390	109/109	368/377	204/204
OSU 704.022	109/109	198/198	109/116	119/122	385/390	109/109	368/377	201/204
Holmskij	112/112	198/198	109/109	119/122	390/394	109/109	368/377	204/204
Crimea	112/112	198/198	112/116	119/119	385/394	109/109	377/377	204/204
Daviana	109/112	198/198	109/116	122/122	389/389	109/109	377/377	204/204

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR368	BR371	BR374	BR375	BR378	BR379	BR381	BR381
Barcelona	204/204	270/270	224/236	256/259	326/326	133/151	127/130	128/131/141
Culpla	204/207	270/273	227/236	256/259	320/329	130/133	127/127	141/141
OSU 612.015	204/204	270/270	236/248	253/259	326/326	115/133	127/130	128/131/141
OSU 675.028	204/204	270/273	236/236	256/259	326/326	115/151	127/127	128/141
OSU 495.072	204/204	273/282	224/224	253/265	326/326	127/133	130/133	131/134/141
OSU 651.011	204/204	270/270	236/236	253/256	320/326/329	149/151	130/130	128/141
OSU 713.068	195/204	270/270	236/248	256/265	320/326/329	115/133	127/127	128/141
Crvenje	204/204	270/270	242/242	256/259	326/326	115/130	127/127	141/141
OSU 679.114	204/204	270/270	224/248	256/256	326/329	115/127	123/129	128/141
OSU 252.146	204/204	273/273	236/236	256/259	326/326	133/133	127/127	128/128
OSU 414.062	204/204	273/273	236/236	256/259	326/326	115/133	127/127	128/128
OSU 372.087	204/204	270/270	228/228	253/265	326/326	115/133	130/130	131/131
OSU 704.022	204/207	270/273	224/236	256/265	326/326	115/151	127/130	141/141
Holmskij	195/204	273/273	236/236	253/253	326/326	115/133	127/127	128/141
Crimea	178/204	270/276	218/242	253/253	326/326	127/133	127/127	128/141
Daviana	178/204	270/273	218/218	253/265	320/326/329	115/145	127/127	128/131/141

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR387	BR389	BR392	BR396	BR397	BR398	BR402	BR406
Barcelona	374/380	320/320	215/221	145/145	238/253	168/168	131/134	212/212
Culpla	365/380	320/320	221/221	145/145	238/253	168/168	128/134	212/212
OSU 612.015	377/377	320/320	215/221	145/145	238/250	168/168	134/134	212/212
OSU 675.028	365/377	320/320	221/221	145/145	238/253	168/168	131/134	212/212
OSU 495.072	365/374	320/320	221/221	145/148	250/250	168/168	131/131	212/212
OSU 651.011	377/377	320/320	221/221	145/145	238/250	168/168	134/134	212/212
OSU 713.068	365/377	320/320	215/221	145/145	250/253	168/168	134/134	212/212
Crvenje	377/377	320/320	221/221	145/145	238/247	168/168	134/134	212/212
OSU 679.114	365/365	320/320	215/221	145/145	247/250	168/168	134/134	212/212
OSU 252.146	374/374	320/320	221/221	145/145	247/253	168/168	128/134	212/212
OSU 414.062	372/372	320/320	215/221	145/145	250/253	168/168	134/134	212/212
OSU 372.087	365/365	329/329	215/221	145/145	250/250	168/168	130/134	212/212
OSU 704.022	365/377	320/320	215/221	139/145	247/253	168/168	130/130	212/212
Holmskij	356/371	320/320	221/221	145/145	250/253	168/172	134/134	212/212
Crimea	365/377	320/320	215/221	145/145	250/250	168/168	128/134	212/215
Daviana	365/365	320/329	221/221	145/145	247/250	168/168	130/130	212/212

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR410	BR411	BR412	BR413	BR414	BR415	BR418	BR420
Barcelona	322/325	121/130	278/287/290/299	242/245	118/121	248/254	122/122	91/94
Culpla	322/325	118/124	290/296	242/242	118/121	248/248	122/130	91/100
OSU 612.015	322/322	118/124	290/302	242/242	118/121	248/254	122/122	91/94
OSU 675.028	322/325	124/124	290/299	242/242	118/121	248/248	122/122	91/91
OSU 495.072	322/322	118/121	278/281	239/242	112/121	248/248	122/122	91/ 94
OSU 651.011	322/325	121/124	290/290	239/242	118/118	248/248	122/122	91/91
OSU 713.068	325/325	118/121	290/302	242/242	118/121	248/254	122/126	91/91
Crvenje	325/325	124/130	299/299	230/242	134/151	248/260	122/122	94/94
OSU 679.114	322/325	124/127	287/290	242/244	118/121	254/254	122/122	91/94
OSU 252.146	322/325	127/127	296/299	242/242	118/121	248/254	126/126	94/94
OSU 414.062	322/325	124/124	278/287/296/305	233/242	118/121	248/254	122/122	94/94
OSU 372.087	322/325	124/127	290/299	233/233	121/121	254/257	122/122	94/94
OSU 704.022	325/325	124/124	278/290	233/242	118/121	254/257	122/122	94/94
Holmskij	322/325	121/124	272/275	242/242	121/121	251/254	122/126	91/94
Crimea	322/322	124/124	290/299	230/242	112/121	248/260	122/122	94/94
Daviana	322/325	124/124	299/299	233/242	121/121	257/257	122/122	91/94

Appendix F. SSR profiles of 16 parents at 125 loci.

	BR421	BR423	BR425	BR426	BR427	BR428	BR430	BR433
Barcelona	127/130	106/115	277/283	296/296	310/310	144/144	263/263	144/150
Culpla	127/127	106/115	277/283	296/296	310/316	128/144	263/263	144/144
OSU 612.015	127/127	115/115	277/283	296/299	310/310	144/144	263/263	144/144
OSU 675.028	122/127	112/112	277/277	296/302	310/310	144/144	263/263	150/150
OSU 495.072	127/127	115/115	277/277	296/296	310/310	144/144	263/266	144/144
OSU 651.011	122/127	112/115	277/277	299/299	310/310	144/144	263/263	144/144
OSU 713.068	127/130	112/115	268/277	296/299	310/316	136/144	263/263	144/144
Crvenje	127/127	106/115	277/277	296/299	316/316	144/144	263/263	139/144
OSU 679.114	127/130	112/115	277/277	296/302	310/316	136/144	263/263	144/144
OSU 252.146	127/130	112/115	277/277	296/302	310/310	136/144	263/263	144/144
OSU 414.062	127/127	106/112	277/277	296/296	310/316	144/144	263/263	150/150
OSU 372.087	127/127	112/115	277/277	296/296	310/316	136/144	263/263	144/144
OSU 704.022	127/127	106/115	277/277	296/296	316/316	144/144	263/263	144/144
Holmskij	127/127	115/115	277/277	296/296	310/316	136/144	263/263	134/144
Crimea	127/127	115/115	268/277	296/302	310/316	144/144	263/266	134/134
Daviana	127/127	112/112	277/283	296/296	316/316	144/144	263/266	144/144

Appendix F. SSR profiles of 16 parents at 125 loci.

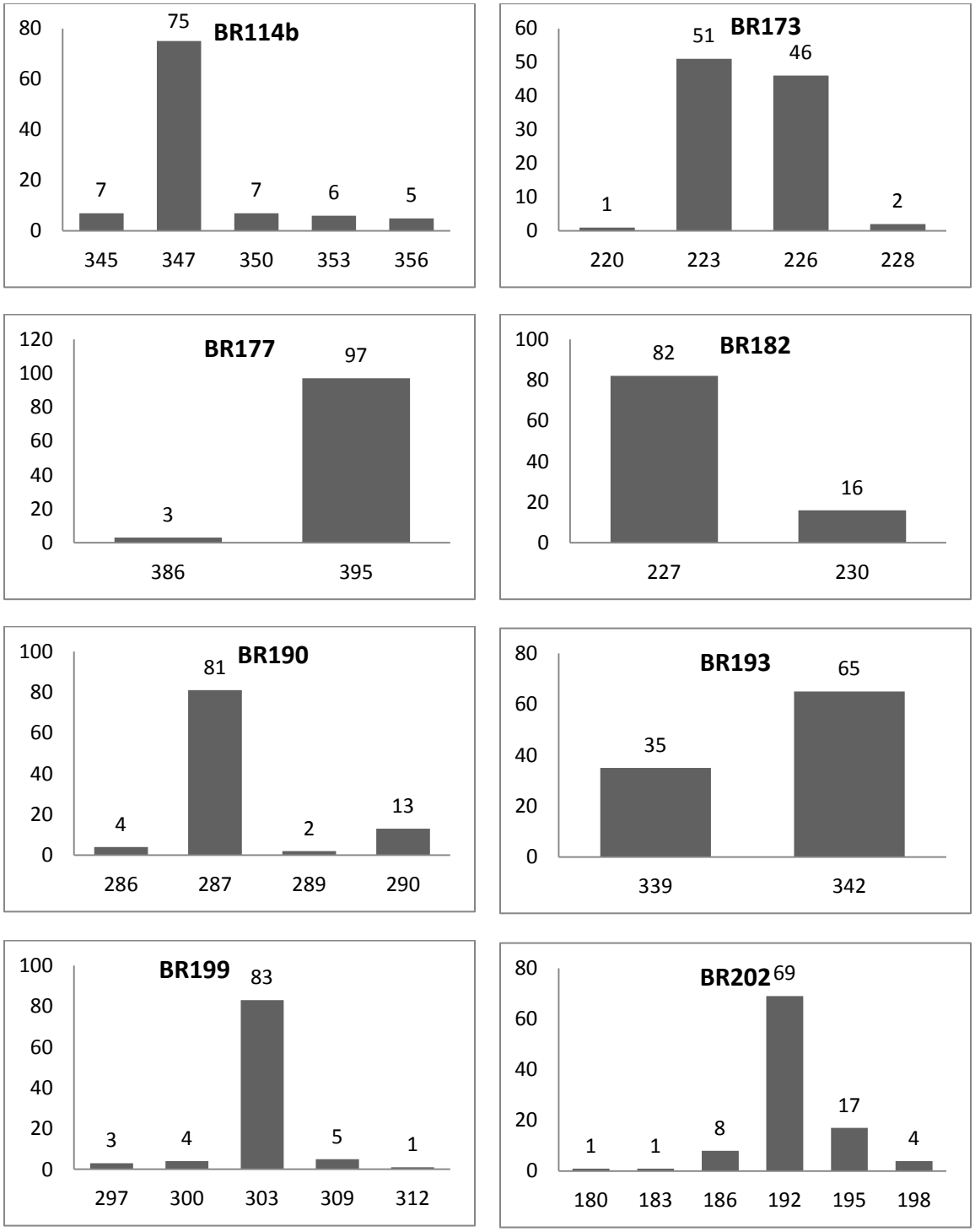
	BR436	BR437	BR438	BR442	BR444	BR446	BR451	BR456
Barcelona	112/112	148/148	191/193	198/200	103/106	153/156	134/134	133/139
Culpla	112/112	148/148	191/193	198/198	103/103	156/159	134/134	139/142
OSU 612.015	112/112	148/148	193/193	198/198	103/106	156/162	134/134	139/142
OSU 675.028	90/112	148/148	191/193	198/200	103/106	153/153	129/134	133/139
OSU 495.072	112/112	148/148	193/197	198/200	103/106	153/156	126/129	139/139
OSU 651.011	112/112	148/148	193/199	198/200	103/106	156/162	134/134	139/139
OSU 713.068	90/112	148/148	193/195	198/198	103/106	153/153	134/134	133/139
Crvenje	90/112	148/148	191/193	225/225	100/103/106	162/162	134/134	137/137
OSU 679.114	112/112	148/148	191/193	198/198	103/106	153/153	134/134	139/142
OSU 252.146	112/112	148/148	191/193	198/200	103/106	153/156	134/134	139/139
OSU 414.062	112/112	148/148	191/193	200/200	103/106	153/156	134/134	139/139
OSU 372.087	112/112	148/148	193/193	198/200	103/106	153/156	134/134	139/142
OSU 704.022	112/112	151/151	193/193	200/200	103/106	153/156	134/134	139/142
Holmskij	112/112	145/148	191/193	200/200	103/106	156/156	134/134	139/142
Crimea	112/112	148/151	191/193	200/200	103/106	162/162	134/134	139/142
Daviana	90/112	148/151	193/193	200/200	103/106	156/162	134/134	139/142

Appendix F. SSR profiles of 16 parents at 125 loci.

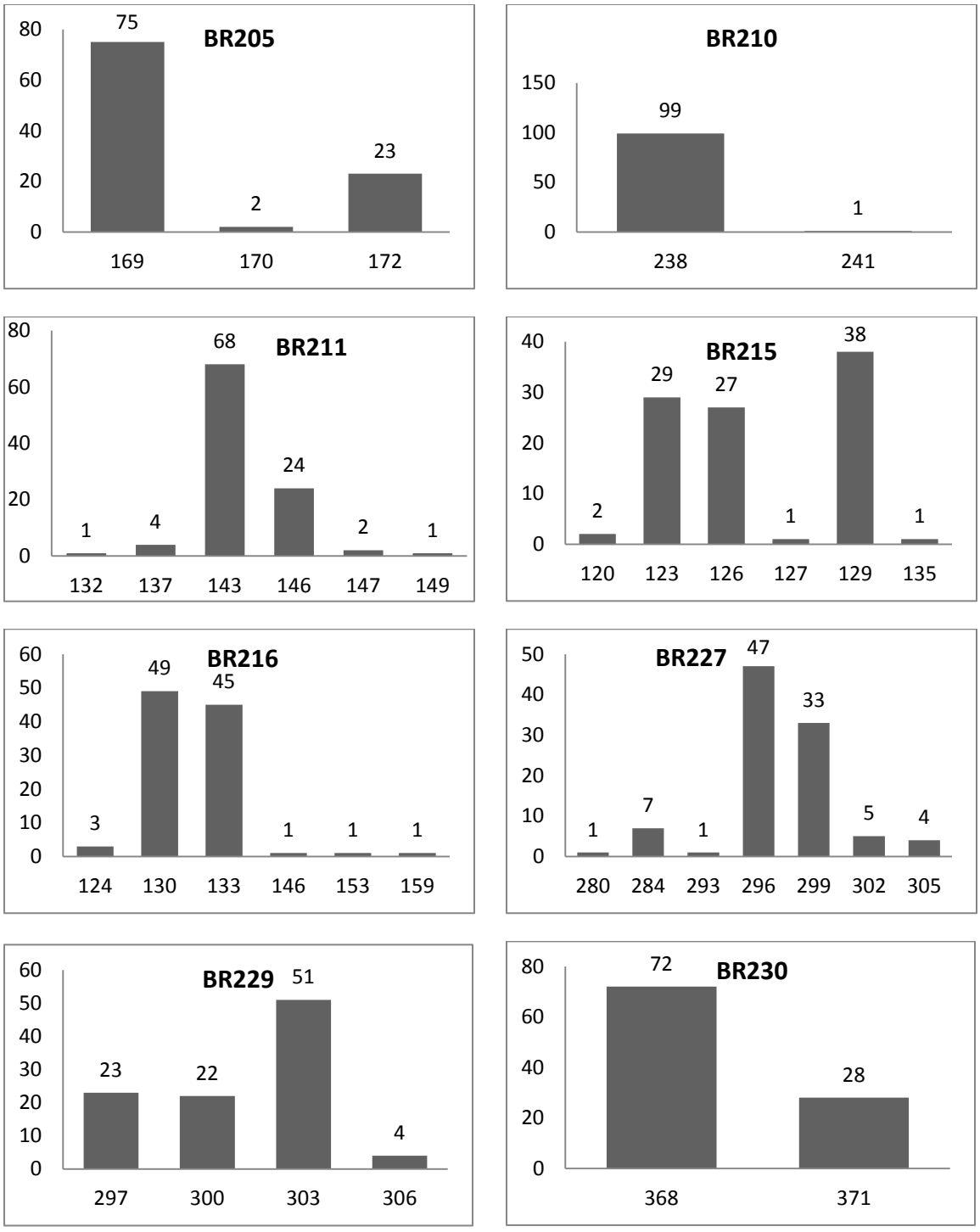
	BR464	BR467	BR468	BR470	BR474	BR475	BR478	BR479
Barcelona	278/284	150/150	373/373	334/334	122/125	237/240	203/205	83/87/89
Culpla	278/284	150/150	373/373	334/334	122/122	237/240	205/207	85/89/93
OSU 612.015	278/284	150/150	373/373	334/334	122/122	237/240	205/207	81/101
OSU 675.028	278/278	150/150	373/376	334/334	122/122	237/237	203/205	89/89
OSU 495.072	281/296	150/150	373/376	334/334	125/125	240/243	205/205	81/89
OSU 651.011	284/284	150/150	373/373	334/334	122/122	237/237	203/205	83/87/89
OSU 713.068	278/278	150/150	373/373	334/334	122/125	237/240	205/205	81/101
Crvenje	278/284	150/150	373/373	334/334	125/125	237/237	203/205	83/89
OSU 679.114	269/278	150/150	373/373	334/334	122/122	237/237	205/205	85/89
OSU 252.146	278/278	150/150	373/373	334/334	122/122	237/240	205/207	83/87/89
OSU 414.062	278/284	150/154	373/373	334/334	122/125	237/240	205/207	81/83/87
OSU 372.087	284/284	150/150	373/373	334/340	122/125	237/240	207/207	83/89
OSU 704.022	278/284	150/150	373/373	334/334	122/122	237/237	207/207	83/89/102
Holmskij	284/296	150/150	361/361	334/334	122/122	237/237	205/205	89/93/106
Crimea	269/279	150/150	373/373	334/334	122/125	237/237	205/207	81/83/87/91/104
Daviana	284/284	141/150	373/373	334/340	122/122	237/237	205/207	87/89/118

Appendix F. SSR profiles of 16 parents at 125 loci.

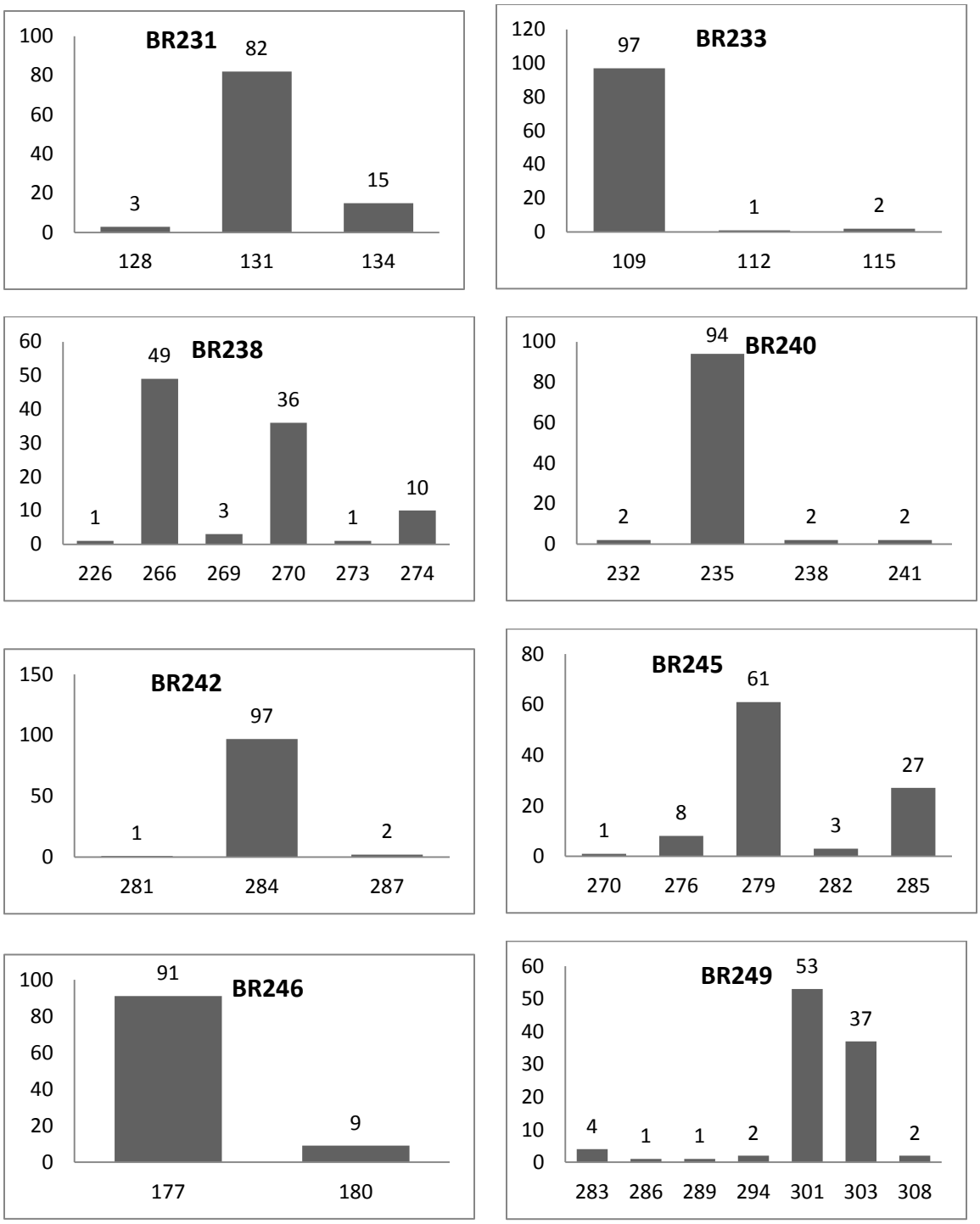
	BR480	BR482	BR483	BR484	BR485	BR487	BR488
Barcelona	132/138	282/286	302/310	367/367	133/135	369/371	264/264
Culpla	132/138	286/286	282/296	367/375	127/131	369/371	264/266
OSU 612.015	134/134	282/286	288/310	367/367	131/133	371/373	264/264
OSU 675.028	132/134	282/286	288/302	363/367	131/131	371/371	264/264
OSU 495.072	134/134	286/286	288/288	367/367	127/131	371/375	266/268
OSU 651.011	132/134	286/286	288/310	367/367	127/135	371/371	264/264
OSU 713.068	132/134	282/288	288/298	367/367	131/133	371/375	264/264
Crvenje	138/138	282/286	292/292	363/367	127/135	371/375	264/264
OSU 679.114	132/138	282/288	288/288	367/367	131/131	371/371	264/264
OSU 252.146	138/138	282/286	292/310	367/367	127/131	371/371	264/264
OSU 414.062	134/136	282/284	302/310	367/367	127/131	371/381	264/264
OSU 372.087	132/132	286/286	298/310	363/367	127/135	381/381	264/266
OSU 704.022	138/138	286/286	288/310	363/367	127/131	375/381	264/264
Holmskij	134/138	286/288	296/298	363/371	127/135	371/373	264/264
Crimea	132/134	286/304	296/306	363/367	127/127	371/377	258/264
Daviana	132/138	286/286	288/310	363/363	127/135	369/381	264/264



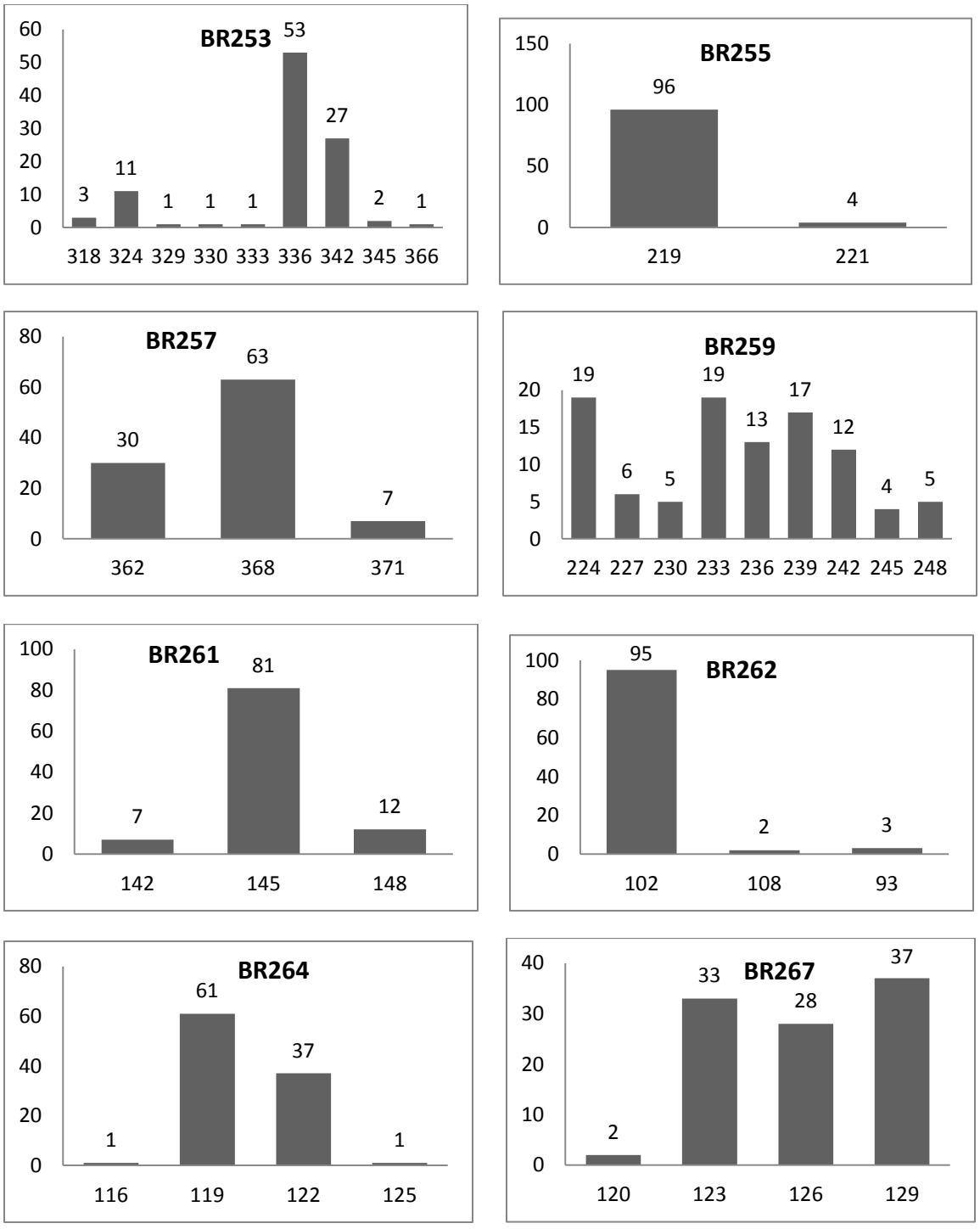
Appendix G. Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



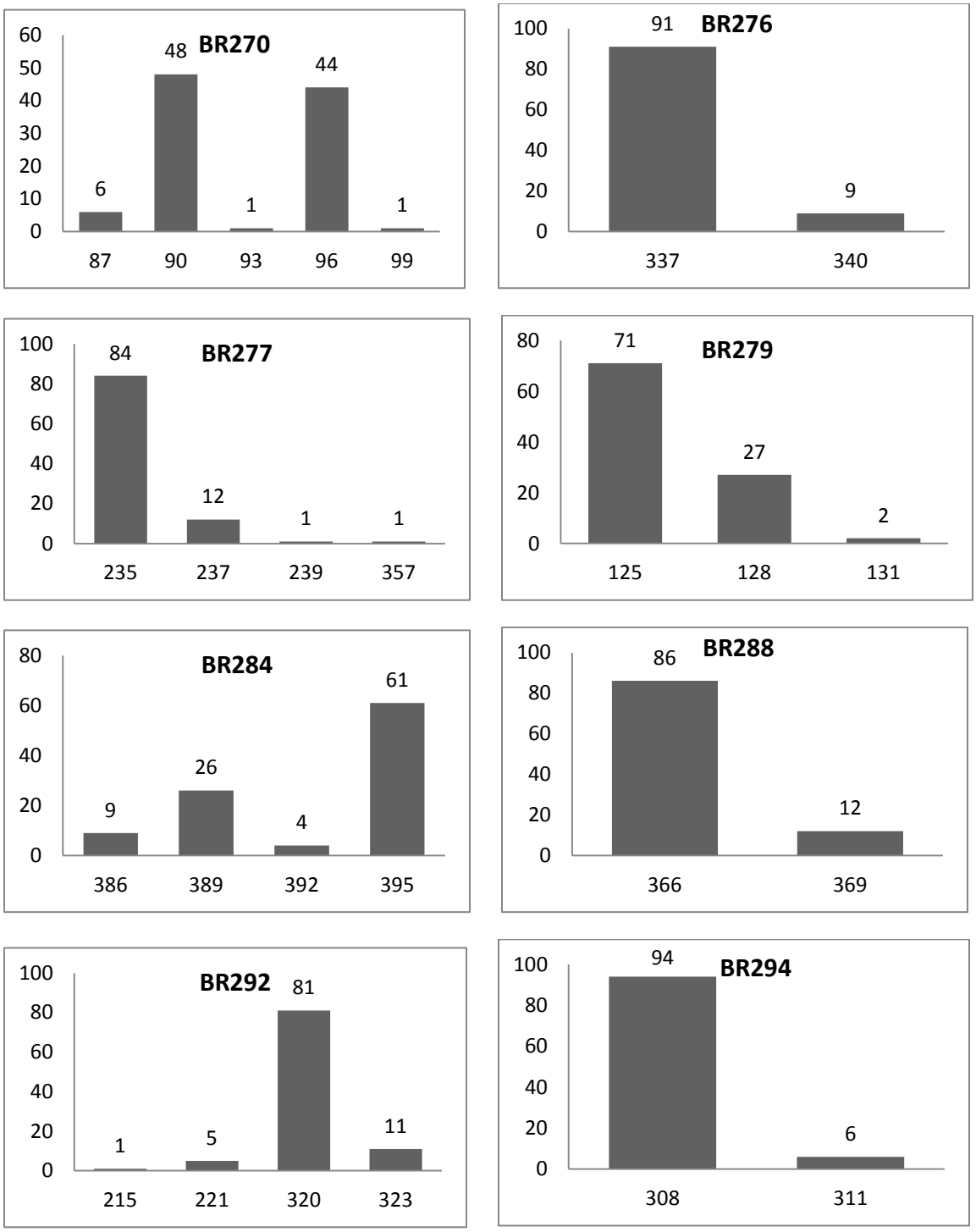
Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



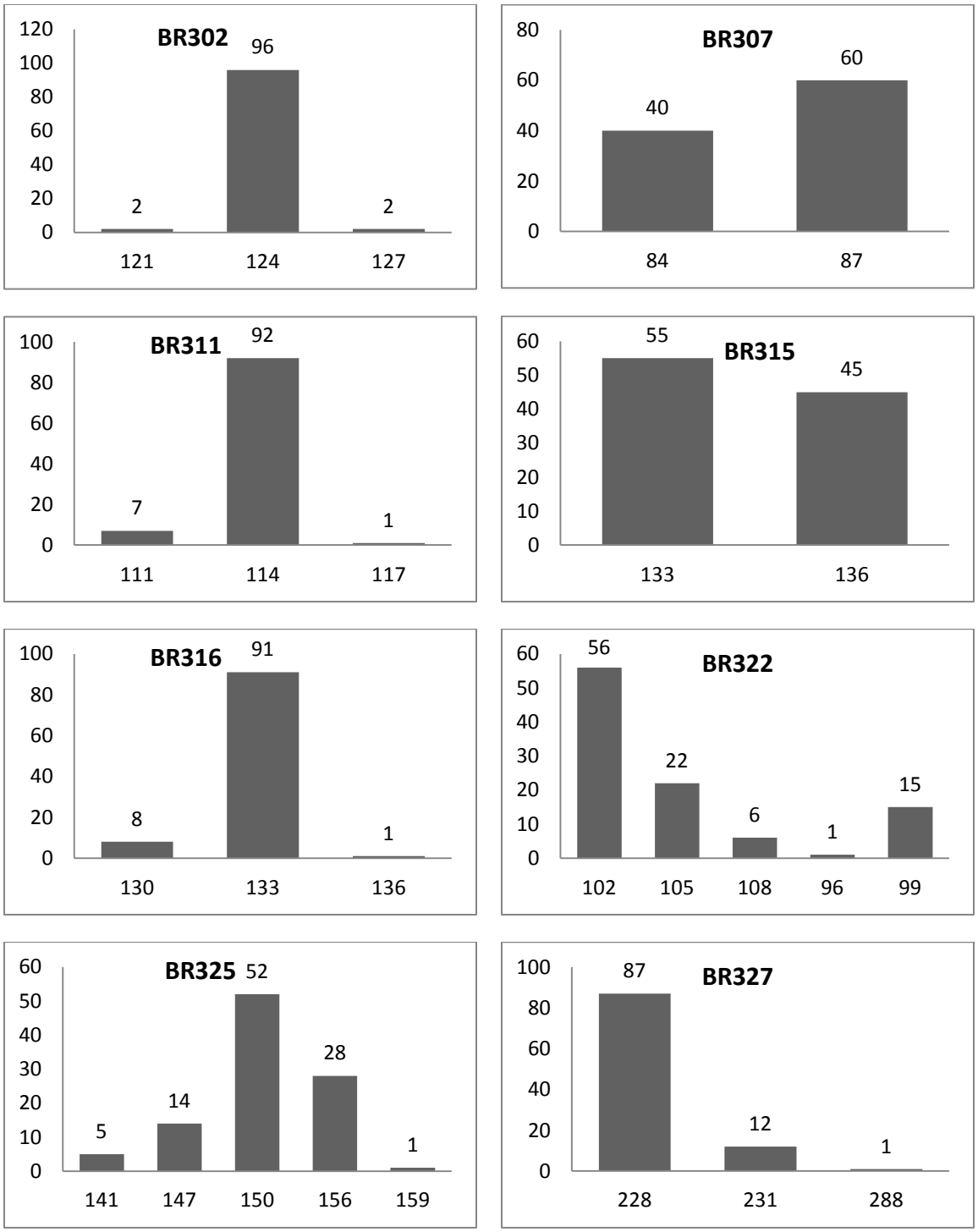
Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



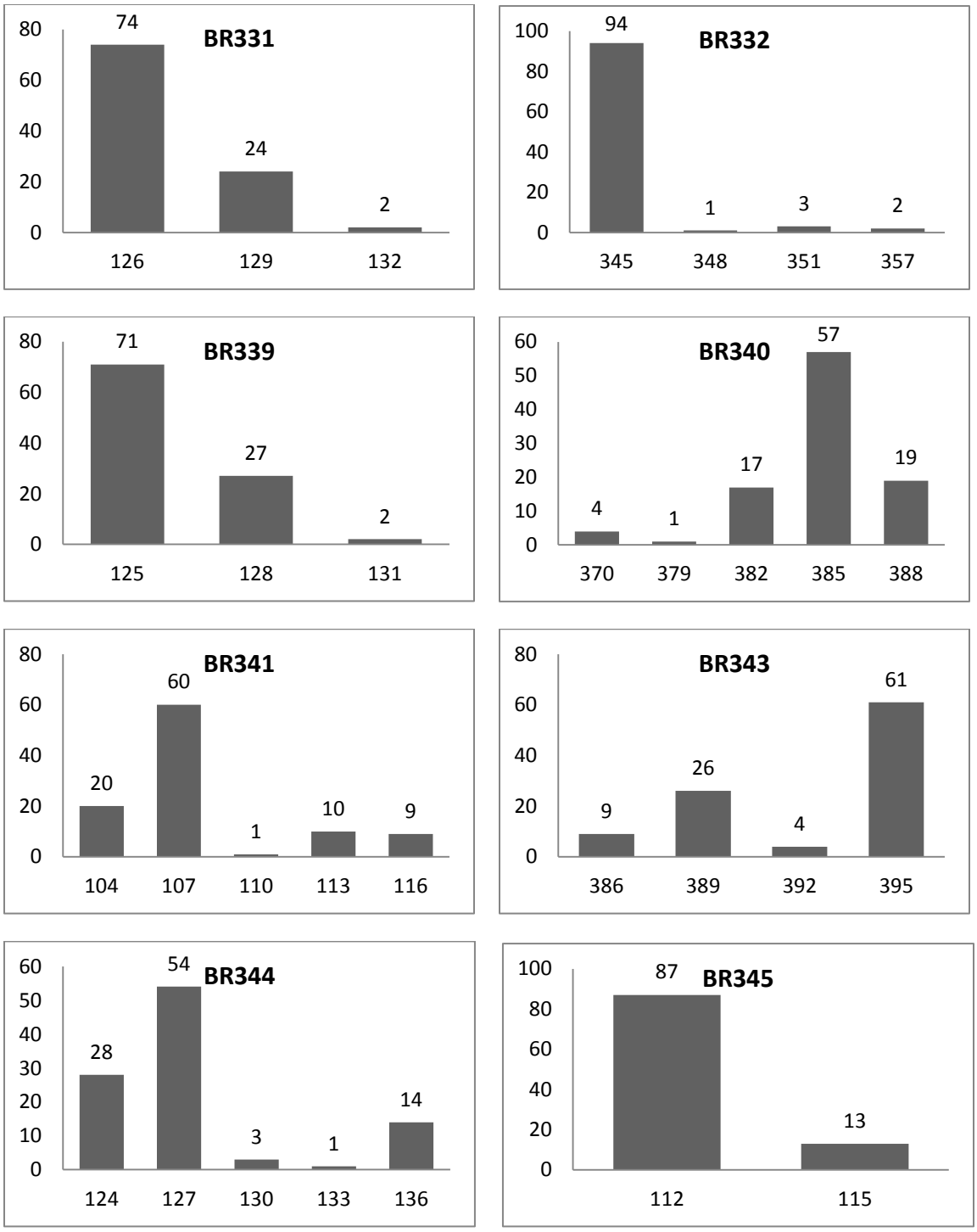
Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



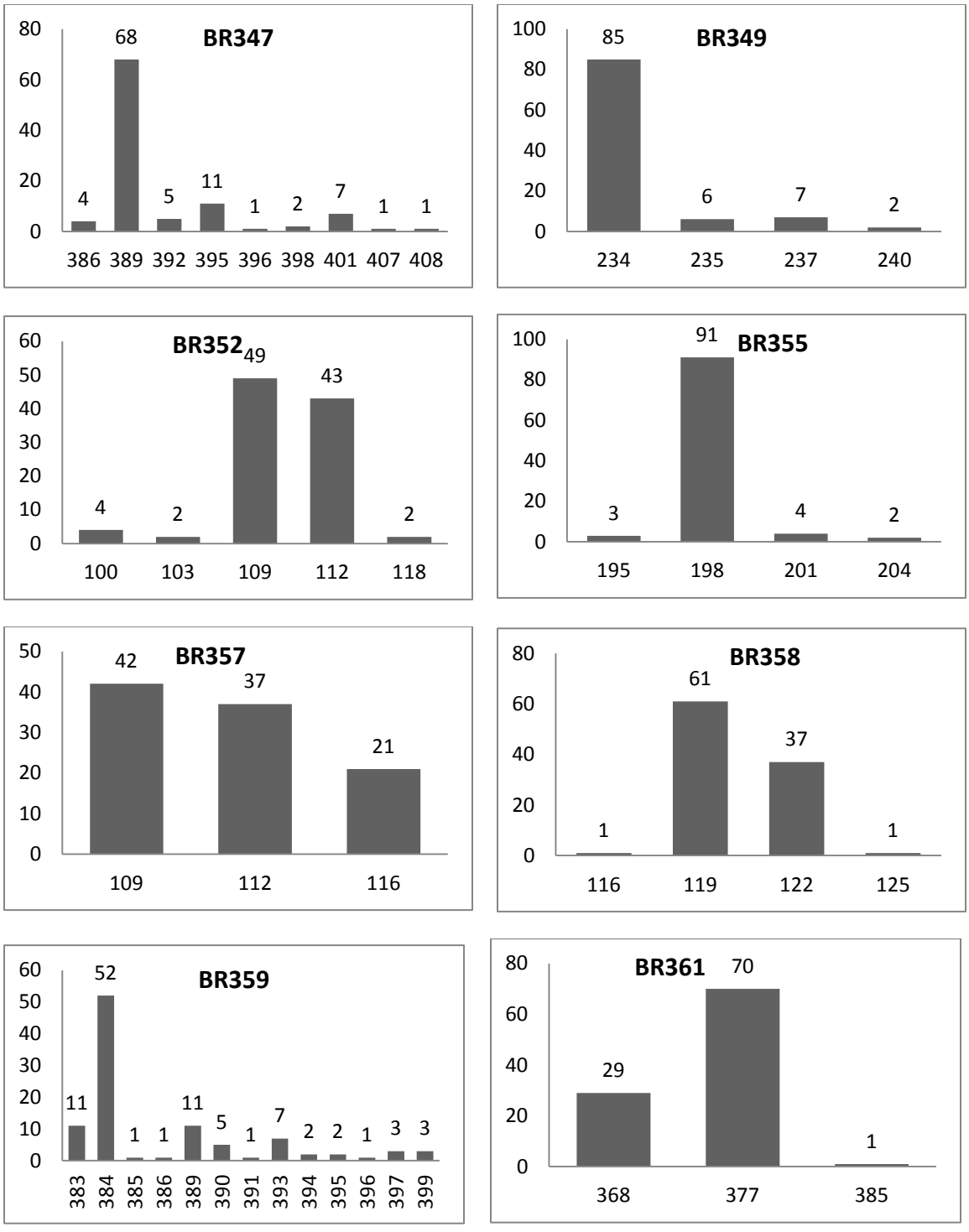
Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



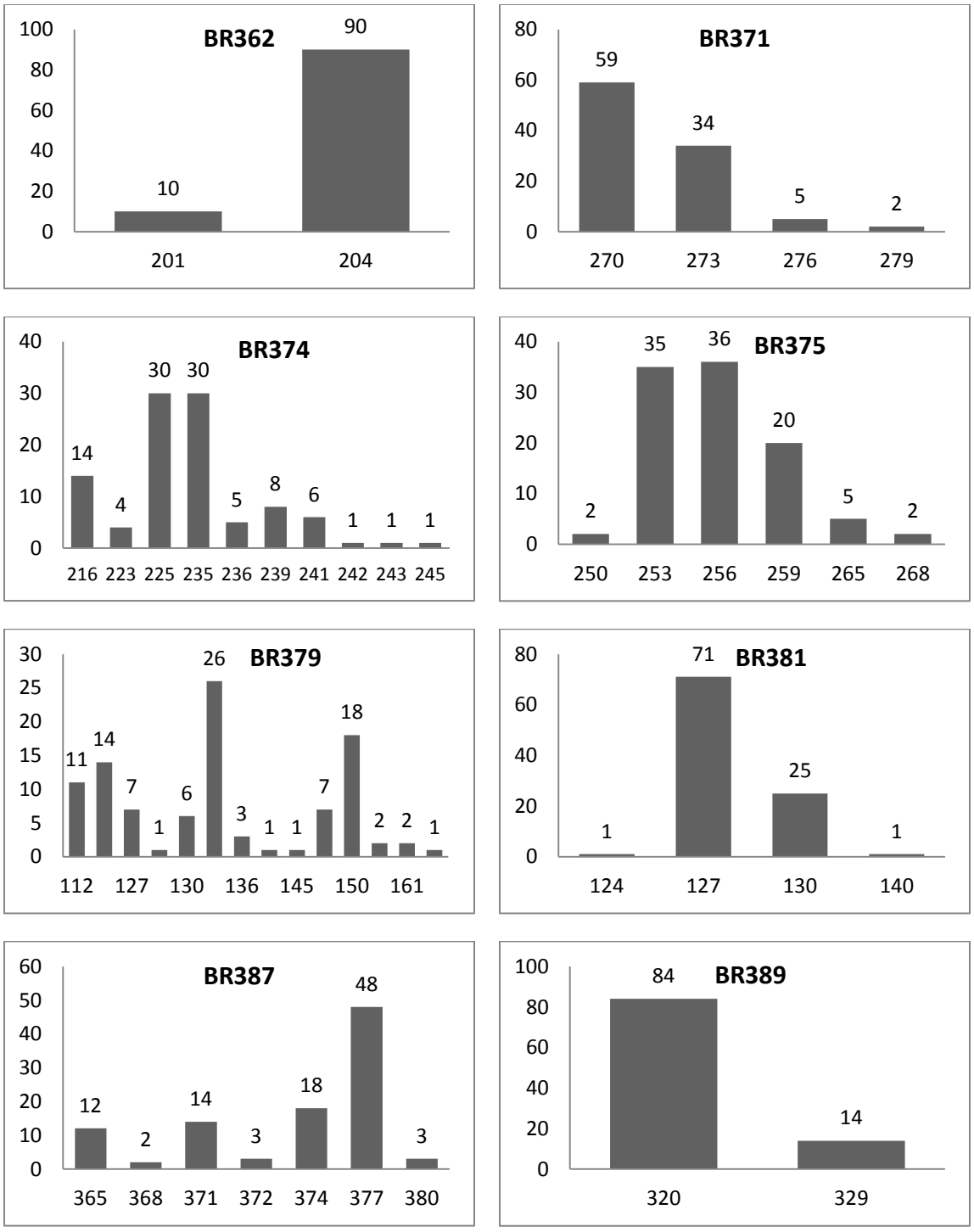
Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



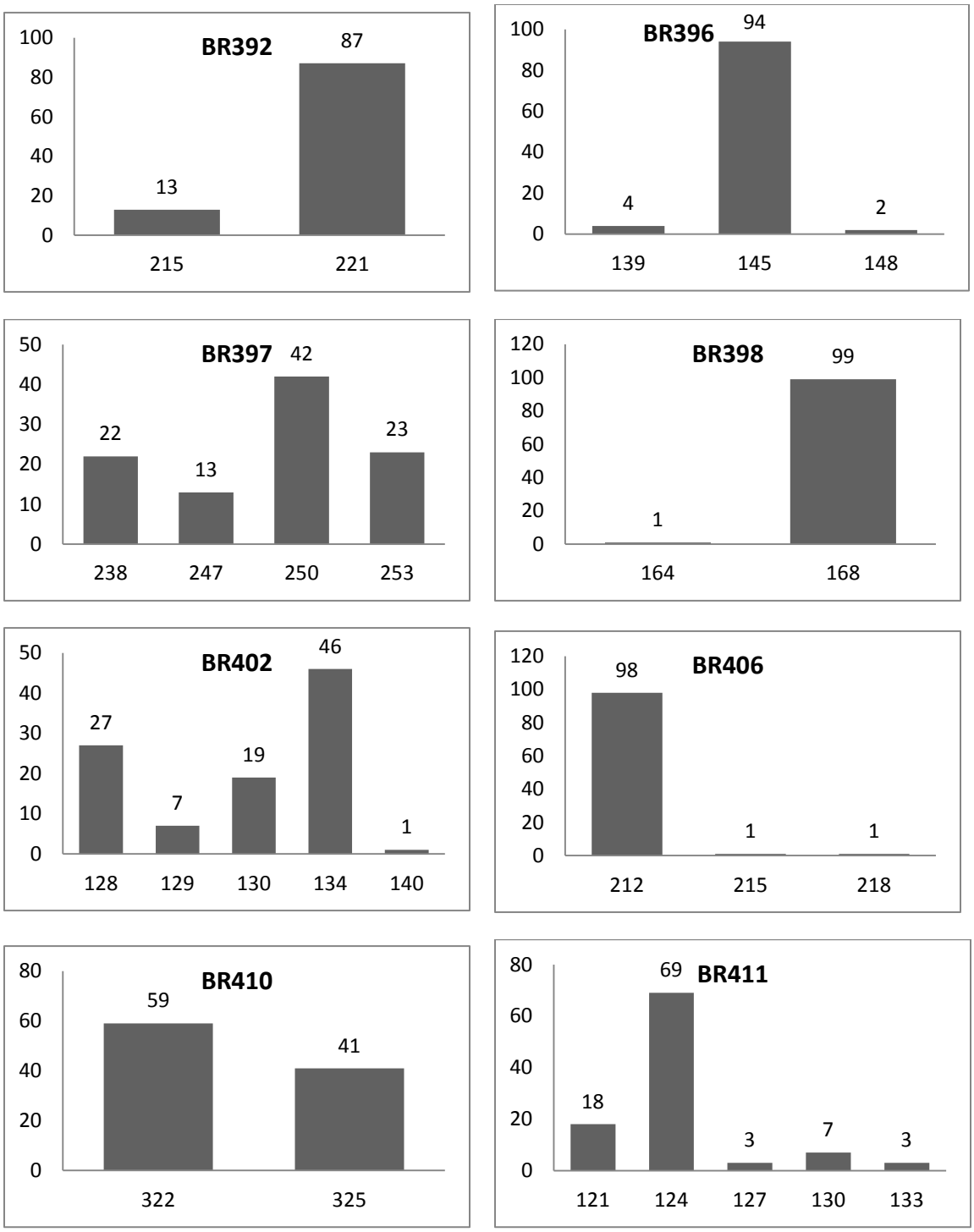
Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



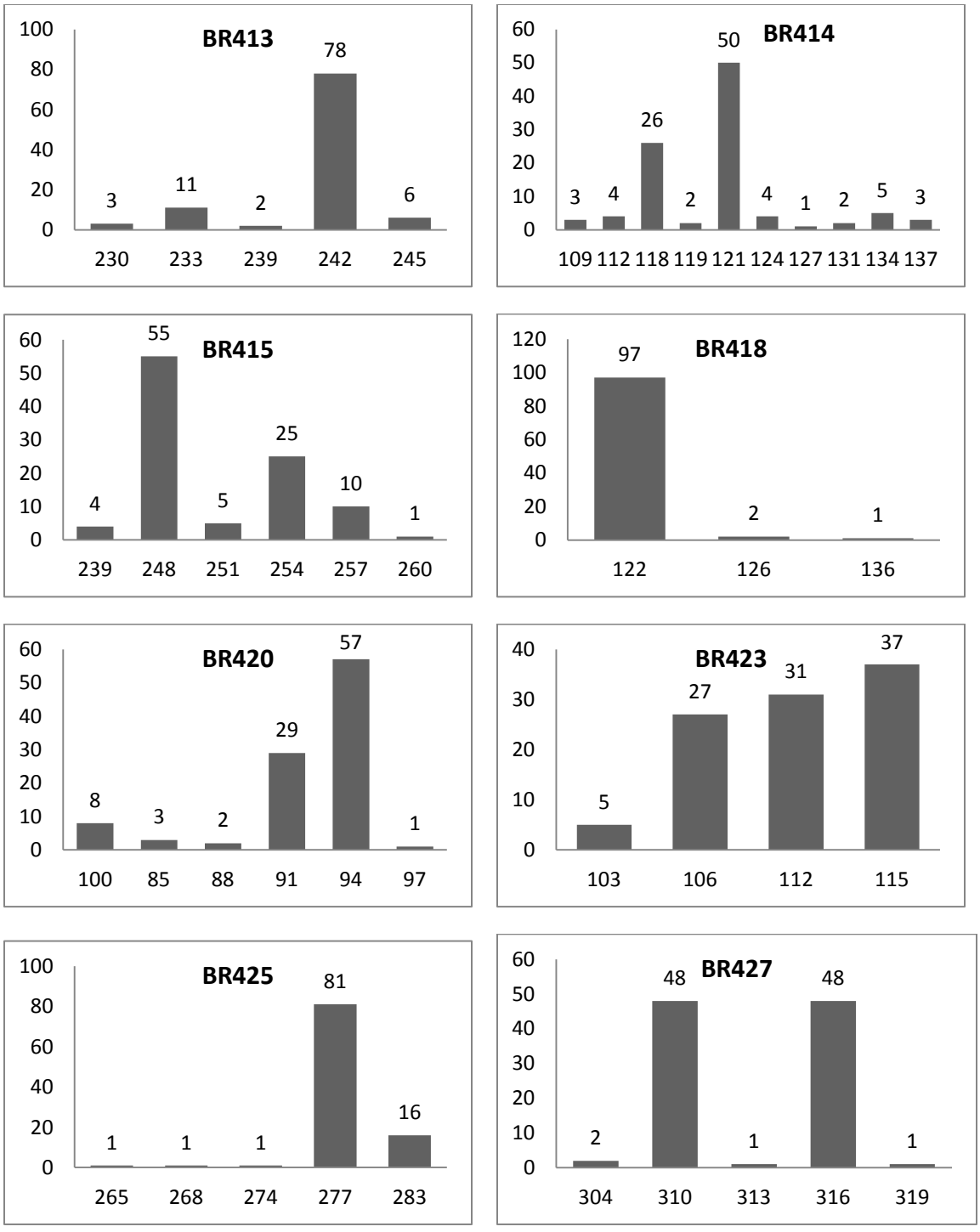
Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



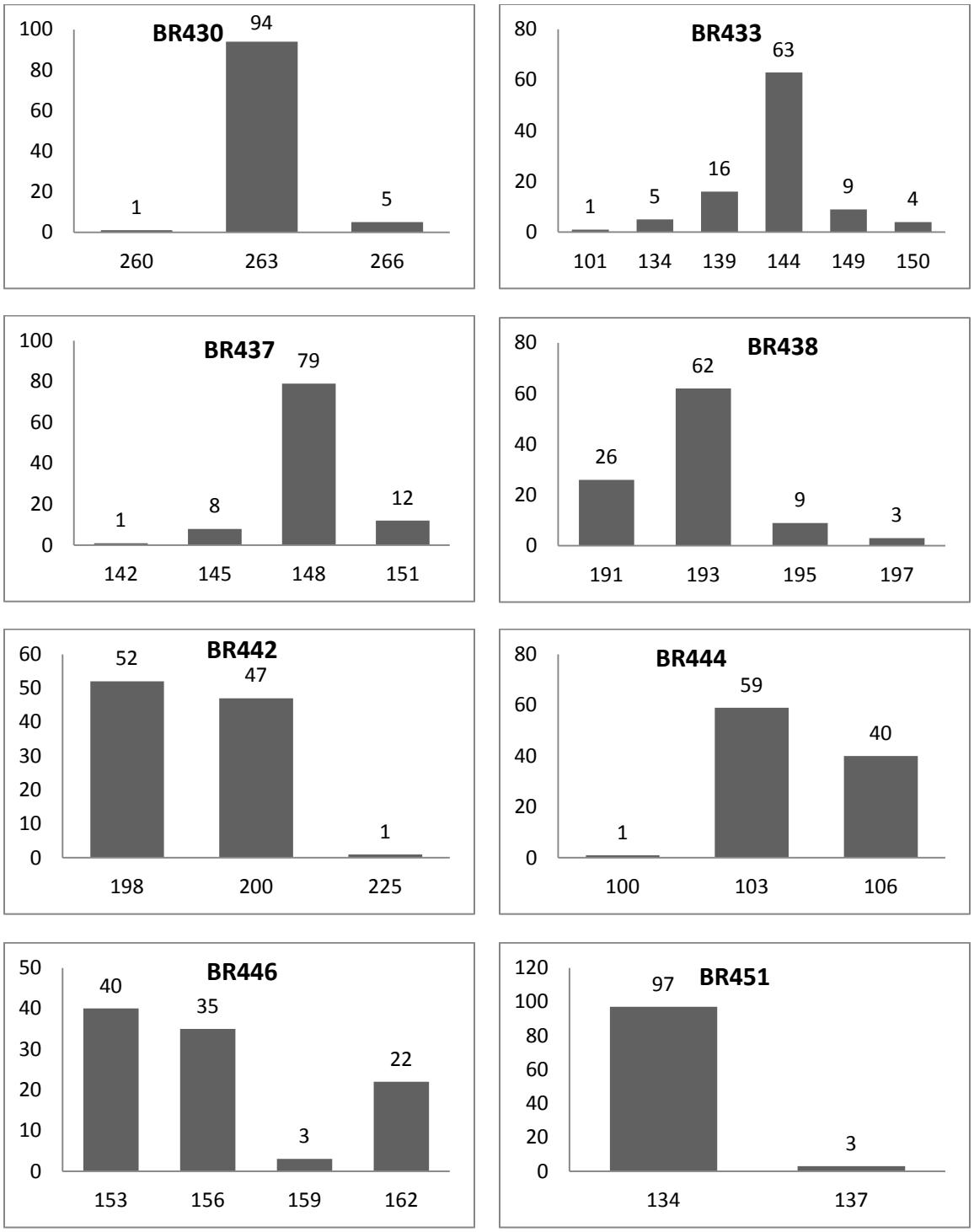
Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



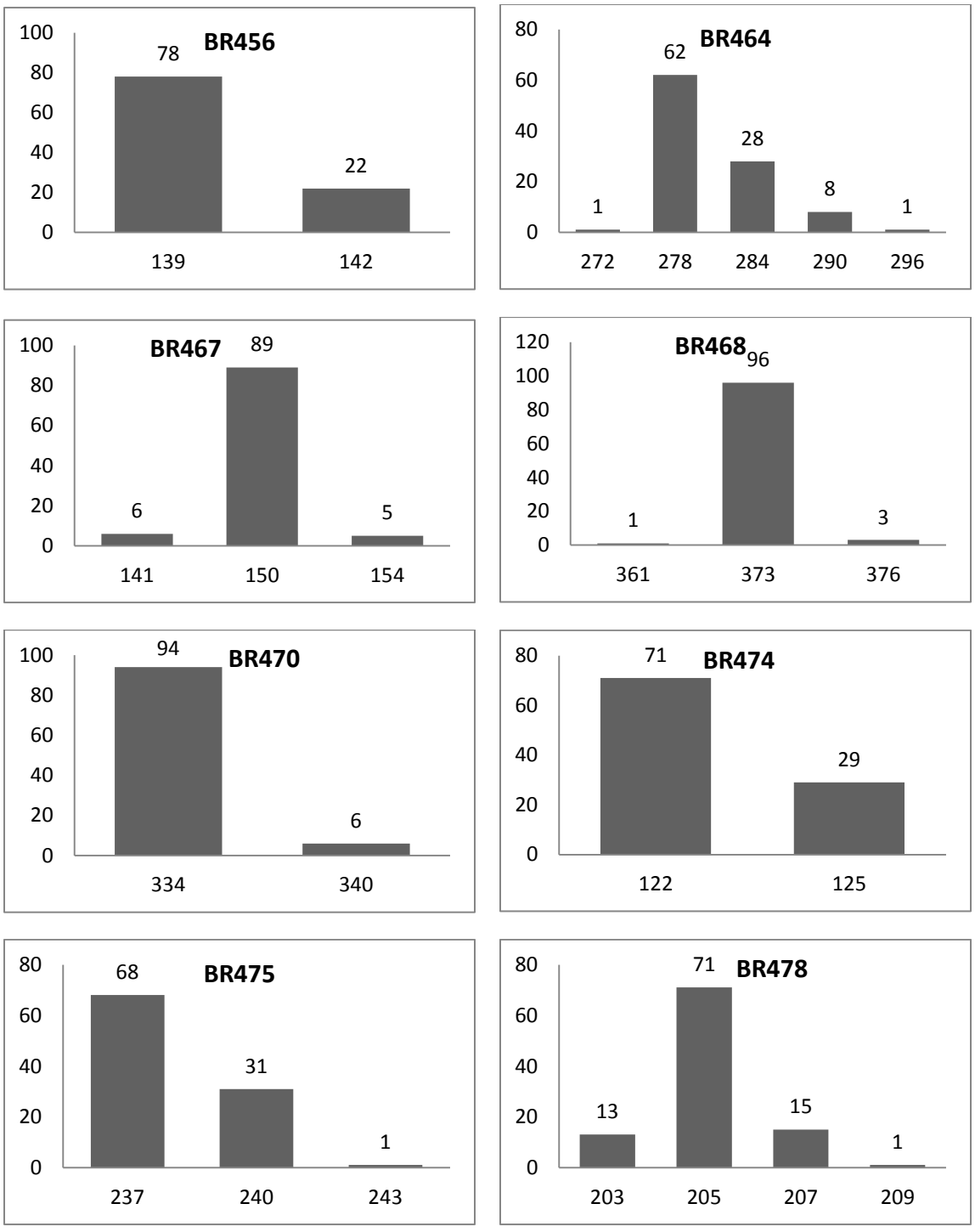
Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



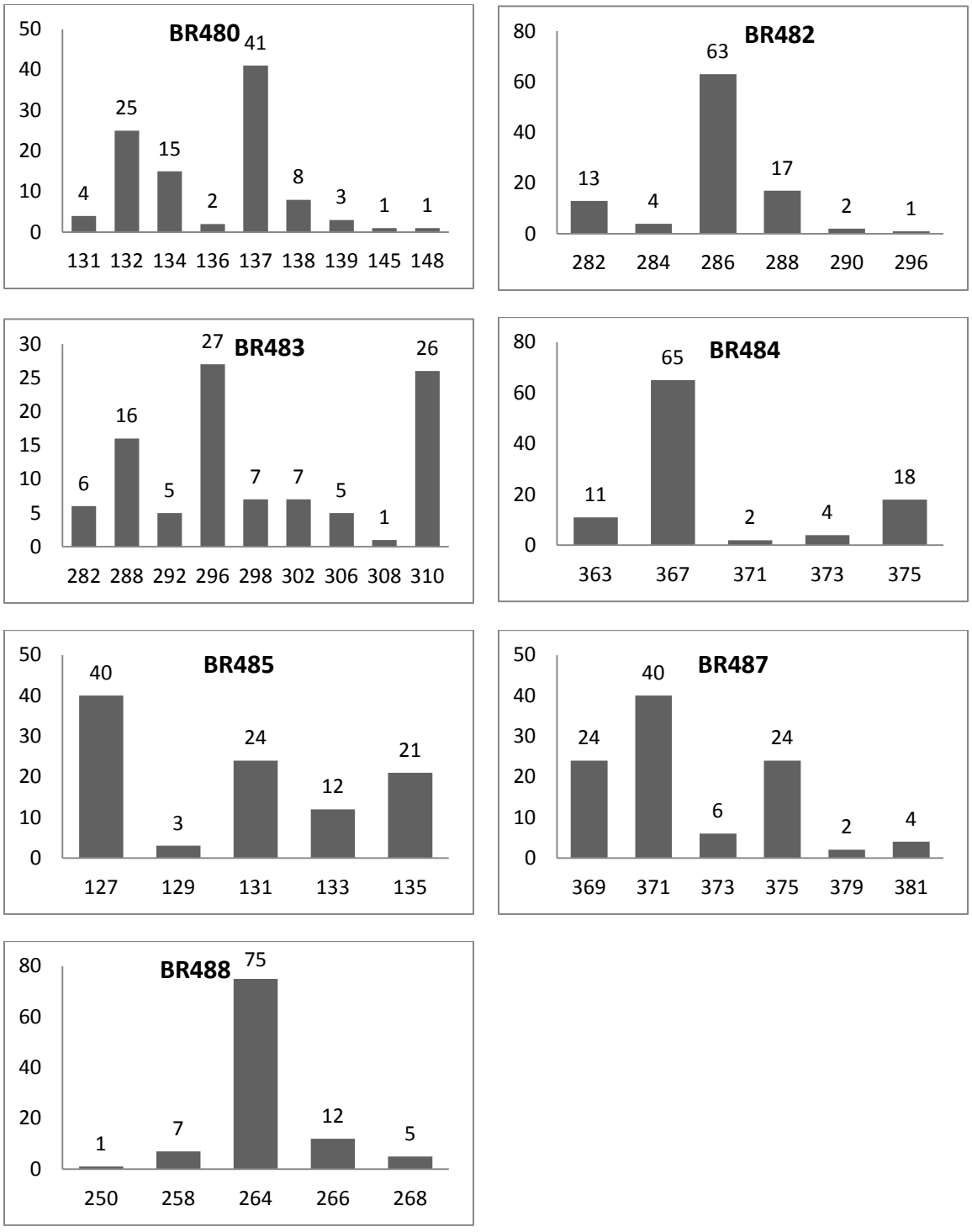
Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.



Appendix G (Cont). Allelic frequencies in 50 hazelnut accessions at 111 SSR loci developed from transcriptome sequences.

Appendix H Allele segregation in markers used to fingerprint LG6 in 5 progenies

Progeny	Marker	Present	Absent	Expected	χ^2	Prob
05024	A614_149	39	40	39.5	0.01266	0.91042
05024	LG610_455	47	49	48	0.04167	0.83826
05024	KG821_249	49	47	48	0.04167	0.83826
05024	LG687_318	50	46	48	0.16667	0.68309
05024	LG688_362	50	46	48	0.16667	0.68309
05024	LG696_382	50	46	48	0.16667	0.68309
05024	Rest-'Culpla'	51	42	46.5	0.87097	0.35069
05024	LG682_384	52	44	48	0.66667	0.41422
05024	LG637_244	49	46	47.5	0.09474	0.75824
05024	LG639_227	50	46	48	0.16667	0.68309
05024	LG628_213	51	45	48	0.375	0.54029
05024	B716_207	52	31	41.5	5.31325	0.02116
05024	KG828_204	52	44	48	0.66667	0.41422

Progeny	Marker	Present	Absent	Expected	χ^2	Prob
05023	A614_150	38	10	24	16.3333	5.3E-05
05023	LG610_455	40	8	24	21.3333	3.9E-06
05023	KG821_248	40	8	24	21.3333	3.9E-06
05023	LG687_327	38	10	24	16.3333	5.3E-05
05023	LG668_176	33	14	23.5	7.68085	0.00558
05023	LG688_362	40	8	24	21.3333	3.9E-06
05023	Rest-'Culpla'	38	10	24	16.3333	5.3E-05
05023	LG682_384	38	10	24	16.3333	5.3E-05
05023	LG637_242	38	10	24	16.3333	5.3E-05
05023	LG628_213	37	11	24	14.0833	0.00017
05023	B716_203	15	9	12	1.5	0.22067
05023	KG828_205	37	10	23.5	15.5106	8.2E-05
05023	B776_148	17	30	23.5	3.59574	0.05793

Progeny	Marker	Present	Absent	Expected	χ^2	Prob
06027	A614_142	44	52	48	0.66667	0.41422
06027	KG821_268	46	50	48	0.16667	0.68309
06027	LG668_164	42	53	47.5	1.27368	0.25908
06027	LG648_235	42	54	48	1.5	0.22067
06027	LG675_247	41	54	47.5	1.77895	0.18228
06027	LG696_395	41	53	47	1.53191	0.21583
06027	Rest-'Crvenje'	43	52	47.5	0.85263	0.35581
06027	LG639_219	43	53	48	1.04167	0.30743
06027	LG628_212	43	53	48	1.04167	0.30743
06027	B716_207	44	52	48	0.66667	0.41422
06027	B776_137	29	52	40.5	6.53086	0.0106

Appendix H (cont.) Allele segregation in markers used to fingerprint LG6 in 5 progenies

Progeny	Marker	Present	Absent	Expected	χ^2	Prob
06028	A614_143	25	23	24	0.08333	0.77283
06028	KG821_268	22	26	24	0.33333	0.5637
06028	LG648_237	22	26	24	0.33333	0.5637
06028	LG675_250	20	26	23	0.78261	0.37634
06028	LG696_396	21	25	23	0.34783	0.55535
06028	Rest-'Crvenje'	21	26	23.5	0.53191	0.4658
06028	LG639_221	22	26	24	0.33333	0.5637
06028	LG628_213	22	26	24	0.33333	0.5637
06028	B716_207	29	19	24	2.08333	0.14891
06028	B776_135	31	17	24	4.08333	0.04331

Progeny	Marker	Present	Absent	Expected	χ^2	Prob
01035	A614_139	45	33	39	1.84615	0.17423
01035	C005_117	60	36	48	6	0.01431
01035	LG610_445	61	35	48	7.04167	0.00796
01035	KG821_257	59	37	48	5.04167	0.02474
01035	LG687_318	59	34	46.5	6.72043	0.00953
01035	LG668_154	61	35	48	7.04167	0.00796
01035	LG648_221	61	35	48	7.04167	0.00796
01035	LG688_362	60	36	48	6	0.01431
01035	LG696_382	59	14	36.5	27.7397	1.4E-07
01035	Rest-OSU495.072	56	34	45	5.37778	0.02039
01035	LG637_232	61	24	42.5	16.1059	6E-05
01035	LG639_217	61	24	42.5	16.1059	6E-05
01035	LG628_215	62	33	47.5	8.85263	0.00293
01035	B716_199d	63	33	48	9.375	0.0022
01035	B776_145	46	34	40	1.8	0.17971