THE USE OF MOLECULAR AND BIOCHEMICAL TOOLS TO ASSIST IN THE BREEDING OF HAZELNUTS (*CORYLUS SPP*.)

By

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ABSTRACT OF THE DISSERTATION

The Use of Molecular and Biochemical Tools to Assist in the Breeding of Hazelnuts (*Corylus* spp.)

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Hazelnuts rank 6th in world tree nut production, with approximately 800,000 metric tons produced per year. Commercial hazelnut production in the United States (the third largest producer of hazelnuts in the world) has been limited, due to the fungal pathogen *Anisogramma anomala*, the causal agent of eastern filbert blight (EFB). Interestingly, *A. anomala* is most deadly to the European hazelnut species (*Corylus avellana*), the only species used for commercial production, but is harbored by and does not cause symptoms in the native American species (*C. americana*). This fungal pathogen invades the vascular system of hazelnuts, girdles branches, and ultimately leads to death of the tree. Control measures to combat EFB are expensive and labor intensive, thus the most cost effective means of combating this disease is the use of disease resistant plant material. The *Corylus* genus holds 10 additional species, many of which carry EFB resistance. Over the past 15 years, extensive germplasm collection trips have been made to develop a broad hazelnut germplasm collection at Rutgers University, the entirely of which has been screened for resistance to EFB. The purpose of this study was to genetically characterize the novel collection of largely EFB resistant germplasm at Rutgers University using simple sequence repeat (SSR) markers. In addition, these same tools were used to further enhance the utility and better direct the use of this germplasm in the breeding program by performing a population structure analysis of *A. anomala* isolates collected from the United States and Canada.

The final aspect of this dissertation investigates additional *Corylus* species by conducting a lipid content and profile analysis of four hazelnut species and interspecific hybrids to determine if there is a species effect on important kernel characteristics. Both the hazelnut germplasm collection and *A. anomala* isolate collection were found to be highly genetically diverse, and the analysis resolved 11 and 22 genetic populations, respectively. It was also found that the lipid content and profiles of hazelnuts will likely not be negatively affected by the introgression of different species into the breeding program. This work has demonstrated that there are a number of diverse sources of resistance in the Rutgers University hazelnut germplasm collection to the exceedingly genetically diverse fungus *A. anomala,* and introgression of sources of resistance in non *C. avellana* species will likely not effect commercially important kernel characteristics.

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Introduction

The genus *Corylus* (2n=2x=22) is widely distributed across temperate regions of the Northern Hemisphere. Various species can be found in Japan, Korea, and China, through Tibet, India, northern Iran, Turkey, and the Caucuses, in addition to much of Europe and North America (Mehlenbacher, 1991). Most taxonomists place *Corylus* in the subfamily Coryloideae of the family Betulaceae, order Fagales, with recent work supporting the inclusion of 11 species placed in four subsections (Bassil et al., 2013; Chen et al., 1999; Erdogan and Mehlenbacher, 2000; Yoo and Wen, 2002). Across the genus, plants range from small, multi-stemmed shrubs to tall, single-trunk trees. Their leaves are simple, alternate, stipulate, doubly serrate, and pubescent (Mehlenbacher, 2003). All species produce edible nuts and are wind-pollinated, monoecious, dichogamous, self-incompatible, and deciduous (Mehlenbacher, 1991). Staminate flowers produce pollen in catkins, while pistillate inflorescences are simple red stigmatic styles that protrude from vegetative buds (Mehlenbacher, 2003). The most well-studied and commercially important member of the genus is the European hazelnut, *C. avellana*, which ranks sixth in world tree nut production behind cashew (*Anacardium occidentale*), almond (*Prunus dulcis*), walnut (*Juglans regia*), chestnut (*Castanea* spp.), and pistachio (*Pistacia vera*). Turkey produces approximately 70% of the world's hazelnut crop (742,997 t in 2011), followed by Italy (≈ 15%) and the U.S. (≈ 5%) (Food and Agriculture Organization of the United Nations, 2017). Ninety-nine percent of the U.S. crop is produced in the Willamette Valley of Oregon.

The lack of commercial hazelnut production in the eastern U.S. is largely due to the disease eastern filbert blight (EFB), caused by the fungus *Anisogramma anomala* (Thompson et al., 1996). This pathogen is found naturally occurring on the wild American hazelnut, *C. americana*, where it causes little damage (Capik and Molnar, 2012; Fuller, 1908; Weschcke, 1954). However, EFB is devastating to most plants of *C. avellana*, on which it causes stem

cankers, branch die-back, and subsequent tree death (Johnson and Pinkerton, 2002). The disease was originally restricted to regions east of the Rocky Mountains, allowing commercial hazelnut production to thrive in the Pacific northwestern U.S. for many decades (Thompson et al., 1996). Unfortunately, *A. anomala* was inadvertently introduced into southwestern Washington in the 1960s and subsequently overwhelmed hazelnut orchards, as control measures had not yet been developed and cultivars in production were generally very susceptible (Davison and Davidson, 1973; Gottwald and Cameron, 1980b). The disease has since spread throughout the Willamette Valley of Oregon.

The fungus *Anisogramma anomala* is an obligate biotrophic ascomycete in the order Diaporthales. It is homothallic and reproduces only by ascospores (Gottwald and Cameron, 1980a), which are moved via rain splash and wind-driven rain to infect young apical meristems (Pinkerton, 1998). Management of EFB adds considerable expense to hazelnut production, due to the need for copious fungicide sprays, scouting for cankers, and pruning of infected wood. Breeding for resistance to EFB is considered to be the most cost-effective means of control (Johnson et al., 1996; Julian et al., 2009; Thompson et al., 1996).

Efforts to Breed for Resistance to Eastern Filbert Blight

Hazelnut breeding in the eastern U.S. began in the early 1900s with the goal of developing better-adapted, cold-hardy, and EFB-resistant plants. These efforts have been previously discussed in detail in Thompson et al. (1996), Molnar et al. (2005), and Molnar (2011). Briefly, most of the breeding efforts were unsuccessful in identifying or developing hazelnuts capable of supporting a commercial hazelnut industry in the eastern U.S., primarily because of a lack of the combination of EFB resistance, cold hardiness, high nut yield, and kernel quality. However, some progress was made in developing improved hybrids from first generation controlled crosses of *C. avellana* and other *Corylus* species, as well as better selections from open-pollinated (OP) seedling populations of those original hybrids (Molnar et al., 2005).

In the Pacific northwestern U.S., the first EFB-resistant cultivar identified was *C. avellana,* 'Gasaway', a late-blooming pollinizer that produces low yields of small nuts. 'Gasaway' was shown to transmit resistance to its offspring in a ratio of one resistant to one susceptible, which is indicative of a dominant allele at a single locus in the heterozygous state (Cameron, 1976; Mehlenbacher et al., 1991). It has since been widely used in the Oregon State University (OSU; Corvallis, OR) hazelnut breeding program, leading to the development of several EFB-resistant cultivars and pollenizers including Santiam (Mehlenbacher et al., 2007), Yamhill (Mehlenbacher et al., 2009), Jefferson (Mehlenbacher et al., 2011), Dorris (Mehlenbacher et al., 2013), and McDonald (Mehlenbacher et al., 2016).

Since the discovery of 'Gasaway', additional *C. avellana* cultivars and seedling selections have been shown to be resistant to EFB in Oregon. These include 'Ratoli' (Lunde et al., 2000; Sathuvalli et al., 2011a) and 'Culpla' (Chen et al., 2007) from Spain; 'Uebov' and 'Crvenje' from Serbia; Moscow #1, 2, 26, 27, and 37 from Moscow, Russia; OSU 495.072, from southern Russia (Sathuvalli et al., 2010); OSU 759.010 from the Republic of Georgia (Sathuvalli et al., 2011b); CCOR 187.001 from Finland (Chen et al., 2007); and OSU 408.040 from the University of Minnesota (Chen et al., 2005; Sathuvalli et al., 2012). Today, in addition to continued use of 'Gasaway', these resistant plants are being incorporated into breeding efforts at OSU (S.A. Mehlenbacher, personal communication).

A hazelnut genetic improvement program was initiated at Rutgers University in 1996. To search for additional sources of EFB resistance in *C. avellana*—the species with the largest nuts and highest quality kernels (Mehlenbacher, 1991)—germplasm collections were made in Russia, Ukraine, and Poland. The resulting seedlings, spanning numerous seed lots across all three countries, were exposed to *A. anomala* at Rutgers University (a subset was also grown at OSU) and later evaluated for their response to the disease. From more than 2400 seedlings, nearly 100 (~4%) EFB-resistant (plants remaining free of disease symptoms) and tolerant (plants with few, small cankers) selections were identified that add to the pool of genetic resources now available

for breeding. Some of these plants have improved nut yield and quality compared to earlier resistant selections, particularly 'Gasaway' (Capik et al., 2013; Molnar et al., 2007).

Adding this new EFB-resistant material to the resistant accessions identified in Oregon and the clonal and seed-propagated interspecific hybrid selections previously developed by eastern U.S. breeders results in a significant amount of disease-resistant germplasm (Capik and Molnar, 2012; Capik et al., 2013). However, with the exception of the known cultivars (Ratoli, Culpla, etc.), much of this material came as seed of unknown or uncertain origin. In regard to many of the selections from early breeding efforts in the eastern U.S., in general, there was little or no control of the pollen parent and records of female parents were often lost, including species designations. Further, due to extensive exchange of plant materials in the U.S., especially among members of the Northern Nut Growers Association (NNGA), selections from different states and provinces likely share the same genetic base. This uncertainty in relationships and genetic backgrounds of the EFB-resistant germplasm presents problems when planning long-term breeding efforts to develop durable resistance in this long-lived, perennial species.

Genetic Characterization of Hazelnut Germplasm to Date

Fortunately, molecular tools are now available to characterize hazelnut germplasm. A number of genetic diversity and cultivar identification/fingerprinting studies have been performed on hazelnuts over the past 18 years. The first genetic study involved the use of random amplification of polymorphic DNA (RAPD) to identify six commonly grown hazelnut cultivars in southern Italy (Galderisi et al., 1999). Microsatellite, or simple sequence repeat (SSR), markers are particularly valuable for fingerprinting accessions, examining relationships, and assessing genetic diversity in many plants (including across species) due to their abundance, polymorphic nature, and co-dominance (Bassil et al., 2005a, 2005b, 2013; Boccacci et al., 2005, 2006, 2008; Gökirmak et al., 2009; Gürcan and Mehlenbacher, 2010a, 2010b; Gürcan et al., 2010a, 2010b). Many of these SSR markers were used in genetic diversity studies and linkage map saturation. This included germplasm collections from Iran (Ghanbari et al., 2005), Italy, and Spain (Boccacci

et al., 2009). Studies by Boccacci et al.(2006) and Botta et al. (2005) resulted in the discovery and/or confirmation of several cases of synonomy between different cultivars. In addition, cluster analysis of 78 *C. avellana* hazelnut cultivars showed genetic differentiation of northern and southern European and Turkish cultivars (Boccacci et al., 2006). In 2009, SSR markers were used to assess the diversity of 270 accessions of *C. avellana* collected from throughout the world (Gokirmak et al., 2009). A considerable significant level of synonomy (26.7%) across named cultivars was observed in this study. On a whole, the germplasm was placed into four main groups which were closely correlated to their geographical origins: Central European, Black Sea, English and Spanish-Italian (Gokirmak et al., 2009). More recent studies showed that germplasm collected from remote locations in Northern Spain was genetically diverse and grouped separately from Spanish/Italian reference cultivars. This work was noted as showing the diversity of *Corylus* at a local level (Ferreira et al., 2010). Lastly, the level of genetic diversity of local germplasm collected from Black Sea countries (Turkey, Georgia, Azerbaijan) was found to be at a similar level as that of germplasm collected from throughout the world (Gurcan et al., 2010).

A collection of the native American hazelnut species *C. americana* and interspecific *C. americana* x *C. avellana* hybrids have also been fingerprinted with SSR markers. Analysis of the allelic data resulted in the plants being placed into several groups including three groups of hybrid material which showed lower diversity than the wild accessions suggesting that breeding work with *C. americana* hybrids in the early 1900s has lead to a genetic bottleneck. By contrast, the three groups of *C. americana* accessions that were resolved from this study were shown to be highly polymorphic (Sathuvalli and Mehlenbacher, 2012). Collectively, these studies have provided significant insight into the genetic diversity and population structure existing in cultivated and wild hazelnuts, including the domestication and spread of cultivated hazelnut in Europe.

The first genetic linkage map and indication of molecular-level variability in *C. avellana* involved the use of isozyme analysis of seedling progenies from several controlled crosses. This

study resulted in the characterization of three linkage groups (Rovira et al., 1993). More recently, a significantly more saturated linkage map was created for *C. avellana* using newly characterized RAPD and SSR markers. Eleven pairs of chromosomes were mapped, which corresponded to the haploid chromosome number of *Corylus* (Mehlenbacher et al., 2006).

Variability of Anisogramma anomala

Resistance to EFB has been shown to have variability depending upon the region. While plants expressing the 'Gasaway' *R*-gene have proven to be resistant to EFB in Oregon for many decades, a report by Molnar et al. (2010a) found that trees of 'Gasaway' and its offspring VR 20-11 developed cankers in the field in New Jersey. A greenhouse study by Molnar et al. (2010b) suggested that pathogentic variability exists in *Anisogramma anomola.* In this study, isolates from 12 locations were used to inoculate trees carrying 'Gasaway', and other sources of resistance. The results showed that trees of 'Gasaway' only developed EFB when exposed to the isolate of *A. anomala* collected from Michigan but 'VR20–11'was infected by isolates from New Jersey, Minnesota, and Michigan. The Michigan isolate also caused the only signs of infection on OSU 408.040 and 'Zimmerman'. These results indicated that the isolate from Michigan was more virulent than the other isolates studied and that variation exists in this pathogen. A follow up field study was performed where additional 'Gasaway'-related plants were exposed to EFB from multiple sources and developed cankers, the same plants had shown no prior disease response in Oregon. These findings further supported the variability of *A. anomala.* Although it was hypothesized that these differential disease responses could be due to pathogenic variation of *A. anomala,* this does not preclude the possibility that these responses were due to environmental variation (climate and disease pressure) differences (Capik and Molnar, 2012).

In a first attempt to characterize the diversity of this fungus on a molecular level, the genome of *A. anomala* was sequenced and SSR markers were identified (Cai et al., 2013). A database of markers was then generated to be used in genetic diversity studies. A total of 236 markers from this database were screened and 23 were found to be polymorphic. Subsequently,

11 markers were used to amplify 30 different isolates of *A. anomala* in a proof of concept study. The study resolved two groups of isolates, one of which were isolates collected from New Jersey and the second included isolates collected from the Great Lakes region and Oregon. This study was the first published proof of genetic differentiation of different isolates of *A. anomala* (Cai et al., 2013).

Breeding for Commercially Desirable Kernel Traits

In addition to breeding for resistance to EFB, a major thrust in hazelnut breeding is to develop plants expressing high-quality kernels. These include medium sized (12 mm diameter), round kernels that are easy to blanch and have thin shells (Mehlenbacher, 1994). Thus far, making selection on biochemical kernel compositions has not been a direct focus in hazelnut breeding programs, despite the fact that the largest component of the dry weight of a hazelnut kernel is oil (62-70%). Of the species characterized (*C. avellana* and *C. americana*), *Corylus* oil has been shown to have a similar fatty acid profile as that of olive oil (Parcerisa, 2000). However, among cultivars there is a relatively significant variation in the amount of oil per weight of kernel, as well as relative proportions of fatty acid profile components. The oil is primarily composed of the monounsaturated fatty acid oleic acid (77.50-82.95%). Linoleic acid content is the second largest component of the fatty acid profile (7.55-13.69%), although it is somewhat variable across cultivars tested (Alasalvar et al., 2009). The percentage of linoleic acid is particularly noteworthy because it confers properties that help prevent oxidation of fatty acids and preserve hazelnut seeds (Bacchetta et al., 2013; Botta et al., 1994; Ebrahem et al., 1994). Thus, opportunities may exist for genetic improvement in these characteristics.

Thesis Overview

The overarching theme of this dissertation is to utilize molecular and biochemical tools to characterize the Rutgers hazelnut germplasm collection and the causal agent of EFB to assist in making informed decisions for the hazelnut breeding program. It is hoped that this work will

help accelerate development of disease resistant hazelnut cultivars with commercial quality kernel characteristics. The first component of this dissertation involves a characterization of the genetic diversity and population structure of a significant portion of the EFB-resistant *Corylus* germplasm collection held at Rutgers University. This material largely originated from seed-based germplasm collections in Russia, Ukraine, and Poland. The subsequent chapter outlines a study involving the phenotyping and genotyping of a new source of seed-based germplasm collected from Turkey, Latvia, and Lithuania, with the goal of identifying and characterizing new sources of resistance to EFB. The third chapter comprises a genetic assessment of the causal agent of EFB, *A. anomala*, to help provide a clearer understanding of the genetic diversity and population structure of the fungus. The final component of this dissertation entails the characterization of oil content and composition of hazelnut kernels from several species of *Corylus* and interspecific hybrids to help discern if interspecific hybridization is likely to affect the oil quantity and quality in hazelnut kernels.

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CHAPTER 1: Characterization of Eastern Filbert Blight-resistant Hazelnut Germplasm Using Microsatellite Markers

ABSTRACT

The development of new cultivars resistant to the disease eastern filbert blight (EFB), caused by *Anisogramma anomala*, is of primary importance to hazelnut (*Corylus* sp.) breeders in North America. Recently, a large number of EFB-resistant cultivars, grower selections, and seedlings from foreign germplasm collections were identified. However, for a significant number of these, little is known of their origin, relationships, or genetic background. In this study, 17 microsatellite markers were used to investigate the genetic diversity and population structure of 323 unique accessions, including EFB-resistant and tolerant germplasm of uncertain origins, in comparison with a panel of known reference accessions representing a wide diversity of Corylus cultivars, breeding selections, and interspecific hybrids. The resulting allelic data were used to construct an unweighted pair group method using arithmetic averages (UPGMA) dendrogram and STRUCTURE diagram to elucidate relationships among the accessions. Results showed 11 consensus groups with EFB-resistant or tolerant accessions in all, providing strong evidence that EFB resistance is relatively widespread across the genus Corylus. Furthermore, open-pollinated seedlings tended to group together with reference accessions of similar geographic origins, providing insight into their genetic backgrounds. The results of this study add to the growing body of knowledge of hazelnut genetic resources and highlight recently introduced EFB-resistant seedling germplasm as new, unrelated genetic pools of resistance.

INTRODUCTION

The genus Corylus (2n = 2x = 22) is widely distributed across temperate regions of the Northern Hemisphere. Various species can be found in Japan, Korea, and China, through Tibet, India, northern Iran, Turkey, and the Caucuses as well as in much of Europe and North America (Mehlenbacher, 1991). Most taxonomists place Corylus in the subfamily Coryloideae of the family Betulaceae, order Fagales, with recent work supporting the inclusion of 11 species placed in four subsections (Bassil et al., 2013; Chen et al., 1999; Erdogan and Mehlenbacher, 2000; Yoo and Wen, 2002). Across the genus, plants range from small, multi stemmed shrubs to tall, single trunk trees. All species produce edible nuts and are wind pollinated, self-incompatible, and deciduous (Mehlenbacher, 1991). The most well-studied and commercially important member of the genus is the european hazelnut (*C. avellana*), which ranks fifth in world tree nut production behind cashew (*Anacardium occidentale*), almond (*Prunus dulcis*), walnut (*Juglans regia*), and chestnut (*Castanea* sp.). Turkey produces 70% of the world's hazelnut crop (742,997 t in 2011) followed by Italy (15%) and the United States (5%) (Food and Agriculture Organization of the United Nations, 2013). Ninety nine percent of U.S. production comes from the Willamette Valley of Oregon.

The lack of commercial hazelnut production in the eastern United States is largely the result of the disease EFB caused by the fungus *Anisogramma anomala* (Thompson et al., 1996). This pathogen is found naturally occurring on the wild american hazelnut (*C. americana*), where it causes little damage (Capik and Molnar, 2012; Fuller, 1908; Weschcke, 1954). However, EFB is devastating to most plants of *C. avellana*, on which it causes stem cankers, branch dieback, and subsequent tree death (Johnson and Pinkerton, 2002). The disease was originally restricted to regions east of the Rocky Mountains, allowing commercial hazelnut production to thrive in the Pacific northwestern United States for many decades (Thompson et al., 1996). Unfortunately, *A. anomala* was inadvertently introduced into southwestern Washington in the 1960s and

subsequently overwhelmed hazelnut orchards, because control measures had not yet been developed and cultivars in production were generally very susceptible (Davison and Davidson, 1973; Gottwald and Cameron, 1980). The disease has since spread throughout the Willamette Valley of Oregon. Its management adds considerable expense to hazelnut production as a result of the need for copious fungicide sprays, scouting for cankers, and pruning of infected wood. Breeding for resistance to EFB is considered to be the most cost-effective means of control (Johnson et al., 1996; Julian et al., 2008, 2009; Thompson et al., 1996).

In the Pacific northwestern United States, the first EFB resistant cultivar identified was *C. avellana* Gasaway, a late blooming pollenizer that produces low yields of small nuts. 'Gasaway' was shown to transmit resistance to its offspring in a ratio of one resistant to one susceptible, which is indicative of a dominant allele at a single locus in the heterozygous state (Cameron, 1976; Mehlenbacher et al., 1991). It has since been widely used in the Oregon State University (OSU, Corvallis, OR) hazelnut breeding program, leading to the development of the EFB-resistant cultivars Santiam (Mehlenbacher et al., 2007), Yamhill (Mehlenbacher et al., 2009), Jefferson (Mehlenbacher et al., 2011), and Dorris (Mehlenbacher et al., 2013). Since the discovery of 'Gasaway', additional *C. avellana* cultivars and seedling selections have been shown to be resistant to EFB in Oregon. These include 'Ratoli' (Lunde et al., 2000; Sathuvalli et al., 2011a) and 'Culpla' (Chen et al., 2007) from Spain; 'Uebov' and 'Crvenje' from Serbia (Sathuvalli et al., 2010); Moscow #1, 2, 26, 27, and 37 from Moscow Province, Russia; OSU 495.072 believed to be from southern Russia (Sathuvalli et al., 2010); OSU 759.010 from the Republic of Georgia (Sathuvalli et al., 2011b); Finland CCOR 187.001 from Finland (Chen et al., 2007); and OSU 408.040 from the University of Minnesota (Chen et al., 2005; Sathuvalli et al., 2012). Today, in addition to continued use of 'Gasaway', these resistant plants are being incorporated into breeding efforts at OSU (S.A. Mehlenbacher, personal communication).

Hazelnut breeding in the eastern United States began in the early 1900s with the goal of developing better-adapted, cold hardy, and EFB-resistant plants. These efforts have been

previously discussed in detail in Molnar (2011), Molnar et al. (2005), and Thompson et al. (1996). Briefly, most of the breeding efforts were unsuccessful in identifying or developing hazelnuts capable of supporting a commercial hazelnut industry in the eastern United States, primarily because of a lack of the combination of EFB resistance, cold-hardiness, high nut yield, and kernel quality. However, some progress was made in developing improved EFB-resistant hybrids from first-generation controlled crosses of *C. avellana* and *C. americana* as well as better selections from open-pollinated (OP) seedling populations of those original hybrids (Molnar et al., 2005).

A hazelnut genetic improvement program was initiated at Rutgers University in 1996. To search for additional sources of EFB resistance in *C. avellana*—the species with the largest nuts and highest quality kernels (Mehlenbacher, 1991)—germplasm collections were made in Russia, Ukraine, and Poland. The resulting seedlings, spanning numerous seed lots across all three countries, were exposed to *A. anomala* at Rutgers University (a subset was also grown at OSU) and later evaluated for their response to the disease. From more than 2400 seedlings, nearly 100 (4%) EFB-resistant (plants remaining free of disease symptoms) and -tolerant (plants with few small cankers) plants were identified that add to the pool of genetic resources now available for breeding. Some of these plants have improved nut yield and quality compared with earlier EFB-resistant selections, particularly ‘Gasaway’ (Capik et al., 2013; Molnar et al., 2007).

Adding this new EFB-resistant material to the resistant accessions identified in Oregon and the clonal and seed propagated interspecific hybrid selections previously developed by eastern U.S. breeders results in a significant amount of disease-resistant germplasm (Capik et al., 2013; Capik and Molnar, 2012). However, with the exception of the known cultivars (Ratoli, Culpla, etc.), much of this material came as seed of unknown or uncertain origin. In regard to many of the grower selections derived from early breeding efforts in the eastern United States, in general, there was little or no control of the pollen parent and records of female parents were often lost, including species designations. Furthermore, as a result of extensive exchange of plant

materials in the United States, especially among members of the Northern Nut Growers Association (NNGA, 2013), selections from different states and provinces likely share the same genetic base. This uncertainty in relationships and genetic backgrounds of the EFB-resistant germplasm presents problems when planning long-term breeding efforts to develop durable resistance in this long-lived, perennial species.

Fortunately, molecular tools are now available to characterize hazelnut germplasm. Microsatellite, or simple sequence repeat (SSR), markers are particularly valuable for fingerprinting accessions, examining relationships, and assessing genetic diversity in hazelnut (including across species) as a result of their abundance, polymorphic nature, and codominance (Bassil et al., 2005a, 2005b, 2013; Boccacci et al., 2005, 2006, 2008; Gökirmak et al., 2009; Gürcan et al., 2010a, 2010b; Gürcan and Mehlenbacher, 2010a, 2010b). Collectively, these studies have provided significant insight into the genetic diversity and population structure existing in cultivated and wild hazelnuts, including the domestication and spread of cultivated hazelnut in Europe. Gökirmak et al. (2009) examined 270 accessions of *C. avellana* spanning the world's production regions, of which 198 were unique. Their analysis organized the accessions into groups based on their SSR profiles and place of origin, revealing four major geographic groups (Central European, Black Sea, English, and Spanish–Italian) with subgroups resolved within them. Their work, as well as that of the others listed previously, provides a framework in which to place previously uninvestigated clonal accessions and seedlings in relation to known cultivars using SSR markers. The objective of our study was to use SSR markers to investigate the genetic diversity and population structure of new EFB-resistant and tolerant hazelnut germplasm in comparison with a wide diversity of known Corylus cultivars, accessions, breeding selections, and interspecific hybrids.

MATERIALS AND METHODS

Plant Material

A total of 323 Corylus accessions from Rutgers University, OSU, and the U.S. Department of Agriculture (USDA) National Clonal Germplasm Repository (NCGR) were examined in this study (Table 1). These accessions include 84 clonal accessions of *C. avellana*, 142 selections derived from OP seed of *C. avellana* from new germplasm introductions, 11 representatives of wild Corylus species, and 86 putative Corylus hybrids. The 84 clonal *C. avellana* accessions comprise a reference panel of known cultivars grown across much of the world's production regions and also include most of the known *C. avellana* sources of EFB resistance. Nearly all of these clonal accessions were previously characterized in Gökirmak et al. (2009) and were selected for incorporation in this study to represent the geographic groups described in their work.

The 142 seed-derived *C. avellana* accessions include 86 EFB-resistant and 33 EFB-tolerant plants identified from germplasm collections made in Russia, Ukraine, and Poland, as described in Capik et al. (2013) and Molnar et al. (2007), with the remainder comprised of susceptible seedlings from these regions as well as Estonia and Moldova. The 86 putative Corylus hybrid accessions consist of EFB-resistant and -tolerant plants developed from earlier U.S. breeding efforts, including that of J. Gellatly, C. Farris, J. Gordon, and E. Grimo. Most of these were selected from seedlings produced by OP (Molnar, 2011; Molnar et al., 2005; Thompson et al., 1996). Collectively, the seed-derived *C. avellana* accessions and the putative Corylus hybrid accessions have largely unknown pedigrees/origins and have not been previously characterized. The 11 wild accessions include plants of *C. americana*, *C. californica*, *C. colurna*, *C. chinensis*, *C. cornuta*, *C. heterophylla*, *C. mandshurica*, and *C. sieboldiana* to serve as outgroup accessions for the phylogenetic analysis and to help place putative hybrids. Finally, three additional accessions (Home Ec Building, Rutgers Passion Puddle, and Morris 32-1379A) have unknown origins and one (Unknown-EFB res.) is an

EFB-resistant grafted *C. avellana* tree that had its identity lost at Rutgers and was included in an attempt to name it.

Genomic DNA Extraction and Microsatellite Genotyping

Young leaves were collected from accessions growing at Rutgers University or the USDA NCGR in Corvallis, OR, during Spring 2011 or 2012 and stored at –80 C until ground in liquid nitrogen. Plant genomic DNA was extracted using the CTAB method adapted from protocols described by Cullings (1992) and Doyle and Doyle (1987). Extracted DNA was quantified with a spectrophotometer (NanoDrop ND-1000; Thermo Scientific, Waltham, MA) and diluted to a concentration of 5 ng/μL^{-1}. Seventeen well-characterized SSR markers (Table 2) were used to genotype all of the hazelnut accessions. These 17 SSR markers were chosen by screening a subset of 35 SSR markers (selected from more than 200 currently available for hazelnut) based on their level of polymorphism in the current data set, coverage of previously determined genetic linkage groups (10 out of 11 represented), high-quality amplification, reproducibility, low frequency of null alleles, and cross-species utility (Bassil et al., 2005a, 2005b; Boccacci et al., 2005; Gökirmak et al., 2009; Gürcan et al., 2010b; Gürcan and Mehlenbacher, 2010a, 2010b; Mehlenbacher et al., 2006). Amplification of all SSR polymerase chain reaction (PCR) products was assumed to be allelic for the current analysis, but this does not preclude the possibility that some amplification products were from paralogous loci. The M13 (-21) 18-bp sequence was added to the 5' end of all forward primers as an economical method for the fluorescent labeling of PCR fragments (Schuelke, 2000), and the ''PIG-tailing'' sequence (GTTTCTT) was added to the 5' end of all reverse primers to reduce uncertainty in scoring ''true'' vs. ''plus-A'' alleles (Brownstein et al., 1996). Primers were synthesized by Integrated DNA Technologies (Coralville, IA). PCR genotyping reactions were performed in 96-well plates in 13-mL reaction volumes. PCR reactions were composed of 5.0 ng genomic DNA, 10·Ramp-Taq PCR buffer (DenvilleScientific, Metuchen, NJ), 2.0 mM $MgCl_2$, 0.25 mM each dNTP (Denville Scientific), 0.5 U Ramp-Taq DNA polymerase (Denville Scientific), 0.5 pmol forward primer with 5' M13 (-21) addition, 1.0 pmol reverse primer with 5' PIG-tail addition,

and 1.0 pmol FAM, NED, PET, or VIC fluorescently labeled M13(-21) primer. PCR cycling was conducted in thermocyclers (GeneAmp 9700; Applied Biosystems, Foster City, CA) using the following parameters: initial denaturation of 94°C for 5 min followed by 30 cycles of 94°C for 30s, 55°C for 45s, 72°C for 45s, followed by 20 cycles of 94°C for 30s, 53°C for 45s, 72°C for 45s, followed by a final extension of 72°C for 10 min. PCR products were run on a capillary electrophoresis genetic analyzer (ABI 3500xl; Applied Biosystems) and were sized using a LIZ600 size standard (Applied Biosystems). Two additional controls were added to each 96-well plate: a sample of *C. avellana* 'Barcelona', a widely grown, EFB-susceptible cultivar in Oregon; and the GeneScan Installation Standard DS33 (Applied Biosystems). Genotyping results were scored and analyzed using Genemapper 4.0 (Applied Biosystems).

Data Analysis

The frequency of null alleles [F (null)] per loci was calculated using Cervus Version 3.0 (Kalinowski et al., 2007). The numbers of alleles for each locus, allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), and polymorphism information content (PIC) values were calculated using Powermarker Version 3.25 (Liu and Muse, 2005). Based on the allele frequencies, a distance matrix was then computed using the same software. A dendrogram based on an UPGMA was then constructed from the frequency-based distance matrix, and bootstrap values for the tree were calculated with a minimum support value of 0.500. The UPGMA dendrogram was visualized using Mega 5.01 (Tamura et al., 2011).

A Bayesian model-based clustering method, STRUCTURE 2.3.3 (Falush et al., 2003; Pritchard et al., 2000), was used to elicit population structure by assigning each accession to a population or populations based on 17-locus genotypes. Software run parameters included the assumption that all loci were independent and in linkage equilibrium. The admixture ancestry model, with correlated allele frequencies, was used for the analysis with a burn-in length of 20,000 iterations followed by 50,000 Markov chain Monte Carlo run iterations at each (K) value. An individual assigned to multiple populations (several colors in its bar) was considered evidence

of admixture or hybridization. A preliminary program run, with (K) set for (K) = 2 through 50, was used to estimate the most parsimonious value of (K) by finding the maximal value of the estimated log probability Pr(X|K) output at each (K) value. The STRUCTURE analysis was then run 10 (K) values above and below the estimated most parsimonious (K) value with 20 replicate runs per (K). The most parsimonious value of (K) was then chosen based on the maximal value of the average estimated log probability Pr(X|K) across all replicates and runs of (K).

Using information derived from the UPGMA dendrogram and the STRUCTURE output, the accessions were assigned to consensus populations. Interpretations of clusters/relationships among accessions in the UPGMA dendrogram were resolved by considering all accessions grouped within a node to be more closely related than to those not included in that node. For the STRUCTURE analysis, accessions were interpreted as belonging to a group/population based on their degree of admixture, where a given accession was considered a group member when exhibiting greater than 50% identity to one group (shown as greater than 50% one solid color). Data for unique accessions in these consensus populations were then subjected to an analysis of molecular variation (AMOVA) using GenAlEx 6.5 (Peakall and Smouse, 2006, 2012). This analysis was used to assess genetic variation within and among the consensus populations and to determine the interpopulation pairwise genetic distance (Fst).

RESULTS

SSR markers

The 17 SSR markers amplified 308 alleles in the current data set. As expected with a diploid species, each of the 17 SSR markers was inherited as a separate multiallelic, codominant locus. The number of alleles for individual SSRs ranged from 12 to 26 alleles per locus with a mean of 18 alleles per locus. The number of genotypes per locus ranged from 27 to 80 with a mean of 53 genotypes per locus. The frequency of null alleles per locus ranged from 0.0071 to 0.2577, which were comparable to previously reported values for hazelnut (Go¨kirmak et al., 2009). Mean values for He, Ho, PIC and inbreeding coefficien twere 0.81, 0.73, 0.79, and 0.103, respectively (Table 3). These results were also similar to previously reported values of He, Ho, PIC, and inbreeding coefficient for hazelnut (Bassil et al., 2005b; Boccacci et al., 2005; Gürcan et al., 2010a; Gürcan and Mehlenbacher, 2010a).

UPGMA clustering

The UPGMA clustering analysis placed the wild Corylus outgroup accessions in the most basal position of the dendrogram in groups that reflected their taxonomic subsections within Corylus (Fig. 1) (Yoo and Wen, 2002). The four ‘‘tree hazel’’ accessions (subsection Colurnaea), *C. colurna* #1, *C. chinensis* #1,and *C. fargesii* #1 and #2,were placed in a group at the very top, most basal position of the dendrogram. Immediately interior to this group was placed a cluster of the four ‘‘beaked-hazel’’ accessions (subsection Siphonochlamys clade). These include C. mandshurica #1, C. sieboldiana #1, C. cornuta ‘Peace River’, and OSU 587.044 (a hybrid of *C. californica* x *C. avellana*). It should be noted that *C. mandshurica* and *C. sieboldiana* are considered synonyms (Mehlenbacher, 1991). Placed interior to this cluster are *C. heterophylla* accessions, which include *C. heterophylla* #1 and #2 and the *C. heterophylla* x C. avellana hybrids China #13, #20, and #23. Also included is OSU 526.041, which is the result of a cross of *C. heterophylla* ‘Ogyoo’ and a mixture of three *C. avellana* pollens (OSU 55.129, Birk 5-6, and

OSU 226.122), where the male parent has yet to be determined (S.A. Mehlenbacher, personal communication).

The remainder of the accessions in the study [all from subsection Corylus (leafy-husk hazels), with the exception of a few putative interspecific hybrids with *C. colurna*] were placed interior to these outgroup accessions. They were divided into 10 distinct groups that largely reflected the origin of the reference cultivars held within them. These groups were given names based on their origins while also taking into account their previous assignment, where applicable, into the major geographic groups described by Gökirmak et al. (2009). The names assigned to groups in the current UPGMA clustering analysis include three previous groups from Gökirmak et al. (2009), the Black Sea group, Spanish–Italian group, Central European group, as well as seven new groups referred to as *C. americana* x *C.avellana* hybrid Groups 1 and 2, Gellatly hybrid group, Moscow group, Mixed group, Gasaway group, and wild *C. avellana* group. Each group is discussed subsequently, in the order in which they appear in the UPGMA dendrogram, starting at the top (Fig. 1).

C. americana x C. avellana Hybrid Group 1

The UPGMA clustering analysis placed both of the wild *C. americana* accessions, 'Rush' from Pennsylvania and 'Winkler' from Iowa, adjacent to one another within a small group near the top of the dendrogram. This group holds several other accessions known to be of *C. americana* interspecific hybrid origin, including 'Skinner'; the National Arbor Day Foundation (NADF) selections #1, #3, and #10; and the Rutgers University selection H3I2R05P05 (Fig. 2). Also placed in this group are several accessions with largely unknown parentage, including Gordon #21 and #24, Grimo Hybrid #3, and 'Purple Haze'.

'Skinner' was selected by F. Ashworth from a cross of a Hudson Bay wild *C. americana* seedling with an OP seedling of *C. avellana* 'Italian Red' (Ashworth, 1970). It is one of the two known F1 *C. americana* x *C. avellana* hybrids included in the study (NY 398 is the other) and is

likely placed close to ‘Rush’ and ‘Winkler’ based on having a high genomic contribution from *C. americana*.

NADF #1, #3, #10, and H3I2R05P05 share a common origin that trace in part to germplasm originating from Badgersett Research Corp., Canton, MN, which in turn traces back to *C. americana* ‘Winkler’. In brief, C. Weschcke of River Falls, WI, used ‘Winkler’ as well as local wild *C. americana* selections in a sizable interspecific hybridization program with *C. avellana* spanning several decades (Weschcke, 1954, 1970). Whereas Weschcke released a number of cultivars, including Carlola, Delores, and Magdalene (Brooks and Olmo, 1997), none proved commercially viable (Weschcke, 1970). However, Weschcke’s germplasm was later used as a foundation for breeding efforts at Badgersett Research Corp. (Rutter, 1987, 1991). Seedlings from Badgersett were then used to plant a 3.6-ha research field at the farm of NADF in Nebraska City, NE, from which the EFB-resistant NADF #1, #3, and #10 were selected (Capik and Molnar, 2012; Hammond, 2006; Xu and Hanna, 2010). H3I2R05P05 was selected from a cross of Rutgers Adel-1, an EFB-resistant seedling selection originating from a plant purchased from Badgersett Research Corp., with *C. avellana* ‘Syrena’ from Poland (Molnar and Capik, 2012).

Sathuvalli and Mehlenbacher (2012) also used ‘Winkler’, ‘Rush’, and many NADF selections, including #1, #3, and #10 (listed as ADF10.050, ADF11.055, and ADF11.051, respectively), in their SSR study of *C. americana* and known interspecific hybrids. In their study, NADF #1, #3, and #10 were placed close together within a larger group of accessions from the NADF, whereas ‘Winkler’ was placed within a second group of NADF accessions. However, ‘Rush’ was placed in a separate clade, comprised largely of its known hybrid offspring.

‘Rush’ has a history of use in breeding interspecific hybrids, which may provide some explanation for the placement of Gordon #21 and #24 in this group. In the early 1900s, ‘Rush’ was crossed with various *C. avellana* cultivars and improved, EFB-resistant F1 hybrids were selected (Crane et al., 1937; Reed, 1936; Slate, 1961). Although several cultivars were released from this work, none was commercially successful. However, progress was made, with some

plants, including ‘Potomac’ and several of the Slate NY numbered selections (e.g., NY 104, NY 398, NY 616), representing improvements over ‘Rush’ (Capik and Molnar, 2012; Coyne et al., 1998; Lunde et al., 2000). In 1963, J. Gordon of Amherst, NY, began planting hundreds of OP seedlings of NY 104 (‘Rush’ x *C. avellana* ‘DuChilly’) and NY 200 (‘Rush’ x *C. avellana* ‘Hall’s Giant’) (Gordon,1993;J.Gordon,personalcommunication). He grew several generations of plants, selecting EFB-resistant individuals to plant successive generations, although few pedigree records were kept. From his efforts, more than 40 EFB resistant clonal selections were established at Rutgers University (Capik and Molnar, 2012) and are included in this study. Thus, the placement of Gordon’s selections in the group with ‘Rush’ is supported by knowledge of his original breeding material.

Grimo Hybrid #3 is an OP seeding of an OP *C. heterophylla* selection. E. Grimo of Niagara-on-the-Lake, Ontario, Canada, grew many ‘Rush’ hybrid selections on his farm, including seedlings of NY 1329 (Grimo, 2011; E. Grimo, personal communication). This led to the selection and release of Grimo 208P. Thus, Grimo Hybrid #3 could be the result of hybridization with a ‘Rush’ seedling, providing support for its inclusion in this group. No prior information is available for ‘Purple Haze’, a red-leaf selection from McKay Nursery, Waterloo, WI.

Black Sea Group

A large group (n = 78) holding nearly all of the reference cultivars originating from the Black Sea region (Turkey, the Republic of Georgia, Azerbaijan, and southern Russia) was revealed by the UPGMA clustering analysis (Fig. 3). This grouping of cultivars is similar to that shown in Gökirmak et al. (2009), although their dendrogram showed a separation of cultivars into two respective groups, Black Sea Group 1 and Black Sea Group 2. These two groups were not clearly resolved in our study. However, two distinct subgroups, one large and one small, were resolved within our Black Sea group.

The larger subgroup holds most of the Black Sea reference cultivars as well as most of the seedlings from Holmskij and Sochi. It was divided into seven distinct clades with the EFB resistant clonal selection OSU 495.072 [believed to have originated from southern Russia (Sathuvalli et al., 2010)] placed in its most basal position.

The clade placed lowest in the dendrogram (Clade 1) contains the reference cultivars Ata Baba and Ashrafi from Azerbaijan as well as OSU 759.010 from the Republic of Georgia. It also holds the seedling accessions 'Ata Baba' OP #1,which originated from the Russian Academy of Agricultural Science Institute of Floriculture and Subtropical Cultures, Sochi, Russia (referred to subsequently as the ''Sochi Institute''), Sochi Market 5 #1, #5, and #6, and Holmskij Market 6 #3.

The clade placed directly above the aforementioned clade (Clade 2) is the largest of the seven and contains Cherkesskii II (the most widely grown cultivar in southern Russia), seven seedlings originating from markets in Holmskij, eight seedlings from Sochi, and nine OP seedlings of Zugdui, a cultivar from the Sochi Institute that is unfamiliar to the authors. The third clade (Clade 3) holds the seedlings 'Adighei' OP #1, from the Chişinău Botanical Garden of the Academy of Sciences of Moldova, and Sochi Market 2 #1 and #4. The fourth clade (Clade 4) holds the reference cultivars from Turkey, Kalinkara, Kudryavchik, Palaz, Sivri Ghiaghli, Tombul (Akcacoca), and Tombul Ghiaghli as well as Ganja from Azerbaijan and two OP seedlings of Abhazki originating from the Sochi Institute. The fifth clade (Clade 5) holds four seedlings from Holmskij markets and 'Kavkas' OP #2, originating from the Sochi Institute. The sixth clade (Clade 6) within the larger Black Sea subgroup holds the reference cultivar Pioneer from southern Russia and four seedlings of President, a cultivar also originating from southern Russia and held at the Sochi Institute. The final clade (Clade 7) within the larger Black Sea subgroup holds the reference cultivar Skorospelka from southern Russia and the EFB-resistant *C. avellana* clonal accession from the Rutgers University collection (Unknown-EFB res.) whose identity was lost. Although the identity of unknown-EFB res. remains unclear, it is valuable to

know its origins are also most likely southern Russia. The seedling selections ZC1, ZC4, and ZC6 were also placed in this group. They are from seed believed to have been collected from the Caucuses region (southern Russia), although the exact location is not known (D. Zaurov, personal communication). The final three seedlings placed in this clade originate from Sochi.

The smaller subgroup of the Black Sea group was placed above and adjacent to the branch point that holds the larger subgroup. It holds the reference cultivars Imperial de Trebizonde from Turkey and Losovskoi Sharovdnii from Harkiv, Ukraine. It also holds four seedlings originating from the Nikita Botanical Gardens, Yalta, Ukraine, and two originating from markets in Simferopol, Ukraine. The remaining three seedlings include one from Holmskij and two from Sochi. This smaller subgroup, while holding several seedlings from southern Russia (Holmskij and Sochi), is the only group within the Black Sea group that holds accessions from Ukraine.

Also included in the Black Sea group is one small cluster of seedling accessions placed in a basal position to the two major Black Sea subgroups described previously. This cluster holds B-X-3 OP #1 and 'Kavkas' OP #1, originating from the Sochi Institute, and 'Badem' OP #3, originating from the Research Institute for Horticulture and Viticulture, Krasnodar, Russia. Their origins, like with all of the accessions placed in the large Black Sea group described previously, support their inclusion in this group.

Gellatly Hybrid Group

Placed below the Black Sea group and above the Moscow group (discussed in the following section) is a small, distinct group of nine accessions (Fig. 4). The known cultivars and clonal selections in this group include the putative *C. colurna* x *C. avellana* hybrids Morrisoka, Laroka, and Eastoka and the EFB-resistant Gellatly Chinese Trazel #6 and #11 (also *C. colurna* despite being named ''Chinese'' trazel). These five accessions were developed by J.U. Gellatly in Westbank, British Columbia, Canada (Gellatly, 1950, 1956, 1964, 1966). Also included are two EFB-resistant OP seedlings of Gellatly Chinese Trazel #6 selected at Rutgers University. The

final two accessions in this group are Gordon hybrids #23 and #32. Although their pedigrees are unknown, the inclusion of these two selections may be because, in addition to the 'Rush' hybrids described earlier, in the 1980s, Gordon used 'Morrisoka', 'Laroka', 'Faroka', and the C. cornuta hybrid Gellatly 502 as parents (Gordon, 1993; J. Gordon, personal communication).

Moscow Group

Placed below the Gellatly hybrid group and a single disparate cluster holding Gordon #13 and Skierniewice mix #9 is the Moscow group. This group is also adjacent to a cluster holding 'Contorta' and seedlings from Simferopol, Ukraine (Fig. 5). This group of 35 plants holds the reference cultivars Early Long Zellernuss (EFB-susceptible), from England, and Auger, the second named OP selection from Gordon, with the remaining accessions believed to have originated from Moscow, Russia. The last clonal accession in the group is the EFB resistant, red-leaf Moscow #2, which originated from scions imported to OSU from the Russian Research Institute of Forestry and Mechanization, Moscow (Sathuvalli et al., 2010). The remaining 32 accessions are EFB-resistant OP seedlings of the red-leaf 'Moskovskii Rubin' or red-leaf 'Kudashovski'. Seeds from both cultivars were collected at the Sochi Institute, although in different years, with both cultivars believed to have originated near Moscow (Capik et al., 2013).

Adjacent to the Moscow group is an unnamed cluster containing the ornamental hazelnut 'Contorta', its offspring 'Red Majestic', and the reference cultivar DuChilly (synonym 'Kentish Cob'), which fell in the Central European group (German section) of Gökirmak et al. (2009) (Fig. 1). The accession Rutgers Passion Puddle, an old, EFB-tolerant *C. avellana* seedling growing on the Rutgers University Cook Campus, New Brunswick, NJ, was also included in this group. The final six accessions placed in this group are EFB-resistant OP seedlings from Simferopol, Ukraine (Simferopol Market 5).

Mixed Group

Placed below the Moscow group is the Mixed group. This group comprises an assortment of 29 accessions that are divided into two subgroups, each of which contain plants from a variety of origins (Fig.6).The subgroup located basally and placed at the bottom of the Mixed group (Subgroup 1) holds the reference cultivar Faroka, a putative hybrid of *C. colurna* developed by J. Gellatly (Gellatly, 1966), and two of its known EFB-resistant offspring: 'Grand Traverse' (Farris, 1989) and Grimo 186M (Grimo, 2011). Also placed in this subgroup are Gordon #1, #31, #33, and #34; 'Estrella #1', a hybrid of *C. heterophylla* var. *sutchuensis* x *C. avellana* 'Holder' (Farris, 1974); and the EFB-resistant seedling 'Kudashovski' OP #13. Besides the 'Kudashovski' OP #13, the accessions in this group share ties through their developers being members of the NNGA who were known to have exchanged germplasm (Capik and Molnar, 2012).

Located interior to and above Subgroup 1 is a larger group (Subgroup 2) that contains the reference cultivars Karol from Poland [Central European group (Gökirmak et al., 2009)], 'Badem' from Turkey [Spanish–Italian group (Gökirmak et al., 2009)], 'Istrski Duguljasti' from Croatia [Black Sea Group 1 (Gökirmak et al., 2009)], Bulgaria X1-8 from Bulgaria [Black Sea Group 1 (Gökirmak et al., 2009)], and 'Barr's Zellernuss' from England [Central European group (Gökirmak et al., 2009)]. Also placed in this subgroup are accessions with uncertain origins, such as 'Henneman 3', 'Ugbrooke', and 'Jeans', as well as a number of additional putative *C. colurna* x *C. avellana* hybrids developed by Gellatly, including Turkish Trazel #3, 'Chinoka', and 'Erioka', and his *C. cornuta* hybrid 'Manoka'. Other clonal accessions with unknown origins placed in this group include Morris 32-1379A, an EFB-resistant *C. avellana* accession from the Morris Arboretum, Philadelphia, PA; Home Ec Building, an EFB tolerant seedling located on the Rutgers University Cook campus, New Brunswick, NJ; and Gordon #30. In addition, the seedling accessions placed in this group include four from Skierniewice, Poland, and one from Simferopol, Crimea, Ukraine. Although some of the accessions placed in this UPGMA group have clear English or central European origins, others span regions from eastern Europe to the Black Sea

region. As such, the reasons for their grouping in the same clade are unclear. Thus, we label this group the Mixed group.

Spanish-Italian Group

Placed interior to and below the Mixed group in the dendrogram is the Spanish–Italian group. Similar to the findings of Gökirmak et al. (2009), nearly all of our reference cultivars previously placed in their Spanish–Italian group were placed in one large group by our UPGMA clustering analysis. These cultivars include Barcelona, Casina, Closca Molla, Culpla (EFB-resistant), Negret, Ratoli (EFB-resistant), Restiello, Sant Juame, Segorbe, Tonda di Giffoni, Tonda Gentile delle Langhe, and Tonda Romana (Fig. 7). Also included in the group, although previously placed in English Group 2 by Gökirmak et al. (2009), are 'Butler', 'Daviana', 'Ennis', 'Gem', 'Royal', and 'Zimmerman'. All of these cultivars besides Daviana (from England), which was placed at the bottom of the group adjacent to Ennis, are believed to share Barcelona as a common parent. Furthermore, 'Butler' and 'Ennis' were shown to be offspring of 'Barcelona' x 'Daviana' (Gökirmak et al., 2009). Several cultivars released from the OSU breeding program with Spanish–Italian cultivars in their pedigrees were also placed in this group. These include 'Clark' (Mehlenbacher et al., 2001), 'Jefferson' (Mehlenbacher et al., 2011), and 'Yamhill' (Mehlenbacher et al., 2009), which each have 'Barcelona' and 'Montebello' (synonym 'Siciliana') in their pedigrees. Although 'Clark' is susceptible to EFB, 'Jefferson' and 'Yamhill' are resistant as a result of a resistance allele from 'Gasaway'. Also included in the group was OSU 541.147, a breeding selection from OSU carrying EFB resistance from *C. americana* 'Rush' (through NY 110) that also has 'Montebello' and 'Barcelona' in its pedigree (S.A. Mehlenbacher, personal communication); EFB-resistant 'Uebov' from Serbia (Sathuvalli et al., 2010); and 'Sodlinger' from the former Yugoslavia. Interestingly, only one seedling accession was placed in this group, EFB-resistant 'Rimski' OP #2, which was selected from OP seedlings of 'Rimski' originating from the Sochi Institute.

Gasaway-Related Group

Placed interior to the Spanish–Italian group, along with a disparate group of the two accessions ‘Freeoka’ and ‘Karloka’, is a group of nine EFB resistant accessions that includes the reference cultivar Gasaway and its offspring VR 20-11 and Santiam (Fig. 8). This group also contains the EFB-resistant clonal accessions Farris 88 BS (a purported OP seedling of the *C. colurna* hybrid ‘Faroka’), Farris Box 1 (unknown pedigree), and Farris G-17 (unknown pedigree). It should be noted that Box 1 and G-17 may be the same genotype because they share common alleles at all 17 loci. Additionally, three seedling accessions with uncertain parentage were included in this group: ‘Badem’ OP #1 and #2, which were both selected from OP seedlings of ‘Badem’ originating from the Research Institute for Horticulture and Viticulture, and ‘Moskovskii Rubin’ OP #10, originating from the Sochi Institute.

‘Gasaway’ is a grower selection of R. Gasaway from Battleground, WA, although little is known of its origin (Thompson et al., 1996). It was not placed in any of the four geographic groups identified by Gökirmak et al. (2009). Its relationship to the Farris selections is unclear, although Farris did use OSU germplasm in his breeding efforts, including plants carrying the ‘Gasaway’ R-gene (Farris, 2000).

Central European Group

Most of the reference cultivars of Polish and German origin were placed together in one large group similar to the Central European group described in Gökirmak et al. (2009). However, our group was divided into two subgroups (Fig. 9), which did not precisely match the Polish and German sections defined in their study. The lower subgroup (Subgroup 1) held the Polish cultivars Acorn Hazel, Little Poland, Lenka 3, and Maria and the ‘‘red-leaf’’ cultivars Rote Zellernuss (German), Red Fortrin, Goc (Poland), and Annie’s Compact Red. This cluster of four red-leaf cultivars suggests they share a common lineage and reinforces the report in Gökirmak et al. (2009) that ‘Rote Zeller’ is a parent of ‘Goc’. The red leaf color in hazelnut is transmitted in a dominant manner (Thompson, 1985). It is also interesting to note that these red-leaf accessions

are placed distant from the UPGMA Moscow group that holds a number of other red-leaf accessions, suggesting a possible unrelated origin for the central European red leaf color compared with that from Moscow.

Several accessions with uncertain parentage are also included in the lower Central European subgroup. These include NY 616 OP #1 [a red-leaf OP seedling of NY 616 (*C. americana* 'Rush' x *C. avellana* 'Barcelona') selected at Rutgers University], 'Slagel' (a named OP selection released by Gordon), and Gordon #19. The inclusion of NY 616 OP #1, which has red leaves from an unknown pollen parent, likely reflects a common lineage with the other red-leaf C. avellana accessions in this group. The reasons for inclusion of 'Slagel' and Gordon #19 in this subgroup are unclear.

The upper subgroup (Subgroup 2) holds a mix of Polish [Frango 2, Lech, Syrena (red leaf), and Volski] and German [Gustav's Zellernuss, its synonym Italian Red (which shares identical alleles at all loci), and Hall's Giant] cultivars as well as Aveline d'Angleterre, which is believed to have originated in France (Gökirmak et al., 2009). Seedlings from Poland were also placed in this group (11 of 21), which includes those grown from OP nuts collected in markets and research stations in Warsaw, Skierniewice, and Konskowli, Poland (Capik et al., 2013). It should be noted that cultivars from the Central European group are known for their cold-hardiness (Thompson et al., 1996). Some of the EFB-resistant and -tolerant Polish seedlings may also express this valuable trait, which warrants investigation. Also included in this subgroup are Gordon#17 and #26. Gordon's use of NY 200 ('Rush' x *C. avellana* 'Hall's Giant') as an early breeding parent provides a link to his selections being placed in this subgroup that holds 'Hall's Giant'.

C. americana x C. avellana Hybrid Group 2

The UPGMA analysis resolved a second group holding known accessions of *C. americana* x *C. avellana* origin (Fig. 10). This group was placed adjacent to the Central European group. It holds the reference accessions NY 398 (*C. americana* 'Rush' x *C. avellana* 'Red

Lambert') and *C. avellana* 'Frango 5' from Poland (Gökirmak et al., 2009). Other clonal accessions placed in this group, although with less certain parentage, are 'Medium Long' from the New York Agricultural Experiment Station, which was previously shown to cluster with 'Rush' in a nuclear and chloroplast SSR analysis by Bassil et al. (2013); Grimo 208P, which is an OP seedling of NY 1329 ('Rush' x *C. avellana* 'Cosford') (Grimo, 2011); and NY 616 OP #2, an OP seedling of NY 616 ('Rush' x *C. avellana* 'Barcelona') selected at Rutgers University.

The remaining accessions in this group have unknown parentages. These accessions include a majority of the Gordon hybrids (25 of 40) as well as Campbell #1 and Campbell #2, which were selected at Rutgers University as being EFB-resistant from a larger group of seedlings purchased from Douglas Campbell Nursery, Niagra-on-the-Lake, Ontario, Canada, under the name ''Turkish tree hazel hybrid seedlings.'' The final three accessions include Farris 188P, an EFB-tolerant clonal selection from C. Farris, and Dabb 2-1 and Dabb 5-6, both EFB-resistant clonal selections obtained from C. Dabbin Ogden, UT. Little is known of their origin. However, Dabb was an active member of the NNGA (as were C. Farris, J. Gordon, and E. Grimo), and he was known to have exchanged nut tree germplasm with members of this organization (Dabb, 1971; L. Dabb, personal communication).

As discussed earlier, the fact that Gordon used 'Rush' hybrids in his breeding pool provides support for a majority of his EFB-resistant selections being included in this group, which holds NY 398 and other accessions known to be related to 'Rush'. Molnar and Capik (2012) suggest that the R-gene from 'Rush' is controlled by a single dominant allele in the heterozygous state, which supports the recovery of a large number of related resistant plants from Gordon's efforts. Furthermore, Gordon's use of NY 200 ('Rush' x *C. avellana* 'Hall's Giant'), as discussed earlier, may also provide support for why *C. americana* x *C. avellana* hybrid Group 2 is located adjacent to the Central European group. D. Campbell was an active member of the NNGA (Campbell, 1996), participating in germplasm exchange along with Gordon, Farris, Grimo, and Dabb, which provides a link for the inclusion of his plant material in this group.

Wild C. avellana Group

A second mixed group of cultivars and seedlings that is notable for holding the only wild *C. avellana* accessions in the study as well as a majority of the seedlings from Simferopol, Ukraine (10 out of 19) (Fig. 11) was placed at the very bottom of the dendrogram. This group is divided into three distinct clusters. The cluster placed lowest in the group (Subgroup1) holds four OP seedlings from Simferopol and one seedling originating from the Vavilov Research Institute of Plant Industry (VIR) in Maykop, Russia (Maykop VIR #1).

The middle cluster (Subgroup 2) holds the reference cultivar Aurea (*C. avellana* var. aurea), a yellow-leaf ornamental hazelnut believed to be from France; Fusco Rubra, a red-leaf cultivar from Germany; Finland CCOR 187, an EFB tolerant wild accession from Finland (Chen et al., 2007); and OSU 408.040, an EFB-resistant seedling selection from Minnesota (Chen et al., 2005). None of these accessions were placed in any of the four major geographic groups by Gökirmak et al. (2009). Subgroup 2 also holds two clonal selections from the Russian Research Institute of Forestry and Mechanization, Moscow (Moscow #28 and a Moscow selection from the same group of introduced plants whose specific accession number was lost at Rutgers University), and an EFB-resistant seedling of 'Kudashovski', which, as mentioned previously, is also believed to have originated from Moscow. The other seedling accessions placed in this group include two wild *C. avellana* accessions from Estonia (Estonia #1 from Agusalu and Estonia #2 from Tartu), six seedlings from Simferopol, one from Maykop, Russia, and one from Poland.

The final cluster (Subgroup 3), placed in a more basal position within this wild *C. avellana* group, holds the reference cultivars Bianca from Italy and Cutleaf from England. It also holds three *C. heterophylla* x *C. avellana* hybrids—China #1, China #14, and China #18. It should be noted that China #14 and China #18 shared the same alleles at all 17 loci, making it likely that they are the same genotype. Although 'Bianca' was placed in the Spanish–Italian subgroup by Gökirmak et al. (2009), 'Cutleaf' was not assigned to one of their four major subgroups. Furthermore, little is known about the background of the hybrid accessions originating

from the Economic Forestry Institute of Liaoning Province, Dalian, China (Capik and Molnar, 2012), to help support their placement in this group.

Overall, the UPGMA wild *C. avellana* group contains a mixture of accessions spanning different origins. However, it is the only group that holds known wild accessions of *C. avellana* (Finland CCOR 187 and the two seedlings from Estonia). In addition, the three accessions from Moscow are believed to be from a breeding program that used local wild *C. avellana* in crosses with southern cultivars to develop cold hardy selections, as discussed in Molnar (2011). Furthermore, OSU 408.040 is a seedling selection derived from an unknown *C. avellana* plant growing in Minnesota, which denotes a level of cold-hardiness not found in most cultivated *C. avellana*. Thus, the UPGMA group was named the wild *C. avellana* group, in part for lack of better resolved genetic associations.

STRUCTURE analysis

The results of the Bayesian model-based clustering analysis (STRUCTURE) are shown in Figure 12. Genotyping data for all samples were imported for STRUCTURE analysis in the order displayed in the UPGMA dendrogram; thus, the first (top) accession in the UPGMA tree is labeled as Accession 1 in the STRUCTURE analysis, etc. The maximum value for the first plateau of the graphical representation of the average estimated log probability Pr(X|K) curve [used to identify the most parsimonious number of clusters/populations (K)] was (K)= 11. Additional (K) values were considered, but (K) = 11 correlated best with the breeding histories, countries of origin, and the results of the UPGMA clustering analysis.

In general, the STRUCTURE results strongly support the groupings depicted in the UPGMA dendrogram. Notable exceptions occurred within the Black Sea group, which is depicted as one large group in the UPGMA clustering analysis that holds two subgroups (large and small) and one basal clade. In the STRUCTURE analysis, the Black Sea group was divided into two genetic groups; however, they did not clearly follow the groupings displayed in the UPGMA dendrogram. Furthermore, the *C. americana* x *C. avellana* hybrid Groups 1 and 2, which are

separated by a relatively wide margin in the UPGMA dendrogram, were combined into one large population in the STRUCTURE analysis. An additional divergence between the UPGMA and STRUCTURE analysis was shown for the Mixed group. Although depicted as one large group in the UPGMA analysis, the Mixed group was dissolved by the STRUCTURE analysis with the included entries subsequently placed in other STRUCTURE groups. Greater details and discussion on the results of the STRUCTURE model-based clustering analysis in comparison with the UPGMA cluster analysis are described for each original UPGMA group below.

Outgroups

The STRUCTURE analysis assigned nearly all of the wild species accessions into unique populations distinct from a great majority of the other accessions in the study. Interestingly, three distinct outgroup populations were resolved, which did not necessarily reflect the groupings revealed by the UPGMA dendrogram. For example, the four accessions placed in the Colurnaea clade of the UPGMA dendrogram were now divided up across three STRUCTURE groups. STRUCTURE Outgroup 1 holds *C. colurna* #1, which was originally placed at the very top of the UPGMA dendrogram in the Colurnaea clade. However, now also placed within this STRUCTURE group are the accessions previously placed within the Siphonochlamys clade of the UPGMA dendrogram (*C. mandshurica* #1, *C. sieboldiana* #1, *C. cornuta* 'Peace River', and OSU 587.044). Next, the two *C. fargesii* accessions were classified as their own STRUCTURE group (Outgroup 2). Finally, *C. chinensis* #1, the last UPGMA Colurnaea member, was placed in STRUCTURE Outgroup 3, which now also holds accessions primarily of *C.heterophylla* origin. It should be noted that four additional *C. heterophylla* hybrid accessions were placed in this group that were not originally included in the *C. heterophylla* UPGMA clade (China #1, #14, #18, and Estrella #1),as well as the hybrid accessions Gordon #21 and NADF #10 (Table 1).

C. americana x C. avellana Hybrid Groups

The STRUCTURE analysis grouped nearly all of the accessions of the UPGMA *C. americana* x *C. avellana* hybrid Groups 1 and 2 into one large population (Table 1). Only six

accessions were moved out from these two UPGMA groups, including 'Frango 5', Farris 188P, and Gordon #5 and #36, which were moved to the Central European group. The accessions Gordon #21 and NADF #10 were both moved to STRUCTURE Outgroup 3. Six accessions were added to the STRUCTURE *C. americana* x *C. avellana* hybrid group, which are also putative *C. americana* x *C. avellana* hybrids. These include NY 616 OP #1 and five Gordon accessions.

Overall, a majority of the accessions placed in this STRUCTURE group can be linked to *C. americana* 'Rush' either directly through known pedigree or indirectly based on germplasm used by their developers. In some cases, their breeding histories corroborate their placement in both their respective UPGMA group and this STRUCTURE population, which provides support for the validity of both analyses. For example, the Gordon hybrids #17, #19, and #26 were previously grouped near *C. avellana* 'Hall's Giant' in the UPGMA Central European group (Fig. 9). This placement supports the report that NY 200 ('Rush' x *C. avellana* 'Hall's Giant') was a component of Gordon's starting material and constitutes part of the genetic background of some of his selections. The fact that the STRUCTURE analysis moved these Gordon accessions into the *C. americana* x *C. avellana* population may suggest that the *C. americana* genetic background is more prominent in them than that of *C. avellana* 'Hall's Giant'. It is interesting to note that when admixture is present in the accessions placed within the *C. americana* x *C. avellana* hybrid group, the colors indicate a strong contribution from the Central European group (Fig. 12).

Nearly all of the accessions in this group are further linked by a shared phenotypic characteristic resistance or high tolerance to EFB. EFB resistance is a relatively rare trait that is not always transmitted at a high level from parents showing resistance (Capik and Molnar, 2012; Molnar and Capik, 2012; Thompson et al., 1996). The presence of a potential dominant R-gene from 'Rush' in germplasm frequently exchanged and accessible to members of the NNGA for more than 50 years would likely lead to a large number of related, EFB-resistant plants being selected by amateur breeders living where the fungus is endemic.

Black Sea Groups

The STRUCTURE analysis largely followed the placement of the known Black Sea accessions resolved in the UPGMA analysis, although it showed deeper resolution of relationships among the accessions in the different subgroups (Table 1). For example, within the UPGMA Black Sea group, two distinct subgroups (one large and one small) and one basal clade were resolved. In the STRUCTURE analysis, a majority of the accessions in the large UPGMA subgroup were placed into one STRUCTURE group (henceforth named STRUCTURE Black Sea Group 1). This group of accessions remained well defined with no additional accessions moved into it by the STRUCTURE analysis. However, a total of 22 accessions were moved out of the UPGMA large subgroup by the STRUCTURE analysis, and of those, 17 were placed into a separate, distinct STRUCTURE group that includes all of the accessions originally found in the small UPGMA Black Sea subgroup and the basal clade (henceforth named the STRUCTURE Black Sea Group 2).The remaining five accessions were moved to the STRUCTURE Spanish–Italian group, discussed subsequently (comprised of the Turkish references cultivars Sivri Ghiaghli, Tombul, Kudryavchik, Tombul Ghiaghli, and Kalinkara). Only three accessions, 'Rimski' OP #2, Maykop VIR #1, and B-X-3 OP #2, were moved into Black Sea Group 2 from across the study.

Although the reason for the differentiation between the two STRUCTURE Black Sea groups is unclear, it should be noted that Black Sea Group 1 holds most of the reference cultivars from the Black Sea region and many seedling accessions from Holmskij and Sochi. While holding some accessions with origins in southern Russia, STRUCTURE Black Sea Group 2 also holds 'Losovskoi Sharovdnii' from Harkiv, Ukraine (northern Ukraine), and seedlings originating from Simferopol and Yalta (Crimean Peninsula), Ukraine. No accessions of Ukrainian origin are found in STRUCTURE Black Sea Group 1.

Gellatly Hybrid Group

The STRUCTURE analysis placed all nine of the UPGMA Gellatly hybrid group accessions in one distinct group, most of which are known *C. colurna* x *C. avellana* hybrids. The analysis also placed an additional 19 accessions in this group, most of which trace their origin directly or indirectly to hybrids developed by Gellatly (Table 1).

Moscow Group

The STRUCTURE analysis placed nearly all of the Moscow seedlings ('Kudashovski' OP and 'Moskovskii Rubin' OP) in a distinct group that is nearly identical to that resolved by the UPGMA dendrogram. The only divergence was that 'Early Long Zeller' (which showed admixture with several other groups) was moved to the Central European group (discussed subsequently) and the Gordon hybrid 'Auger' was moved to the STRUCTURE *C. americana* x *C. avellana* group. Furthermore, only one accession was moved into the STRUCTURE Moscow group, 'Moskovskii Rubin' OP #10, which joins the vast majority of its half-sibs included in the study.

Mixed Group

The STRUCTURE analysis dissolved the large UPGMA Mixed group into several other STRUCTURE groups, some of which are discussed in more detail subsequently. In respect to the two original subgroups of the Mixed group revealed by the UPGMA analysis, differing levels of admixture was observed within them, which merits some discussion. Six of the nine total accessions in UPGMA Subgroup 1 show only minor admixture with other groups and were clearly placed in the Gellatly hybrid STRUCTURE group. The three remaining accessions show a much greater level of admixture. These include the *C. heterophylla* hybrid 'Estrella #1', which was placed in STRUCTURE Outgroup 3, and 'Kudashovski' OP #13 and Gordon #33, which were placed in the STRUCTURE Central European group.

Subgroup 2 of the UPGMA Mixed group holds accessions from a variety of origins with many exhibiting significant amount of admixture across multiple STRUCTURE groups (Fig. 12).

Of the 20 total accessions, eight were also placed in the STRUCTURE Gellatly hybrid group. Of the remaining 12 accessions, ‘Karol’ (from Poland) and three seedlings from Skierniewice, Poland, were placed in the STRUCTURE Central European group. ‘Karol’ was placed in a similar group in Gökirmak et al. (2009). Furthermore, ‘Ugbrooke’, ‘Barrs Zellernuss’, Skierniewice mix #5, and Simferopol Market 3 #2 were placed in the STRUCTURE wild *C. avellana* group. Lastly, ‘Henneman 3’, ‘Istrski Duguljasti’, ‘Jeans’, and ‘Badem’ were placed in the STRUCTURE Spanish–Italian group; of these, only ‘Badem’ was placed in a similar group in Gökirmak et al. (2009).

Spanish-Italian Group

The STRUCTURE analysis largely followed the placement of the known Spanish–Italian accessions resolved in the UPGMA analysis. Of the 25 accessions held in the UPGMA Spanish–Italian group, only five were moved to different STRUCTURE groups. These include ‘Negret’, ‘Segorbe’, and ‘Zimmerman’, which were placed in the wild *C. avellana* group, as well as ‘Tonda Romana’ and ‘Rimski’ OP #2, which were placed in the Gellately hybrid group and Black Sea Group 2, respectively. ‘Segorbe’ and ‘Negret’ are from Spain and were placed in the Spanish–Italian group in Gökirmak et al. (2009). Thus, it is not clear why they were moved in our study; however, the STRUCTURE analysis shows they both contains significant admixture between a number of groups [Fig. 12 (Accession 205)], which may have led to its new placement. ‘Tonda Romana’ and ‘Rimski’ OP #2 both also show a high level of admixture between several groups [Fig. 12 (Accessions 192 and 203, respectively)].

Accessions moved into the STRUCTURE Spanish–Italian group from other UPGMA groups include ‘Sivri Ghiaghli’, ‘Tombul’, Kudryavchik’, ‘Tombul Ghiaghli’, ‘Kalinkara’, ‘Henneman 3’, ‘Istrski Duguljasti’, ‘Badem’, ‘Jeans’, and ‘Bianca’ (Table 1).

Gasaway-Related Group

The STRUCTURE analysis dissolved the UPGMA Gasaway-related group. Although all nine accessions showed kinship with the STRUCTURE wild *C. avellana* group (discussed

subsequently), only ‘Gasaway’, VR 20-11, and Santiam were placed there. The three Farris accessions (88 BS, Box 1, and G-17) and the two ‘Badem’ OP accessions (#1 and #2) were moved into the STRUCTURE Central European group (also discussed subsequently). As a result of a lack of pedigree information, it is unclear why these accessions would be placed in either the UPGMA ‘Gasaway’ or Central European groups. The final accession, ‘Moskovskii Rubin’ OP #10, was moved into the STRUCTURE Moscow group as mentioned earlier.

Central European Group

The STRUCTURE analysis largely followed the placement of the known Central European accessions resolved in the UPGMA analysis. Of the 36 accessions originally placed in this group, only four were moved to other groups. However, the STRUCTURE analysis moved 18 additional accessions into the Central European group (Table 1). These include six accessions originally from the dissolved UPGMA Mixed group, five from the dissolved Gasaway group, one from the Moscow group, four from the C. americana · C. avellana hybrid group, one from the wild C. avellana group, and finally ‘Karloka’, which was not included in a UPGMA group. A common thread found in nearly all of the 18 added accessions is a relatively high degree of admixture per each accession, which may help explain their divergent placements between the two analyses (Fig. 12).

Wild C. avellana Group

The STRUCTURE analysis largely followed the placement of the wild *C. avellana* group accessions resolved in the UPGMA analysis. Of the 27 accessions placed in the UPGMA group, only six were placed elsewhere by the STRUCTURE analysis. Three *C. heterophylla* hybrid accessions (China #1, #14, and #18) were moved to STRUCTURE Outgroup 3; ‘Fusco Rubra’ was moved to the Central European group; Maykop VIR #1 was moved to STRUCTURE Black Sea Group 2; and ‘Bianca’ was moved to the STRUCTURE Spanish–Italian group.

Eighteen additional accessions were moved into the wild *C. avellana* group by the STRUCTURE analysis. Most notably, this includes ‘Gasaway’ and its offspring ‘Santiam’, VR

20-11, and ‘Zimmerman’. Also now included are ‘Segorbe’, Skierniewice mix #9, ‘Ugbrooke’, ‘Barrs Zellernuss’, Simferopol Market 3 #2, and Skierniewice mix #5. It is hard to draw conclusions on the inclusion of these accessions in the *C. avellana* wild group. However, as mentioned in the UPGMA discussion, a number of the included reference accessions placed in this group fell outside of the major groups in Gökirmak et al. (2009) and were labeled in their study as the most genetically divergent. This also included ‘Gasaway’, which is now placed in this group. However, the STRUCTURE analysis for these reference accessions and the rest of the somewhat unknown accessions placed in the group shows a relatively uniform genetic relationship despite a number of them having disparate origins. For example, the group includes accessions spanning France (‘Aurea’), England (‘Cutleaf’), Finland (CCOR 187), Estonia (two seedlings), Russia (two clonal accessions), and Ukraine (17 seedlings), all with limited admixture with other STRUCTURE groups (Fig. 12).

Restructured (consensus) populations

The results of the UPGMA and STRUCTURE analysis are broadly similar to previous research on hazelnut genetic resources (e.g., Bassil et al., 2013; Gökirmak et al., 2009; Gürcan et al., 2010b) and are generally well supported by the known breeding histories and collection origins of a vast majority of the accessions. Overall, the reference cultivars provide a useful framework on which to place the unknown grower selections and OP seedlings from foreign germplasm collections. Seedlings from similar collection origins tend to group closely together with the collection origins of a majority of them corresponding to that of the reference cultivars with which they were grouped. Although the UPGMA clustering analysis and STRUCTURE results are largely in agreement with each other, the STRUCTURE results seem to better reflect the known, biologically relevant major and minor details of relationships between and among the accessions in the study, including their species background, reported breeding histories, and/or geographic origins.

Thus, based primarily on the STRUCTURE results, most accessions were decisively placed into one of 11 consensus groups/populations. However, the results of the STRUCTURE analysis were inconclusive for some accessions as a result of high levels of admixture between multiple groups, sometimes resulting in less than 50% identity for any single group. This occurred for the following entries: 'Fusco Rubra', 'Jeans', 'Tonda Romana', 'Kalinkara', 'Uebov', 'Daviana', 'Barrs Zellernuss', VR 20-11, OSU 495.072, Gordon #32, Holmskij Market 3 #2, B-X-3 OP #2, Nikita Botanical Garden 1 #3, 'Kudashovski' OP #13, and Skierniewice mix #6. In these cases, group assignment for AMOVA was based on a combination of the UPGMA clustering and STRUCTURE results as well as known breeding histories and/or geographic origins of the accessions. Using this approach, all of the accessions in the study were then clearly assigned into 11 consensus groups for the AMOVA.

The AMOVA showed that 89% of the genetic variation of the accessions was attributable to within-population variance, whereas 11% was attributed to among-population variance.The within-population variance was then partitioned into each of the 11 populations, and the percentage of variance contributed by each population was calculated using the total within population variance. The consensus populations, excluding the outgroups, had variance percentages ranging from 9.05% to 15.22%, indicating a range of levels of variation found across populations. The highest within-population variance was found in the STRUCTURE Central European group (15.22%) followed by the *C. americana* x *C. avellana* hybrid group (14.29%). Comparatively, the lowest within population variance was found in the STRUCTURE Gellatly hybrid group (9.05%) followed by the Spanish–Italian group (9.15%) (Table 4).

The pairwise F_{ST} values derived from the AMOVA indicate a large degree of genetic differentiation between consensus groups/populations. The AMOVA results also denote that each consensus group is statistically different from every other consensus group ($P < 0.05$) (Table 5). These results indicate that the consensus groups constitute an accurate representation of the genetic relationships between the groups/populations.

Presence of eastern filbert blight-resistant accessions

As shown in Table 1 and Figure 12, each of the 11 final consensus groups holds accessions known to express resistance or tolerance to EFB, providing strong evidence that EFB resistance is a relatively widespread phenomenon across the Corylus genus. Although this study cannot address whether there are different resistance genes present in the different groups, it does show that EFB resistance is present in hazelnuts of many different genetic backgrounds. Furthermore, a number of the EFB-resistant OP seedling accessions from the new germplasm introductions were placed in groups where none or very few of the known EFB-resistant reference accessions were placed, suggesting that they represent new pools of resistant plant material. Thus, these new plants may represent potential targets for revealing novel R-genes in future studies, of which a first step would be investigating inheritance of resistance in progeny and mapping R-genes to the hazelnut linkage map (Mehlenbacher et al., 2006). For example, the final Black Sea Group 2 holds eight resistant and two tolerant OP seedling accessions with no EFB-resistant reference accessions placed in this group (Fig.1; Table 1). The Moscow group holds 33 new EFB-resistant OP seedling selections and is only joined by one EFB-resistant clonal selection, Moscow #2, a new introduction from the Russian Research Institute of Forestry and Mechanization, Moscow, Russia, of which little pedigree background is known. Furthermore, seven EFB-resistant OP seedling accessions were placed in the large Central European group, which holds no EFB-resistant reference accessions besides 'Slagel', a named hybrid selection from Gordon of whose pedigree is unknown. Also placed in the Central European group were several clonal, EFB-resistant, interspecific hybrid accessions from Farris and Gordon that have unclear origins. Based on these examples and others represented in this study, it is reasonable to assume that further collection and disease screening efforts from different regions of the world may lead to the identification of additional resistant plants, possibly from different genetic populations than those included in our study. Having access to a very wide diversity of EFB-resistant germplasm should help breeders maintain genetic diversity in their breeding lines as they

strive to develop broad-based, durable resistance to infection by *A. anomala* in combination with many other important traits of commercial and ecological value.

The results of this study also provide a substantial reference tool to help manage and reduce the population size of EFB resistant seedling plants and clonal accessions held in the Rutgers University germplasm collection. For example, before this study, seedlings and clonal germplasm accessions at Rutgers University were organized and maintained in rows in the field largely by collection origin (e.g., John Gordon Nursery) or by individual seed lot of introduced germplasm. In an attempt to maintain the potential genetic diversity present, the best plants of every seed lot (in terms of EFB-resistance and nut and kernel characteristics) were maintained (Capik et al., 2013). A similar approach was used for preserving clonal accessions obtained from private breeders and plant enthusiasts. This approach resulted in the generation of a field collection of more than 200 large (over 4 m) trees from just a few collection trips and private breeder contributions (Capik et al., 2013; Capik and Molnar, 2012;Molnar et al.,2007),which uses a large area of field space and is expensive to maintain. Fortunately, the SSR results provide the ability to use the consensus groups, in addition to phenotypic traits, as a decision tool to reduce the number of accessions held in field collections and to better target breeding and research efforts. For example, 34 OP seedling accessions spanning 12 different seed lots, all of which are currently being maintained in the field, were merged into Black Sea Group 1. Using the new genetic relationship information and phenotypic data (as discussed in Capik et al.,2013), the number of trees can be substantially reduced, where each seed lot need not be represented while still maintaining adequate representation from this genetic group. As a further example, the 40 total John Gordon hybrid clones were placed into four of the consensus groups, although a majority (29 of 40) were placed in the final *C. americana* x *C. avellana* hybrid group. This large number of accessions can be substantially reduced based on this finding by maintaining the best two to three accessions from each of the genetic groups represented, effectively reducing 40 large trees down to less than 10. Following this approach, a more refined, select group of EFB-resistant

accessions can be used as the foundation for breeding genetically diverse, new, EFB-resistant cultivars for the creation of genetic mapping populations and advanced molecular genetic research projects aimed at the discovery of novel R-genes, etc. We expect that selecting plants for use in breeding based on their SSR-derived relationships in addition to other traits should prove much more effective in terms of maintaining genetic diversity than selecting accessions based on phenotype or collection origin alone.

DISCUSSION

As discussed previously, the results of the UPGMA cluster and STRUCTURE analysis are largely similar and are also congruent with previous research on hazelnut genetic resources. Overall, the results support the known breeding histories and collection origins of a vast majority of the accessions. Furthermore, the reference cultivars, especially those selected to represent the groupings resolved in Gökirmak et al. (2009), provide a useful framework on which to place the unknown accessions.

EFB-resistant or -tolerant accessions were found in each of the final 11 consensus groups resolved in the study, providing strong evidence that EFB resistance is widespread across the Corylus genus. This finding provides support that breeding for resistance to EFB need not be equated with the narrowing of genetic diversity in future breeding. Furthermore, the SSR results, in combination with phenotypic characteristics, will allow us to narrow our germplasm collection at Rutgers University to the most interesting and unique accessions within each of the consensus populations, saving considerable field space and reducing maintenance expenses. As future evaluations and improvement goals dictate, the remaining pool of accessions can be used to enhance breeding efforts to develop commercial-quality, EFB-resistant cultivars while striving to maintain a high level of genetic diversity. Some of these new accessions should also be preserved in the USDA National Clonal Germplasm Repository, as resources and field space permit. Finally, this project contributes to the growing body of evidence showing that the Corylus genus is very diverse and that hazelnut breeders have access to a substantial gene pool from which to continue genetic improvement efforts for EFB resistance and a multitude of other traits of commercial value and scientific and ecological interest.

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Table 1. Accession name, identification code, unweighted pair group method using arithmetic averages (UPGMA) group, STRUCTURE (Falush et al., 2003; Pritchard et al., 2000) identification (ID) number, consensus ID number, and eastern filbert blight (EFB) response of 323 Corylus accessions examined using simple sequence repeat markers . Accessions are organized by Consensus Population groups (1-11) in ascending order.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	[z]EFB response
Corylus colurna (col.) #1	H3BR07P03, Burnt Ridge Nursery, Onalaska, WA	Colurnaea	1	1	res.
C. mandshurica #1	H3FR09P28, Holden Arboretum unknown,Kirkland, OH	Siphonochlamys	5	1	res.
C. sieboldiana #1	Dawes Arboretum D1996-0541.002, Newark, OH	Siphonochlamys	6	1	res.
'Peace River' (*C. cornuta*)	PI 637852, Peace River, Alberta, Canada (J. Gellatly selection)	Siphonochlamys	7	1	res.
OSU 587.044 [*C. californica* (calif.) × C. ave.]	Oregon State University (OSU) *C. californica* B0509 × C. avellana OSU 278.113	Siphonochlamys	8	1	tolerant
C. fargesii #2	Holden Arboretum, Kirtland, OH. 97-298-C, Shaanxi or Gansu province, China	Colurnaea	3	2	res.
C. fargesii #1	Morris Arboretum, Philadelphia, PA. 96-574-G, Shaanxi or Gansu province, China	Colurnaea	4	2	res.
C. chinensis #1	H3DR01P01, Lawyer Nursery, Olympia, WA	Colurnaea	2	3	res.
China #23 (*C. het.* × *C. ave*.)	Dalian, China via University of Nebraska, Lincoln (UNL)	*C. heterophylla*	9	3	res.
OSU 526.041(*C. het* × *C. ave.*)	OSU 526.041, *C. heterophylla* 'Ogyoo' × C. avellana	*C. heterophylla*	10	3	res.

[z] EFB response is described as resistant (res.) tolerant, susceptible (susc.), or unknown.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
C. heterophylla #1	H1R15P78, Lawyer Nursery, Olympia, WA	*C. heterophylla*	11	3	res.
C. heterophylla #2	H1R15P59, Lawyer Nursery, Olympia, WA	*C. heterophylla*	12	3	res.
China #13 (*C. het*. × *C. ave*.)	Dalian, China via UNL	*C. heterophylla*	13	3	res.
China #20 (*C. het*. × *C. ave*.)	Dalian, China via UNL	*C. heterophylla*	14	3	res.
Gordon #21 [*Corylus* hybrid (hyb.)]	Gordon selection R21P01, John Gordon Nursery, Amherst, NY	*C. americana* × *C. avellana Hybrid Group 1*	15	3	tolerant
NADF #10 (11-55) (*C. amer* × *C. ave.)*	National Arbor Day Foundation, Nebraska City, NE	*C. americana* × *C. avellana Hybrid Group 1*	19	3	res.
'Estrella #1'[*C. heterophylla* (*het.*)×*C. avellana* (*ave*.)]	PI 557350, C. Farris, Michigan, USA,	Mixed Group	185	3	res.
China #1 (*C. het.* × *C. ave.*)	Dalian, China via UNL	Wild *C. avellana* group	299	3	res.
China #18 (*C. het.* × *C. ave.*)	Dalian, China via UNL	Wild *C. avellana* group	300	3	susc.
China #14 (*C. het.* × *C. ave.*)	Dalian, China via UNL	Wild *C. avellana* group	301	3	susc.
'Purple Haze' (*Corylus* hyb.)	McKay Nursery, Waterloo, WI	*C. americana* × *C. avellana Hybrid Group 1*	16	4	susc.
'Skinner'[*C. americana* (*amer*.) × *C. ave*])	Hudson Bay *C. americana* selection × C. avellana 'Italian Red' Open Pollinated (OP)	*C. americana* × *C. avellana Hybrid Group 1*	17	4	tolerant
H3I2R05P05 (*C. amer* × *C. ave*.)	Rutgers University Adel-1 (Badgersett hybrid) × *C. avellana* 'Syrena'	*C. americana* × *C. avellana Hybrid Group 1*	18	4	tolerant

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
NADF #3 (11-51) (*C. amer* × *C. ave.)*	National Arbor Day Foundation, Nebraska City, NE	*C. americana* × *C. avellana* *Hybrid Group 1*	20	4	res.
'Winkler' (*C. amer*.)	PI 557019, Iowa, USA	*C. americana* × *C. avellana* *Hybrid Group 1*	21	4	res.
'Rush' (*C. amer*.)	PI 557022, Pennsylvania, USA	*C. americana* × *C. avellana* *Hybrid Group 1*	22	4	res.
NADF #1 (10-50) (*C. amer* × *C. ave*.)	National Arbor Day Foundation, Nebraska City, NE	*C. americana* × *C. avellana* *Hybrid Group 1*	23	4	res.
Gordon #24 (*Corylus* hyb.)	Gordon selection R24P01, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 1*	24	4	res.
Grimo Hybrid #3 (*C. het* × *C. ave.*)	Grimo Het. Hazel Hybrid #3, Grimo Nut Nursery, Niagara-on-the-lake,Ontario, Canada	*C. americana* × *C. avellana* *Hybrid Group 1*	25	4	res.
Gordon #13 (*Corylus* hyb.)	Gordon selection R12DP02, John Gordon Nursery	No Group	113	4	res.
'Auger' (*Corylus* hyb.)	John Gordon Nursery	Moscow Group	115	4	res.
Gordon #17 (*Corylus* hyb.)	Gordon selection R15P02, John Gordon Nursery	Central European Group	238	4	res.
Gordon #26 (*Corylus* hyb.)	Gordon selection R27P02, John Gordon Nursery	Central European Group	239	4	res.
NY 616 OP #1 (*C. amer* × *C. ave*.)	H2R07P39, NY 616 OP (red leaf)	Central European Group	252	4	susc.
Gordon #19 (*Corylus* hyb.)	Gordon selection R17P04, John Gordon Nursery	Central European Group	259	4	res.
Gordon #7 (*Corylus* hyb.)	Gordon selection R06P02, John Gordon Nursery	*C. americana* × *C. avellana* Hybrid Group 2	262	4	res.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB Response
Gordon #15 (*Corylus* hyb.)	Gordon selection R13P01, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	263	4	res.
Gordon #10 (*Corylus* hyb.)	Gordon selection R10P01, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	264	4	res.
Gordon #4 (*Corylus* hyb.)	Gordon selection R03P01, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	265	4	res.
Gordon #3 (*Corylus* hyb.)	Gordon selection R02P01, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	266	4	res.
Gordon #20 (*Corylus* hyb.)	Gordon selection R18P01, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	267	4	res.
Gordon #39 (*Corylus* hyb.)	Gordon selection R45P02, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	268	4	res.
Gordon #12 (*Corylus* hyb.)	Gordon selection R12DP01, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	269	4	res.
Gordon #25 (*Corylus* hyb.)	Gordon selection R26P01, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	270	4	res.
Gordon #8 (*Corylus* hyb.)	Gordon selection R08DP02, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	271	4	res.
Gordon #9 (*Corylus* hyb.)	Gordon selection R09P01, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	272	4	res.
Gordon #11 (*Corylus* hyb.)	Gordon selection R10P01, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	273	4	res.
Gordon #27 (*Corylus* hyb.)	Gordon selection R28P01, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	274	4	res.
NY 398 (*C. amer* × *C. ave*.)	PI 557382, 'Rush' × *C. avellana* 'Red Lambert'	*C. americana* × *C. avellana* *Hybrid Group 2*	275	4	res.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
Gordon #28 (*Corylus* hyb.)	Gordon selection R29P02, John Gordon Nursery	*C. americana* × *C. avellana* Hybrid Group 2	276	4	res.
Gordon #16 (*Corylus* hyb.)	Gordon selection R15P01, John Gordon Nursery	*C. americana* × *C. avellana* Hybrid Group 2	277	4	res.
Grimo 208P (*C. amer* × *C. ave*.)	NY 1329 ('Rush' × *C. avellana* 'Cosford') × OP	*C. americana* × *C. avellana* Hybrid Group 2	278	4	res.
Dabb 2-1 (*Corylus* hyb.)	C. Dabb selection, Utah, USA	*C. americana* × *C. avellana* Hybrid Group 2	279	4	res.
Gordon #37 (*Corylus* hyb.)	Gordon selection R39P01, John Gordon Nursery	*C. americana* × *C. avellana* Hybrid Group 2	280	4	res.
Gordon #38 (*Corylus* hyb.)	Gordon selection R40P03, John Gordon Nursery	*C. americana* × *C. avellana* Hybrid Group 2	281	4	res.
Dabb 5-6 (*Corylus* hyb.)	C. Dabb selection, Utah, USA	*C. americana* × *C. avellana* Hybrid Group 2	282	4	res.
'Medium Long' (*C. amer* × *C. ave*.)	PI 617265, likely from New York Agri. Exp. Station	*C. americana* × *C. avellana* Hybrid Group 2	283	4	res.
Gordon #18 (*Corylus* hyb.)	Gordon selection R17P02, John Gordon Nursery	*C. americana* × *C. avellana* Hybrid Group 2	284	4	res.
Campbell #2 (*Corylus* hyb.)	H3HR02P19, Doug Campbell Nursery, Ontario, Canada	*C. americana* × *C. avellana* Hybrid Group 2	288	4	res.
Gordon #14 (*Corylus* hyb.)	Gordon selection R12DP03, John Gordon Nursery	*C. americana* × *C. avellana* Hybrid Group 2	290	4	res.
Gordon #35 (*Corylus* hyb.)	Gordon selection R38P01, John Gordon Nursery	*C. americana* × *C. avellana* Hybrid Group 2	291	4	res.
NY 616 OP #2 (*C. amer* × *C. ave*.)	H3R17P01, NY 616 OP	*C. americana* × *C. avellana* Hybrid Group 2	292	4	res.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
Campbell #1 (*Corylus* hyb.)	H3HR02P15, Doug Campbell Nursery, Ontario, Canada	*C. americana* × *C. avellana* *Hybrid Group 2*	293	4	res.
Gordon #29 (*Corylus* hyb.)	Gordon selection R30DP02, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	294	4	tolerant
Gordon #22 (*Corylus* hyb.)	Gordon selection R22P01, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	295	4	res.
Gordon #2 (*Corylus* hyb.)	Gordon selection Neighbor N, John Gordon Nursery	*C. americana* × *C. avellana* *Hybrid Group 2*	296	4	res.
'Skorospelka'	PI 617175, Russia (southern),	Black Sea Group	43	5	unknown
Holmskij Market 5 #4	H3R4P30 (RUS 13), Holmskij Market 5, Holmskij, Russia	Black Sea Group	55	5	res.
Holmskij Market 3 #2	CRXR15P50 (RUS 11), Holmskij Market 3, Holmskij, Russia	Black Sea Group	56	5	res.
Holmskij Market 5 #2	H3R04P23 (RUS 13), Holmskij Market 5, Holmskij, Russia	Black Sea Group	57	5	res.
'Abhazki' OP #2	CRR04P96 (04029 R), 'Abhazki' OP, Sochi Inst., Russia	Black Sea Group	59	5	susc
'Abhazki' OP #1	CRR04P80 (04029 R), 'Abhazki' OP, Sochi Inst., Russia	Black Sea Group	64	5	susc.
'Palaz'	PI 304632, Turkey (Ordu)	Black Sea Group	65	5	susc.
'Ganja'	PI 634202, Azerbaijan	Black Sea Group	66	5	susc.
'Adighei' OP #1	H4AR26P103 (07586), 'Adighei' OP, Moldova Botanical Garden, Chișinău, Botanica, Moldova	Black Sea Group	68	5	susc.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
Sochi Market 2 #1	CRXR14P34 (RUS 4), Sochi Market 2, Sochi, Russia	Black Sea Group	69	5	res.
Sochi Market 2 #4	CRXR14P47 (RUS 4), Sochi Market 2, Sochi, Russia	Black Sea Group	70	5	res.
Zugdui OP #7	CRR06P09 (04024 R), 'Zugdui' OP, Sochi Inst., Russia	Black Sea Group	71	5	tolerant
Zugdui OP #3	CRR06P02 (04024 R), 'Zugdui' OP, Sochi Inst., Russia	Black Sea Group	72	5	res.
Zugdui OP #1	CRR05P92 (04024 R), 'Zugdui' OP, Sochi Inst., Russia	Black Sea Group	73	5	tolerant
Zugdui OP #4	CRR06P03 (04024 R), 'Zugdui' OP, Sochi Inst., Russia	Black Sea Group	74	5	susc.
Sochi Market 5 #4	CRXR14P117 (RUS 7), Sochi Market 5, Sochi, Russia	Black Sea Group	75	5	res.
Zugdui OP #8	CRR06P19 (04024 R), 'Zugdui' OP, Sochi Inst., Russia	Black Sea Group	76	5	tolerant
Zugdui OP #5	CRR06P05 (04024 R), 'Zugdui' OP, Sochi Inst., Russia	Black Sea Group	77	5	res.
Zugdui OP #9	CRR06P33 (04024 R), 'Zugdui' OP, Sochi Inst., Russia	Black Sea Group	78	5	tolerant
Sochi Market 2 #2	CRXR14P42 (RUS 4), Sochi Market 2, Sochi, Russia	Black Sea Group	79	5	res.
Zugdui OP #2	CRR05P94 (04024 R), 'Zugdui' OP, Sochi Inst., Russia	Black Sea Group	80	5	tolerant
Sochi Market 5 #2	CRXR14P112 (RUS 7), Sochi Market 5, Sochi, Russia	Black Sea Group	81	5	tolerant

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
Zugdui OP #6	CRR06P06 (04024 R), 'Zugdui' OP, Sochi Inst., Russia	Black Sea Group	82	5	tolerant
Holmskij Market 4 #1	CRXR15P65 (RUS 12), Holmskij Market 4, Holmskij, Russia	Black Sea Group	83	5	tolerant
Holmskij Market 4 #2	H3R07P25 (RUS 12), Holmskij Market 4, Holmskij, Russia	Black Sea Group	84	5	res.
Sochi unknown mix #1	CRR03P97 (04032R), unknown mixture, Sochi region, Russia	Black Sea Group	85	5	susc.
Holmskij Market 3 #1	CRXR15P40 (RUS 11), Holmskij Market 3, Holmskij, Russia	Black Sea Group	87	5	res.
Holmskij Market 3 #3	CRXR15P59 (RUS 11), Holmskij Market 3, Holmskij, Russia	Black Sea Group	88	5	res.
Holmskij Market 6 #2	CRXR17P22 (RUS 14), Holmskij Market 6, Holmskij, Russia	Black Sea Group	89	5	Tolerant
Sochi Institute Mix #1	CRXR13P13 (Rus 1), Sochi Inst.mixture of OP seeds.	Black Sea Group	91	5	susc.
'Cherkesskii II'	PI 617176, Russia, North Caucasus	Black Sea Group	92	5	susc.
Holmskij Market 5 #3	H3R4P28 (RUS 13), Holmskij Market 5, Holmskij, Russia	Black Sea Group	93	5	Tolerant
Sochi Market 2 #3	CRXR14P44 (RUS 4), Sochi Market 2, Sochi, Russia	Black Sea Group	94	5	res.
Holmskij Market 6 #1	CRXR17P21 (RUS 14), Holmskij Market 6, Holmskij, Russia	Black Sea Group	95	5	Tolerant
'Ata Baba' OP #1	CRRR07P05 (04019 R), 'Ata Baba' OP, Sochi Inst., Russia	Black Sea Group	96	5	susc.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
'Ata Baba'	PI 557422, Azerbaijan	Black Sea Group	97	5	susc.
Sochi Market 5 #5	H3R10P05 (RUS 7), Sochi Market 5, Sochi, Russia	Black Sea Group	98	5	Tolerant
Sochi Market 5 #6	H3R10P09 (RUS 7), Sochi Market 5, Sochi, Russia	Black Sea Group	99	5	res.
Sochi Market 5 #1	CRXR14P105 (RUS 7), Sochi Market 5, Sochi, Russia	Black Sea Group	100	5	susc.
'Ashrafi'	PI 641127, Azerbaijan	Black Sea Group	101	5	susc.
Holmskij Market 6 #3	H3R10P14 (RUS 14), Holmskij Market #6, Holmskij, Russia	Black Sea Group	102	5	Tolerant
OSU 759.010	OSU breeding selection imported as 'Tskhenis Dzudzu',Republic of Georgia	Black Sea Group	103	5	Tolerant
B-X-3 OP #1	CRR01P116 (04041 R), B-X-3 OP, Sochi Institute (Inst.), Russia	Black Sea Group	26	6	res.
'Badem' OP #3	H3R03P12 (RUS 16), 'Badem' OP, Inst. of Orchard and Wine Production, Krasnodar, Russia	Black Sea Group	27	6	res.
'Kavkas' OP #1	CRR04P107 (04028 R), 'Kavkas' OP, Sochi Inst., Russia	Black Sea Group	28	6	res.
Nikita Botanical (Bot.) Garden (Gard.) 1 #3	H3R10P88 (Rus 28), Nikita Botanical Garden 1, Yalta, Crimea, Ukraine	Black Sea Group	29	6	res
Holmskij Market 1 #1	H3R13P40 (RUS 9) Holmskij Market 1, Holmskij, Russia	Black Sea Group	30	6	res
Nikita Bot. Gard. 2 #1	CRXR16P35 (RUS 29), Nikita Botanical Garden 2, Yalta, Crimea, Ukraine	Black Sea Group	31	6	susc.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
Simferopol Market 1B #1	H3R14P26 (RUS 22), Simferopol Roadside Market 1B, Simferopol, Ukraine	Black Sea Group	32	6	res.
'Imperial de Trebizonde'	PI 271105,Turkey	Black Sea Group	33	6	susc.
Simferopol Market 4 #3	H3R4P09 (RUS 25), Simferopol Roadside Market 4, Simferopol, Ukraine	Black Sea Group	34	6	res.
Nikita Bot. Gard. 1 #1	CRXR16P57 (RUS 28), Nikita Botanical Garden 1, Yalta, Crimea, Ukraine	Black Sea Group	35	6	tolerant
'Rimski' OP #3	CRR02P44 (04040R), 'Rimski' OP, Sochi Inst., Russia	Black Sea Group	36	6	susc.
Nikita Bot. Gard. 1 #2	CRXR16P86 (RUS 28), Nikita Botanical Garden 1, Yalta, Crimea, Ukraine	Black Sea Group	37	6	susc.
Sochi Institute Mix #2	H3R10P94 (Rus 1), Sochi Inst.mixture of OP seeds.	Black Sea Group	38	6	res.
'Losovskoi Sharovdnii'	Harkiv, Ukaine	Black Sea Group	39	6	susc.
OSU 495.072	OSU breeding selection, southern Russia	Black Sea Group	40	6	res.
Sochi unknown #2	CRR05P32 (04026 R), unknown, Sochi region, Russia	Black Sea Group	41	6	res.
ZC6 Ukraine	unknown seeding, D. Zaurov, Caucasus region, Russia	Black Sea Group	42	6	res.
ZC4 Ukraine	unknown seeding, D. Zaurov , Caucasus region, Russia	Black Sea Group	44	6	res.
Sochi Market 4 #1	CRXR14P97 (RUS 6), Sochi Market 4, Sochi, Russia	Black Sea Group	45	6	tolerant

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
Unknown EFB-res.	unknown (identity lost)	Black Sea Group	46	6	res.
ZC1 Ukraine	unknown seeding, D. Zaurov, Caucasus region, Russia	Black Sea Group	47	6	res.
Sochi unknown #1	CRR03P09 (04034R), unknown, Sochi region, Russia	Black Sea Group	48	6	susc.
'President' OP #1	CRR06P43 (04022 R), 'President' OP, Sochi Inst., Russia	Black Sea Group	49	6	tolerant
'President' OP #3	CRR06P50 (04022 R), 'President' OP, Sochi Inst., Russia	Black Sea Group	50	6	res.
'President' OP #4	CRR06P53, 04022 R, 'President' OP, Sochi Inst., Russia	Black Sea Group	51	6	res.
'President' OP #2	CRR06P47, 04022 R, 'President' OP, Sochi Inst., Russia	Black Sea Group	52	6	tolerant
'Pioneer'	PI 617718, Russia (southerm)	Black Sea Group	53	6	suc.
'Kavkas' OP #2	CRR04P116, 04028 R, 'Kavkas' OP, Sochi Inst., Russia	Black Sea Group	54	6	res.
Holmskij Market 5 #1	CRXR15P77, RUS 13, Holmskij Market 5, Holmskij, Russia	Black Sea Group	58	6	susc
Sochi Market 5 #3	CRXR14P115 (RUS 7), Sochi Market 5, Sochi, Russia	Black Sea Group	86	6	susc.
Sochi Market 3 #1	H3R05P09 (RUS 5), Sochi Market 3, Sochi, Russia	Black Sea Group	90	6	tolerant
'Rimski' OP #2	CRRR02P41 (04040R),'Rimski' OP, Sochi Inst., Russia	Spanish-Italian Group	203	6	res.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
B-X-3 OP #2	CRR02P96 (04038 R), B-X-3 OP, Sochi Inst., Russia	No Group	225	6	res.
Maykop VIR #1	CRXR17P48 (RUS 15), Mix, Vavilov VIR Breeding Station, Maykop, Russia	*Wild C. avellana* group	319	6	res.
'Morrisoka' (*C. col.* × *C. ave.*)	PI 557331, J. Gellatly, British Columbia, Canada	Gellatly Hybrid Group	104	7	susc.
'Eastoka' (*C. col.* × *C. ave.*)	PI 557357, J. Gellatly, British Columbia, Canada	Gellatly Hybrid Group	105	7	susc.
'Laroka' (*C. col.* × *C. ave.*)	PI 557333, J. Gellatly, British Columbia, Canada	Gellatly Hybrid Group	106	7	susc.
Gordon #23 (*Corylus* hyb.)	Gordon selection R24DP01, John Gordon Nursery	Gellatly Hybrid Group	107	7	res.
Gordon #32 (*Corylus* hyb.)	Gordon selection R35P01, John Gordon Nursery	Gellatly Hybrid Group	108	7	res.
Chinese Trazel #6 OP #2	H02R08P61, OP seed of Chinese Trazel #6 from USDA NCGR	Gellatly Hybrid Group	109	7	res.
Chinese Trazel #6 OP #1	H02R05P21, OP seed of Chinese Trazel #6 from U.S. Department of Agriculture (USDA) National Clonal Germplasm Repository (NCGR)	Gellatly Hybrid Group	110	7	res.
Chinese Trazel #11 (*C. col* × *C. ave.*)	PI 557264, J. Gellatly, British Columbia, Canada	Gellatly Hybrid Group	111	7	res.
Chinese Trazel #6 (*C. col* × *C. ave.*)	PI 557261, J. Gellatly, British Columbia, Canada	Gellatly Hybrid Group	112	7	res.
'Red Majestic'	Netherlands, Plant Patent #16048 (red leaves, contorted stems)	No Group	156	7	susc.
'Contorta'	PI 557049, England	No Group	157	7	susc.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
Gordon #30 (*Corylus* hyb.)	Gordon selection R32P02, John Gordon Nursery	Mixed Group	161	7	res.
'Manoka' (*C. cornuta* × *C. ave*.)	PI 617186, J. Gellatly, British Columbia, Canada	Mixed Group	164	7	unknown
Home Ec Building	Cook Campus, Rutgers University, unknown origin	Mixed Group	166	7	susc.
Bulgaria XI-8	PI 557219, Bulgaria	Mixed Group	167	7	tolerant
Morris 32-1379A	Morris Arboretum32-1379A, PI 660747, unknown origin,	Mixed Group	168	7	res.
Turk Trazel #3 (*C. col.* × *C. ave*.)	PI 557395, J. Gellatly, British Columbia, Canada	Mixed Group	174	7	res.
'Erioka' (*C. col.* × *C. ave*.)	PI 557389, J. Gellatly, British Columbia, Canada	Mixed Group	177	7	susc.
'Chinoka' (*C. col.* × *C. ave*.)	PI 557387, J. Gellatly, British Columbia, Canada	Mixed Group	178	7	susc.
Gordon #34 (*Corylus* hyb.)	Gordon selection R37P01, John Gordon Nursery	Mixed Group	180	7	res.
Grand Traverse (*C. col.* × *C. ave*.)	PI 617185, C. Farris, Michigan, offspring of 'Faroka',	Mixed Group	181	7	res.
Grimo 186M (*C. col.* × *C. ave*.)	Grimo Nut Nursery selection, offspring of 'Faroka'	Mixed Group	182	7	res.
'Faroka' (*C. col.* × *C. ave*.)	PI 557393, J. Gellatly, British Columbia, Canada	Mixed Group	183	7	res.
Gordon #1 (*Corylus* hyb.)	Gordon selection #8V, John Gordon Nursery	Mixed Group	186	7	res.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
Gordon #31 (*Corylus* hyb.)	Gordon selection R34P22, John Gordon Nursery	Mixed Group	188	7	res.
'Tonda Romana'	PI 557025, Italy (Lazio)	Spanish-Italian Group	192	7	susc.
'Freeoka' (*C. col.* × *C. ave.*)	PI 557362, J. Gellatly, British Columbia, Canada	No Group	214	7	susc.
Skierniewice mix #9	H4AR18P84 (06054 P), Unknown seed mixture, Skierniewice, Poland	No Group	114	8	res.
Simferopol Market 5 #4	CRXR19P10 (RUS 26), Roadside Market 5, near Simferopol, Ukraine	No Group	150	8	res.
Simferopol Market 5 #3	CRXR19P05(RUS 26), Roadside Market 5, near Simferopol, Ukraine	No Group	151	8	res.
Simferopol Market 5 #7	H3R7P9 (RUS 26), Roadside Market 5, near Simferopol, Ukraine	No Group	152	8	res.
Simferopol Market 5 #1	CRXR19P02 (RUS 26), Roadside Market 5, near Simferopol, Ukraine	No Group	153	8	res.
Simferopol Market 5 #6	H3R7P11 (RUS 26), Roadside Market 5, near Simferopol, Ukraine	No Group	154	8	res.
Simferopol Market 5 #2	CRXR19P04 (RUS 26), Roadside Market 5, near Simferopol, Ukraine	No Group	155	8	res.
Rutgers Passion Puddle	Cook Campus, Rutgers University, unknown origin	No Group	158	8	susc.
Simferopol Market 3 #2	H3R10P24 (RUS 24), Roadside Market #3, Simferopol, Ukraine	Mixed Group	160	8	tolerant
'Barrs Zellernuss'	PI 557158, England	Mixed Group	163	8	susc.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
Skierniewice mix #5	H4AR18P66 (06054 P), Unknown seed mixture, Skierniewice, Poland	Mixed Group	172	8	tolerant
'Ugbrooke'	PI 557100, New Zealand	Mixed Group	176	8	susc.
'Segorbe'	PI 557046, Spain, Castellon de la Plana	Spanish-Italian Group	205	8	susc.
'Santiam'	OSU 249.159 × VR 17-15	Gasaway Group	216	8	res.
VR20-11	('Barcelona' × 'Compton') × 'Gasaway',OSU, Oregon, USA	Gasaway Group	223	8	res.
'Gasaway'	PI 557042, Washington, USA	Gasaway Group	224	8	res.
'Cutleaf'	PI 557306, England	*Wild C. avellana* group	298	8	susc.
Estonia #2	H4AR27P06 (07591), Tartu, Estonia	*Wild C. avellana* group	302	8	susc.
Finland CCOR 187	PI 557080, Finland	*Wild C. avellana* group	305	8	tolerant
Simferopol Market 2 #2	H3R12P58 (RUS 23), Simferopol Roadside Market 2, Simferopol, Ukraine	*Wild C. avellana* group	306	8	res.
Simferopol Market 1A #2	CRXR18P08 (RUS 21), Roadside Market 1A, Simferopol,Ukraine	Wild *C. avellana* group	307	8	res.
Simferopol Market 4 #2	CRXR19P30 (RUS 25), Roadside Market 4, Simferopol, Ukraine	Wild *C. avellana* group	308	8	tolerant
Simferopol Market 2 #1	CRXR19P39b (RUS 23), Roadside Market 2, Simferopol,Ukraine	Wild *C. avellana* group	309	8	susc.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
Simferopol Market 3 #1	CRXR18P39a (RUS 24), Roadside Market 3, Simferopol, Ukraine	Wild *C. avellana* group	310	8	susc.
Estonia #1	H4AR26P18 (07589), Agusalu, Estonia	Wild *C. avellana* group	311	8	susc.
'Aurea'	PI 557050, France	Wild *C. avellana* group	312	8	susc.
Moscow selection (unknown)	Russian Res. Inst. Forestry and Mech., Moscow, Russia	Wild *C. avellana* group	313	8	res.
Maykop VIR #2	CRXR17P64 (RUS 15), Mixture of seed from Vavilov Institute of Plant Industry (VIR) Breeding Station, Maykop, Russia	Wild *C. avellana* group	314	8	tolerant
Simferopol Market 4 #1	CRXR19P21 (RUS 25), Roadside Market 4, Simferopol, Ukraine	Wild *C. avellana* group	315	8	res.
Skierniewice mix #8	H4AR18P83 (06054 P), Unknown seed mixture, Skierniewice, Poland	Wild *C. avellana* group	316	8	tolerant
OSU 408.040	PI 617266, Minnesota, USA	Wild *C. avellana* group	317	8	res.
Moscow #28	Russian Res. Inst. Forestry and Mech., Moscow, Russia	Wild *C. avellana* group	318	8	susc.
Simferopol Market 2 #3	H3R12P62 (RUS 23), Simferopol Roadside Market 2, Simferopol, Ukraine	Wild *C. avellana* group	320	8	res.
Simferopol Market 5 #5	CRXR19P15 (RUS 26), Roadside Market 5, near Simferopol, Ukraine	Wild *C. avellana* group	321	8	susc.
Simferopol Market 1A #3	H3R04P12 (RUS 21), Simferopol Roadside Market #1A, Simferopol, Ukraine	Wild *C. avellana* group	322	8	res.
Simferopol Market 1A #1	CRXR18P05 (RUS 21), Roadside Market 1A, Simferopol, Ukraine	Wild *C. avellana* group	323	8	res.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
'Sivri Ghiaghli'	PI 304633, Turkey	Black Sea Group	60	9	susc.
'Tombul'	PI 318463, Turkey (Akcacoca)	Black Sea Group	61	9	Unknown
'Kudryavchik'	PI 671177, Russia (southern)	Black Sea Group	62	9	Unknown
'Tombul Ghiaghli'	PI 304634, Turkey	Black Sea Group	63	9	susc.
'Kalinkara'	PI 557240 ,Turkey	Black Sea Group	67	9	Tolerant
'DuChilly'	PI 557099, England	No Group	159	9	susc.
'Henneman 3'	PI 557427, Oregon, USA	Mixed Group	165	9	susc.
'Istrski Duguljasti'	PI 557400, Croatia	Mixed Group	169	9	susc.
'Badem'	PI 304630, Turkey	Mixed Group	170	9	susc.
'Jeans'	PI 557116, Unknown,	Mixed Group	171	9	susc.
'Casina'	PI 557033, Spain (Asturias)	Spanish-Italian Group	189	9	susc.
'Zimmerman'	'Barcelona' × 'Gasaway', Oregon, USA	Spanish-Italian Group	190	9	res.
'Sant Jaume'	PI 557103, Spain	Spanish-Italian Group	191	9	susc.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
'Negret'	PI 270340, Spain (Tarragona)	Spanish-Italian Group	193	9	susc.
'Culpla'	PI 557107, Spain (Tarragona)	Spanish-Italian Group	194	9	res.
'Tonda Gentile delle Langhe'	PI 557075, Italy (Piemonte)	Spanish-Italian Group	195	9	susc.
'Ratoli'	PI 557167, Spain (Tarragona)	Spanish-Italian Group	196	9	res.
'Closca Molla'	PI 557109, Spain (Tarragona)	Spanish-Italian Group	197	9	Tolerant
'Restiello'	PI 557129, Spain	Spanish-Italian Group	198	9	susc.
'Sodlinger'	PI 557212, Yugoslavia	Spanish-Italian Group	199	9	susc.
'Royal'	PI 557052, 'Barcelona' × 'Cosford', Oregon, USA	Spanish-Italian Group	200	9	susc.
'Gem'	PI 557029, Washington, USA	Spanish-Italian Group	201	9	susc.
'Barcelona'	PI 557037, Spain (Tarragona)	Spanish-Italian Group	202	9	susc.
'Tonda di Giffoni'	PI 296207, Italy (Campania)	Spanish-Italian Group	204	9	Tolerant
'Uebov'	Serbia	Spanish-Italian Group	206	9	res.
OSU 541.147 (*C. amer.* × *C. ave*.)	NY 110 (*C. amer.* 'Rush' × *C. ave.* 'DuChilly') × OSU 226.118	Spanish-Italian Group	207	9	res.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
'Yamhill'	OSU 296.082 × VR 8-32 (Gasaway res.), Oregon, USA,	Spanish-Italian Group	208	9	res.
'Jefferson'	OSU 252.146 × OSU 414.062 (Gasaway res.)	Spanish-Italian Group	209	9	res.
'Clark'	PI 617268, 'Tombul Ghiaghli × 'Willamette', Oregon, USA,	Spanish-Italian Group	210	9	susc.
'Butler'	PI 557077, 'Barcelona' × 'Daviana', Oregon, USA	Spanish-Italian Group	211	9	susc.
'Daviana'	PI 557040, England	Spanish-Italian Group	212	9	susc.
'Ennis'	PI 557045, 'Barcelona' × 'Daviana', Washington, USA	Spanish-Italian Group	213	9	susc.
'Bianca'	PI 557182, Italy (Campania)	Wild *C. avellana* group	297	9	unknown
'Kudashovski' OP #19	CRXR15P04 (RUS 2), 'Kudashovski' OP, Sochi Inst., Russia	Moscow Group	116	10	res.
'Moskovskii Rubin' OP #7	CRR04P28 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Moscow Group	117	10	res.
'Moskovskii Rubin' OP #11	CRR04P48 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Moscow Group	118	10	res.
'Kudashovski' OP #5	CRXR13P83 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	119	10	res.
'Moskovskii Rubin' OP #9	CRR04P33 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Moscow Group	120	10	res.
'Moskovskii Rubin' OP #12	CRR04P52 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Moscow Group	121	10	res.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
'Moskovskii Rubin' OP #3	CRR04P19 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Moscow Group	122	10	res.
'Kudashovski' OP #17	CRXR14P11 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	123	10	res.
'Moskovskii Rubin' OP #4	CRR04P22 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Moscow Group	124	10	res.
'Kudashovski' OP #12	CRXR13P103 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	125	10	res.
'Kudashovski' OP #14	CRXR13P124 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	126	10	res.
'Kudashovski' OP #22	CRXR15P11 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	127	10	res.
'Kudashovski' OP #7	CRXR13P91 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	128	10	res.
'Kudashovski' OP #8	CRXR13P95 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	129	10	res.
'Kudashovski' OP #18	CRXR14P14 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	130	10	res.
'Kudashovski' OP #1	CRXR13P78 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	131	10	res.
'Kudashovski' OP #2	CRXR13P79 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	132	10	res.
'Moskovskii Rubin' OP #13	CRRR04P65 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Moscow Group	133	10	res.
'Kudashovski' OP #15	CRXR13P125 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	134	10	res.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
'Moskovskii Rubin' OP #8	CRR04P32 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Moscow Group	135	10	res.
'Kudashovski' OP #21	CRXR15P08 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	136	10	res.
'Kudashovski' OP #6	CRXR13P89 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	137	10	res.
'Kudashovski' OP #4	CRXR13P82 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	138	10	res.
'Kudashovski' OP #9	CRXR13P96 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	139	10	res.
'Kudashovski' OP #3	CRXR13P81 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	140	10	res.
'Kudashovski' OP #16	CRXR14P08 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	141	10	res.
'Moskovskii Rubin' OP #1	CRR04P17 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Moscow Group	142	10	res.
'Moskovskii Rubin' OP #6	CRR04P27 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Moscow Group	143	10	res.
'Kudashovski' OP #10	CRXR13P97 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	144	10	res.
'Moskovskii Rubin' OP #5	CRR04P25 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Moscow Group	145	10	res.
'Moskovskii Rubin' OP #2	CRR04P18 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Moscow Group	147	10	res.
Moscow #2	Russian Res. Inst. Forestry and Mech., Moscow, Russia	Moscow Group	148	10	res.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
'Kudashovski' OP #11	CRXR13P102 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Moscow Group	149	10	res.
'Moskovskii Rubin' OP #10	CRR04P37 (04030R), 'Moskovskii Rubin' OP, Sochi Inst., Sochi, Russia	Gasaway Group	222	10	res.
'Early Long Zeller'	PI 557161, England	Moscow Group	146	11	susc.
Skierniewice mix #6	H4AR18P72 (06054 P), Unknown seed mixture, Skierniewice, Poland	Mixed Group	162	11	tolerant
Skierniewice mix #2	H4AR18P02 (06054 P), Unknown seed mixture, Skierniewice, Poland	Mixed Group	173	11	tolerant
Skierniewice mix #4	H4AR18P30 (06054 P), Unknown seed mixture, Skierniewice, Poland	Mixed Group	175	11	tolerant
'Karol'	PI 617231, Poland	Mixed Group	179	11	susc.
Gordon #33 (*Corylus* hyb.)	Gordon selection R35P02, John Gordon Nursery	Mixed Group	184	11	res.
'Kudashovski' OP #13	CRXR13P108 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Mixed Group	187	11	res.
'Karloka' (*C. col.* × *C. ave.*)	PI 557394, J. Gellatly, British Columbia, Canada	No Group	215	11	susc.
Farris 88 BS (*C. col.* × *C. ave.*)	PI 617191, 'Faroka' OP, C. Farris, Michigan, USA	Gasaway Group	217	11	res.
Farris Box 1 (*Corylus* hyb.)	C. Farris selection, Michigan, USA via UNL	Gasaway Group	218	11	res.
Farris G-17 (*Corylus* hyb.)	C. Farris selection, Michigan, USA via UNL	Gasaway Group	219	11	res.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
'Badem' OP #2	CRXR18P102 red leaf (RUS 16), 'Badem' OP, Inst. Orch. Wine Prod., Krasnodar, Russia	Gasaway Group	220	11	tolerant
'Badem' OP #1	CRXR18P102 green leaf (RUS 16), 'Badem' OP, Inst. Orch. Wine Prod., Krasnodar, Russia	Gasaway Group	221	11	tolerant
'Garibaldi' OP #1	H4AR20P34 (06050 P), 'Garibaldi' OP, Konskowli, Poland	Central European Group	226	11	tolerant
Skierniewice mix #7	H4AR18P73 (06054 P), Unknown seed mixture, Skierniewice, Poland	Central European Group	227	11	res.
'Katalonski' OP #1	H4AR20P132 (06053 P), 'Katalonski' OP, Konskowli, Poland	Central European Group	228	11	tolerant
Warsaw mix #2	H4AR21P05 (06085 P), Unknown seed mixture, Warsaw, Poland	Central European Group	229	11	res.
Warsaw mix #1	H4AR21P03 (06085 P), Unknown seed mixture, Warsaw, Poland	Central European Group	230	11	res.
'Syrena'	PI 617175, Poland	Central European Group	231	11	susc.
Warsaw Market 4 #1	H4AR20P88 (06080 P), Warsaw Market 4, Warsaw, Poland	Central European Group	232	11	res.
Skierniewice mix #1	H4AR18P01 (06054 P), Unknown seed mixture, Skierniewice, Poland	Central European Group	233	11	susc.
Skierniewice mix #10	H4AR19P16 (06054 P), Unknown seed mixture, Skierniewice, Poland	Central European Group	234	11	susc.
Warsaw mix #3	H4AR21P43 (06085 P), Unknown seed mixture, Warsaw, Poland	Central European Group	235	11	res.
Skierniewice mix #3	H4AR18P130 (06054 P), Unknown seed mixture, Skierniewice, Poland	Central European Group	236	11	tolerant

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
‘Aveline d'Angleterre’	PI 557194, England (or France)	Central European Group	237	11	susc.
‘Volski’	PI 617238, Poland	Central European Group	240	11	unknown
‘Halls Giant’ OP #1	H4AR17P02fe (06052 P), ‘Hall's Giant’ OP, Konskowli, Poland	Central European Group	241	11	res.
Warsaw Market 3 #1	H4AR19P34 (06079 P), Warsaw Market 3, Warsaw, Poland	Central European Group	242	11	tolerant
‘Webba’ OP #1	H4AR19P120 (06051 P), ‘Webba’ OP, Konskowli, Poland	Central European Group	243	11	susc.
‘Frango 2’	PI 617227, Poland	Central European Group	244	11	unknown
‘Lech’	PI 617232, Poland	Central European Group	245	11	unknown
‘Halls Giant’ OP #2	H4AR17P03fe(06052 P), ‘Hall's Giant’ OP, Konskowli, Poland	Central European Group	246	11	tolerant
‘Warsaw Market’ 8 #1	H4AR20P51 (06084 P), Warsaw Market 8, Warsaw, Poland	Central European Group	247	11	unknown
‘Hall's Giant’	PI 557027, Germany/France	Central European Group	248	11	susc.
‘Italian Red’	PI 557034, Germany	Central European Group	249	11	susc
‘Gustav's Zellernuss’	PI 557085, Germany	Central European Group	250	11	suc.
‘Slagel’ (Corylus hyb.)	John Gordon Nursery	Central European Group	251	11	res.

Accession name and species	Identification code (seed lot), origin, and/or parentage	UPGMA group	STRUCTURE ID no.	Consensus populations	EFB response
'Goc'	PI 617230, Poland	Central European Group	253	11	susc.
'Red Fortin'	PI 617182, Washington, USA	Central European Group	254	11	susc.
'Annie's Compact Red'	Burnt Ridge Nursery, Onalaska, WA	Central European Group	255	11	unknown
'Rote Zellernuss'	PI 271280, Netherlands	Central European Group	256	11	susc.
'Lenka 3'	PI 617233, Poland	Central European Group	257	11	unknown
'Maria'	PI 617236, Poland	Central European Group	258	11	susc.
Acorn hazelnut	PI 617226, Poland	Central European Group	260	11	unknown
'Little Poland'	PI 617235, Poland	Central European Group	261	11	unknown
Gordon #5(Corylus hyb.)	Gordon selection R06P01, John Gordon Nursery	*C. americana* × *C. avellana* Hybrid Group 2	285	11	res.
'Frango 5'	PI 617229, Poland	*C. americana* × *C. avellana* Hybrid Group 2	286	11	unknown
Farris 188P (Corylus hyb.)	C. Farris selection, Michigan, USA via UNL	*C. americana* × *C. avellana* Hybrid Group 2	287	11	tolerant
Gordon #36 (Corylus hyb.)	Gordon selection R38P02, John Gordon Nursery	*C. americana* × *C. avellana* Hybrid Group 2	289	11	res.
'Fusco Rubra'	PI 557047, Germany	Wild *C. avellana* group	303	11	susc.
'Kudashovski' OP #20	CRXR15P07 (RUS 2), 'Kudashovski' OP, Sochi Inst., Sochi, Russia	Wild *C. avellana* group	304	11	res.

Table 2. Characteristics of the 17 simple sequence repeat markers used to assess the genetic diversity and relationships of 323 *Corylus* accessions.

Primer	Source	Linkage group no.	Primer Sequence	Motif	Product size range (bp)
A640	Gürcan et al., 2010a, 2010b	10	F-TGCCTCTGCAGTTAGTCATCAAATGTAGG R-CGCCATATAATTGGGATGCTTGTTG	$(CT)_{15}(CA)_{13}$	354-378
B005	Bassil et al., 2005a, 2005b	2	F-CAAACTTATGATAGGCATGCAA R-TGTCACTTTGGAAGACAAGAGA	$(GA)_{22}$	267-301
B502	Boccacci et al., 2005	10	F-CTCATGACTGCCCATTTCTCG R-AGGCATGCAGGCTTCACAC	$(GA)_1GC(GA)_2GC(GA)_{14}$	185-214
B634	Gürcan et al., 2010a, 2010b	4	F-CCTGCATCCAGGACTCATTA R-GTGCAGAGGTTGCACTCAAA	$(AG)_{15}$	218-238
B657	Gürcan et al., 2010a, 2010b	11	F-GAGAGTGCGTCTTCCTCTGG R-AGCCTCACCTCCAACGAAC	$(AG)_{15}$	210-228
B665	Gürcan et al., 2010a, 2010b	8	F-GCAACCACCAAATTGCACTA R-GCTTTTAAAGTCCACGCATGA	$(CT)_{17}$	177-203
B671	Gürcan et al., 2010a, 2010b	9	F-TTGCCAGTGCATACTCTGATG R-ACCAGCTCTGGGCTTAACAC	$(AG)_6NN(GA)_{17}$	221-249
B733	Gürcan et al., 2010a, 2010b	7	F-CACCCTCTTCACCACCTCAT R-CATCCCCTGTTGGAGTTTTC	$(TC)_{15}$	161-183
B749	Gürcan et al., 2010a, 2010b	1	F-GGCTGACAACACAGCAGAAA R-TCGGCTAGGGTTAGGGTTTT	$(TC)_{12}$	200-210
B751	Gürcan et al., 2010a, 2010b	7	F-AGCTGGTTCTTCGACATTCC R-AAACTCAAATAAAACCCCTGCTC	$(GA)_{15}$	141-153
B753	Gürcan et al., 2010a, 2010b	7	F-AAGGGTTGTTACCCATGCAC R-GGTGCATTTAGTGCTTCTGG	$(GA)_{15}$	224-254
B774	Gürcan et al., 2010a, 2010b	5	F-GTTTTGCGAGCTCATTGTCA R-TGTGTGTGGTCTGTAGGCACT	$(AG)_{15}$	195-213
B776	Gürcan et al., 2010a, 2010b	6	F-TGTATGTACACACGGAGAGAGAGA R-TGAGGGGAAGAGGTTTGATG	$(GA)_{17}$	134-148

Primer	Source	Linkage group no.	Primer Sequence	Motif	Product size range (bp)
B789	Gürcan et al., 2010a, 2010b	2	F-GCCACGTCCAGAATCAAAAT R-CCTCAGGGCTGAGAAGTTGA	$(AG)_{16}$	158-186
KG807	Gürcan and Mehlenbacher, 2010a	11	F-AAGCAAGAAAGGGATGGT R-CTTACAGATAAATGGCTCAAA	UNKNOWN	226-248
KG810	Gürcan and Mehlenbacher, 2010a	4	F-TCCTCACCAATCACACTATTT R-TTATTCCACCAAAGTCTACCTC	$(AG)_{15}$	366-392
KG830	Gürcan and Mehlenbacher, 2010a	9	F-TGGAGGAAGTTTTGAATGGTAGTAGAGGA R-AAAGCAACTCATAGCTGAAGTCCAATCA	$(CT)_{13}GTATT(CA)_3$	279-311

Table 3. Summary statistics, including observed heterozygosity (Ho), expected heterozygosity (He), polymorphism information content (PIC), and inbreeding coefficient (f), for 323 hazelnut (*Corylus* sp.) accessions assessed with 17 simple sequence repeat markers.

Locus	Allele (no.)	Major allele frequency	Genotypes (no.)	Ho	He	PIC	f	Frequency of null alleles
A640	16	0.24	52	0.83	0.85	0.83	0.06	0.0106
B005	15	0.32	40	0.64	0.78	0.75	0.184	0.1050
B502	17	0.33	58	0.66	0.82	0.80	0.196	0.1061
B634	19	0.29	53	0.80	0.83	0.81	0.038	0.0189
B657	20	0.18	55	0.86	0.88	0.87	0.027	0.0120
B665	20	0.19	64	0.52	0.89	0.87	0.41	0.2577
B671	21	0.24	67	0.81	0.86	0.85	0.065	0.0299
B733	12	0.32	39	0.73	0.77	0.74	0.058	0.0297
B749	18	0.39	40	0.60	0.71	0.67	0.171	0.0921
B751	13	0.39	38	0.72	0.78	0.76	0.079	0.0418
B753	22	0.28	75	0.83	0.87	0.86	0.043	0.0191
B774	17	0.33	47	0.79	0.82	0.80	0.046	0.0189
B776	21	0.47	47	0.57	0.71	0.68	0.202	0.1205
B789	26	0.36	71	0.80	0.81	0.80	0.018	0.0071
KG807	13	0.37	27	0.67	0.74	0.70	0.104	0.0472
KG810	21	0.26	80	0.80	0.86	0.84	0.076	0.0379
KG830	17	0.31	50	0.80	0.82	0.80	0.029	0.0120
Mean	18.12	0.31	53.11	0.73	0.81	0.79	0.103	___

Table 4. Population partitioning of total within population variance across 348 (340 unique) hazelnut (*Corylus* sp.) accessions.

Source	N	df	Sum of squares within populations	Variance components within populations	Proportion of total variance (%)
Outgroup 1	10	9	65	7.22	1.63
Outgroup 2	4	3	6.75	2.25	.169
Outgroup 3	24	23	162.75	7.08	4.08
C. americana x C. avellana	94	93	570.64	6.14	14.29
Black Sea Group 1	84	83	493.04	5.94	12.35
Black Sea Group 2	68	67	443.88	6.63	11.12
Gellatly hybrid Group	54	53	349.06	6.59	8.74
Wild *C. avellana* Group	74	73	481.01	6.59	12.05
Spanish-Italian Group	66	65	405.58	6.24	10.16
Moscow Group	68	67	407.71	6.09	10.21
Central European Group	100	99	607.61	6.14	15.22

Table 5. Matrix of pairwise AMOVA FST values (below the diagonal) and *P*-values above the diagonal with variation within each group of *Corylus* accessions along the principle diagonal.

Group	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11
G1[z]	——	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
G2[y]	0.326	——	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
G3[x]	0.104	0.308	——	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
G4[w]	0.179	0.353	0.108	——	0.001	0.001	0.001	0.001	0.001	0.001	0.001
G5[v]	0.179	0.356	0.133	0.173	——	0.001	0.001	0.001	0.001	0.001	0.001
G6[u]	0.138	0.300	0.076	0.121	0.069	——	0.001	0.001	0.001	0.001	0.001
G7[t]	0.128	0.307	0.083	0.099	0.130	0.090	——	0.001	0.001	0.001	0.001
G8[s]	0.150	0.327	0.076	0.102	0.136	0.063	0.069	——	0.001	0.001	0.001
G9[r]	0.164	0.348	0.095	0.109	0.078	0.085	0.081	0.083	——	0.001	0.001
G10[q]	0.182	0.342	0.122	0.123	0.173	0.112	0.102	0.087	0.138	——	0.001
G11[p]	0.178	0.356	0.101	0.061	0.154	0.096	0.071	0.058	0.0904	0.102	——

[z]Outgroup 1

[y]Outgroup 2

[x]Outgroup 3

[w]*C. americana* × *C. avellana* hybrid Group

[v]Black Sea Group 1

[u]Black Sea Group 2

[t]Gellatly hybrid Group

[s]Wild *C. avellana* Group

[r]Spanish-Italian Group

[q]Moscow Group

[p]Central European Group

Figure 1. Unweighted pair group method using arithmetic averages (UPGMA) dendogram with 10 of the major groups, excluding outgroups, collapsed. All accessions are *Corylus avellana* unless otherwise noted. In reference to the hybrid accessions, *C. ave.*, *C. het*, *C. amer.*, and *C. col*., correspond to *C. avellana*, *C. heterophylla, C. americana*, and *C. colurna*, respectively. The abbreviations hyb. And OP indicate hybrid and open-pollinated, respectively. Accessions marked with an asterisk (*) are resistant to eastern filbert blight.

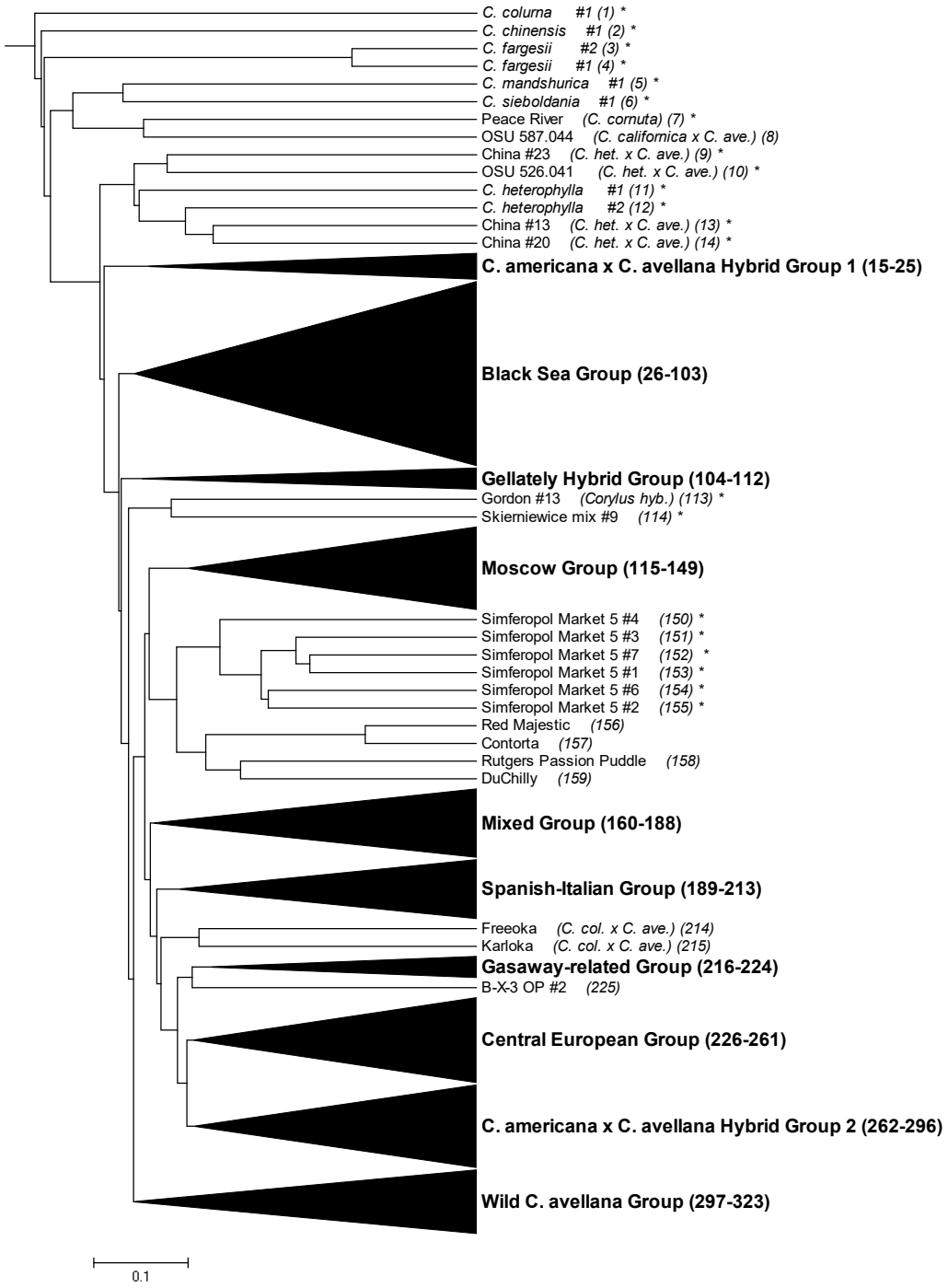


Figure 2. Uncollapsed *Corylus americana* × *C. avellana* hybrid group 1 node of the unweighted pair group method using arithmetic averages (UPGMA) dendrogram. *C. ave.*, *C. amer.,* and *C. het*. correspond to *C. avellana*, *C. americana*, and *C. heterophylla* respectively. The abbreviation hyb. Indicates hybrid. Accessions marked with an asterisk (*) are resistant to eastern filbert blight.

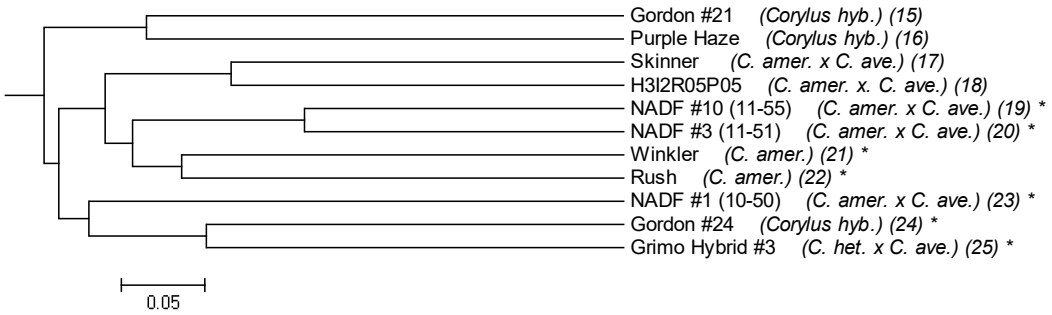


Figure 3. Uncollapsed Black Sea group node of the unweighted pair group method using arithmetic averages (UPGMA) dendrogram. All accessions are *Corylus avellana*. The abbreviation OP indicates open-pollinated, Res. Indicates resistant, EFB indicates eastern filbert blight, and Nikita Bot. Gard. indicates Nikita Botanical Garden (Yalta, Ukraine). Accessions marked with an asterisk (*) are resistant to eastern filbert blight.

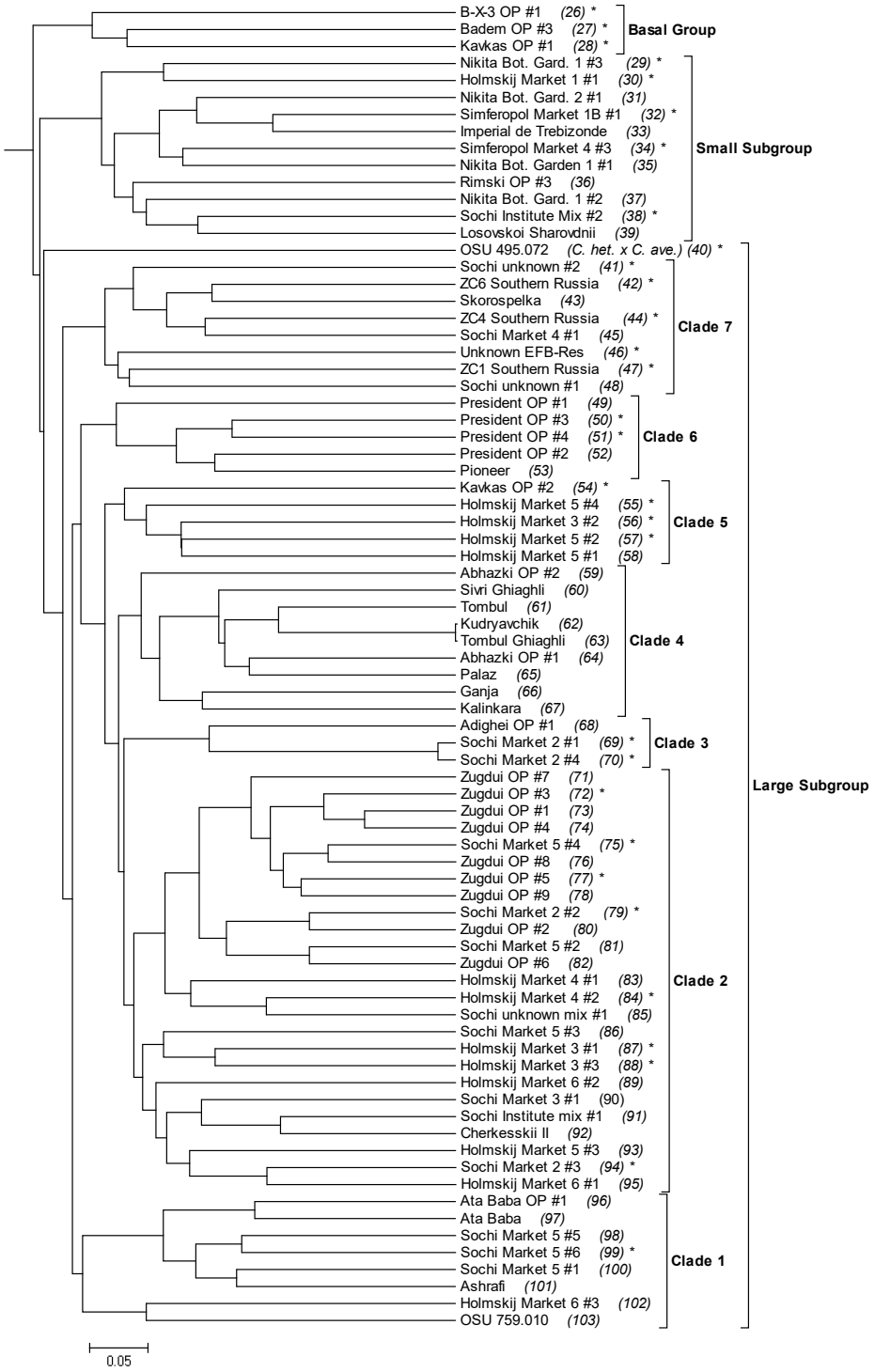


Figure 4. Uncollapsed Gellately hybrid group node of the unweighted pair group method using arithmetic averages (UPGMA) dendrogram. In reference to the hybrid accessions, *Corylus avellana* and *C. colurna* are represented by *C. ave.* and *C. col.*, respectively. Accessions marked with an asterisk (*) are resistant to eastern filbert blight.

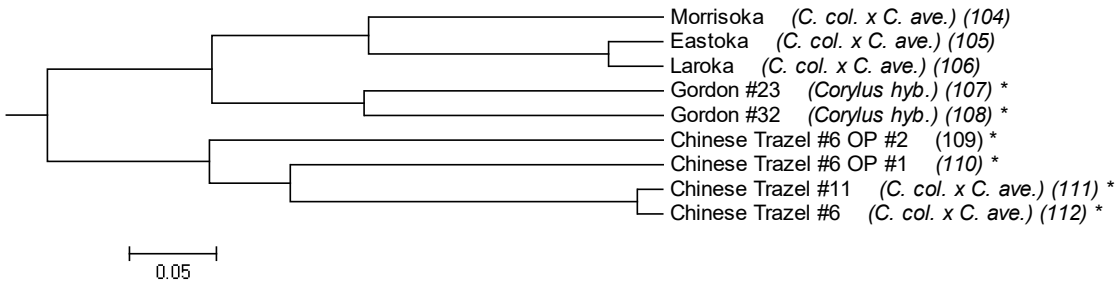


Figure 5. Uncollapsed Moscow group node of the unweighted pair group method using arithmetic averages (UPGMA) dendogram. All accessions included in this group are *Corylus avellana* unless otherwise noted. The abbreviations hyb. And OP indicate hybrid and open-pollinated, respectively. Accessions marked with an asterisk (*) are resistant to eastern filbert blight.

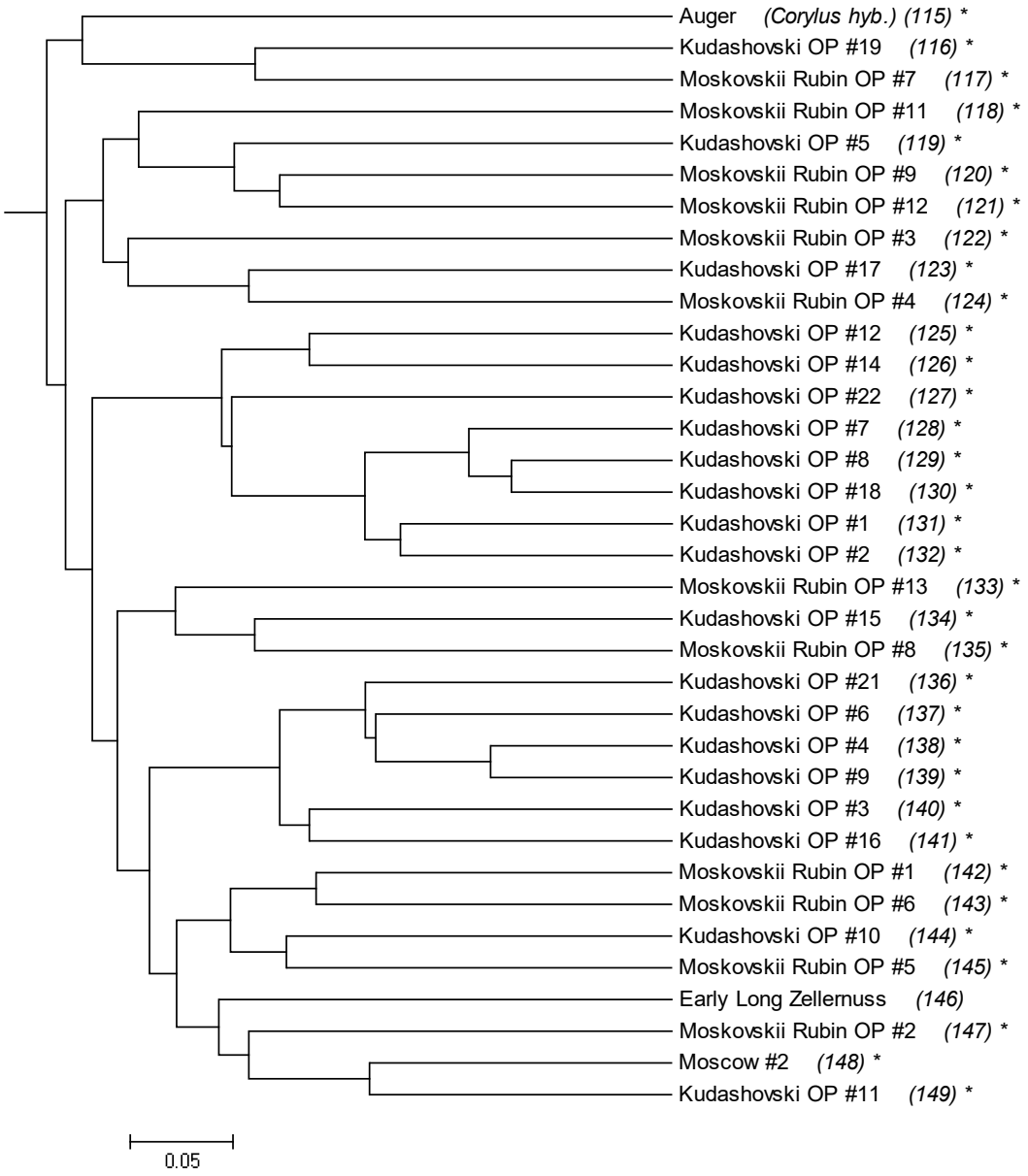


Figure 6. Uncollapsed Mixed group node of the unweighted pair group method using arithmetic averages (UPGMA) dendogram. All accessions are *Corylus avellana* unless otherwise noted. In reference to the hybrid accessions, *C. ave*., *C. het.*, and *C. col.* correspond to *C. avellana*, *C. heterophylla,* and *C. colurna*, respectively. The abbreviations hyb. And OP indicate hybrid and open-pollinated, respectively. Accessions marked with an asterisk (*) are resistant to eastern filbert blight.

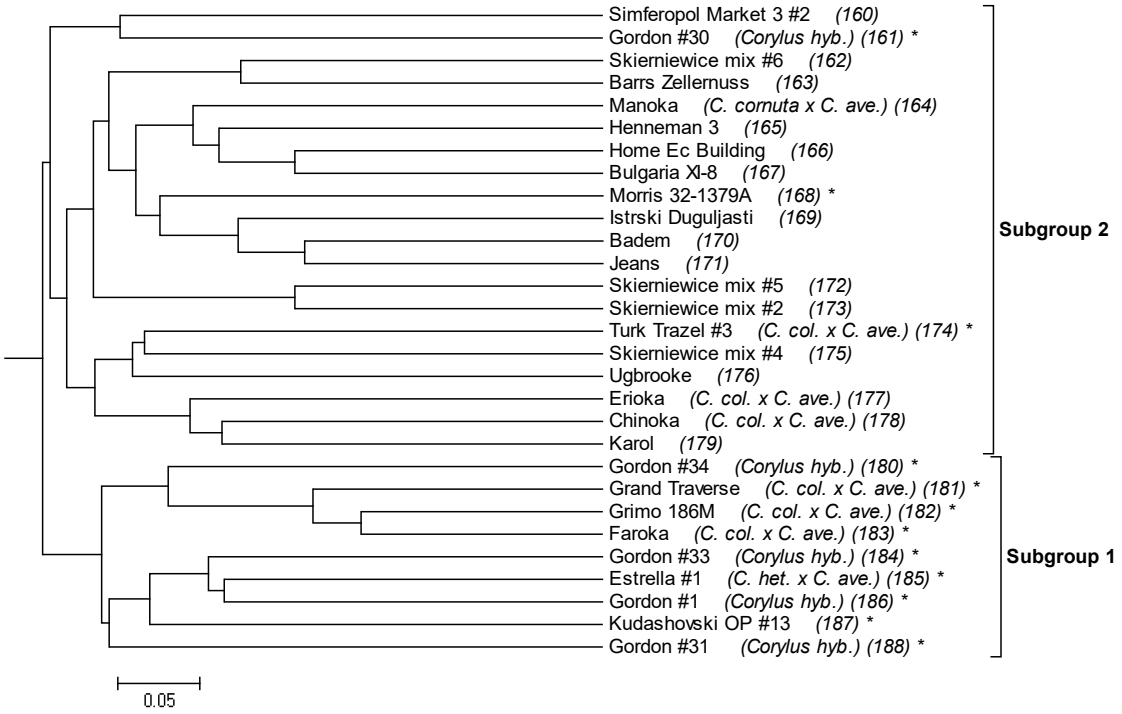


Figure 7. Uncollapsed Spanish-Italian group node of the unweighted pair group metod using arithmetic averages (UPGMA) dendrogram. All accessions are *Corylus avellana* unless otherwise noted. In reference to the hybrid accessions, *C. ave.* and *C. amer.* correspond to *C. avellana* and *C. americana*, respectively. The abbreviations OP indicates open-pollinated. Accessions marked with an asterisk (*) are resistant to eastern filbert blight.

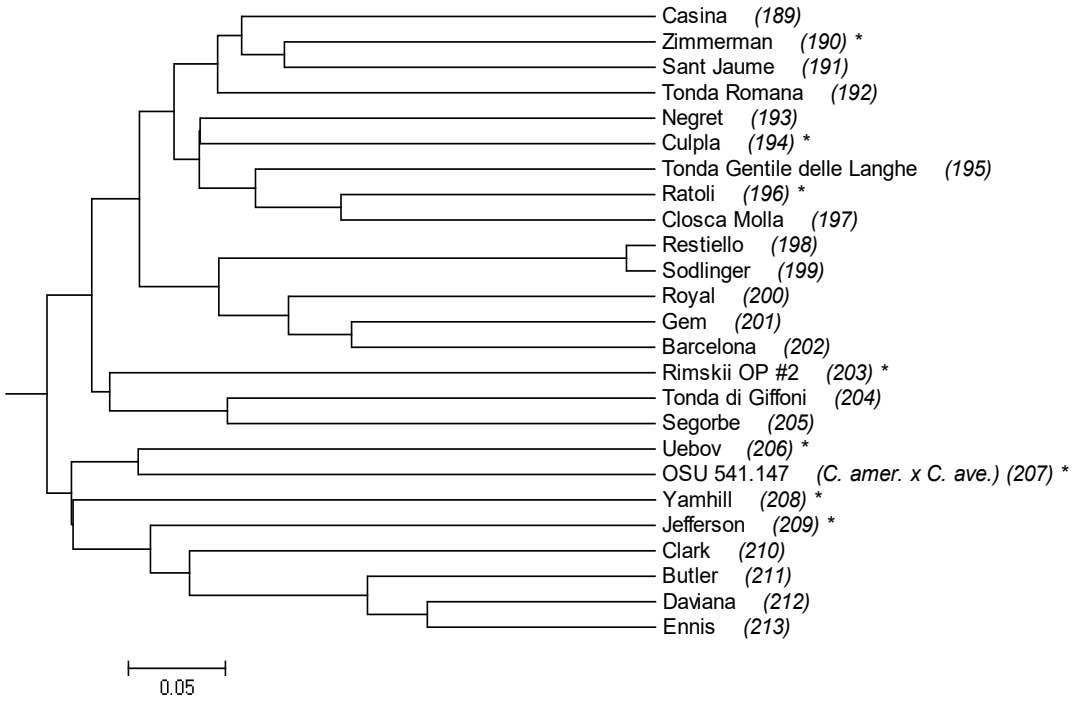


Figure 8. Uncollapsed Gasaway group node of the unweighted pair group method using arithmetic averages (UPGMA) dendrogram. All accessions are C. avellana unless otherwise noted. In reference to the hybrid accessions, *C. ave.* and *C. col.* Correspond to *C. avellana* and *C. colurna*, respectively. The abbreviations hyb. and OP indicate hybrid and open-pollinated, respectively. Accessions marked with an asterisk (*) are resistant to eastern filbert blight.

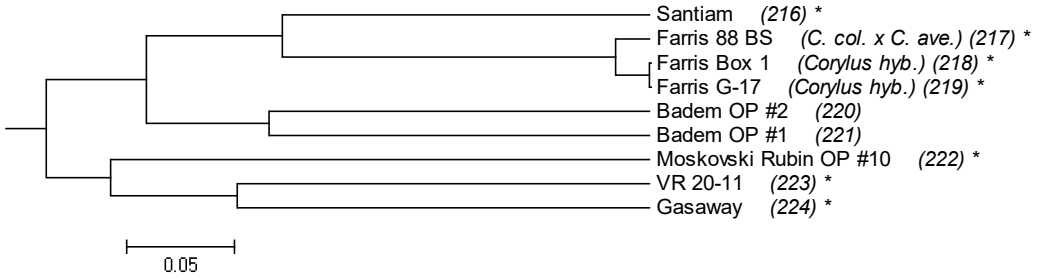


Figure 9. Uncollapsed Central European group node of the unweighted pair group method using arithmetic averages (UPGMA) dendrogram. All accessions are Corylus avellana unless otherwise noted. In reference to hybrid accessions, *C. ave.* and *C. amer.* correspond to *C. avellana* and *C. americana*, respectively. The abbreviations hyb. and OP indicate hybrid and open-pollinated, respectively. Accessions marked with an asterisk (*) are resistant to eastern filbert blight.

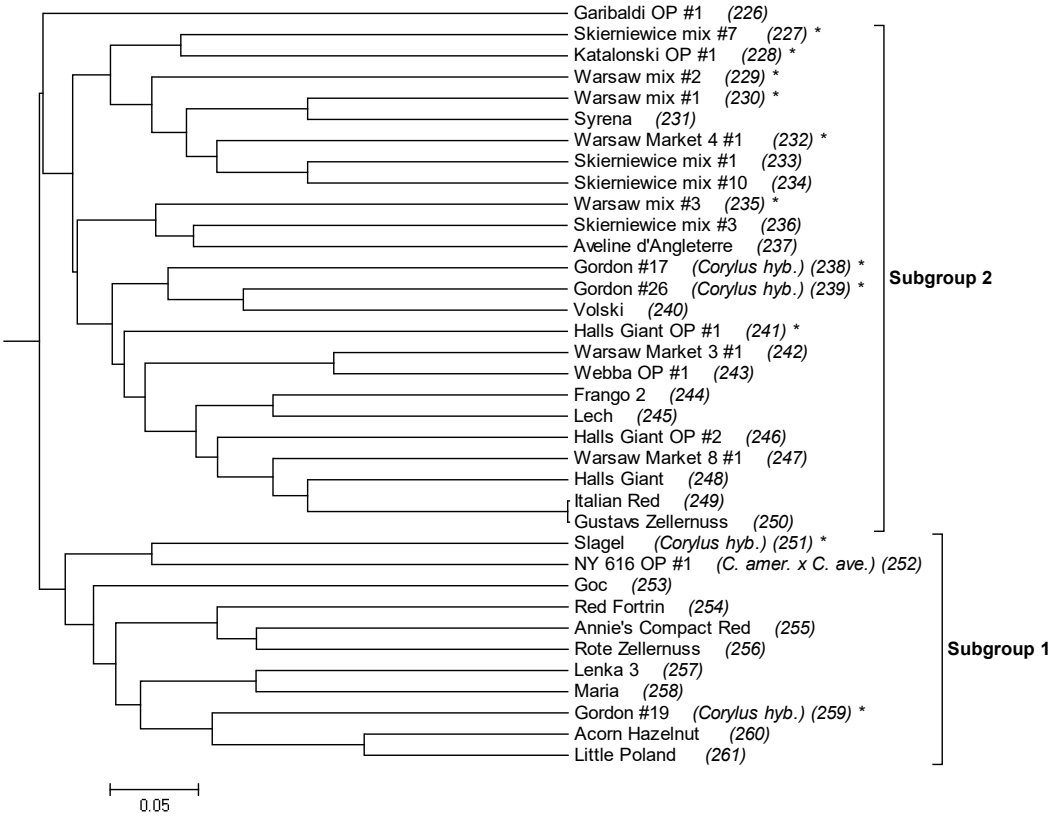


Figure 10. Uncollapsed *Corylus americana* x *C. avellana* hybrid Group 2 node of the unweighted pair group method using arithmetic averages (UPGMA) dendrogram. All accessions are *C. avellana* unless otherwise noted. In reference to the hybrid accessions, *C. ave.* and *C. amer.* correspond to *C. avellana* and *C. americana*, respectively. The abbreviations hyb. and OP indicate hybrid and open-pollinated, respectively. Accessions marked with an asterisk (*) are resistant to eastern filbert blight.

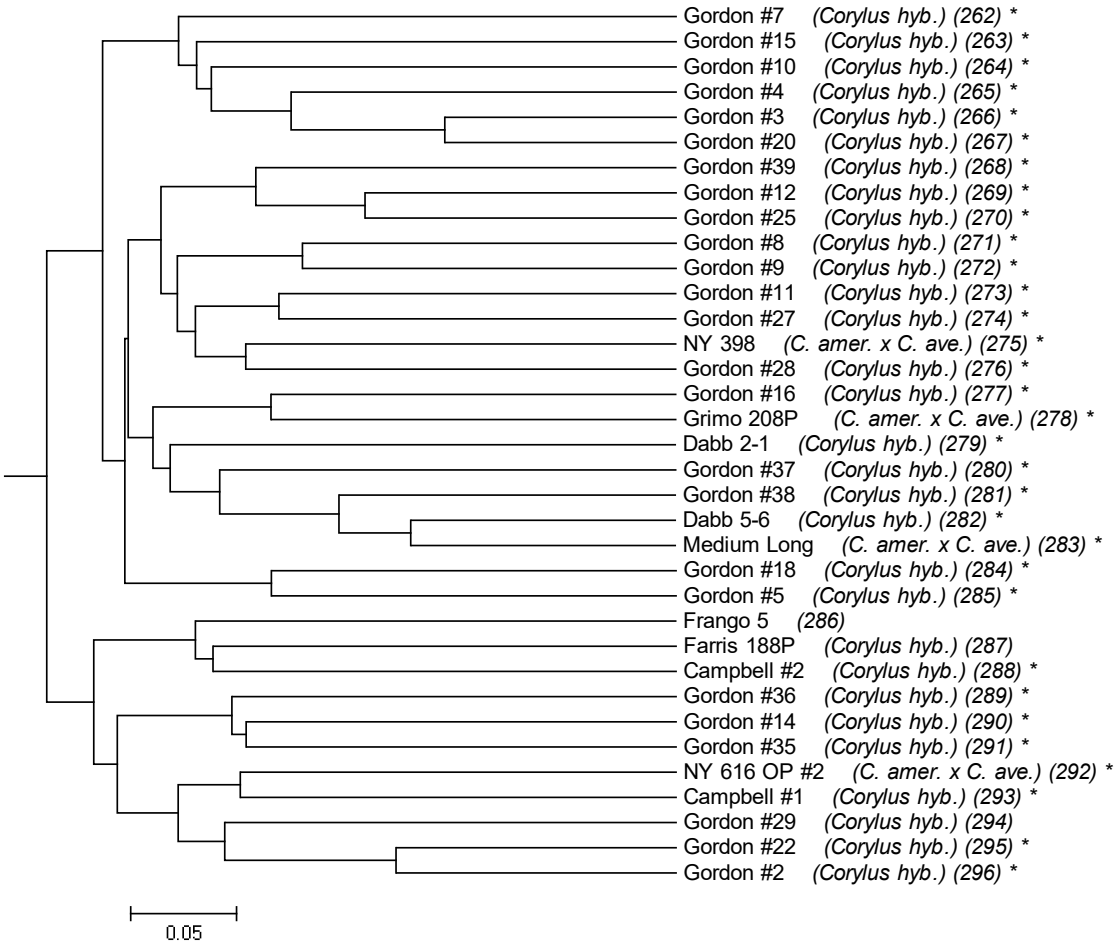


Figure 11. Uncollapsed wild *Corylus avellana* group node of the unweighted pair group method using arithmetic averages (UPGMA) dendogram. All accessions are *C. avellana* unless otherwise noted. In reference to the hybrid accessions, *C. ave.* and *C. het.* correspond to *C. avellana* and *C. heterophylla,* respectively. The abbreviation OP indicates open-pollinated. Accessions marked with an asterisk (*) are resistant to eastern filbert blight.

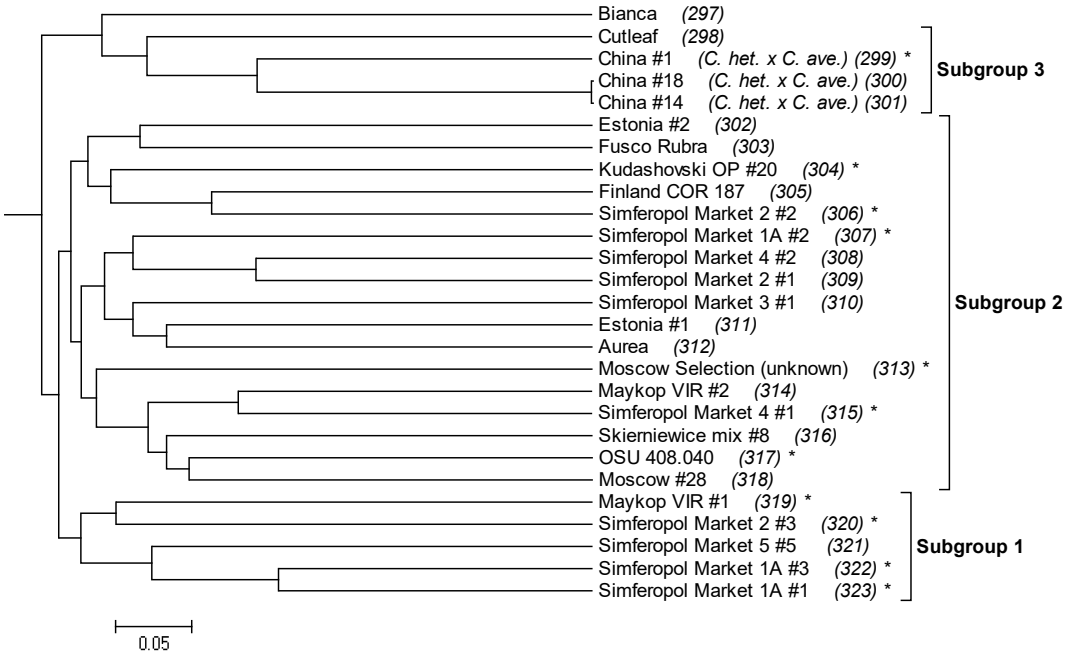
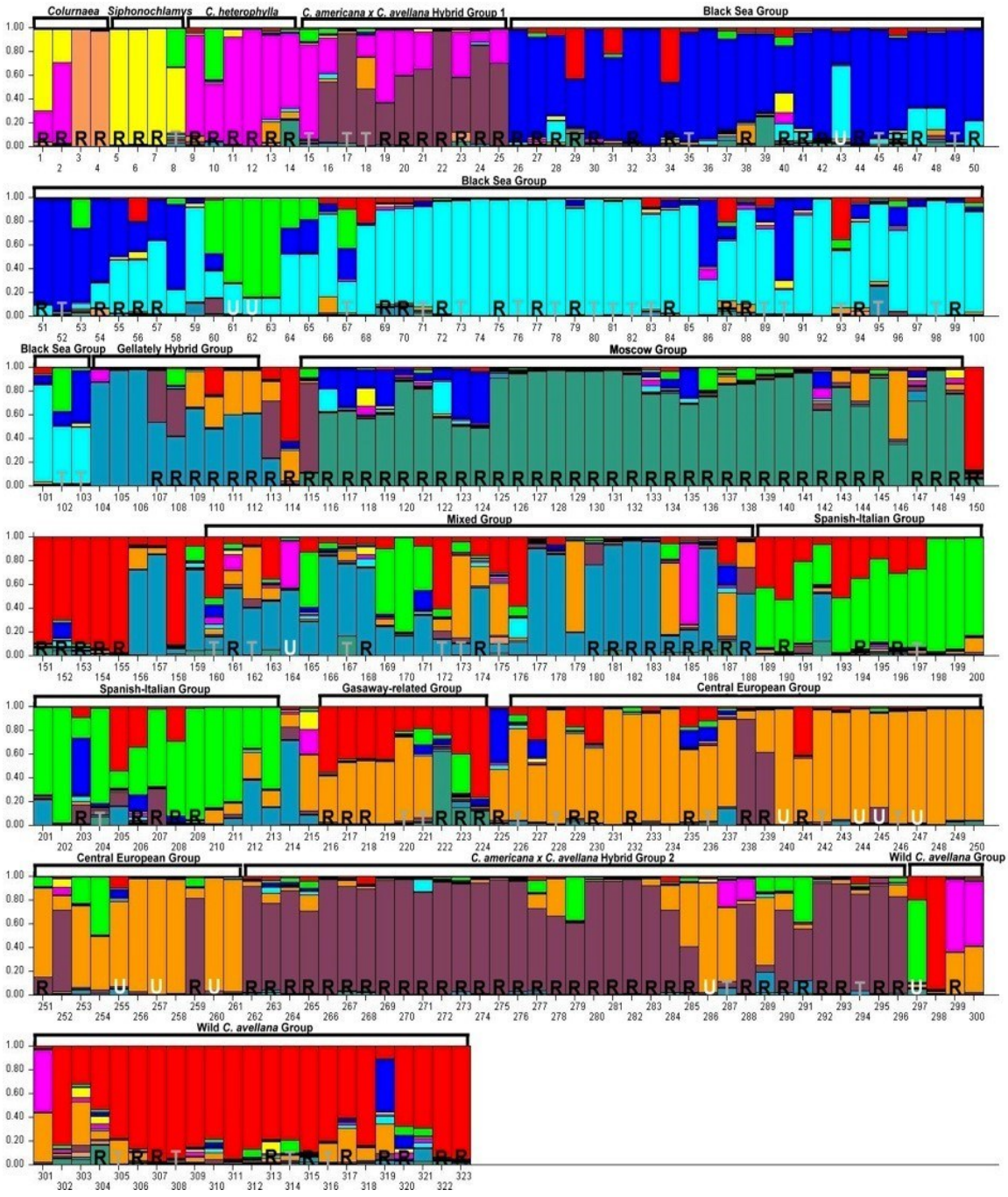


Figure 12. STRUCTURE analysis output resulting in the most parsimonious number of populations (K) =11 (Falush et al., 2003; Pritchard et al., 2000). Accessions are susceptible to eastern filbert blight (EFB) unless otherwise marked where R represents EFB-resistant accessions, T represents EFB-tolerant accessions, and U represents unknown EFB resistance. The STRUCTURE accessions are labeled in accordance with their placement in the UPGMA dendrogram (Figure 1), where accession number 1 is placed at the very top of the UPGMA dendrogram.



Chapter 2: Genetic Diversity of Eastern Filbert Blight Resistant Hazelnuts Collected from Turkey, Latvia and Lithuania

ABSTRACT

Hazelnuts ranks 6th in world tree nut production, and 4% of this crop is grown in the United States. Unfortunately, the disease eastern filbert blight (EFB), native to the Eastern US has historically limited production in the US the Pacific North West. Methods to control EFB are costly and labor intensive, and thus the most cost effective means of combating this disease is to breed resistant cultivars. Resistance to EFB has been characterized in a handful of accessions, although the most commonly used gene for resistance 'Gasaway', has begun to breakdown. In an effort to find new sources of resistance, germplasm collection trips have been made throughout the native range of *C. avellana*. In this study 842 seedlings were collected from Turkey, Latvia, and Lithuania, germinated, and later screened for resistance to EFB). A total of 45 plants found to be resistant or tolerant were subsequently genetically fingerprinted and compared to the previously characterized EFB resistant and tolerant germplasm. Phylogenetic cluster and population structure analysis were both performed. The results indicated that seedlings collected from Turkey represented a novel population of EFB resistant germplasm. By contrast, nearly all of the Latvian and Lithuanian seedlings showed kinship to previously characterized EFB resistant germplasm collected from northern Europe. This study has resulted in the characterization of novel EFB resistant plant material, and could represent new source(s) of resistance for use in the Rutgers Hazelnut breeding program, or at minimum help maintain genetic diversity in EFB resistant breeding lines.

INTRODUCTION

Hazelnuts (*Corylus avellana*) are one of the world's major tree nut crops. They are ranked sixth in world tree nut production behind cashew, walnut, almond, chestnut, and pistachio (FAO, 2017). The United States (US) is the third largest contributor to the world hazelnut market with 32,659 tons (t) of dry, in-shell nuts produced in 2014, behind Turkey, the world leader with 450,000 t, and Italy with 111,538 t (Food and Agriculture Organization of the United Nations, 2017). Today, 99% of the US hazelnut crop is produced in the Willamette Valley of Oregon (Mehlenbacher and Olsen, 1997). Production in the US has been limited to the Pacific Northwest (PNW) largely due to the disease eastern filbert blight (EFB) caused by the ascomycete fungus *Anisogramma anomala* (Johnson and Pinkerton, 2002). Early attempts to establish a hazelnut industry east of the Rocky Mountains failed due to this disease, which is endemic to the eastern US where it is harbored by the native American hazelnut *C. americana* (Barss, 1921, 1930; Fuller, 1908; Pinkerton et al., 1993; Weschcke 1954). It should be noted that *A. anomala* is not present outside of North America (Johnson and Pinkerton, 2002).

Eastern filbert blight is a perennial stem canker disease. The fungus reproduces solely by ascospores that are ejected during periods of prolonged branch wetness and spread via rain splash and wind. The ascospores adhere to and infect young, actively growing hazelnut shoots in the spring. After infection, a 16-18 month latent period occurs during which time the tree shows no signs or symptoms of disease despite a proliferation of hyphal growth in susceptible trees. However, after a winter dormancy period is completed the fungus enters its reproductive phase, which leads to the development of stromata-lined cankers that erupt from stems and girdle branches. Susceptible trees generally die five years after infection in New Jersey (Capik and Molnar, 2012).

The PNW remained free of EFB until the 1960s, when the disease was inadvertently introduced into southwest Washington where it devastated orchards since no control methods had

been developed (Davison and Davidson, 1973). By 1986, the disease could be found in the northern part of the Willamette Valley of Oregon (Pinkerton et al., 1992), and from there subsequently spread through the remainder of the hazelnut production region (Pscheidt et al., 2012). Unlike many other commercial fruit and nut tree crops, hazelnuts are valued for their reduced input requirements and relatively low cost of production. Thus, the introduction of EFB has had a significant impact on hazelnut production in Oregon due to the costs associated with managing the disease (Gottwald and Cameron, 1980; Mehlenbacher, 2005; Pinkerton et al., 1992). Management strategies include intense scouting, pruning of infected stems, and fungicide applications. Unfortunately, these methods are costly and/or labor intensive and not always effective (Johnson et al., 1996). Thus, the most sustainable and cost effective method of control has been host resistance (Johnson et al., 1996; Julian et al., 2008, 2009).

In 1976, resistance to EFB was first observed in a late-shedding pollenizer named 'Gasaway' (*C. avellana*). Trees of 'Gasaway' were found to be free of disease free in a hazelnut orchard of susceptible cultivars overcome by EFB (Cameron, 1976). Subsequent research showed that resistance to EFB in 'Gasaway' was controlled by a dominant allele in the heterozygous state at a single locus (Coyne et al., 1998; Mehlenbacher and Thompson, 1991). Based on this finding, significant breeding efforts were undertaken to introgress 'Gasaway' resistance into cultivars with commercially suitable yields and nut attributes. After more than 20 years of breeding, resistant, commercial-quality cultivars were released, including Jefferson, Yamhill, Dorris, Wepster, McDonald, and several associated pollinizers (Mehlenbacher et al., 2009, 2011, 2013, 2016). Today, these new resistant cultivars have been credited with reviving the Oregon hazelnut industry where it has expanded from 11,700 ha in 2009 to 24,000 ha today (S. Mehlenbacher, personal communication).

Prior to the release of commercially suitable cultivars expressing EFB resistance derived from crosses from 'Gasaway', several selections with quantitative sources of resistance (high level of tolerance) and desirable nut characteristics were also developed at OSU. This includes

'Clark', 'Lewis', and 'Sacajawea' (Mehlenbacher et al., 2000, 2001, 2008). More recently, a number of additional sources of resistance have been identified in *C. avellana* as well as other *Corylus* species; *C. avellana* sources include 'Ratoli' and 'Culpla' from Spain, OSU 495.072 from southern Russia, OSU 759.010 from Georgia, OSU 408.040 from Minnesota, and 'Uebov' from Serbia; *C. americana* resistance consists of 'Rush' from Pennsylvania, Yoder#5 from Ohio, and 'Winkler' from Iowa; *C. colurna* sources are comprised of 'Grand Traverse' from Michigan and the Gellatly Trazel series from British Columbia, Canada; and the *C. heterophylla* source includes 'Oygoo' from Japan and 'Estrella #1' from Michigan. A number of these new sources of resistance have been studied in segregating populations with several placed on the hazelnut genetic linkage map at OSU based on cosegregation with linked SSR and/or RAPD markers (Bhattarai et al., 2017; Chen et al., 2007; Lunde et al., 2000, 2006; Sathuvalli et al., 2011a; Sathuvalli et al., 2011b).

European hazelnut, the hazelnut of commerce, has been grown for centuries throughout Europe, the Caucuses, and Turkey. Its native species range is extensive, extending from coastal Norway and Finland south into Morocco, then east into the Ural Mountains of Russia and west to the Atlantic Ocean (Molnar, 2011). Some of the most important cultivars of hazelnut to the commercial hazelnut industry have been selected from local wild populations of *C. avellana* in these regions. These include, for example, the cultivars Tombul, Palaz, and Sivri Ghiaghli in Turkey and Cherkeskii II, Skorospelka, and Ata-Baba in other parts of the Black Sea Region (Thompson et al., 1996). To expand the genetic diversity of hazelnut resources available to breeders in the US, a number of seed-based germplasm collection trips have been made across the native range of *C. avellana* over the past 15 years. At Rutgers nearly 10,000 new seedlings have been germinated and grown in the greenhouse and field for evaluations largely to identify new genotypes resistant to EFB (T. Molnar, personal communication). After multiple years of exposure to high disease pressure, resistance to EFB was identified in plant material introduced from Russia, Crimea, Ukraine, Poland, and Georgia (Capik et al., 2013; Leadbetter et al., 2015,

2016; Molnar et al., 2007). These new sources of resistance are now being used in breeding and further studies.

Although breeding efforts are underway to develop commercial quality plants with other resistance sources, the 'Gasaway' *R*-gene remains the primary source of EFB resistance in the US; the entire Oregon hazelnut industry is currently protected by this single source of resistance. This is primarily due to the long generation times associated with woody plant breeding and the high nut quality expectations of hazelnut growers in Oregon. Unfortunately, trees expressing the 'Gasaway' *R*-gene have been shown to develop EFB cankers in the field in New Jersey, and also when using isolates collected from Michigan, New Jersey, and Minnesota in greenhouse inoculations (Capik and Molnar, 2012; Molnar et al., 2010a, 2010b). This variable disease response provides evidence for pathogenic variation in *A. anomala* (Molnar et al., 2010b), which could present a danger for the current US hazelnut industry if new isolates are introduced into the PNW and introduces larger challenges for establishing production in the East.

Molecular studies undertaken by Cai et al. (2013) and Muehlbauer et al. (unpublished) corroborate the field and greenhouse studies as they have shown that significant genetic diversity exists among isolates collected from different locations throughout the US and Canada. The genetic and potentially pathogenic variability observed in *A. anomala* substantiates the need to continue to search for and study new sources of EFB resistance in hazelnuts with the end goal of developing plants with durable resistance. Fortunately, over the past decade extensive work has been done to develop and characterize hundreds of simple sequence repeat (SSRs) markers specific to *Corylus* that have been used to describe relationships, population structure, and genetic diversity (Bassil et al., 2005; Gurcan et al., 2009, 2010; Gurcan and Mehlenbacher, 2010). These markers have been used in a variety of studies including development of the first linkage map of hazelnut (Mehlenbacher et al., 2006), further saturation of the linkage map (Colburn, 2017), and cultivar identification (Akin, 2016). In addition, multiple studies have used SSRs to characterize the genetic backgrounds of hazelnut germplasm. These have included the study of genetic

relationships of *C. avellana* germplasm collected throughout different hazelnut growing regions and native ranges such as Ireland, northern Spain, the Black Sea region, Germany and North America (Boccacci et al., 2008, 2009; Brown, 2016; Campa et al., 2011; Ferreira et al., 2010; Gurcan et al., 2010; Leineman, 2013; Sathuvalli and Mehlenbacher, 2011). Of these studies, one of the most extensive was undertaken by Gökirmak et al. (2009) to assess the diversity of a large collection of *C. avellana* representing a majority of the world's cultivars. In total, 270 unique accessions were characterized using 21 SSRs. The results showed that *C. avellana* in general is highly genetically diverse and that most germplasm could be partitioned into four major groups that were closely linked to their known geographic origins. This study was used as a reference for a more recent study (Muehlbauer et al., 2014) where 323 accessions, including a large pool of new EFB-resistant seedling selections from new germplasm introductions, and a reference panel of accessions representative of the *Corylus* genus, were genotyped using 17 SSR markers. In summary, eleven genetic (consensus) groupings were resolved from this analysis, each populated largely with accessions (both reference panel cultivars and new germplasm introductions) with similar geographic and species origins. The results were consistent with the findings of Gökirmak et al. (2009) in respect to the placement and grouping of most of the reference panel accesions, which provided additoinal support for the placement of plants with unknown pedigrees. Further, accessions conferring resistance to EFB were found included in all 11 consensus groups, indicating that EFB resistance can be found widely across the *Corylus* genus. These results and those from the other studies previously mentioned strongly suggest that breeders should be able to select for plants resistant to EFB and other traits without narrowing genetic diversity.

In this study, new germplasm collected from Turkey, Latvia, and Lithuania was exposed to EFB in the field in New Jersey and examined for its response. A subset of this germplasm collection was then selected based on its EFB response and subjected to characterization using 16 SSR markers to study genetic diversity and relationships. Further, the existing SSR-based allelic data set that included 323 accessions generated by Muehlbauer et al. (2014) was used as a

framework to compare the new accessions, to help better examine their relationships to a wider body of germplasm, especially plants expressing resistance to EFB.

MATERIALS AND METHODS

Plant Material

Two separate collection trips were made to obtain the germplasm characterized in this study. The first was made by Shawn Mehlenbacher to Turkey in 2004. During this trip, open pollinated (OP) nuts were harvested from 50 different *C. avellana* accessions held at the Horticultural Research Institute in Giresun, Turkey. The nuts were collected from trees established by Engin Cetiner, who had collected scion wood from select trees originating in the old production regions of Turkey (Ordu to Trabzon). Descriptions of these trees were noted in Caliskan and Cetiner (1997) and used by S. Mehlenbacher as a reference to make the collections from specific accessions.

The second germplasm collection, which totaled 11 separate seed lots, was made by Thomas Molnar and David Zaurov in Latvia and Lithuania in 2005. Six seed lots were collected in Lithuania from trees growing at the Lithuanian Research Center of Agriculture and Forestry (LRCAF) in Babtai and the remaining five were collected from Latvia: one from the Latvia Institute of Fruit Growing in Dobele, constituting a mixture of seeds harvested from multiple trees in their research collection, and four from nuts purchased at the Central Market in Riga. During both collection trips, care was taken to obtain nuts with commercially desirable phenotypes (round kernels, high kernel to shell ratio, minimal defects, etc.). All seed lots included in this study are listed in Table 1.

Seeds were stratified in damp peat moss at 4°C for 5 months. They were then germinated in wooden planting boxes filled with peat-based growing medium (Promix® BX; Premier Horticulture, Riviére-du-Loup, Québec, Canada) in a greenhouse maintained at 24°C/18°C day/night with 16 h daylengths. After 4-6 weeks, seedlings were transplanted to individual 3.7-L plastic pots with the same growing medium and top-dressed with 5 g of a 5-6 month time-release fertilizer (Osmocote Plus 15N-9P_2O_5-12K_2O with micronutrients; The Scotts Co., Marysville,

OH). All trees in this study were planted in the field in October 2006 at the Rutgers Cream Ridge Fruit Research Center, Cream Ridge, NJ. While the Turkish seed lots were obtained a year earlier than those from Latvia and Lithuania, the Turkish plants were maintained in their containers over the first growing season and then planted at the same time as the Latvian/Lithuanian trees. Trees were planted in rows 6 m apart with a spacing of 1 m between trees. The Turkish trees were planted in a completely random design and the Latvian and Lithuanian were grouped in the row by seed lot. While no pesticides or fungicides were applied, weed control and fertility amendments followed common practice used in the hazelnut breeding program at Rutgers University.

Field Inoculations

Prior to budbreak during the first three years after planting, trees were inoculated in the field with *A. anomala* by tying 10-15 cm pieces of infected branches into the canopy of each tree (Molnar et al., 2007). This was done to ensure high disease pressure was maintained and to reduce the possibility of susceptible plants escaping infection. Infected branches were collected each winter from adjacent fields at the Rutgers University research farm and stored at -20°C in polyethylene bags until needed. In later years, infections within the planting as well as nearby heavily infected hazelnut plots contributed to a steady influx of inoculum.

Evaluation of Disease Response

EFB was first observed on some seedlings in 2008 (data not shown) but ratings did not begin until 2010, with a final disease rating made in Dec. 2015. Ratings were conducted using a scale of 0 to 5 following an index developed by Pinkerton et al. (1992): 0=no detectable EFB; 1=single canker; 2=multiple cankers on a single branch; 3=multiple branches with cankers; 4=greater than 50% of branches contain cankers; 5=all branches contain cankers, excepting basal sprouts. In terms of examining trees for breeding purposes and selection for inclusion in genetic diversity analysis (described subsequently), trees rated as a 0 were considered “resistant” to EFB. Ratings of 1 or 2 were termed “highly tolerant” to disease, where these trees will typically not

develop sufficient cankers to impede long term growth or production. A rating of 3 was considered "moderately tolerant" because the tree is unlikely to be killed, although branch dieback will likely lead to yield loss over time. Ratings of 4 and 5 were considered to be "susceptible" and these trees will show highly reduced growth in 2 years and will likely die within 5-7 years.

Genetic Diversity Analysis

Young leaf tissue was collected in May 2013 from 45 EFB resistant or tolerant hazelnut accessions selected from the Turkish, Latvian, and Lithuanian seed lots to be included in the genetic diversity analysis (Table 2). In addition to these selections, 35 cultivars and/or previously characterized breeding selections were included as control genotypes, which represent each consensus group resolved in Muehlbauer et al. (2014) and function as a diversity panel for *C. avellana*. These 35 genotypes also allow for a means to compare and adjust SSR allele sizing to make direct comparisons between the new data and data generated from the earlier study (Table 3).

Following leaf collection, tissue was flash frozen in liquid nitrogen and then ground with a mortar and pestle. Genomic DNA was extracted using a CTAB protocol adapted from methods described by (Cullings 1992; Doyle and Doyle, 1987). The DNA was then quantified using a spectrophotometer (NanoDrop ND-1000; Thermo Scientific, Waltham, MA) and diluted to a concentration of 5 ng/μL^{-1}. Sixteen thoroughly characterized SSRs (Table 4), originally published by Bassil et al. (2005), Gurcan et al. (2010b), and Gurcan and Mehlenbacher (2010) were used to genotype the 46 seedlings and the 35 controls. The M13 (-21) 18-bp sequence was added to the 5' end of all forward primers as an economical method to fluorescently label the PCR fragments (Schuelke 2000) and the "PIG-tailing" sequence (GTTTCTT) was added to the 5' end of all reverse primers to lessen the uncertainty in scoring "true" vs. "plus-A" alleles (Brownstein et al., 1996). The primers were synthesized by Integrated DNA Technologies (Coraville, IA). PCR genotyping reactions consisted of approximately 5 ng genomic DNA,

10xRamp-Taq PCR buffer (Denville Scientific, Metuchen, NJ), 2.0 mM $MgCl_2$, 0.25 mM each dNTP (Denville Scientific), 0.5 U Ramp-Taq DNA polymerase (Denville Scientific), 0.5 pmol forward primer with 5' M13 (-21) addition, 1.0 pmol reverse primer with 5' PIG-tail addition, and 1.0 pmol FAM, NED, PET or VIC fluorescently labeled M13 (-21) primer. PCR cycling was performed in GeneAmp 9700 thermalcyclers (Applied Biosystems, Foster City, CA) using the following parameters: initial denaturation of 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 55°C for 45 s, 72°C for 45 s, followed by 20 cycles of 94°C for 30 s, 53°C for 45 s, 72°C for 45 s, followed by a final extension of 72°C for 10 min.

PCR products were run on a capillary electrophoresis genetic analyzer (ABI 3500xl; Applied Biosystems) and were sized using a LIZ600 size standard (Applied Biosystems). A GeneScan Installation Standard DS-33 (Applied Biosystems) was added to each 96-well plate to serve as control for each run on the genetic analyzer. SSR genotyping results were then analyzed using Genemapper 4.0 (Applied Biosystems). SSR genotyping results from the 45 new selections were merged with genotype data from Muehlbauer et al. (2014) to examine relationships among the material as well as in comparison to a much broader base of germplasm. To do so, SSR profiles of the 35 control genotypes were first cross-checked with the SSR profiles generated in Muehlbauer et al. (2014). Allele bins were adjusted to account for any allele shifting between the old and new dataset to ensure the two datasets were comparable.

Genotypic Data analysis

Summary statistics were calculated individually for the Turkish accessions and combined Latvian/and Lithuanian accessions using the program Powermarker v3.25. These values included number of alleles per loci, major allele frequency, number of genotypes per loci, observed and expected heterozygosity values, polymorphism information content (PIC), and inbreeding coefficient (f). In addition, the program was used to calculate allele frequencies to create a genetic distance matrix. The distance matrix was subsequently used to construct a UPGMA

dendrogram, which was visualized in MEGA 6 (Tamura et al., 2011). Bootstrap support for the tree was calculated in Powermarker, with a minimum support value of 0.5.

Accessions were reordered based upon the UPGMA dendrogram and inputted into the program STRUCTURE v 2.3.4 (Falush et al., 2003; Pritchard et al., 2000). This program utilizes a Bayesian model-based clustering method to perform a population structure analysis, where each accession is assigned to a population(s) based upon its SSR profile. The run parameters assumed all loci were independent and in linkage equilibrium, and it used an admixture ancestry model with correlated allele frequencies. A burn-in of 20,000 iterations and MCMC of 50,000 were used at each K value. Individuals assigned to multiple populations (multiple colors in a bar) were considered to be indicative of admixture or hybridization. The STRUCTURE analysis was run 5 K values above (K=5) and 5 K values below (K=16) the most parsimonious K value found in Muehlbauer et al. (2014) (K=11). The most parsimonious K value for this dataset was chosen based upon the maximal value of the average estimated log probability Pr(X|K) across all replicate runs of K, using the program STRUCTURE Harvester (Earl & vonHoldt, 2011; Evanno et al., 2005).

RESULTS

EFB response

A total of 508 Turkish, 40 Lithuanian, and 304 Latvia seedlings were planted in the field in 2006; final disease ratings were made in Dec. 2015 (Table 1). As expected based on previous studies, most trees were found to be highly susceptible to EFB. Of the Turkish seedlings, 9 trees were rated as 0; 2 trees were rated as 1; 2 trees were rated as 2; 16 trees were rated as 3; 57 trees were rated as 4; and 422 trees were rated as 5. It should be noted that the nine trees rated as 0 originated from a total of eight different seed lots. In respect to the Lithuanian seedlings, 1 tree was rated as 0; no trees were rated as 1, 2, or 3; 6 trees rated as 4; and 33 trees rated as 5. The resistant Latvian seedling originated from an OP seedling of the cultivar Luisa growing at the Lithuanian Institute of Horticulture. Finally, of the Latvian seedlings, 7 trees rated as 0; 0 trees rated as 1; 2 trees rated as 2; 3 trees rated as 3; 8 trees rated as 4; and 284 trees rated as 5. Of the seven Latvian seedlings rated 0, five originated from the collection made at the Latvia State Institute of Fruit Growing (seed lot 05010) and the remaining two originated from the Central Market in Riga, Latvia (Riga #3).

Genetic Diversity Analysis

Summary Statistics

Summary statistics calculated across all loci of the two EFB resistant/tolerant seedling populations (Turkish and Latvia/Lithuania) were indicative of the presence of genetic diversity (Table 5). Each of the two populations showed relatively similar major allele frequencies, observed and expected heterozygosity, PIC values, and inbreeding coefficients to each other. They also showed similarity to the data published in Muehlbauer et al. (2014) (Table 5). However, differences were observed in the total number of alleles and genotypes per loci when comparing the three datasets (Turkish, Latvian/Lithuanian, and Muehlbauer et al. [2014]). The Turkish population (across all loci) resulted in approximately half the total number of alleles

compared to that reported in Muehlbauer et al. (2014) but with the exception of one locus, had more alleles per loci than the Latvian/Lithuanian. The greatest difference in any summary statistic was observed in the number of genotypes per population, where the data reported in Muehlbauer et al. (2014) showed much greater genotype numbers than either of the new seedling populations, although the Turkish population resulted in a larger number of genotypes than the Latvian/Lithuanian population across all loci (Table 5).

Cluster and STRUCTURE Analysis

In general, the cluster analysis resulted in a similar topology as that of the core groupings resolved in Muehlbauer et al. (2014) with minor movement of accessions largely within groups. With the addition of the 45 new accessions into the previous dataset from Muehlbauer et al. (2014), the UPGMA analysis resulted in similar clustering of 11 of the previous 13 groups (exceptions were Mixed and Gellately group from Muehlbauer et al. [2014]) in the combined dendrogram. Of the 29 Turkish accessions, 26 fell into one large basal subgroup of the dendrogram (Cluster V) (Figure 1). Of the previously characterized accessions that also fell into this group, the majority were placed into the Black Sea Group in Muehlbauer et al. (2014). Thus, this expanded group kept the name Black Sea Group in this study. Of the additional Turkish accessions, one fell into an unnamed cluster (Cluster III) alongside several other accessions that also previously fell into the Black Sea Group of the dendrogram (Muehlbauer et al. 2014). The final two Turkish seedlings fell into a separate undefined cluster (Cluster VI), which was a sister clade to the Black Sea Large Subgroup and Basal Group of the dendrogram (Figure 1). The seedlings in Cluster VI grouped together with several accessions formerly represented in the Black Sea dendrogram group (Muehlbauer et al. 2014). Although the Turkish seedlings did not exclusively group together in one dendrogram cluster, they all clustered with accessions previously placed in the Black Sea Group of the dendrogram illustrated in Muehlbauer et al. (2014).

In respect to the Latvian/Lithuanian seedlings, four accessions fell into 3 dendrogram clusters that held plants previously classified as the Black Sea, Central European, and Spanish-Italian dendrogram groups respectively as described in Muehlbauer et al (2014)(Figure 1). The remaining seven accessions were spread across four unnamed dendrogram clusters.

The seedling Latvia Institute Mix #8 fell into Cluster VI of the dendrogram (Figure 1) alongside accessions formerly showing kinship to the Black Sea Group of the dendrogram in Muehlbauer et al. (2014). Nine seedlings fell in Cluster II, and although they were not contained within them, they fell alongside both the Central European Group and the Gasaway Group. The majority of the accessions that these nines eedlings fell amongst, had fallen into the Central European Group of the previous study (Muehlbauer et al. 2014). The final two seedlings, Latvia Institute Mix #4 and 'Muskovos Rubinas' OP #1, both fell in Cluster I of the dendrogram alongside the Wild *C. avellana* subgroups 1, 2, and 3 (Figure 1). Although there were several exceptions, most of the accessions included in this cluster had previously grouped into the Wild *C. avellana* clade (Muehlbauer et al. 2014).

The STRUCTURE analysis and subsequent STRUCTURE harvester output resulted in a most parsimonious K value of 8. Thus, several of the 11 STRUCTURE groups described in Muehlbauer et al. (2014) collapsed as a result of the combined population structure analysis. This included outgroups 1, 2, and 3 of the prior study, which fell together into one group. The wild *C. avellana* and Central European group also combined into one group along with most of the Latvian and Lithuanian seedlings. The analysis resulted in reorganization of the Black Sea groups, such that the new Black Sea Group 1 encompassed both of the previous Black Sea groups (Muehlbauer et al., 2014). In addition, a new group was formed, which primarily held the new Turkish accessions along with a few previously characterized Turkish cultivars previously held in Black Sea Group 2. Of the six remaining structure populations, the Moscow, Spanish-Italian, *C. americana* × *C. avellana* hybrid, and Gellately group are closely aligned with the structure groups described in Muehlbauer et al. (2014) (Figure 2).

Placement of New Accessions into Consensus Populations

The dendrogram cluster placement and population structure profiles of the new accessions were analyzed individually, together, and then in comparison to the resulting placement of nearby accessions into consensus populations described in Muehlbauer et al. (2014). The results were used to determine which consensus (genetic) population each of the new resistant/tolerant Turkish, Latvian and Lithuanian seedlings most closely aligned with.

Turkish Accessions

Nearly all of the Turkish accessions included in the study fell into the Black Sea Large Subgroup and Basal Group of the dendrogram (Figure 3). These 26 Turkish seedlings showed kinship to plants previously classified as the Black Sea dendrogram group and Black Sea Group 2 of the STRUCTURE analysis in Muehlbauer et al. (2014). In addition, these accessions fell alongside several cultivars that were placed in consensus group 6 (Black Sea group 2) of the previous study (Muehlbauer et al. 2014). Thus, all 26 Turkish accessions within this cluster were placed in the new Black Sea group 2 (Figure 3).

A single Turkish accession (Turkey #29) exhibiting complete resistance to EFB did not fall in any previously characterized dendrogram cluster, although it did cluster near several accessions that had previously grouped in the Black Sea dendrogram group (Muehlbauer et al. 2014). In addition, it fell in the newly formed STRUCTURE Black Sea group 2. It also grouped alongside several accessions placed in consensus group 6 (Black Sea group 2) in the previous study. Thus, it was placed with the 26 aforementioned Turkish seedlings into the newly characterized Black Sea group 2 (Figure 4).

The final two Turkish accessions, one of which showed complete resistance to EFB (Turkey #15), fell together in close proximity to several accessions previously placed in the Black Sea dendrogram group (Figure 5). The STRUCTURE analysis showed that the majority of the genetic background of both accessions was similar to the new structure Black Sea group 2, thus it

was placed in the same consensus group (Black Sea group 2) as the remainder of the Turkish accessions in this study (Figure 5).

Latvian/Lithuanian Accessions

A single Latvian accession Riga Market 2 #5, grouped in the Black Sea Large Subgroup and Basal Group dendrogram cluster, but showed close kinship with plants previously classified as the Wild *C. avellana*/Central European structure group (Figure 3). This seedling was ultimately placed in the combined Wild *C. avellana*/Central European consensus group. One single accession Latvia Institute Mix #8 fell in the same uncharacterized dendrogram cluster as Turkey #15, but its structure profile aligned with the Wild *C. avellana*/Central European structure group. The seedling was placed in the Wild *C. avellana*/Central European consensus group (Figure 5).

Two Latvian seedlings, Latvia Institute Mix #1 and Riga Market #1 (Central European dendrogram cluster), fell into the Central European dendrogram cluster. An additional nine seedlings Riga Market 2 #1, #2, #3, and #4 and Latvia Institute Mix #2, #3, #5, #6, and #7, fell in dendrogram clusters close to the Central European dendrogram group, specifically close to a number of known accession that originated from Poland. The population structure profiles of all of these seedlings aligned with the new Wild *C. avellana*/Central European structure group. Thus, all 11 of the aforementioned accessions were placed in the newly formed Wild *C. avellana*/Central European consensus group of this study (Figure 6).

The Lithuanian accession 'Muskovos Rubinas' OP and Latvia Institute Mix #4 both fell in a cluster between the combined wild *C. avellana* subgroup 1 and 2 and wild *C. avellana* subgroup 3 clusters in the dendrogram. Both of these accessions were considered to have genetic profiles that aligned most closely with the newly formed Wild *C. avellana*/Central European Structure group. Thus these seedlings were placed with all of the other Latvian/Lithuanian seedlings in the Wild C. avellana/Central European consensus group (Figure 7).

One seedling collected from the cultivar ‘Luisa’ grouped with plants in the previously characterized Spanish-Italian dendrogram cluster. The STRUCTURE analysis corroborated this and showed this accession as having close kinship with the Spanish-Italian structure group. This newly characterized accession was placed in the Spanish-Italian consensus group of this analysis.

DISCUSSION

The first Turkish, Latvian, and Lithuanian seedlings with resistance to EFB have been identified, characterized with SSR markers, and compared to a large allelic data set containing over 300 unique hazelnut accessions. Few sources of EFB resistance are currently available from these regions, thus these plants may represent valuable breeding material useful towards the goal of developing plants with durable resistance. On a whole, despite the addition of 45 new accessions, the UPGMA cluster analysis closely approximated 11 of the 13 main groupings previously reported by Muehlbauer et al. (2014) (Figure 1). This provided for the ability to place many of the new seedlings into genetic populations that were previously elucidated from the larger data set of Muehlbauer et al. (2014). Similarly, the STRUCTURE output approximated 8 of the 11 previously characterized STRUCTURE groupings from Muehlbauer et al. (2014) (Figure 2), thus both the new and old analysis were congruent, which provided additional support between the outputs of both studies and allowed for discussion on the placement of the new accessions within the larger body of germplasm.

Both the Turkish and Latvian/Lithuanian groups were shown to be genetically diverse as can be seen in summary statistics (Table 5). Interestingly, the Turkish seedlings, when added to the larger data set, attributed to a reorganization of the Black Sea Groups previously resolved in Muehlbauer et al. (2014), and were ultimately placed into a novel genetic grouping by the STRUCTURE analysis. This new grouping held only a few previously characterized cultivars, none of which were resistant to EFB, providing support that the new EFB-resistant germplasm is likely unique and of possible direct value for breeding. In contrast, the Latvian/Lithuanian seedlings were placed alongside accessions previously classified as part of either the wild *C. avellana* or Central European consensus groups; many were grouped close to known accessions from Poland in the UPGMA dendrogram and STRUCTURE analysis, several of which are known to be resistant to EFB. Thus, while having unique SSR profiles, they did not stand out as

significantly unique genetic sources, unlike a majority of the Turkish accessions, and consideration for use in breeding should likely be based more strongly on other traits important to cultivar development beyond their genetic origin and EFB resistance. For example, it remains a strong possibility that they are related to Polish cultivars or seedlings, or at least share a common ancestry, and should be compared side-by-side to other EFB-resistant Polish plants in the Rutgers germplasm collection before selecting them for use in breeding. Whereas, the Turkish material appears significantly more novel and would only likely need to be compared within the group of EFB-resistant Turkish accessions for selection of breeding parents. In closing, this study, as with other prior genetic diversity studies (Boccacci et al., 2008; Boccacci et al., 2009; Colburn et al., 2017; Gokirmak et al., 2009; Muehlbauer et al., 2014), shows hazelnuts to be highly genetically diverse and provides a better understanding of the origins of EFB resistant plant material. Based on our growing knowledge of hazelnut genetic resources, breeders can be confident that they can select for traits like EFB-resistance without narrowing genetic diversity.

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Table 1. Summary of response of Corylus avellana germplasm from Turkey, Latvia, and Lithuania to eastern filbert blight (EFB) caused by Anisogramma anomala in New Jersey, USA, after 9 years of exposure.

			Eastern Filbert Blight Rating[Z]							
Rutgers Code	Collection Location	Total Number of Trees	Mean	0	1	2	3	4	5	Number of Dead Trees[Y]
04102	Giresun, Turkey	13	4.7	0	0	1	0	0	10	2
04103	Giresun, Turkey	12	4.3	0	2	0	0	0	7	3
04104	Giresun, Turkey	10	4.6	0	0	0	1	2	7	0
04105	Giresun, Turkey	7	5	0	0	0	0	0	7	0
04106	Giresun, Turkey	7	5	0	0	0	0	0	6	1
04107	Giresun, Turkey	20	4.6	0	0	0	3	2	15	0
04108	Giresun, Turkey	10	4.2	1	0	0	1	1	5	2
04109	Giresun, Turkey	16	4.81	0	0	0	0	3	11	2
04110	Giresun, Turkey	13	4.92	0	0	0	0	1	5	7
04112	Giresun, Turkey	8	5	0	0	0	0	0	5	3
04113	Giresun, Turkey	5	4.6	0	0	0	0	2	2	1
04115	Giresun, Turkey	12	4.42	0	0	0	2	3	6	1
04117	Giresun, Turkey	12	4.67	0	0	0	0	4	6	2
04118	Giresun, Turkey	20	4.95	0	0	0	0	1	16	3
04119	Giresun, Turkey	19	4.42	1	0	0	2	2	12	2
04120	Giresun, Turkey	13	4.69	0	0	0	1	2	7	3
04121	Giresun, Turkey	15	4.47	1	0	0	0	3	11	0
04122	Giresun, Turkey	27	4.96	0	0	0	0	1	24	2
04124	Giresun, Turkey	1	5	0	0	0	0	0	1	0
04125	Giresun, Turkey	5	4.6	0	0	0	0	2	3	0
04126	Giresun, Turkey	15	4.87	0	0	0	1	0	12	2
04128	Giresun, Turkey	11	4.82	0	0	0	1	0	9	1
04129	Giresun, Turkey	4	4.5	0	0	0	1	0	2	1
04130	Giresun, Turkey	20	5.4	0	0	0	0	6	12	2
04131	Giresun, Turkey	21	4.67	1	0	0	0	2	13	5
04136	Giresun, Turkey	5	5	0	0	0	0	0	5	0
04137	Giresun, Turkey	8	5	0	0	0	0	0	8	0
04138	Giresun, Turkey	4	4.5	0	0	0	0	2	1	1
04140	Giresun, Turkey	15	4.67	1	0	0	0	0	11	3
04143	Giresun, Turkey	6	4.33	0	0	0	1	2	3	0
04145	Giresun, Turkey	6	4.33	0	0	0	0	0	5	1
04146	Giresun, Turkey	1	5	0	0	0	0	0	1	0
04147	Giresun, Turkey	4	4.75	0	0	0	0	1	3	0
04149	Giresun, Turkey	27	4.44	2	0	0	1	3	15	6
04150	Giresun, Turkey	3	5	0	0	0	0	0	3	0
04151	Giresun, Turkey	5	4	1	0	0	0	0	4	0

			Eastern Filbert Blight Rating[Z]							
Rutgers Code	**Collection Location**	**Total Number of Trees**	**Mean**	**0**	**1**	**2**	**3**	**4**	**5**	**Number of Dead Trees[Y]**
04152	Giresun, Turkey	6	4.83	0	0	0	0	1	2	3
04154	Giresun, Turkey	4	4.75	0	0	0	0	1	2	1
04155	Giresun, Turkey	18	4.94	0	0	0	0	1	17	0
04156	Giresun, Turkey	3	4.67	0	0	0	0	1	2	0
04157	Giresun, Turkey	7	3.86	0	0	1	0	0	5	1
04158	Giresun, Turkey	8	4.5	0	0	0	0	4	3	1
04159	Giresun, Turkey	26	4.73	1	0	0	0	2	21	2
04160	Giresun, Turkey	9	5	0	0	0	0	0	9	0
04165	Giresun, Turkey	2	5	0	0	0	0	0	1	1
04168	Giresun, Turkey	6	4.5	0	0	0	1	1	3	1
04174	Giresun, Turkey	2	5	0	0	0	0	0	2	0
04175	Giresun, Turkey	13	4.92	0	0	0	0	1	9	3
04176	Giresun, Turkey	3	5	0	0	0	0	0	1	2
04179	Giresun, Turkey	1	5	0	0	0	0	0	1	0
Totals for Trees from Giresun, Turkey		**508**	**4.71**	**9**	**2**	**2**	**16**	**57**	**351**	**71**
05004	Lithuania, 'Tombul' OP	8	4.88	0	0	0	0	1	7	0
05005	Lithuania, 'Moskvos Rubinas' OP	7	4.14	0	0	0	0	3	4	0
05006	Lithuania Mix near Institute	5	4.8	0	0	0	0	1	4	0
05007	Lithuania, 'Palaz' OP	6	5	0	0	0	0	0	6	0
05008	Lithuania, 'Luisa' OP	11	4.89	1	0	0	0	1	8	1
05009	Lithuania, 'K824' OP	3	5	0	0	0	0	0	3	0
Totals for Trees from Lithuania		**40**	**4.73**	**1**	**0**	**0**	**0**	**6**	**32**	**1**
05010	Doeble Mix, Latvia	156	4.77	5	0	1	2	4	128	16
Riga 1	Riga, Lativa Market #1	49	4.98	0	0	0	0	1	48	0
Riga 2	Riga, Lativa Market #2	50	4.98	0	0	0	0	1	49	0
Riga 3	Riga, Lativa Market #3	46	4.63	2	0	1	1	2	38	2
Riga 4	Riga, Lativa Market #4	3	5	0	0	0	0	0	1	2
Totals for Trees from Latvia		304	4.82	7	0	2	3	8	264	20
Grand Total		**852**	**4.75**	**17**	**2**	**4**	**19**	**71**	**647**	**92**

[Z]Disease responses were recorded using the following scale: 0=no detectable EFB; 1=single canker; 2=multiple cankers on a single branch; 3=multiple branches with cankers; 4=greater than 50% of branches contain cankers; 5=all branches contain cankers, except basal sprouts.
[Y]Trees that had already been killed by EFB prior to Dec. 2015 were recorded as 5.

Table 2. List of new seedlings analyzed in this study, their seed lot, the city and country they were collected from, their eastern filbert blight resistance rating, and the population they grouped with [labeled according to consensus population in the previous study Muehlbauer et al. (2014)].

Accession Name	Identification code (seed lot), origin and/or parentage	EFB response	Population
Latvia Institute Mix #1	CRTR04P166 (05010), Latvia State Institute of Fruit-Growing	0	Combined Wild *C. avellana* and Central European Group
Latvia Institute Mix #2	CRTR05P14 (05010), Latvia State Institute of Fruit-Growing	0	Combined Wild *C. avellana* and Central European Group
'Luisa' OP	CRTR05P48 (05008), Lithuanian Institute of Horticulture	0	Spanish Italian Group
Latvia Institute Mix #4	CRTR05P87 (05010), Latvia State Institute of Fruit-Growing	0	Combined Wild *C. avellana* and Central European Group
Latvia Institute Mix #5	CRTR05P97 (05010), Latvia State Institute of Fruit-Growing	0	Combined Wild *C. avellana* and Central European Group
Latvia Institute Mix #8	CRTR05P140 (05010), Latvia State Institute of Fruit-Growing	0	Combined Wild *C. avellana* and Central European Group
Riga Market 2 #2	CRTR05P95 (Riga 3), Riga Roadside Market 2, Riga, Latvia	0	Combined Wild *C. avellana* and Central European Group
Riga Market 2 #3	CRTR06P100 (Riga 3), Riga Roadside Market 2, Riga, Latvia	0	Combined Wild *C. avellana* and Central European Group
Latvia Institute Mix #3	CRTR05P85 (05010), Latvia State Institute of Fruit-Growing	2	Combined Wild *C. avellana* and Central European Group
Riga Market 2 #5	CRTR06P121 (Riga 3), Riga Roadside Market 2, Riga, Latvia	2	Combined Wild *C. avellana* and Central European Group
Latvia Instituite Mix #6	CRTR05P112 (05010), Latvia State Institute of Fruit-Growing	3	Combined Wild *C. avellana* and Central European Group
Latvia Institute Mix #7	CRTR05P124 (05010), Latvia State Institute of Fruit-Growing	3	Combined Wild *C. avellana* and Central European Group
'Muskovos Rubinas' OP	CRTR05P58 (05005), Lithuanian Insitute of Horticulture	4	Combined Wild *C. avellana* and Central European Group
Riga Market 1 #1	CRTR06P38 (Riga 2), Riga Roadside Market 1, Riga, Latvia	4	Combined Wild *C. avellana* and Central European Group
Riga Market 2 #1	CRTR06P90 (Riga 3), Riga Roadside Market 2, Riga, Latvia	4	Combined Wild *C. avellana* and Central European Group
Riga Market 2 #4	CRTR06P90 (Riga 3), Riga Roadside Market 2, Riga, Latvia	4	Combined Wild *C. avellana* and Central European Group
Turkey #1	CRTR02P033, Giresun 233, (OSU 04131) Hazelnut Research Inst., Giresun, Turkey	0	Black Sea Group 2
Turkey #7	CRTR02P68, Giresun 362 (OSU 04149) Hazelnut Research Inst., Giresun, Turkey	0	Black Sea Group 2
Turkey #8	CRTR02P71, Giresun 362, (OSU 04149) Hazelnut Research Inst., Giresun, Turkey	0	Black Sea Group 2
Turkey #13	CRTR02P131, Giresun 453, (OSU 04159) Hazelnut Research Inst., Giresun, Turkey	0	Black Sea Group 2

Accession Name	Identification code (seed lot), origin and/or parentage	EFB response	Population
Turkey #14	CRTR02P134, Giresun 112, (OSU 04103) Hazelnut Research Inst., Giresun, Turkey	0	Black Sea Group 2
Turkey #15	CRTR02P138, Giresun 115, (OSU 04104) Hazelnut Research Inst., Giresun, Turkey	0	Black Sea Group 2
Turkey #18	CRTR02P186, Giresun 146, (OSU 04108) Hazelnut Research Inst., Giresun, Turkey	0	Black Sea Group 2
Turkey #22	CRTR03P158, Giresun 380, (OSU 04151) Hazelnut Research Inst., Giresun, Turkey	0	Black Sea Group 2
Turkey #27	CRTR04P14, Giresun 286, (OSU 04140) Hazelnut Research Inst., Giresun, Turkey	0	Black Sea Group 2
Turkey #29	CRTR04P124,Giresun 194, (OSU 04121) Hazelnut Research Inst., Giresun, Turkey	0	Black Sea Group 2
Turkey #3	CRTR02P13, Giresun 109, (OSU 04102) Hazelnut Research Inst., Giresun, Turkey	2	Black Sea Group 2
Turkey #28	CRTR04P108, Giresun 429, (OSU 04157) Hazelnut Research Inst., Giresun, Turkey	2	Black Sea Group 2
Turkey #2	CRTR02P10, Giresun 328, (OSU 04143) Hazelnut Research Inst., Giresun, Turkey	3	Black Sea Group 2
Turkey #4	CRTR02P25, Giresun 227, (OSU 04129) Hazelnut Research Inst., Giresun, Turkey	3	Black Sea Group 2
Turkey #9	CRTR02P83, Giresun 188, (OSU 04119) Hazelnut Research Inst., Giresun, Turkey	3	Black Sea Group 2
Turkey #10	CRTR02P84, Giresun 188, (OSU 04119) Hazelnut Research Inst., Giresun, Turkey	3	Black Sea Group 2
Turkey #16	CRTR02P149, Giresun 362, (OSU 04149) Hazelnut Research Inst., Giresun, Turkey	3	Black Sea Group 2
Turkey #19	CRTR03P01, Giresun 191, (OSU 04120) Hazelnut Research Inst., Giresun, Turkey	3	Black Sea Group 2
Turkey #24	CRTR04P03, Giresun 173, (OSU 04115) Hazelnut Research Inst., Giresun, Turkey	3	Black Sea Group 2
Turkey #25	CRTR04P04, Giresun 173, (OSU 04115) Hazelnut Research Inst., Giresun, Turkey	3	Black Sea Group 2
Turkey #5	CRTR02P32, Giresun 230, (OSU 04130) Hazelnut Research Inst., Giresun, Turkey	4	Black Sea Group 2
Turkey #6	CRTR02P42, Giresun 230, (OSU 04130) Hazelnut Research Inst., Giresun, Turkey	4	Black Sea Group 2
Turkey #12	CRTR02P128, Giresun 362, (OSU 04159) Hazelnut Research Inst., Giresun, Turkey	4	Black Sea Group 2
Turkey #17	CRTR02P171, Giresun 191, (OSU 04120) Hazelnut Research Inst., Giresun, Turkey	4	Black Sea Group 2
Turkey #20	CRTR03P99, Giresun 447, (OSU 04158) Hazelnut Research Inst., Giresun, Turkey	4	Black Sea Group 2
Turkey #21	CRTR03P128, Giresun 135, (OSU 04107) Hazelnut Research Inst., Giresun, Turkey	4	Black Sea Group 2
Turkey #23	CRTR03P183, Giresun 447, (OSU 04158) Hazelnut Research Inst., Giresun, Turkey	4	Black Sea Group 2
Turkey #26	CRTR04P13, Gireseun 194, (OSU 04121) Hazelnut Research Inst., Giresun, Turkey	4	Black Sea Group 2
Turkey #30	CRTR04P127, Giresun 173, (OSU 04115) Hazelnut Research Inst., Giresun, Turkey	4	Black Sea Group 2

Table 3. Summary of accessions included in this study as controls, which were all re-run alongside the new seedlings to ensure that the new data could be lined up and compared to the dataset used in Muehlbauer et al. (2014).

Accession name and species	Identification code (seed lots), origin, and/or parentage	Consensus Population[Z]
'Rush' *(C. americana)*	[Y]PI 557022, Pennsylvania	*C. americana x C. avellana* hybrid group
'Ata Baba'	PI 557422, Azerbaijan	Black Sea Group 1
'Cherkesskii II'	PI 617176, Russia, North Caucasus	Black Sea Group 1
'Ganja'	PI 634202, Azerbaijan	Black Sea Group 1
'Palaz'	PI 304632, Turkey (Ordu)	Black Sea Group 1
'Skorospelka'	PI 617175, Russia (southern)	Black Sea Group 1
Sochi Institute Mix 2	H3R10P94 (Rus 1), Sochi Institute mixture of OP seeds	Black Sea Group 2
B-X-3 OP #1	CRR01P116 (04041 R), B-X-3 OP, Sochi Institute	Black Sea Group 2
Holmskij Market 1 #1	H3R13P40 (RUS 9) Holmskij Market 1, Holmskij, Russia	Black Sea Group 2
'Imperial de Trebizonde'	PI 271105, Turkey	Black Sea Group 2
Kavakas OP #1	CRR04P107 (04028 R), 'Kavkas' OP, Sochi Institute	Black Sea Group 2
'Pioneer'	PI 617718, Russia (southern)	Black Sea Group 2
'Aurea'	PI 557050, France	Wild *C. avellana* group
'Cutleaf'	PI 557306, England	Wild *C. avellana* group
Estonia #1	H4R26P18 (07589), Agusalu, Estonia	Wild *C. avellana* group
Estonia #2	H4AR27P06 (07591), Tartu, Estonia	Wild *C. avellana* group
Finland CCOR 187	PI 557080, Finland	Wild *C. avellana* group
'Gasaway'	PI 557042, Washington	Wild *C. avellana* group
'Badem'	PI 304630, Turkey	Spanish-Italian group
'Barcelona'	PI 557037, Spain (Tarragona)	Spanish-Italian group
'Closca Molla'	PI 557109, Spain (Tarragona)	Spanish-Italian group
'Culpla'	PI 557107, Spain (Tarragona)	Spanish-Italian group
'Kalinkara'	PI 557240, Turkey	Spanish-Italian group
'Kudryavchik'	PI 671177, Russia (southern)	Spanish-Italian group
'Ratoli'	PI 557167, Spain (Tarragona)	Spanish-Italian group
'Sivri Ghiagli'	PI 304633, Turkey	Spanish-Italian group
'Tombul'	PI 318436 (Akcacoca)	Spanish-Italian group
'Tombul Ghiaghli'	PI 304634, Turkey	Spanish-Italian group
'Tonda di Giffoni'	PI 296207, Italy (Campania)	Spanish-Italian group
'Kudashovski' OP #1	CRXR13P78 (RUS 2), 'Kudashovski' OP, Sochi Institute	Moscow group
'Halls Giant'	PI 557027, Germaany/France	Central European group
Karol	PI 617231, Poland	Central European group
'Syrena'	PI 617175, Poland	Central European group
'Volski'	PI 617238, Poland	Central European group
Warsaw #1	H4AR21P03 (06085 P), unknown seed mixture, Warsaw , Poland	Central European group

[Z]Consensus populations from Muehlbauer et al. (2014).
[Y]Plant introduction (PI) number for plants found in the US National Plants Germplasm System.

Table 4. Summary of simple sequence repeat markers used in this study, their original publication, the linkage group each is located on, the forward and reverse sequences for each marker, and the repeat motif.

Marker Name	Source	Linkage Group no.	Primer Sequence	Motif
A640	Gürcan et al., 2010a, 2010b	10	F-TGCCTCTGCAGTTAGTCATCAAATGTAGG R-CGCCATATAATTGGGATGCTTGTTG	$(CT)_{15}(CA)_{13}$
B005	Bassil et al., 2005	2	F-CAAACTTATGATAGGCATGCA R-TGTCACTTTGGAAGACAAGAGA	$(GA)_{22}$
B634	Gürcan et al., 2010a, 2010b	4	F-CCTGCATCCAGGACTCATTA R-GTGCAGAGGTTGCACTCAAA	$(AG)_{15}$
B751	Gürcan et al., 2010a, 2010b	7	F-AGCTGGTTCTTCGACATTC R-AAACTCAAATAAAACCCCTGCTC	$(GA)_{15}$
B657	Gürcan et al., 2010a, 2010b	11	F-GAGAGTGCGTCTTCCTCTGG R-AGCCTCACCTCCAACGAAC	$(AG)_{15}$
B665	Gürcan et al., 2010a, 2010b	8	F-GCAACCACCAAATTGCACTA R-GCTTTTAAAGTCCACGCATGA	$(CT)_{17}$
B671	Gürcan et al., 2010a, 2010b	9	F-TTGCCAGTGCATACTCTGATG R-ACCAGCTCTGGGCTTAACAC	$(AG)_6NN(GA)_{17}$
KG830	Gürcan and Mehlenbacher, 2010a	9	F-TGGAGGAAGTTTTGAATGGTAGTAGAGGA R-AAAGCAACTCATAGCTGAAGTCCAATCA	$(CT)_{13}GTATT(CA)_3$
B774	Gürcan et al., 2010a, 2010b	5	F-GTTTTGCGAGCTCATTGTCA R-TGTGTGTGGTCTGTAGGCACT	$(AG)_{15}$
B776	Gürcan et al., 2010a, 2010b	6	F-TGTATGTACACACGGAGAGAGAGA R-TGAGGGGAAGAGGTTTGATG	$(GA)_{17}$
B753	Gürcan et al., 2010a, 2010b	7	F-AAGGGTTGTTACCCATGCAC R-GGTGCATTTAGTGCTTCTGG	$(GA)_{15}$
B749	Gürcan et al., 2010a, 2010b	1	F-GGCTGACAACACAGCAGAAA R-TCGGCTAGGGTTAGGGTTTT	$(TC)_{12}$
B789	Gürcan et al., 2010a, 2010b	2	F-GCCACGTCCAGAATCAAAAT R-CCTCAGGGCTGAGAAGTTGA	$(AG)_{16}$
KG807	Gürcan and Mehlenbacher, 2010a	11	F-AAGCAAGAAAGGGATGGT R-CTTACAGATAAATGGCTCAAA	UNKNOWN
KG810	Gürcan and Mehlenbacher, 2010a	4	F-TCCTCACCAATCACACTATTT R-TTATTCCACCAAAGTCTACCTC	$(AG)_{15}$
B733	Gürcan et al., 2010a, 2010b	7	F-CACCCTCTTCACCACCTCAT R-CATCCCCTGTTGGAGTTTTC	$(TC)_{15}$

Table 5. Summary statistics including observed heterozygosity (H_o), polymorphism information content (PIC), and inbreeding coefficient (f) for the Turkish, Latvian/Lithuanian, and previously reported hazelnut germplasm collection (Muehlbauer et al., 2014) assessed with 17 simple sequence repeat markers.

Locus	Alleles (no.)	Major allele frequency	Genotypes (no.)	H_o	H_e	PIC	f
loc_A640 (Turkish Accessions)	9	0.36	13	0.84	0.78	0.75	-0.056
loc_A640 (Latvian/Lithuanian Accessions)	7	0.32	7	1	0.76	0.73	-0.264
loc_A640 (Muehlbauer et al. 2014)	16	0.24	52	0.83	0.85	0.83	0.026
loc_B005 (Turkish Accessions)	7	0.44	11	0.74	0.72	0.68	-0.015
loc_B005 (Latvian/Lithuanian Accessions)	4	0.64	4	0.55	0.55	0.50	0.048
loc_B005 (Muehlbauer et al. 2014)	15	0.32	40	0.64	0.78	0.75	0.184
loc_B634 (Turkish Accessions)	10	0.64	10	0.57	0.56	0.54	-0.004
loc_B634 (Latvian/Lithuanian Accessions)	6	0.45	6	0.55	0.73	0.70	0.294
loc_B634 (Muehlbauer et al. 2014)	19	0.29	53	0.80	0.83	0.81	0.038
loc_B751 (Turkish Accessions)	9	0.34	17	0.68	0.80	0.77	0.165
loc_B751 (Latvian/Lithuanian Accessions)	7	0.32	9	0.64	0.79	0.76	0.223
loc_B751 (Muehlbauer et al. 2014)	13	0.39	38	0.72	0.78	0.76	0.079
loc_B657 (Turkish Accessions)	7	0.26	15	0.86	0.80	0.77	-0.055
loc_B657 (Latvian/Lithuanian Accessions)	5	0.35	7	0.69	0.72	0.68	0.085
loc_B657 (Muehlbauer et al. 2014)	20	0.18	55	0.86	0.88	0.87	0.027
loc_B665 (Turkish Accessions)	10	0.23	16	0.43	0.86	0.84	0.513
loc_B665 (Latvian/Lithuanian Accessions)	8.	0.29	11	0.79	0.81	0.79	0.068
loc_B665 (Muehlbauer et al. 2014)	20	0.19	64	0.52	0.89	0.87	0.410
loc_B671 (Turkish Accessions)	8	0.50	12	0.48	0.70	0.66	0.326
loc_B671 (Latvian/Lithuanian Accessions)	8	0.38	10	0.46	0.78	0.75	0.438
loc_B671 (Muehlbauer et al. 2014)	21	0.24	67	0.81	0.86	0.85	0.065
loc_KG830 (Turkish Accessions)	10	0.50	15	0.75	0.71	0.69	-0.057
loc_KG830 (Latvian/Lithuanian Accessions)	6	0.36	9	0.71	0.76	0.72	0.097
loc_KG830 (Muehlbauer et al. 2014)	17	0.31	50	0.80	0.82	0.80	0.029

Locus	Alleles (no.)	Major allele frequency	Genotypes (no.)	H_o	H_e	PIC	f
loc_B774 (Turkish Accessions)	7	0.45	14	0.57	0.73	0.70	0.235
loc_B774 (Latvian/Lithuanian Accessions)	5	0.46	6	0.54	0.65	0.59	0.211
loc_B774 (Muehlbauer et al. 2014)	17	0.33	47	0.79	0.82	0.80	0.046
loc_B776 (Turkish Accessions)	8	0.36	12	0.76	0.73	0.68	-0.028
loc_B776 (Latvian/Lithuanian Accessions)	5	0.61	6	0.57	0.57	0.53	0.037
loc_B776 (Muehlbauer et al. 2014)	21	0.47	47	0.57	0.71	0.68	0.202
loc_B753 (Turkish Accessions)	7	0.60	9	0.15	0.61	0.59	0.757
loc_B753 (Latvian/Lithuanian Accessions)	5	0.56	5	0.13	0.63	0.60	0.825
loc_B753 (Muehlbauer et al. 2014)	22	0.28	75	0.83	0.87	0.86	0.043
loc_B749 (Turkish Accessions)	6	0.55	7	0.21	0.62	0.56	0.673
loc_B749 (Latvian/Lithuanian Accessions)	4	0.50	6	0.40	0.66	0.60	0.433
loc_B749 (Muehlbauer et al. 2014)	18	0.39	40	0.60	0.71	0.67	0.171
loc_B789 (Turkish Accessions)	12	0.38	15	0.75	0.75	0.72	0.022
loc_B789 (Latvian/Lithuanian Accessions)	10	0.38	10	0.67	0.80	0.78	0.207
loc_B789 (Muehlbauer et al. 2014)	26	0.36	71	0.80	0.81	0.80	0.018
loc_KG807 (Turkish Accessions)	5	0.63	6	0.37	0.55	0.50	0.338
loc_KG807 (Latvian/Lithuanian Accessions)	3	0.50	4	0.33	0.57	0.48	0.450
loc_KG807 (Muehlbauer et al. 2014)	13	0.37	27	0.67	0.74	0.70	0.104
loc_KG810 (Turkish Accessions)	9	0.29	14	0.93	0.79	0.76	-0.158
loc_KG810 (Latvian/Lithuanian Accessions)	6	0.58	7	0.54	0.62	0.59	0.172
loc_KG810 (Muehlbauer et al. 2014)	21	0.26	80	0.80	0.86	0.84	0.076
loc_B733 (Turkish Accessions)	9	0.52	11	0.52	0.64	0.59	0.205
loc_B733 (Latvian/Lithuanian Accessions)	6	0.46	7	0.71	0.63	0.55	-0.106
loc_B733 (Muehlbauer et al. 2014)	12	0.32	39	0.73	0.77	0.74	0.058
Mean (Turkish Accessions)	**8.31**	**0.44**	**12.31**	**0.60**	**0.71**	**0.68**	**0.170**
Mean (Latvian/Lithuanian Accessions)	**5.94**	**0.45**	**7.13**	**0.58**	**0.69**	**0.65**	**0.199**
Mean (Muehlbauer et al. 2014)	**18.12**	**0.31**	**53.11**	**0.73**	**0.81**	**0.79**	**0.103**

Figure 1. Dendrogram resulting from the cluster analysis of previously analyzed accessions (Muehlbauer et al., 2014) and all new seedlings included in this study. Clades described in greater detail in subsequent figures are indicated by roman numerals (I-VI).

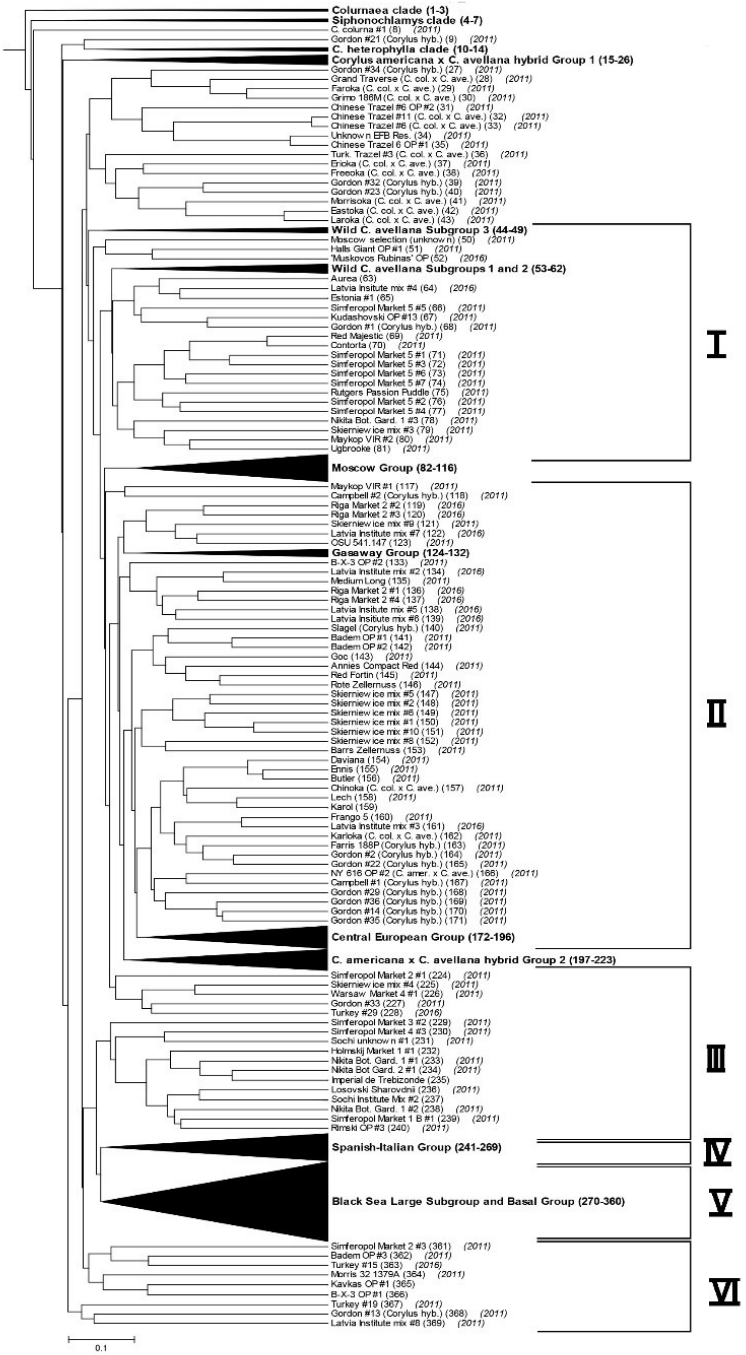


Figure 2. STRUCTURE diagram resulting from the population structure analysis of all accession included in the study. They were ordered in accordance with the dendrogram (Fig. 1).

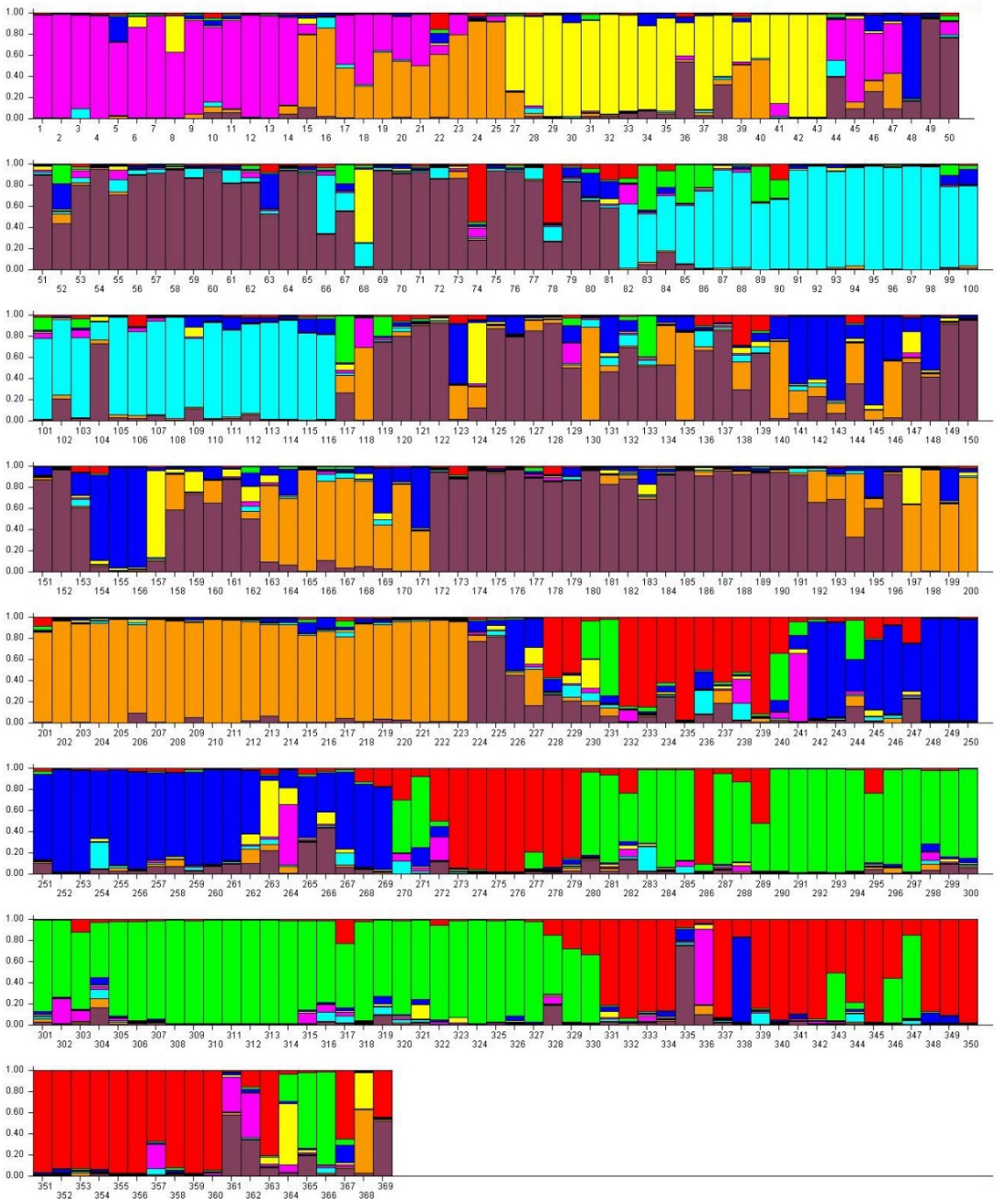
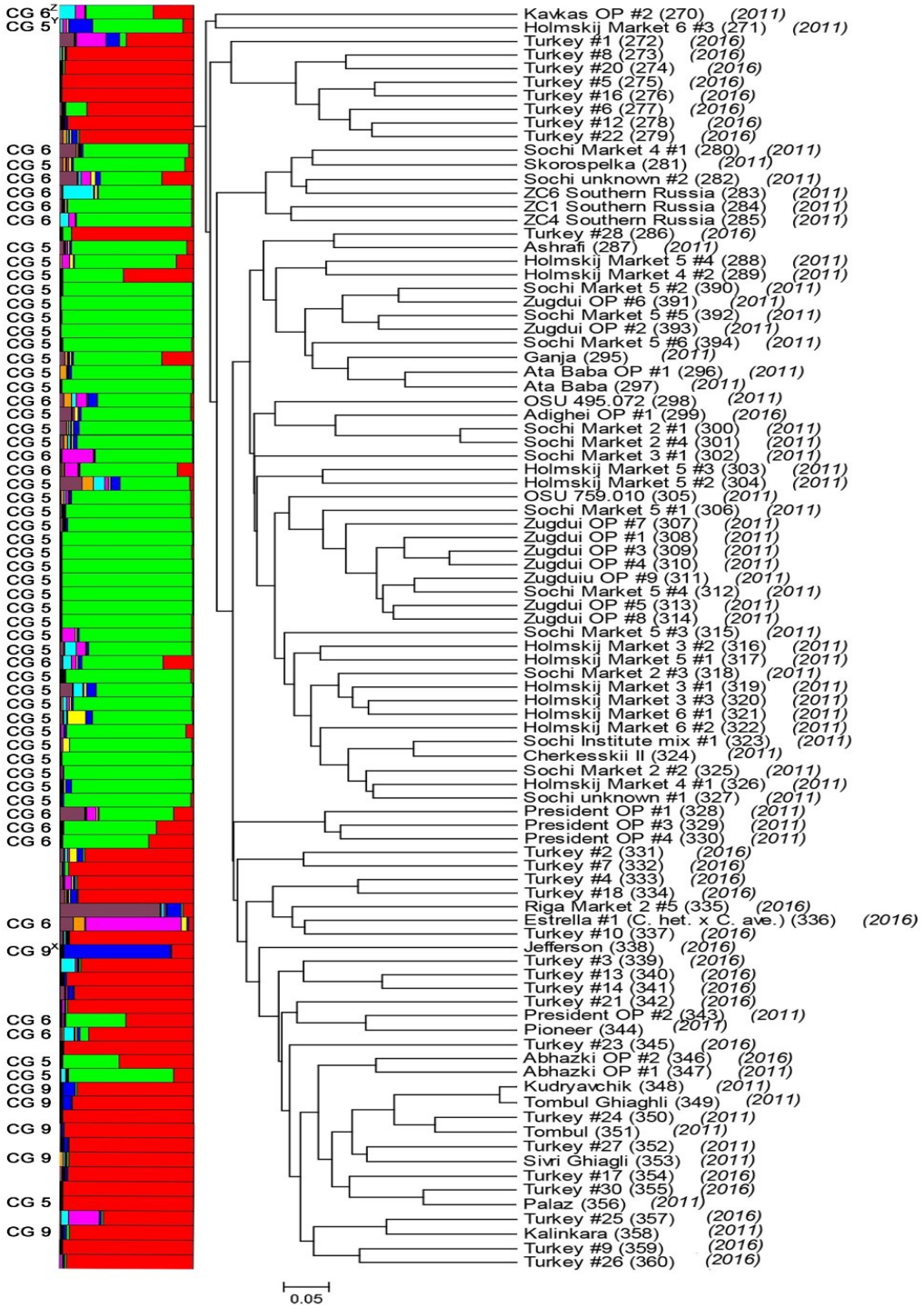
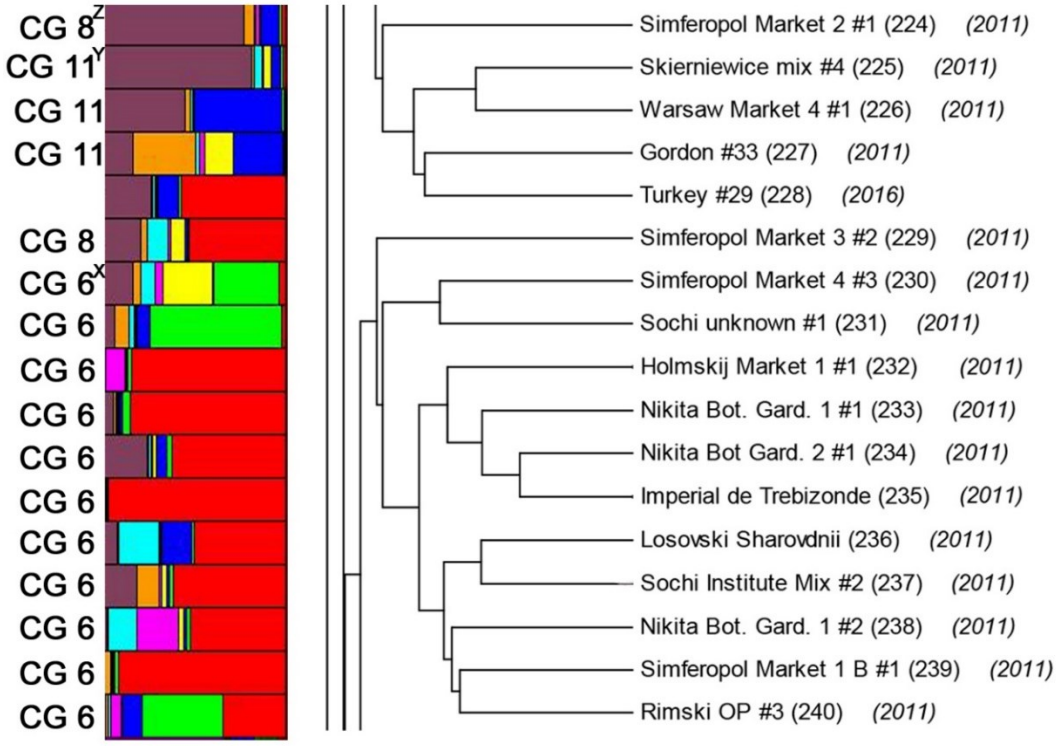


Figure 3. Cluster V, Black Sea Large Subgroup and Basal Group, of the dendogram and the corresponding component of the STRUCTURE analysis output, both of which include 26 newly characterized Turkish, one Latvian seedling, and closely related hazelnut germplasm.



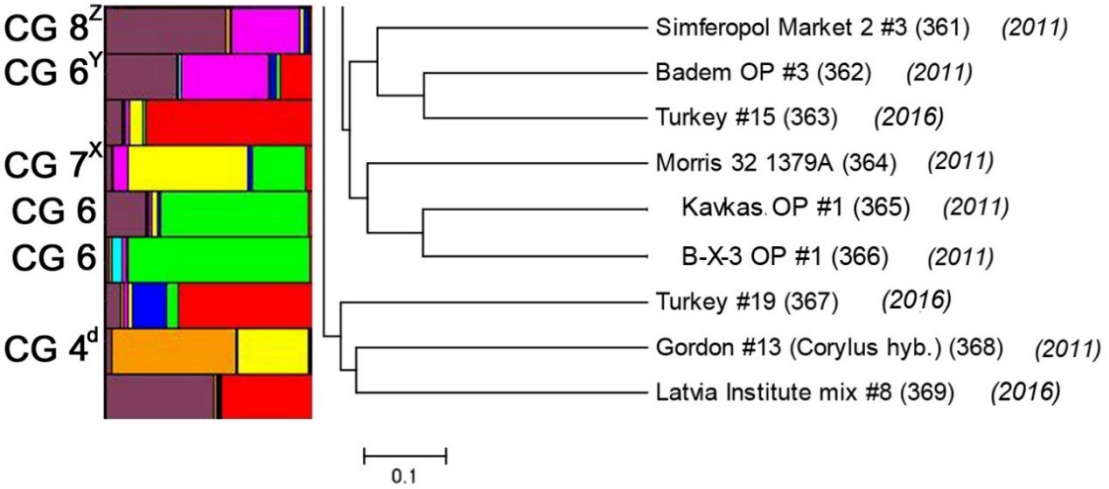
[Z]CG 6 corresponds to the consensus group (Black Sea Group 2) from Muehlbauer et al. (2014).
[Y]CG 5 corresponds to the consensus group (Black Sea Group 1) from Muehlbauer et al. (2014).
[X]CG 9 corresponds to the consensus group (Spanish-Italian group) from Muehlbauer et al. (2014).

Figure 4. Cluster III of the dendogram and the corresponding component of the STRUCTURE analysis output, both of which include one newly characterized Turkish seedling and closely related hazelnut germplasm.



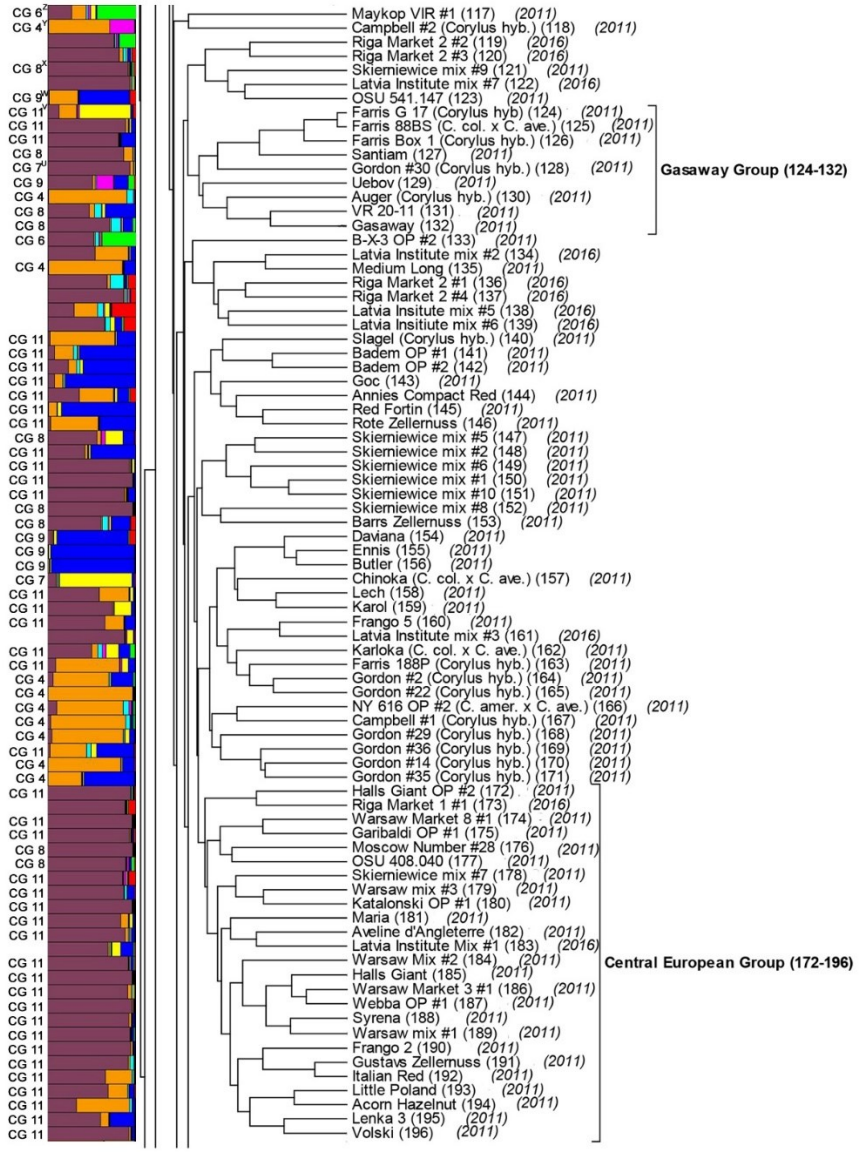
[Z]CG 8 corresponds to the consensus group (Wild *C. avellana* group) from Muehlbauer et al. (2014).
[Y]CG 11 corresponds to the consensus group (Central European group) from Muehlbauer et al. (2014).
[X]CG 6 corresponds to the consensus group (Black Sea Group 2) from Muehlbauer et al. (2014).

Figure 5. Cluster VI of the dendogram and the corresponding component of the STRUCTURE analysis output, both of which include 2 newly characterized Turkish, 1 Latvian seedling, and closely related hazelnut germplasm.



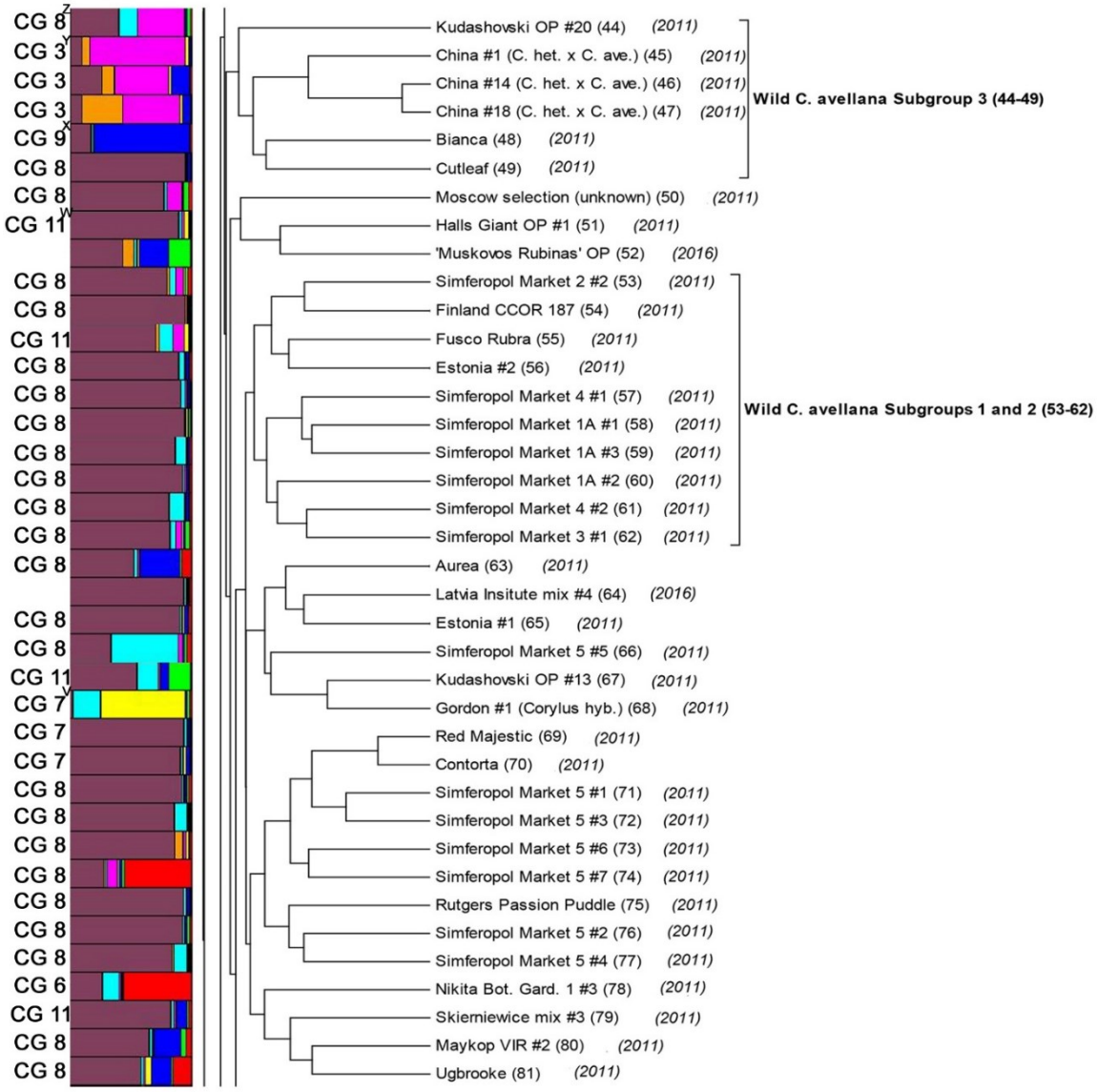
ZCG 8 corresponds to the consensus group (Wild *C. avellana* group) from Muehlbauer et al. (2014).
YCG 6 corresponds to the consensus group (Black Sea Group 2) from Muehlbauer et al. (2014).
XCG 7 corresponds to the consensus group (Gellately hybrid group) from Muehlbauer et al. (2014).
WCG 4 corresponds to the consensus group (*C. americana x C. avellana* hybrid group) from Muehlbauer et al. (2014).

Figure 6. Cluster II of the dendogram and the corresponding component of the STRUCTURE analysis output, both of which include eleven newly characterized seedlings from Eastern Europe and closely related hazelnut germplasm.



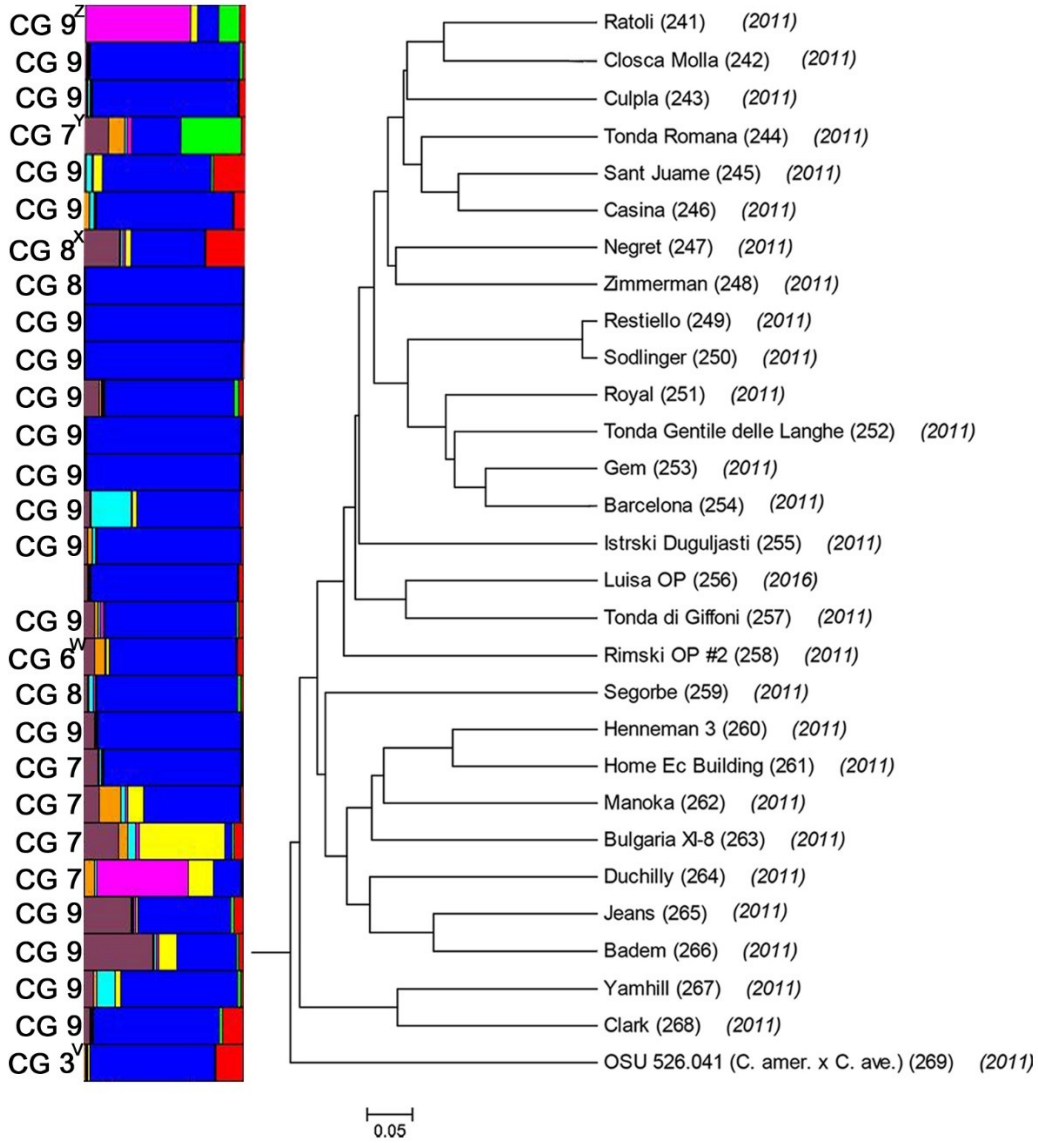
[Z]CG 6 corresponds to the consensus group (Black Sea Group 2) from Muehlbauer et al. (2014).
[Y]CG 4 corresponds to the consensus group (*C. americana x C. avellana* hybrid group) from Muehlbauer et al. (2014).
[X]CG 8 corresponds to the consensus group (Wild *C. avellana* group) from Muehlbauer et al. (2014).
[W]CG 9 corresponds to the consensus group (Spanish-Italian group) from Muehlbauer et al. (2014).
[V]CG 11 corresponds to the consensus group (Central European group) from Muehlbauer et al. (2014).
[U]CG 7 corresponds to the consensus group (Gellately hybrid group) from Muehlbauer et al. (2014).

Figure 7. Cluster I of the dendogram and the corresponding component of the STRUCTURE analysis output, both of which include two newly characterized seedlings from Eastern Europe and closely related hazelnut germplasm.



[Z]CG 8 corresponds to consensus group 8 (Wild *C. avellana* group) from Muehlbauer et al. (2014).
[Y]CG 3 corresponds to consensus group 3 (Outgroup 3) from Muehlbauer et al. (2014).
[X]CG 9 corresponds to consensus group 9 (Spanish-Italian group) from Muehlbauer et al. (2014).
[W]CG 11 corresponds to consensus group 11 (Central European group) from Muehlbauer et al. (2014).
[V]CG 7 corresponds to consensus group 7 (Gellately hybrid group) from Muehlbauer et al. (2014).

Figure 8. Cluster IV of the dendogram, Spanish-Italian Group, and the corresponding component of the STRUCTURE analysis output, both of which include one newly characterized Lithuanian seedling and closely related hazelnut germplasm.



[Z]CG 9 corresponds to the consensus group (Spanish-Italian group) from Muehlbauer et al. (2014).
[Y]CG 7 corresponds to the consensus group (Gellately hybrid group) from Muehlbauer et al. (2014).
[X]CG 8 corresponds to the consensus group (Wild *C. avellana* group) from Muehlbauer et al. (2014).
[W]CG 6 corresponds to the consensus group (Black Sea Group 2) from Muehlbauer et al.(2014).
[V]CG 3 corresponds to consensus group 3 (Outgroup 3) from Muehlbauer et al. (2014).

Chapter 3: Characterization of genetic diversity and population structure of Anisogramma anomala using microsatellite markers

ABSTRACT

Anisogramma anomala (Peck) E. Müller, a biotrophic ascomycete in the order Diaporthales, causes eastern filbert blight (EFB) of hazelnuts (*Corylus*). Until recently, little has been documented on its genetic diversity and population structure. In this study, 18 simple sequence repeat markers were used to fingerprint 182 isolates of the fungus from across North America. Our results, based on summary statistics of the allelic data, a UPGMA dendrogram, population STRUCTURE analysis, and analysis of multilocus genotypes show that *A. anomala* exhibits significant genetic diversity across multiple populations. Isolates were placed into three major clades by the UPGMA analysis, and the STRUCTURE output showed K=42. When considering both analyses, 22 consensus groups were designated via an analysis of molecular variance ($p \leq 0.01$). Dendrogram topology and STRUCTURE assignment was generally correlated with collection origin; isolates collected in close proximity (53, 224, and 396 km) tended to cluster together and be genetically similar. However, some locations held populations that were diverse and some populations with a high degree of similarity had disparate origins suggesting movement by humans. Overall, the results demonstrate the presence of multiple, genetically distinct populations of *A. anomala* in North America and serve as a reference to assist in understanding and managing EFB.

INTRODUCTION

Anisogramma anomala (Peck) E. Müller is the causal agent of the disease eastern filbert blight (EFB) of hazelnuts (*Corylus spp.*). It is an obligate, biotrophic ascomycete in the order Diaporthales, and is native to a wide expanse of eastern North America where it is found associated with its natural host, the wild American hazelnut (*Corylus americana* Marshall) (Gottwald and Cameron 1979, 1980a; Pinkerton et al. 1995). While the fungus causes little to no damage to *C. americana*, it causes large perennial stem cankers, branch die-back, and ultimately tree death of most cultivars of the commercially important European hazelnut (*Corylus avellana* L.) (Capik and Molnar 2012; Fuller 1908; Johnson and Pinkerton 2002; Pinkerton et al. 1993; Weschcke 1954). EFB is considered to be the primary limiting factor of hazelnut production in eastern North America (Thompson et al. 1996) and its management causes significant expense in Oregon, where 99% of the U.S. commercial crop is grown (Julian et al. 2009). To date, the fungus has not been found outside of North America (Anonymous 2012).

The first descriptions and reports of the EFB pathogen were made in the northeastern United States (U.S.) in the late 1800s to early 1900s (Ellis and Everhart 1892; Fuller 1908; Peck 1874). It was recognized as a serious threat to the burgeoning hazelnut industry in the Pacific Northwestern U.S. where the fungus was nonexistent, and a strict quarantine was established on the movement of *Corylus* plants west of the Rocky Mountains to prevent its spread (Barss 1921, 1930; Pinkerton et al. 1992). Despite the quarantine, EFB was inadvertently introduced into southwest Washington in the 1960s (Davison and Davidson 1973) where it devastated local production. By 1979, EFB was found in 49 orchards in Washington, and by 1986 it was present in the northeast corner of the Willamette Valley of Oregon (Pinkerton et al. 1992). By 2001, EFB could be found as far as ~60 km south of the original point of detection (Pinkerton et al. 2001). Today, it is present across the entire Willamette Valley where it imparts a significant economic impact on *C. avellana* production in the U.S. (Julian et al. 2009; Pscheidt 2014).

A. anomala is reported to be homothallic and reproduces only by ascospores (Gottwald and Cameron 1979) which are released from November to May (Gottwald and Cameron 1980b). Liberated ascospores are then moved by rain splash and wind-driven rain (Pinkerton et al. 1998). The discharge and dispersal model for EFB has been likened to that of another member of the Diaporthales, *Venturia inaequalis* (Cooke) G. Wint. (Aylor 1995, 1998; Pinkerton et al. 2001). Similarities are evident in their disease progression models, which indicate directional spread by wind, leading to a higher infection gradient in trees downwind than those located upwind of an inoculum source (Gottwald and Cameron 1980a; Pinkerton et al. 1992, 2001).

Due to the high costs associated with chemical-based control, the development and utilization of resistant cultivars is considered to be the best EFB management strategy (Julian et al. 2009). In support of this approach, considerable research has been done over the past two decades to identify sources of resistance and subsequently introgress resistance genes into improved breeding lines (Mehlenbacher 1994; Molnar et al. 2005). The first source of resistance identified was 'Gasaway' (*C. avellana*), a late shedding pollinizer from Washington found to confer resistance through a dominant allele in the heterozygous state at a single locus (Mehlenbacher et al. 1991, 2004). Despite the poor nut production traits of 'Gasaway', breeders at Oregon State University (OSU), Corvallis, OR, have used it to develop a number of EFB-resistant, commercial-quality cultivars (Mehlenbacher et al. 2009, 2011, 2013, 2014), which have provided the basis for the expansion of Oregon's hazelnut industry by ~6,000 ha over the past five years (S. Mehlenbacher, *personal communication*).

Concerns over the long term durability of a single *R*-gene have motivated researchers to find additional sources of resistance. Over the past fifteen years, many hundreds of cultivars and thousands of seedling accessions of *C. avellana* have been screened, resulting in the identification of a large number of additional EFB-resistant plants. The most notable are 'Crevenje' and 'Uebov' from Serbia, 'Culpla' and 'Ratoli' from Spain, and several selected seedlings and clones

originating from Russia, Crimea, the Republic of Georgia, Finland, Chile, Minnesota (USA), and Turkey. A significant number of resistant accessions and interspecific hybrids from other *Corylus* species have also been identified. Many trees in this collective group are currently being utilized in research and breeding efforts at Rutgers University and OSU (Chen et al. 2005, 2007; Colburn et al. 2015; Coyne et al. 1998; Lunde et al. 2000; Sathuvalli et al. 2009, 2010, 2011a, 2011b) (Capik et al. 2013; Leadbetter et al. 2015, 2016; Molnar et al. 2007; Muehlbauer et al., 2014a).

While a significant collection of EFB-resistant germplasm is now at the disposal of plant breeders, several studies have suggested that pathogenic variation may exist in *A. anomala.* In the first examination of this topic, Osterbauer (1996) used greenhouse-based inoculations on clonal trees (VR6-28) carrying the 'Gasaway' *R*-gene. She found that the plants developed small, sunken lesions (although lacking stromata) when challenged with isolates of *A. anomala* from Minnesota and Ontario, but developed no signs or symptoms of the disease when challenged with isolates from Oregon and four other regions. Later, Molnar et al. (2010) expanded on this greenhouse inoculation work to include isolates from 10 different locations. These isolates were used to challenge trees of 'Gasaway' as well as 11 additional clonal accessions shown to be resistant to EFB in Oregon. Their results showed that trees of 'Gasaway' developed EFB only when exposed to an isolate of *A. anomala* from Michigan. Interestingly, this Michigan isolate was also found to be the only isolate able to infect 'Zimmerman' ('Gasaway' × 'Barcelona') and OSU 408.040 (a *C. avellana* selection from Minnesota), while also infecting a greater number of trees (replications) across all genotypes than any of the other isolates tested. Thus, the results strongly suggested that the isolate from Michigan was more virulent than the others tested, and more broadly, that variation exists within the pathogen. Additionally, in the same study, VR20-11, another offspring of 'Gasaway', [('Barcelona'×'Compton') × 'Gasaway'], developed EFB when exposed to isolates from Minnesota and New Jersey in addition to Michigan. Thus,

questions remained unanswered on the interactions of different isolates of *A. anomala* and plants containing the 'Gasaway' *R*-gene.

EFB was also observed on trees of 'Gasaway' and VR20-11 growing in the field in New Jersey under natural conditions (Molnar et al. 2010b). This finding, like the greenhouse study described previously, was in contrast to the disease response observed in Oregon for 'Gasaway' and VR20-11, where these trees remained free of EFB after several decades of exposure to *A. anomala*. Capik and Molnar (2012) later examined a larger number of trees with 'Gasaway' in their background and other cultivars and accessions shown to be resistant to EFB in Oregon. They again found a differential response to EFB across a number of the cultivars when grown in New Jersey, particularly those protected by the 'Gasaway' *R*-gene. Their results further suggested that isolates of the fungus present in New Jersey were different than those in Oregon. However, since their findings were based only on phenotypic responses, environmental variation such as climate and disease pressure remained as confounding factors in the field-based study. Through understanding of the genetic diversity and variation of *A. anomala* and its population structure is critical for support of breeding efforts to develop cultivars expressing durable forms of resistance.

In the first attempt to study molecular genetic variation of *A. anomala*, Osterbauer (1996) sequenced and compared the ITS region of 67 isolates from five locations in Oregon and Washington, but found little sequence divergence among them. She also examined 33 isolates collected from multiple locations across North America with Random Amplified Polymorphic DNA (RAPD) markers, although the results were again mostly inconclusive. However, her work did show an apparent genetic profile similarity between isolates from Oregon, Washington, New York, and Ontario, Canada. These isolates were grouped together and shown to be different than the remainder of the isolates in the study.

More recently, the Illumina sequencing platform, coupled with a novel "SEQ-Assembly-SSR" approach, was used to identify simple sequence repeat (SSRs) markers for *A. anomala* (Cai et al. 2013). A database of 39,361 microsatellite loci for *A. anomala* was generated, from which 236 loci were screened and 23 were found to be polymorphic (Cai et al., 2013). SSRs hold great utility for genetic diversity studies since they are highly polymorphic and easily reproducible. They have been successfully used to elucidate fungal population structure and diversity as well as identify genetic bottlenecks resulting from the introduction of disease resistant plant material (Breuillin et al. 2006; Tenzer et al. 1999; Zhang et al. 2002). They have also been used to help pinpoint centers of disease epidemics and new introductions of fungal pathogens (Tenzer et al. 1999; Zhang et al. 2002).

To confirm the utility of the new SSRs for *A. anomala*, Cai et al. (2013) used 11 loci to investigate diversity of 30 different isolates from a wide geographic area. Results showed that the isolates were distinct and resolved two major groups: one consisting almost entirely of isolates from New Jersey, and the other holding isolates from the Great Lakes region and Oregon. Their analysis confirmed that the fungus was genetically diverse and laid the framework for a more in-depth study. The objective of the current study is to expand upon the work of Cai et al. (2013) using 18 SSRs to investigate the genetic diversity and population structure of 182 isolates of *A. anomala* originating from 43 locations across North America.

MATERIALS AND METHODS

Anisogramma anomala Isolates and DNA Extractions

Infected hazelnut (*C. avellana*) stems containing mature *A. anomala* stromata were collected between January 2011 and March 2013 from trees growing at 43 different locations spanning 12 states and one Canadian Province (Supplemental Table 1). When possible, the infected stems were collected from different trees at each location to obtain samples derived from separate infection events. If only one infected tree was available, stems were collected far apart in the canopy in an attempt to avoid collecting cankers from the same infection event (and potentially the same isolate). Stems were placed in polyethylene bags and stored at -20 ºC until use. While the EFB cankers on individual stems each contained multiple stromata, only a single stromata was used to obtain ascospores for DNA extractions. We defined the extractions from these single, disparate stromata from individual stems as our "isolates", of which a total of 182 from 43 locations were obtained and examined in this study (Supplemental Table 1).

To maintain consistency, the isolates used for SSR primer testing (described in the subsequent section) were the same ones used in Cai et al. (2013). They were obtained from locations in New Jersey (NJ_Cream_Ridge_6), Ohio (OH_Newark_11), Oregon (OR_Corvallis_1), and Wisconsin (WI_Moquah_1), and were chosen to represent geographically divergent regions of the U.S. For these isolates, ascospores were germinated and grown in axenic culture (Stone et al. 1994), to obtain sufficient DNA for primer testing. DNA was extracted using a DNeasy plant kit (Qiagen) (Cai et al. 2013). We used this previously extracted DNA to identify additional polymorphic SSR primers for their inclusion in the broader diversity study.

DNA extractions of the remaining 178 isolates were modified from that described in Cai et al. (2013). Instead of culturing the ascospores, the perithecial matrix from a single stromata was obtained and transferred to a 1.5 mL microcentrifuge tube containing 1 ml of sterile water. The ascospore suspension was then filtered through a 40 µm nylon mesh cell strainer, centrifuged

at 5,000 rpm for 2 min, and then the water was removed with a pipette which left behind a concentrated pellet of spores. The spore pellets were stored in microcentrifuge tubes at -80 ºC. DNA was extracted from the stored spore pellets using a modified MoBio Ultra Clean Plant DNA Isolation kit (MoBio, Carlsbad, CA).

SSR Primers, Amplification, and Scoring

A total of 18 SSRs (Supplemental Table 2) were used to genotype the 182 isolates, five of which were previously described [Aa00689, Aa02342, Aa16574, Aa03927 and Aa02944 (Cai et al. 2013)]. Briefly, 96 SSR loci were chosen from the genome-wide microsatellite database (39,361 loci) generated by Cai et. al. (2013). The set included 66 di-, 27 tri-, and 8 tetra-nucleotide repeat motifs. The primers were amplified in the four "tester" isolates, and examined for polymorphic alleles. Primers that resolved at least three different alleles per locus were considered targets for final selection and use in the current diversity study. (It should be noted that *A. anomala* is a homothallic fungus; single stromata extractions yield single genotype isolations consisting of haploid ascospores, and these haploid ascospore isolates yield one allele at each locus (Cai et al. 2013), thus the maximum number of alleles across the four "tester" isolates would be four).

The following amplification protocol was used for both primer testing and genotyping the isolates. The M13 (-21) 18-bp sequence was added to the 5' end of all forward primers as a cost effective means of fluorescent labeling of PCR fragments (Schuelke 2000), and the "PIG-tailing" sequence (GTTTCTT) was included at the 5' end of all reverse primers to reduce uncertainty in scoring "true" vs. "plus-A" alleles (Brownstein et al. 1996). Primers were synthesized by Integrated DNA Technologies (Coralville, IA). PCR genotyping was performed in 96-well plates in 13-µL reaction volumes. PCR reactions consisted of approximately 5 ng genomic DNA, 10xRamp-Taq PCR buffer (Denville Scientific, Metuchen, NJ), 2.0 mM $MgCl_2$, 0.25 mM each dNTP (Denville Scientific), 0.5 U Ramp-Taq DNA polymerase (Denville Scientific), 0.5 pmol forward primer with 5' M13 (-21) addition, 1.0 pmol reverse primer with 5' PIG-tail addition, and

1.0 pmol FAM, NED, PET or VIC fluorescently labeled M13 (-21) primer. PCR cycling was performed in GeneAmp 9700 thermalcyclers (Applied Biosystems, Foster City, CA) using the following parameters: initial denaturation of 94ºC for 5 min followed by 30 cycles of 94 ºC for 30 s, 55 ºC for 45 s, 72 ºC for 45 s, followed by 20 cycles of 94 ºC for 30 s, 53 ºC for 45 s, 72 ºC for 45 s, followed by a final extension of 72 ºC for 10 min.

PCR products were separated on a capillary electrophoresis genetic analyzer (ABI 3500xl; Applied Biosystems) and sized using a LIZ600 size standard (Applied Biosystems). Two additional controls were added to each 96-well plate for the genotyping portion of the experiment: isolate OR_Corvallis_2 from OSU, originally sequenced by Cai et al. (2013); and the GeneScan Installation Standard DS-33 (Applied Biosystems). SSR marker testing and genotyping results were analyzed using Genemapper 4.0 (Applied Biosystems).

Data Analysis

Summary statistics were calculated for each SSR using the program Powermarker 3.25 (Liu & Muse 2005); parameters calculated included allele number per loci, frequency of the most common allele, gene diversity (expected heterozygosity, the probability that two randomly chosen alleles in a population are different), and polymorphism information content (PIC) value. Allele frequencies were generated by the same program and used to construct a genetic distance matrix. An unweighted pair group method with arithmetic mean (UPGMA) dendrogram was then constructed from a frequency-based distance matrix using the Nei (1982) coefficient. A bootstrap analysis was performed with a minimum support value of 0.5, and a majority rule consensus tree was visualized in the CONSENSE component of the PHYLIP v. 3.696 package. (Felenstein 2005). The final UPGMA dendrogram was visualized using Mega 5.01 (Tamura et al. 2011).

A Bayesian model-based clustering method, STRUCTURE 2.3.3 (Flaush et al. 2003; Prichard et al. 2000), was used to discern population structure through assignment of each isolate into a population or populations according to their 18-locus haplotypes. Software run simulation

parameters assumed all loci were independent and in linkage equilibrium. The admixture ancestry model with correlated allele frequencies was used for the analysis with a burn-in length of 20,000 iterations followed by 50,000 Markov chain Monte Carlo run iterations at each (*K*) value. If an individual was assigned to more than one population (several colors in a bar), it was considered evidence of admixture. The STRUCTURE analysis was run at 20 replicates per (*K*), where (*K*) was set to (*K*) = 2 through 50. Results from each run were imported into the program Structure Harvester (Earl & vanHoldt et al. 2012), which provided the maximal value of ΔK, an ad hoc statistic based upon the rate of change in the log probability of sequential *K* values, and correlates to the most parsimonious value of (*K*). (Evanno et al., 2005)

The results of the UPGMA and STRUCTURE outputs were compared and, based upon points of congruency between the analyses, isolates were then assigned to consensus populations (described more fully in the Results section). The full dataset was then analyzed using the program MLGsim (Sternberg et al. 2003) to determine the number of multilocus genotypes (MLGs) present in each consensus population. Concurrently, a simulation approach (1000 simulations) was used in MLGsim to calculate the statistical significance of the likelihood statistic (P_{sex}) that any MLGs observed more than once in a population were the result of clonal reproduction. If a P_{sex} value was found to be significantly low ($P \leq 0.001$), the corresponding MLG was considered as likely to have arose by clonal reproduction. Then, using the results of this analysis, a clone-corrected data set was created, where for every location only one isolate was included for each MLG, if clonal MLGs (as indicated by significantly low P_{sex} values) were deemed to be present at a location. Both the full and clone-corrected datasets were then used in a subsequent analysis to determine the effect of possible clonal reproduction and homothallism on population structure and genetic diversity.

MLG assessment and analysis of linkage disequilibrium were conducted using the program MultiLocus v1.2 (Agapow and Burt 2001). These analyses were performed to assess the degree of recombination occurring in both the consensus populations and within the entire

collection of isolates, using both the complete and clone corrected datasets. The populations were analyzed for total number of MLGs, frequency of the most commonly occurring MLG, as well as the genotypic diversity (probability that two individuals taken at random will have the same MLG). In addition, two linkage disequilibrium indices were calculated, the Index of Association (I_A) and a modified version of I_A, $\bar{r}_d$, which is different from I_A in that it is not correlated to the number of loci amplified. Both statistics were analyzed by 1,000 permutations per population data set. Values that were found to be significantly different from 0 indicated linkage disequilibrium or clonal reproduction in that population.

An analysis of molecular variance (AMOVA) was performed to assess whether the consensus groups (with and without clonal isolates) were statistically different from each other using Φ_{PT} statistics in the program GenAlex 6.502 (Peakall and Smouse, 2006, 2012). Inter-population pairwise Φ_{PT} values were calculated between all of the consensus populations with statistical significance at $P \leq 0.01$. Results from the AMOVA analysis were also utilized to determine the variance within and among populations of isolates, as well as to determine how much variance each of the consensus populations contributed to the total within population variance.

Spatial autocorrelation analysis was performed using the program GenAlEx 6.5 (Peakall and Smouse 2006 and 2012) to examine the arrangement of isolate genotypes over geographic space and to assess whether isolate genotypes can be correlated to collection sites. The degree of autocorrelation was calculated using the autocorrelation coefficient (r), which is a measure of genetic similarity between pairs of isolates in a particular distance class. Distance classes were chosen such that an even number of samples were included in each class, and total geographic distance spanned 0 – 4146 km. The r values ranged from -1 (negative spatial autocorrelation) to + 1 (positive spatial autocorrelation), and the significance of r values was calculated by comparing the observed value with those obtained from 999 permutations of the samples. Using

the permuted correlograms, a 95% confidence interval was constructed, and when r fell above the confidence interval (positive r value), significant spatial genetic structure was inferred.

RESULTS

Primer Screening

The 96 SSRs were amplified in each of the four "tester" isolates. Results showed that loci representing all three nucleotide motif types (di, tri, or tetra repeats) resulted in polymorphic alleles with the number of SSR markers resulting in (1, 2, 3, and 4 products) for each repeat type shown in Figure 1. Overall, 34 of the 96 total loci yielded three or four alleles each, with the di-nucleotide repeats resulting in the greatest proportion of polymorphic loci. From these 34 polymorphic SSR markers, 13 were selected for use in this study, along with five additional SSR primers previously tested by Cai et al. (2013). The SSR marker motifs, primer sequences, and expected product size ranges are listed in Supplemental Table 2.

Summary Statistics

Summary statistics for each of the 18 SSR markers used for the population diversity study are listed in Table 1. Major allele frequencies ranged from 0.240-0.624 with a mean value of 0.411. The number of alleles per loci ranged from 8-14 with a mean of 9.5, and the gene diversity values ranged from 0.567-0.868 with a mean of 0.731. The PIC values ranged from 0.520-0.855.

Population Structure Analyses

Phylogenetic analysis

The UPGMA dendrogram placed the isolates into three major clades (Clades I, II, and III) and a basal subgroup of six isolates (Fig. 2).

Clade I (Fig. 3): Clade I primarily holds isolates from the Midwestern U.S. and Ontario, Canada. However, a few isolates from New Jersey, Pennsylvania, Oregon, and Maine are also included. A cluster of isolates numbered 7-13 falls in the most basal position within the clade and consists of all of the isolates collected from New Franklin, MO, and a single isolate from East Lansing, MI (University of Michigan). Strong bootstrap support (0.882) indicates they are

closely related. Interior to this first basal group, and in the most basal position within the remainder of Clade 1, is a group of nine isolates representing a diversity of backgrounds. These span collection origins of Illinois, New Jersey, Michigan, and Ontario, Canada. While Isolates 14 and 15 (Illinois and New Jersey, respectively) did not fall in a statistically supported cluster, they grouped alongside isolates 16 and 17, which have strong bootstrap support, and were collected from Leslie and Merrill, MI, respectively. Adjacent to this isolate pair is another statistically significant clade of isolates (18-22) collected from East Lansing, MI [n=3], and Sparta, Ontario [n=2].

The remainder of the isolates in Clade 1 are interior to those discussed above and are broken up into smaller clusters many with significant bootstrap support. Starting in the topmost position, Isolates 23-36 form a strongly supported (bootstrap value of 0.837) group of 14 isolates from very disparate geographic origins including four isolates from Harrisburg, PA, four from Leslie, MI, two from Boothbay, ME, and the four isolates from Oregon (two from Corvallis and two from Wilsonville). The sister clade to this one contain mostly isolates from New York and Ontario and is divided into three small groups. The upper most group holds Isolates 37-42, which span origins of Toronto, Canada, Western, MN, and Belmont, NY. Adjacent to this group are Isolates 43-54 (bootstrap support of .539), which includes the four isolates from Angelica, NY, and four from Belmont, NY, representing orchards located ~10 km apart. In addition, the three isolates from Niagara-on-the-lake, Ontario, were placed in this group (Niagara-on-the-lake is ~180 km from the NY locations), as well as one isolate from Montevideo, MN. The third small group within this cluster contains Isolates 55-57, which consists of the two isolates from Tustin, MI, and an isolate from Canton, MN.

The remaining isolates within Clade 1 (n=19) are all from Wisconsin and Minnesota (n = 5), except Isolate 65 from East Lansing, MI. They fell into several small, statistically supported groups placed, as a whole, in a sister position to the isolates described previously.

Clade II (Fig. 4): Clade II holds isolates primarily from New Jersey (n=35) and Massachusetts (n=20). A basal cluster holds five isolates (Isolates 77-81) from Jamaica Plain, MA (bootstrap support of 0.890), while the remaining isolates are held in two relatively large interior groups placed in sister positions. The topmost interior group is divided into two main clusters, the uppermost holding Isolates 82-95, which originate from four locations in New Jersey and show very strong bootstrap support (0.964). The lower interior group holds isolates from New Jersey and Massachusetts. It includes two statistically supported isolate pairs: isolates 96 and 97 and isolates 98 and 99, all from New Brunswick, New Jersey. Interior to these are a group of isolates (Isolates 102-107; bootstrap support of 0.887), five of which were collected from Massachusetts and one from New Jersey.

Sister to the group of isolates discussed above (Isolates 82-117), is a group holding Isolates 118-134. These are primarily from New Jersey, although two isolates from Alburtis, PA (Isolates 122 and 126) and one from Findley Lake, NY (Isolate 130) were also placed into this group.

Clade III: (Fig. 5) Clade III contains isolates largely from Ohio, Pennsylvania, and New Jersey, although there are a small number of isolates obtained from a wide region scattered throughout this clade. Eight statistically supported groupings were identified within Clade III, although not all of the isolates fell in statistically supported groups. Four isolates were placed in the most basal position, with Isolates 135 and 136 of this basal group (from Western, MN, and Leslie, MI, respectively), falling together with strong support (.988). The remainder of the isolates of Clade III were placed interior to this basal group and divided into two sister clusters, the upper holding Isolates 139 to 169 and lower holding Isolates 170 to 182.

The upper group is divided into three main clusters, one in a more basal position and two placed interior and sister to each other. The basal cluster holds isolates 139 and 140 from Findley Lake, NY, and New Brunswick, NJ (bootstrap support .948), Isolates 141 and 142 from Sparta, Ontario, and Isolates 143 and 144 from Molt and East Lansing, MI, respectively (bootstrap

support .641). The uppermost of the interior sister clusters holds all of the isolates collected from Newark, OH (Dawes Arboretum) clustered together (Isolates 145-155), although only a subset fell in one of two statistically supported groupings. The lower cluster holds isolates 156-169, which originate from New Brunswick, NJ, as well as Etters and Harrisburg, PA (<20 km apart).

The lower interior sister group of Clade III holds isolates from a diversity of locations including Massachusetts, Pennsylvania, Minnesota, Illinois, and Cortland, Ontario. Statistically supported groups include two isolates from Minnesota and Illinois (Isolates 172 and 173) and all eight isolates from Hop Bottom, PA (175-182).

STRUCTURE analysis

Population genetic analysis using the program STRUCTURE separated the isolates into 42 genetically distinct populations (K=42). The K value was discerned from the graph of Δ K, which was plotted against the K values. The maximal Δ K was 42, which corresponds to the most parsimonious value of K (Supplemental Fig. 1) (Evanno et al., 2005; Earl & vanHoldt et al. 2012). Of the 42 populations, 20 were clearly represented as solid blocks of color in the structure output, which was the criteria used to define a Structure Grouping. The remaining 22 populations were comprised of appreciable levels of admixture across 39 isolates (Supplemental Fig. 2-4). The 20 well-defined STRUCTURE groups are described briefly below.

STRUCTURE groups 1-9 (Supplemental Fig. 2): Isolates 2-6, which were obtained from Courtland, Ontario, McGraw, NY, and Ino, WI, resulted in a solid block of color in the STRUCTURE analysis and comprise STRUCTURE Group 1 (SG 1). Following this approach for designation, STRUCTURE Group 2 (SG 2) includes Isolates 7-13, all but one of which were collected from Franklin, MI. Isolates 15-22 primarily originate from Michigan and Sparta, Ontario, with the exception of one isolate from New Brunswick, NJ, and comprise SG 3. It is worth noting that Isolates 15-17 displayed some admixture, although the vast majority of their genetic backgrounds aligned with SG 3. Next, SG 4 is comprised of Isolates 23-36 whose origins span Boothbay, ME, Leslie, MI, Harrisburg, PA, and two locations in Oregon. SG 5 is comprised

of Isolates 37-42 which originate from Toronto, Ontario, Belmont, NY, and Montevideo and Western, MN. Eight isolates from NY, three from Niagara-on-the-Lake, Ontario, and a single isolate from Minnesota (Isolates 43-54) define SG 6. Further, SG 7 holds eight isolates from throughout Wisconsin. A small group of just three isolates (63-65), two from Wycoff, MN, and one from East Lansing, MI, comprise SG 8. And lastly, SG 9 consists of five isolates collected from Wisconsin (Isolates 72-76).

STRUCTURE groups 10-15 (Supplemental Fig. 3): Isolates 77-81, all of which were collected from Jamaica Plain, MA, comprise SG 10. The entirety of SG 11 holds isolates collected throughout New Jersey (Isolates 82-95), and displayed slight admixture. Further, SG 12 holds just two isolates from New Jersey (Isolates 96-97), both of which also exhibited some admixture. Isolates 102-107, all from Jamaica Plan, MA, except one, were placed in SG 13. SG 14 consists of isolates (Isolates 109-117) also only from Jamaica Plain, MA. Lastly, SG 15 exclusively held isolates collected in New Jersey (New Brunswick and Adelphia)(Isolates118-121).

STRUCTURE groups 16-20 (Supplemental Fig. 4): SG 16 holds only two isolates (135 and 136) from Western, MN and Leslie, MI. SG 17 is comprised of two isolates (139 and 140) collected from New Brunswick, NJ, and Findley Lake, NY. All 11 isolates collected from Newark, OH (145-155) were grouped in SG 18. A mixture of isolates with origins in New Brunswick, NJ, and Etters and Harrisburg, PA, were placed together in SG 19 and displayed minor admixture. Minimal admixture was also resolved in SG 20, which held isolates (175-182), all of which were collected in Hop Bottom, PA.

CONSENSUS groups (Congruence between dendrogram and STRUCTURE analyses)

When comparing the results of the UPGMA clustering analysis (dendrogram) to the results of the STRUCTURE analysis, there were clear instances of congruence, where the STRUCTURE population assignment matched the grouping architecture of the dendrogram (Figs. 3-5). These occurred where all or a portion of each of the 20 STRUCTURE populations/groups

(color patterns) closely matched the majority of the 30 statistically supported clusters of the UPGMA diagram, and provided a means to resolve 22 "consensus" populations based upon the combined analyses (Fig. 3-5). In total, 13 STRUCTURE groups (100 isolates) were fully corroborated by statistically supported dendrogram clusters. The remaining seven STRUCTURE groups were partially corroborated by one or more dendrogram clusters. In contrast, six statistically supported dendrogram clusters were shown to have highly admixed genetic profiles, thus were not supported by a STRUCTURE grouping.

When considering a combination of the two analysis methods, there are at least 13 distinct populations of *A. anomala* sampled in this study. However, this may be an underestimate, as the STRUCTURE analysis and, although to a lesser extent, the dendrogram clustering results, indicate that there are likely at least nine additional possible genetic populations that were not statistically supported independently by both analysis. The high level of genetic diversity and admixture resolved by the markers across the isolates made it difficult to clearly discern the total number of populations of *A. anomala*. Additionally, two main types of population level admixture, which accounted for the remaining 22 STRUCTURE populations, seemed prevalent across a number of isolates in the study, including: 1) isolates exhibiting significant levels of admixture in combination with a plurality population assignment (color block) that matched one of the 22 "consensus" groups (CGs) (e.g. isolates 1, 14, 35, 36, 55, 56, 57…); and, 2) isolates that were entirely comprised of high levels of similar patterns of population admixture (e.g. isolates 170 – 174). In both cases, the admixture pattern as well as a plurality population assignment in a STRUCTURE population/group (when present) helped to visually clarify the genetic relationships depicted in the UPGMA dendrogram (Fig 3–5). For example, isolates 2–6 exhibited a relatively low level of admixture in the STRUCTURE analysis and showed bootstrap support as a group in the UPGMA dendrogram. These five isolates would clearly belong to a consensus group when visualizing the congruence between the two analyses. Isolate 1 appears to be sister to isolates 2–6 in the UPGMA dendrogram, but lacks statistical

support for inclusion in this grouping based solely on the UPGMA analysis. Although Isolate 1 exhibits high levels of admixture, a clear genetic relationship exists between Isolate 1 and Isolates 2–6 in the STRUCTURE analysis (based on the plurality population assignment in the STRUCTURE output), providing justification for combining all of the isolates into a single CG, labeled CG 1. Isolates 170 – 174 could also be combined into a single CG named CG 21 (despite the fact that these isolates lacked a predominant STRUCTURE population assignment), because these isolates clearly shared a very similar pattern of population admixture.

Within Clade I, four Structure Groups (2, 4, 6, and 9) were substantiated by statistically supported dendrogram groupings, and thus named CG 2, 4, 6 and 9. Additional CGs resolved by congruence between the UPGMA dendrogram and the STRUCTURE analysis within Clade I (Fig. 3) included CG3 (isolates 14-22), which was the result of the merging of two statistically supported dendrogram groups with two previously ungrouped isolates (14 and 15 - both of which showed high levels of admixture). CG 5 (isolates 37 – 42 and 55 – 57) was the result of two merged dendrogram pairs and five un-grouped isolates, where only three of the nine isolates (isolates 55 – 57) showed significant levels of admixture. CG7 included isolates 58-61 and 66-69, seven of which had fallen into three separately supported dendrogram groupings. Two statistically supported dendrogram groupings (Isolates 64-65 and 70-71), together with isolates 62-63, were placed in CG8, where all but one of the isolates were collected in Wycoff, MN. Despite the majority of their kinship to SG8, isolates 70 and 71 from Wycoff, MN, showed a distinct pattern of admixture, which could explain their separation from the other four isolates in the dendrogram.

In Clade II, congruence between the UPGMA dendrogram and the STRUCTURE analysis resulted in seven CGs. Statistically supported dendrogram isolate clusters, (Isolates 77-81, Isolates 82-95, and Isolates 102-107 were corroborated by identical STRUCTURE analysis resulting in CG 10, CG 11, and CG 13, respectively. CG12 consisted of Isolates 96-101 as well as Isolates 127-128; this grouping was formed because Isolates 98-101 and 127-128 had similar

admixture profiles, a significant portion of which matched the predominant STRUCTURE population assignment of Isolates 96 and 97. The statistically supported isolate pair 113 and 114 showed a high degree of STRUCTURE population similarity with the surrounding isolates (Isolates 109-117) forming CG14. Despite a high level of admixture, Isolate 108 was also included in CG14 because the majority of the admixture profile matched this CG. CG15 consisted of the dendrogram isolate cluster 118-121, as well as isolates 129 and 131-134, where these latter isolates all showed similar admixture profiles and the plurality of the admixture profile matched that of Isolates 118 - 121.

The highest degree of admixture in Clade II was seen in isolates placed in CG16, none of which were members of a statistically supported dendrogram grouping. In addition, the primary component of the admixture profile of all isolates included in the group (122-126 and 130) was the same STRUCTURE population.

Clade III holds of six CGs. The STRUCTURE analysis fully corroborated two statistically supported dendrogram clades, resulting in CG 20 and CG 22. The remaining CGs were the result of merging smaller statistically supported groups and single isolates. This includes CG17, comprised of Isolates 135-136 and Isolates 137-138, the latter of which displayed significant admixture. Despite the high levels of admixture in the STRUCTURE profiles of Isolates 137 and 138, these isolates showed greater kinship to CG17 than any other STRUCTURE group. Isolate clusters 139-140 and 143-144 were combined with Isolates 141 and 142 to form CG18. Appreciable admixture was observed in Isolates 141-144, although one STRUCTURE population best matched the plurality component of the genetic backgrounds of Isolates 139-140. CG 19 contains two statistically supported dendrogram groupings and three ungrouped isolates. Isolates 145-155 (CG 19) displayed relatively low admixture, and consisted of all of the isolates collected from Newark, OH. As previously mentioned, isolates 170-174 showed high levels of admixture in the STRUCTURE analysis. The nearly identical admixture

profiles set this group of isolates apart from all other isolates and resulted in their own CG (CG21).

Multilocus Genotype (MLG) and Linkage Disequilibrium Analysis

The MLG analysis showed that, in total, the collection of 181 isolates consisted of 141 unique MLGs. Eleven of the consensus populations held at least one pair of clonal isolates from either the same location or a different location, while seven of the consensus populations held at minimum two clonal isolates from the same collection location. Clonal isolates at identical collection sites were identified and subsequently used to create the clone corrected dataset, where all but one isolate per MLG was eliminated for each collection site. A total of 43 clonal isolates accounted for 13 MLGs across the 7 consensus populations (Table 2), indicating that the clonal isolates were spread relatively uniformly among these MLG's. The P_{sex} values for each of the 13 clonal MLGs were found to be significantly low, thus each of the clonal MLGs were likely the result of spread via mycelium within the interconnected canopy of the same tree or an effect of the homothallic nature of *A. anomala* (ascospores from single stroma were found to be haploid with monomorphic SSR profiles) and corresponding to the collection of isolates from related infection events or lineages.

The number of individuals per population in the full dataset ranged from 4 to 14. In the clone corrected dataset (described prior), the number of individuals per population ranged from 4-11. In both the full and clone corrected data set, the total number of MLGs ranged from 3-9 per population across all 22 consensus populations. The frequency of the most common genotype in the complete dataset was seven, but fell to three in the clone corrected data set. The lowest frequency of the most common genotype in any population across both datasets was 1. The multilocus genotypic diversity (probability that two random isolates in a population will have different genotypes) ranged from 0.6-1 across populations in the complete dataset, and 0.73-1 across populations for the clone corrected dataset. The genotypic diversity of the entire complete dataset was 0.9945, while the genotypic diversity of the entire clone-corrected dataset was 0.9982.

Multilocus linkage disequilibrium was assessed for both datasets based on the index of association (I_A) and modified version of I_A, $\bar{r}_d$ (Table 2). Both the complete and clone corrected datasets resulted in significant linkage disequilibrium (clonal reproduction) as shown by the I_A and modified I_A values at $p \leq 0.05$ for the following populations; 1, 2, 3, 4, 5, 7, 8, 12, 14, 15, 17, 18, 19, 20. The remaining eight populations resulted in of I_A and $\bar{r}_d$ values that were not significantly different from zero, thus were not considered to be in linkage disequilibrium.

AMOVA analysis

An AMOVA was performed on both the full and clone corrected data set (Table 3). Both AMOVAs were analyzed based upon the CG assignments, where the clone corrected dataset held fewer isolates in 6 of the populations. In total, 22 consensus groups of isolates were analyzed to determine if they were statistically distinct populations. In both AMOVA outputs, the pairwise ΦPT statistics indicated that each of the 22 populations were found to be statistically different from each other at the $p \leq 0.01$ level of significance. This result illustrates that there are at least 22 statistically supported genetic populations of *A. anomala* in the isolate collection.

In addition to illustrating that the isolate populations were statistically distinct, the AMOVA provided the partitioned population variance. A total of 64% of the variance was apportioned among populations and the remaining 36% was within populations, although this shifted slightly for the clone corrected dataset where interpopulation variance decreased to 61% and within population variance increased to 39%. The within population variance was further partitioned into each of the 22 populations, and found to be fairly similar within each isolate group (Table 3). On a whole, the variance for each of the populations ranged from 1.11% (Population 11) to 10.4% (Population 20) for both the complete and clone corrected datasets.

Spatial Auto-Correlation

A correlogram (Fig. 6) was used to discern the geographic distances (distance classes) at which genetic population structure of *A. anomala* was statistically correlated to collection

location. The extent of genetic structure, or patch size at which intra-population structure was found, was the distance class of 53. Thus, isolates within 53 km of each other tended to have similar or closely related genotypes. In addition, oscillation of the autocorrelation coefficient (r) above and below 0 was observed through the ~400 km distance class and significantly positive correlation between genotypes and location was found at distance classes 224 and 396, which is indicative of micro-spatial structure. This micro-spatial structure implies that there is patchiness (clustering) of isolates with similar genotypes, where (beyond 53 km) small clusters of isolates with similar genotypes tended to aggregate at distances of 224 and 396 km apart.

DISCUSSION

A total of 42 genetic populations were identified through the STRUCTURE analysis (Supplementary Fig. 1). The dendrogram topology and STRUCTURE analysis were compared, and 22 consensus groups (populations) were discerned. These 22 populations were statistically supported by the AMOVA analysis. In addition, further individual and multi-locus genotype analysis of the population structure of our collection of 182 isolates of *A. anomala* from 43 collection sites corroborated the high level of genetic diversity illustrated in both the STRUCTURE and cluster analysis. Overall, these results bolster support of earlier work showing that *A. anomala* is genetically diverse (Cai et al., 2013). Additionally, due to the scope of the sampling range of this study, we were able to resolve clustering of many highly similar genotypes at a regional level.

Based upon the SSR marker statistics, the *A. anomala* isolates sampled in our study were found to be genetically diverse in comparison to other fungal diversity studies using a similar approach and marker system. For example, the mean number of alleles per locus was found to be 5.2 for *Metarhizium anisopliae* var. *anisopliae* (Velásquez 2007), 7.9 for *Magnaporthe grisea* (Adreit et al. 2007), and 9.3 for *Beauveria bassiana* (Wang et al. 2005), all of which was less than the average number of alleles per loci for *A. anomala* (9.5). Further, the mean gene diversity value of *A. anomala* (0.7308) was found to be considerably higher than that of the related ascomycete, *Venturia inaequalis,* where Tenzer et al. (1999) reported that the mean gene diversity across seven SSR loci was 0.46. It was also found to be significantly higher than that of *Magnapothe grisea* (.447) (Adreit, 2007) and *Phytopthora infestans* (0.46) (Lees 2006), the latter of which was analyzed as a diploid.

In addition to loci level genetic diversity, MLG statistics were also indicative of genotype diversity. Of the 141 MLGs identified 128 were represented by one single isolate, thus 70% of the isolates sampled for this study were considered to be genetically unique. The remaining 30%

of the (clonal) isolates were found to be evenly dispersed across nearly half of the consensus populations. The frequency of the most common genotype in the dataset was found to be 7, thus the greatest representation of one MLG in the entire population (dataset) was less than 4%. In addition, multilocus genotypic and genotypic diversity values of the dataset were both nearly 1. High proportions of unique MLGs and low representation of any one genotype further corroborate the presence of high genetic diversity within *A. anomala*. While these results showing high genetic diversity were initially unexpected based on the reproductive morphology of *A. anomala*, other researchers have shown high genotypic diversity is not uncommon for a homothallic fungi. As stated by Taylor et al. 1999, reproductive modes of fungi (i.e. clonal or recombination) are not necessarily exclusively linked to one single reproductive morphology (homothallism or heterothallism). Recombinant genotypes could be due to high marker mutation rates, which is particularly true of SSRs (Li et al. 2002 and Lynch et al. 2008). Unfortunately, there is currently no statistical method to discern the mechanism of recombination for *A. anomala* (Taylor 1999).

Beyond the existence of high genetic diversity within the collection of isolates, statistical analysis of the individual CGs indicated relationships also exists between genotype and geographic collection location. As previously stated, the dendrogram and STRUCTURE analysis were used to guide the development of 22 CGs. Twelve of the consensus groups were ≥ 50% comprised of isolates collected from one location. Thus, isolates collected from the same location tended to cluster together based upon their genotypes. The resulting AMOVA of these consensus groups showed that the variation among all 22 CGs was high (61-64%; complete and clone corrected data set respectively). In comparison, the sum of the diversity within each of these populations was nearly half of the inter-population diversity (36-39%) and was evenly partitioned among all 22 consensus populations (1.1-10.4%). Statistically significant differentiation of these populations as illustrated by ΦPT values (Table 3) show that isolates in consensus groups (many of which were often collected from the same location) were more related to each other than other

isolates from other CGs. The autocorrelation analysis corroborated this finding in that it showed isolates collected within certain proximities (53, 224 and 396 km apart) are more genetically similar than those found further than 443 km apart. The steep peaks and oscillation of the rc values of the correlogram are indicative of microspatial genetic structure, where high density clusters of similar genotypes are separated by intervening gaps of low densities of similar genotypes (Peakall et al 2003, Sokal and Wartenbert 1983, and Smouse and Peakall 1999)

I_A and $\bar{r}_d$ values both lent further insight into the genetic relationships of isolates within each consensus population. The majority of the consensus populations (14 of 22) resulted in significantly high I_A and $\bar{r}_d$ values, which illustrated that those isolate populations were in linkage disequilibrium. Linkage disequilibrium in these populations is indicative of non-random mating (Milgroom 1996). Of the 14 consensus groups containing a large proportion of isolates with the same genotype, the majority of the isolates in each of seven of them were collected from single locations.

The regional genotypic specificity exhibited though the population structure of *A. anomala* supports anecdotal evidence of isolate movement and proliferation throughout the collection sites in North America. One example can be visualized in Fig. 3, which depicts a cluster of isolates collected in Oregon (two from Corvallis and two from Wilsonville). This location is of particular interest because it is a place where the fungus is not endemic and was introduced in the 1960s from an unknown source (Davison and Davidson, 1973). There is significant bootstrap support for this grouping .837, as well as a significantly high I_A value indicating presence of only one isolate genotype. Although the two sample locations in Oregon (Corvallis and Wilsonville) are over 100 km apart, both are represented by nearly identical isolates, which supports previous studies suggesting that infection in the Pacific Northwest originated from a single point introduction (Gottwald and Cameron 1980b; Pinkerton et al 1992). However, additional isolates are needed to substantiate this claim, work which is currently being completed by the authors. Other isolates included in this cluster were collected from Boothbay,

ME, Leslie, MI, and Harrisburg PA. These isolates that were shown to have very similar genotypes, but were collected from disparate locations could be indicative of movement of plant material by humans to or from Oregon, likely through shipments of nursery stock harboring latent infections. As further support, two of the collection sites have record of ordering hazelnut plant material from the same mail order nursery located in Oregon.

In summary, the results of this study shed new light on the diversity and population structure of *A. anomala,* the causal agent of EFB; a disease that is considered to be the greatest limiting factor to hazelnut production in North America. Based on the isolates sampled in our study, *A. anomala* appears to be a very genetically diverse organism that has some isolate specificity on a regional and local scale. Both of these findings can have important implications for managing disease in hazelnut orchards and when attempting to breed hazelnut plants resistant to EFB. For example, two public hazelnut breeding programs in the United States (Oregon State University and Rutgers University) have goals of developing plants that express high, durable resistance to this disease (Mehlenbacher, 1994; Molnar et al., 2005). In our study, the isolates collected from Oregon fell into one genetic population indicating that trees grown in that region may be exposed to only a limited pool of *A. anomala* genotypes (previous work reports *A. anomala* was introduced from single point in 1960s [Gottwald and Cameron1980b]. If existing quarantine measures hold strong in the Pacific Northwest where movement of plants of the *Corylus* genus are restricted from the East (Barss 1930), one could speculate that the limited isolate diversity in the region may result in the longevity of the single R-gene currently being used to protect plants in the region (Mehlenbacher et al 1991). However, on the alternative side of this discussion, breeders screening plants in the region may be limited to working with only a small representation of the fungi, reducing their effectiveness in selecting plants expressing resistance to a wider diversity of isolates (earlier work suggests *A. anomala* expresses pathogenic variation [Capik and Molnar 2012; Molnar et al. 2010a Molnar et al., 2010b]).

In contrast to the scenario in Oregon, isolates collected in New Brunswick, NJ, fell into eight different genetic populations suggesting trees grown in this region are likely to encounter a greater diversity of *A. anomala* isolates. Further, no quarantine measures are in place to prevent the movement of isolates from other regions. This wider diversity may present a more challenging scenario for managing EFB in orchards, many of which are expected to last >35 yrs, as well as identifying and/or developing through breeding trees that express long-lived resistance to EFB. Thus, with the new knowledge that *A. anomala* appears to be a very diverse organism with some regional population structure, a bolstered breeding approach should be considered to develop plants expressing durable forms of resistance. Single gene resistance should be vetted thoroughly across regions (and/or through controlled exposure to known isolates), with multigenic (quantitative) sources of resistance including that from North American native hazelnut *Corylus americana* as well as R-gene pyramiding explored and considered as part of a long-term breeding objectives. Additional breeding strategies should include strengthened regional collaboration among breeding programs to share germplasm and assess resistance to the pathogen across a wide diversity of isolates and genetic backgrounds of the host plants.

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Table 1. Summary statistics for each simple sequence repeat used in the analysis. Summary statistics include Major allele frequency, allele number, gene diversity and Polymorphism Information Content (PIC).

Marker	Major Allele Frequency	Allele Number	Gene Diversity	PIC
Aa02944	0.4598	8	0.7141	0.6792
Aa29706	0.3107	9	0.7836	0.7518
Aa31542	0.5200	9	0.6662	0.6298
Aa22416	0.2400	13	0.8680	0.8549
Aa23178	0.2824	14	0.8592	0.8463
Aa24810	0.4167	10	0.7523	0.7237
Aa18854	0.3729	15	0.8144	0.7997
Aa13813	0.3353	9	0.7583	0.7218
Aa24840	0.5361	7	0.6645	0.6351
Aa32255	0.3394	11	0.7528	0.7175
Aa23930	0.6236	6	0.5634	0.5253
Aa01237	0.4286	8	0.7304	0.6952
Aa26466	0.5000	8	0.6640	0.6189
Aa38820	0.3333	13	0.7718	0.7381
Aa02342	0.4886	7	0.6663	0.6164
Aa00689	0.3333	6	0.7204	0.6683
Aa03927	0.2697	12	0.8385	0.8201
Aa16574	0.6089	6	0.5670	0.5199
Mean	**0.4111**	**9.5**	**0.7308**	**0.6979**

Table 2. Multilocus Genotype and Linkage Disequilibrium analysis results for each of the 22 consensus populations, and full data set with and without clonal genotypes.

Population	No. Isolates (*N*)	No. MLG (*g*)	Frequency of the most common MLG	$\hat{G}^Z$	I_A^Y	$\bar{r}_d^X$	Isolates with repeated MLGs
1	6	6	1	1	1.72***	0.12***	
2	7	6	2	0.95	0.86*	0.21*	
3	9	9	1	1	1.31**	0.11**	
4	14	9	3	0.91	0.90**	0.13**	(OR_Corvallis_2; OR_Wilsonville_1; OR_Wilsonville_2)(PA_Harrisburg_3; PA_Harrisburg_4; PA_Harrisburg_6)
4[nc]	11	9	2	0.95	0.67*	0.10*	
5	9	7	2	0.94	1.38***	0.17***	
6	12	5	6	0.74	0.417[ns]	0.14[ns]	(Ontario_Niagra_on_the_Lake_2; Ontario_Niagra_on_the_Lake_3)(NY_Belmont_3; NY_Belmont_5)(NY_Angelica_1; NY_Angelica_2; NY_Angelica_3; NY_Angelica_4)
6[nc]	7	5	3	0.86	0.32[ns]	0.11[ns]	
7	8	8	1	1	0.57*	0.04*	
8	6	6	1	1	1.45**	0.15**	
9	5	5	1	1	-0.05[ns]	-0.02[ns]	
10	5	5	1	1	0.43[ns]	0.11[ns]	
11	14	3	7	0.60	0[ns]	0[ns]	(NJ_New_Brunswick_3; NJ_New_Brunswick_14; NJ_New_Brunswick_15; NJ_New_Brunswick_34; NJ_Rahway_1; NJ_Rahway_2)(NJ_Cream_Ridge_5; NJ_New_Brunswick_1; NJ_New_Brunswick_9; NJ_New_Brunswick_18; NJ_New_Brunswick_35; NJ_Rahway_3; NJ_Rahway_4)
11[nc]	6	3	3	0.73	0[ns]	0[ns]	

[nc] Indicates populations where the all but one clonal isolate per location was taken out.

[Z] The probability that two random individuals in a population have a different MLG.

[Y] Index of association where P values are (ns= not significant, *= p≤ 0.05, **= p≤ 0.01, ***= p≤ 0.001)

[X] $\bar{r}_d$ is a modified I_A statistic, where the value is independent of the number of loci analyzed. P values are ([ns]= not significant, *= p≤ 0.05, **= p≤ 0.01, ***= p≤ 0.001)

Population	No. Isolates (*N*)	No. MLG (*g*)	Frequency of the most common MLG	$\hat{G}$[a]	L_A[b]	$\bar{r}_d$[c]	Isolates with repeated MLGs
12	8	7	2	0.96	1.06**	0.10**	
13	6	3	3	0.73	0.694 ns	0.23 ns	MA_Jamaica_Plain_8; NJ_New_Brunswick_24
13nc	6	3	3	0.73	0.69 ns	0.23 ns	
14	10	9	2	0.98	1.01**	0.158**	MA_Jamaica_Plain_15; MA_Jamaica_Plain_17
14nc	9	9	1	1	0.87*	0.13*	
15	9	9	1	1	1.45***	0.13***	
16	6	6	1	1	0 ns	0 ns	
17	4	4	1	1	1.3*	0.17*	
18	6	6	1	1	1.45***	0.14***	
19	11	10	2	0.96	0.73*	0.08*	
20	14	6	5	0.81	1.14**	0.17**	(NJ_New_Brunswick_23; NJ_New_Brunswick_30; NJ_New_Brunswick_31; NJ_New_Brunswick_32; NJ_New_Brunswick_33) (NJ_New_Brunswick_10; PA_Etters_1; and PA_Etters_3) (NJ_New_Brunswick_20; NJ_New_Brunswick_21)
20nc	8	6	3	0.89	1.18**	0.17**	
21	5	5	1	1	0.61 ns	0.04 ns	
22	8	7	2	0.96	0.06 ns	0.01 ns	PA_Hop_Bottom_4; PA_Hop_Bottom_6
22nc	**7**	**7**	**1**	**1**	**-0.02 ns**	**0 ns**	
Total Population	**182**	**141**	**7**	**0.9945**	**2.07*** **	**0.12*** **	
Total Populationnc	**158**	**141**	**3**	**0.9982**	**1.71*** **	**0.10*** **	

Table 3. Analysis of molecular variance (AMOVA) and apportionment of total within population variance into 22 populations, with and without clonal isolates.

Population	No	Df	Sum of Squares within Populations	Variance Components within Populations	Proportion of Total Variance
1	6	5	26	5.2	6.51
2	7	6	9.429	1.57	2.36
3	9	8	24.778	3.097	6.2
4	14	13	23.786	1.83	5.96
4[nc]	11	10	20.182	2.02	5.09
5	9	8	21.111	2.64	5.29
6	12	11	7.667	0.698	1.92
6[nc]	7	6	7.667	1.28	1.9
7	8	7	29.875	4.268	7.48
8	6	5	21.667	4.33	5.43
9	5	4	6.2	1.55	1.55
10	5	4	9.6	2.4	2.4
11	14	13	4.429	0.34	1.11
11[nc]	6	5	4.429	0.89	1.12
12	8	7	23.75	3.39	5.95
13	6	5	4.833	0.967	1.21
13[nc]	6	5	4.833	0.97	1.22
14	10	9	17.1	1.9	4.28
14[nc]	9	8	17.1	2.14	4.31
15	9	8	26.444	3.31	6.62
16	6	5	19	3.8	4.76
17	4	3	14	4.67	3.5
18	6	5	21.167	4.23	5.3
19	11	10	10.727	1.07	2.69
20	14	13	41.5	3.19	10.4
20[nc]	8	7	41.5	5.93	10.46
21	5	4	24.4	6.1	6.11
22	8	7	11.75	1.68	2.94
22[nc]	7	6	11.75	1.96	2.96

[nc] Indicates populations where the all but one clonal isolate per location was taken out.

Figure 1. Results of screening of 96 simple sequence repeat markers with pooled DNA from four *Anisogramma anomala* isolates and the number of products resolved for each repeat type.

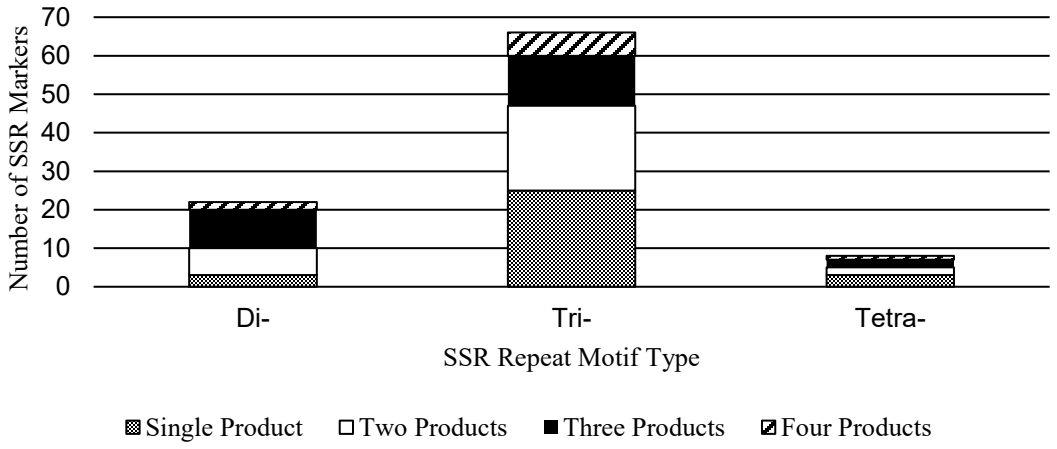


Figure 2. Overview of the collapsed UPGMA dendrogram clades and basal subgroup. Statically supported nodes (Bootstrap support of 1000 iterations) are noted where appropriate.

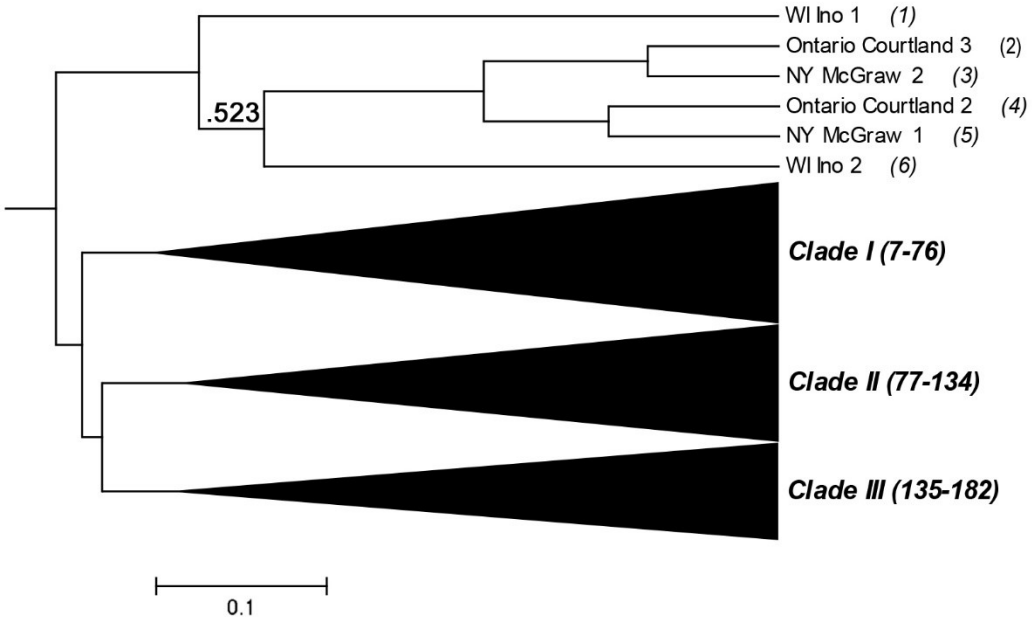


Figure 3. Expansion of Clade I (Midwest clade) of the UPGMA dendrogram alongside the STRUCTURE analysis output for the accessions shown. Consens us groups are labeled CG. Statically supported nodes (Bootstrap support of 1000 iterations) are noted where appropriate.

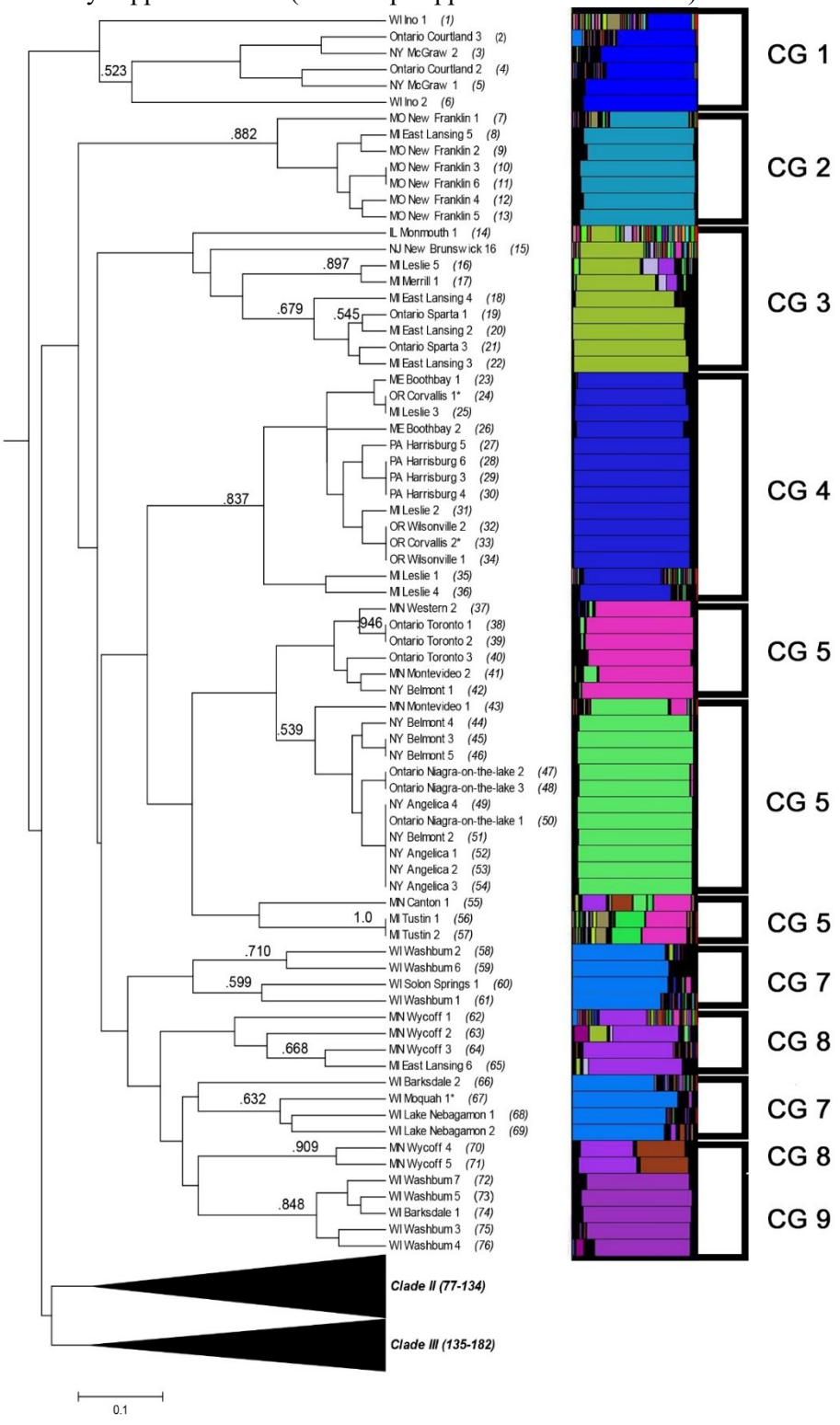


Figure 4. Expansion of Clade II (Northeastern clade) of the UPGMA dendrogram alongside the STRUCTURE analysis output for the accessions shown. Consensus groups are labeled CG. Statically supported nodes (Bootstrap support of 1000 iterations) are noted where appropriate.

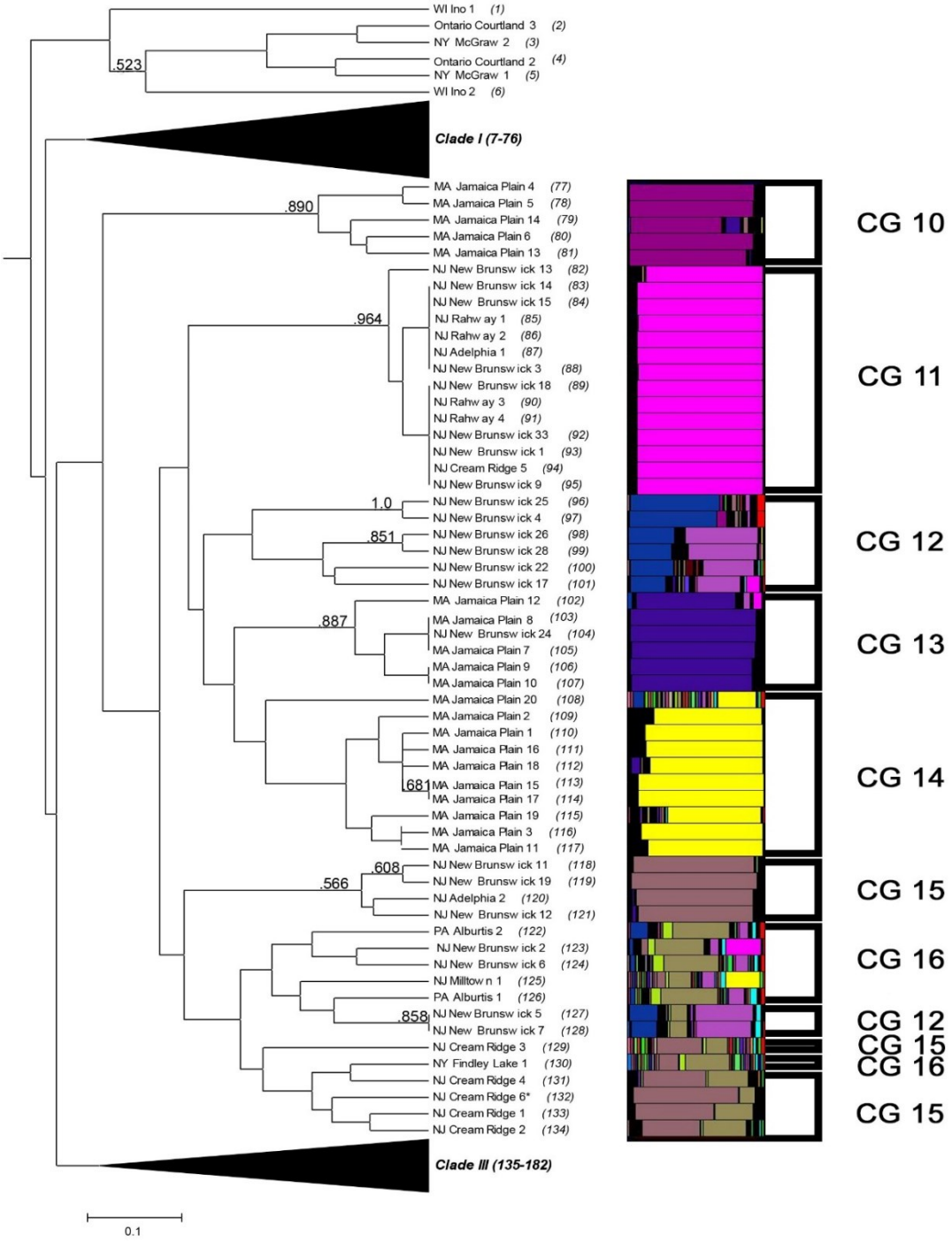


Figure 5. Expansion of Clade III (Central states clade) of the UPGMA dendrogram alongside the structure analysis output for the accessions shown. Consensus groups are labeled CG. Statically supported nodes (Bootstrap support of 1000 iterations) are noted where appropriate.

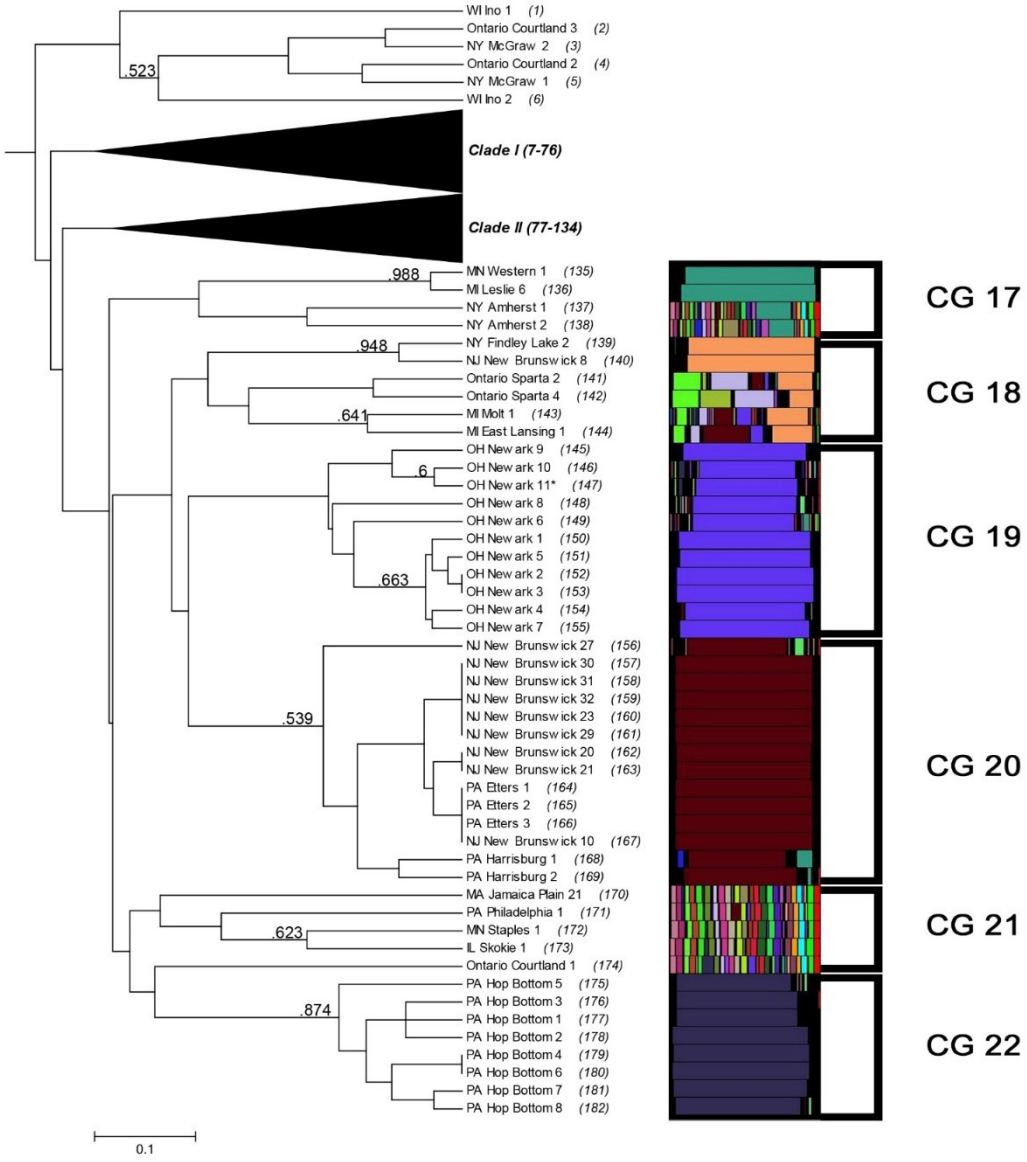
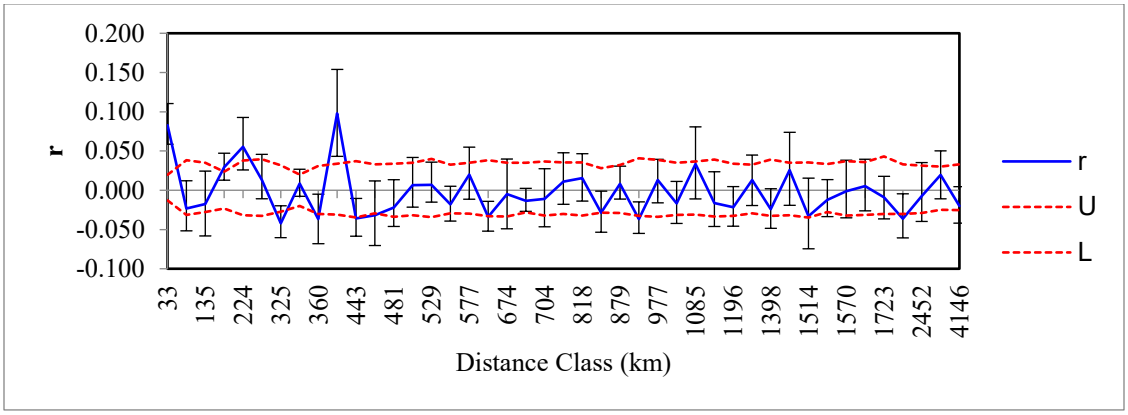


Figure 6. A spatial correlogram, depicting the correlation between the genetic and physical distance between isolates. The correlation coefficient is denoted as (r) on the vertical axis. The upper and lower boundaries of statitical significance at the P<.001 level are denoted by the boundary lines U and L.



Chapter 4: Lipid profiles of a diverse collection of Corylus species and interspecific hybrids

ABSTRACT

Hazelnuts (*Corylus* sp.) are a low-input, perennial crop that hold potential as a source of healthy culinary oil and feedstock for biofuels. Until recently, production of hazelnuts in the eastern United States was limited due to the fungal disease eastern filbert blight (EFB). Over the past several years, a number of sources of resistance to EFB have been identified and are being used in breeding. To date, however, little has been done to characterize the oil content and lipid profiles of these EFB-resistant breeding parents. In this study, the total kernel oil content and fatty acid profiles of four *C. avellana* cultivars were compared to nine diverse, EFB-resistant *Corylus* accessions representing three wild species (*C. americana, C. heterophylla,* and *C. colurna*) and various interspecific hybrids with *C. avellana*. Results showed that, on average, the wild/interspecific hybrid accessions had a lower kernel oil content—60.3%—than the pure *C. avellana* cultivars—67.2%—although there was some variation across accessions. Results also showed that the 13 accessions had a relatively uniform fatty acid profile, which was comprised mostly of oleic acid (~80%) and linoleic acid (~11%). Linoleic acid content was found to be more variable than oleic acid content. The relatively high kernel oil content and consistent fatty acid profile (high unsaturated/saturated FA ratio) suggest that multiple hazelnut species may be ideal for use as both a bio-fuel feedstock and a culinary oil. Minor (or simple) selection of breeding parents would help overcome the slight oil content deficiencies and allow for the use of wild species in the development of new, more widely adapted cultivars.

INTRODUCTION

Hazelnuts (*Corylus* sp.) are a widely adapted tree nut crop that hold potential as a low-input perennial feedstock for biofuels and other oleochemicals, in addition to being a healthy culinary oil. Estimates of hazelnut oil yields are ~1000 kg/ha, which is significantly higher than current soybean oil yields of ~500 kg/ha (soybean oil is the primary biodiesel feedstock in the United States) (Hanna et al., 2005; Xu and Hanna, 2009a, 2009b). Furthermore, hazelnut oil contains ~80-85% monosaturated oleic acid, in comparison to only ~20% found in soybean oil, which increases its value for a number of chemical and food applications. (Alasalvar et al., 2009; Demchick et al., 2014; Ebrahem et al., 1994; Hanna et al., 2005; Özdemir et al., 2001; Parcerisa et al., 1998; Xu et al., 2007). Hazelnut oil was also shown to have a ~12 ˚C higher onset oxidative temperature (higher oxidative stability) than soybean oil (Xu et al., 2007), making it even more attractive as a biofuel feedstock.

The fungal disease eastern filbert blight (EFB; *Anisogramma anomala*) has limited the regions in North America where hazelnuts could be grown successfully without expensive control measures (Johnson et al., 1996; Thompson et al., 1996). Over the past decade, a number of sources of resistance to this disease have been identified, including multiple *Corylus* species and interspecific hybrids as described in Capik and Molnar (2012) and Muehlbauer et al. (2014). Many of these sources of resistance are now being used in breeding efforts to develop new, EFB-resistant cultivars.

In this study, the total kernel oil content and fatty acid profiles of four *C. avellana* cultivars were compared to nine diverse, EFB-resistant *Corylus* accessions representing three wild species (*C. americana, C. heterophylla,* and *C. colurna*) and various interspecific hybrids with *C. avellana*. The information generated will be used to better understand the oil characteristics of the wild species available for use in breeding, which may guide future efforts to improve the oil content and fatty acid profiles of hazelnuts adapted to regions where EFB is present.

MATERIALS AND METHODS

The 13 hazelnut accessions evaluated in the study are listed in Table 1. Mature nuts were collected in fall 2013 from Rutgers University, New Brunswick, NJ, and Oregon State University (OSU), Corvallis, OR, USA. The nuts were washed and then air dried for 4 weeks in mesh bags and subsequently stored in a cooler at 4 ˚C. Fifty nuts were sampled from each accession. Kernels were removed from their shells and finely ground using a mortar and pestle. Approximately 21 g of ground kernel meal were obtained from each accession and divided equally into three sub-samples. The exact weight of each sub-sample was recorded and oil was extracted from each using 150 ml of hexane in a soxhlet extractor for eight hours. Following the extraction, hexane was evaporated in a Buchi Rotary Evaporator until the oil weight was consistent. The remaining hazelnut oil was then weighed. The following formula was used to derive the total oil percentage: (Total oil content [g]/ Total fresh weight of hazelnuts [g]) × 100 = total percentage of oil.

Oil samples from each accession were transesterified to produce fatty acid methyl esters (FAME) as described by Sukhij and Palmquist (1988), in preparation for analysis on the gas chromatograph (GC). Approximately 50 mg of oil was added to each of three vials per accession and was methylated using methanolic HCL following an adaption of the protocol by Sukhij and Palmquist (1988). Samples were run on a GC using a Thermo Scientific™ TRACE™ TR-WAX GC Column with a Supelco 37 Component Fatty Acid Methyl Ester Mixture (Sigma Aldrich). A 4 mg heptadecanoic acid standard was added to each sample. The GC-FID analysis for FAMES was as follows. Injection: 0.5 µL injection volume, 1:25 split injection; Column: Alltech EC-WAX, 30 m, 0.25 mm I.D., 0.25 µm film; Inlet: 220 ˚C, 71.8 kPa, 23.7 mL/min, He: 0.5 mL/min; Gradient -Initial: 60 ˚C, hold 1 min; 10 ˚C/min to 220˚C, hold 23 mins; 5˚C/min to 240 ˚C, hold for 16 minutes. Each accession sub-sample was analyzed three times and an average was taken for the final results.

The Waller-Duncan test was used to evaluate the differences in percent oil content and individual fatty acid components using the statistical software SAS 9.4 (SAS Institute., Cary, NC, USA). Data were then subjected to a one-way analysis of variance to examine whether total percent oil content across accessions was statistically significant.

RESULTS

In general, the wild/interspecific hybrid accessions had a lower kernel oil content than the *C. avellana* cultivars, although there was some measurable variation across accessions (Figure 1). The average total oil content of the four *C. avellana* cultivars was 67.2%, whereas the average of the wild/interspecific hybrid accessions was 60.3%. More specifically, the average of the three accessions related to *C. americana* (CCOR 715.001, OSU 531.016, and National Arbor Day Foundation [NADF] #10) was 57.2%. The average of the five *C. heterophylla* accessions was 62%. The single *C. colurna* × *C. avellana* hybrid Chinese Trazel #6 was 61%. Across the study, *C. avellana* 'Sacajawea' had the highest oil content (69.4%), with *C. americana* CCOR 715.001 from Michigan having the lowest (45.3%). It is notable, however, that *C. americana* OSU 531.016 had a kernel oil content of 67%, which was not significantly different from any of the *C. avellana* accessions at the p=.05 level.

All 13 accessions had similar proportions of fatty acids but varying individual percentages of fatty acids within their fatty acid profiles, which were comprised mostly of the monounsaturated fatty acid oleic acid (~80%; 18:1) and polyunsaturated linoleic acid (~11%; 18:2). Small amounts of saturated palmitic acid (16:0) and stearic acid (18:0) were also found for each accession (Table 2). The average oleic acid content of the four *C. avellana* cultivars was 80.0%, which was similar to the average for the nine wild/hybrid accessions of 82.1%. More specifically, the oleic acid percentages of the accessions ranged from a high of 85.2% (*C. heterophylla* OSU 404.026) to a low of 77.7% (*C. heterophylla* × *C. avellana* hybrid 'Estrella #1'). Interestingly, while the relative oleic acid contents appeared similar across all accessions, most were able to be differentiated as unique by the Waller-Duncan test (p=.05).

The results showed that linoleic acid content was more variable than oleic acid content. The average percentage of linoleic acid in the four *C. avellana* cultivars was 12.3% and the average of the nine remaining wild/interspecific hybrid accessions was 11.4%. The accession

with the highest percentage of linoleic acid was the *C. heterophylla* hybrid China #18 (20.3%), while the lowest was the *C. colurna* × *C. avellana* hybrid Chinese Trazel #6 with 6%.

DISCUSSION

Our study reflected other published work reporting that hazelnut kernels contain between 60-65% oil by weight (Ebrahem et al., 1994; Özdemir et al., 2001; Parcerisa et al., 1998; Xu et al., 2007; Xu and Hanna, 2009a, 2009b, 2010). Our results showed that, on average, the wild/hybrid accessions have a lower percent kernel oil content compared to the *C. avellana* cultivars, although several accessions had oil contents approaching that of the cultivars. While most published work is focused on the commercially important *C. avellana*, Xu and Hanna (2009b) found that the overall average oil content of 20 different NADF hybrids (*C. americana* × *C. avellana*) over two years was 58.2%. Their finding was similar to the 60.23% found for our accessions related to *C. americana*. However, they also found variation within accessions ranging from 46.4 to 67.2%. While a much larger study including more than one year of harvest must be completed, our results suggest that significant variation exists in kernel oil content in the wild species' and interspecific hybrids. Thus, when breeding goals include developing new EFB-resistant cultivars with high oil yields, it may be important to evaluate the wild accessions for their percent oil content prior to use in breeding. It should be mentioned, however, that the heritability of oil traits in hazelnut has yet to be studied in detail.

Also reflecting other published studies on hazelnut, our results showed that there are four main fatty acid components of the oil (Demchick et al., 2014; Ebrahem et al., 1994; Hanna et al., 2005; Özdemir et al., 2001; Parcerisa et al., 1998, Xu et al., 2007; Xu and Hanna, 2009b, 2010). The vast majority (over 90%) of the fatty acids present in hazelnut oil are unsaturated oleic and linoleic acid, with nearly all of the accessions containing ~80% oleic acid (although some minor variation was observed). More relative variation was observed in the linoleic acid content than the oleic acid content. Depending on the downstream application of the oil, it may be of value to select for breeding parents with lower linoleic acid; however, as discussed in a number of the papers referenced above, the current fatty acid profile of hazelnut oil (including the minor variation present) still represents an improvement over many other oil seed crops in current use.

In conclusion, the relatively high kernel oil contents and consistent fatty acid profiles (high unsaturated/saturated FA ratio) suggest that multiple hazelnut species are ideal for use as both a bio-fuel feedstock and as a source of healthy culinary oil. With some minor selection of parents beforehand, our results suggest that the use of wild species should present a viable means to develop new, more widely adapted oil-producing cultivars.

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Table 1. Cultivar/accession name, species, and origin of the hazelnut (*Corylus* sp.) accession evaluated in this study.

Cultivar/accession	Species/parentage	Origin
Barcelona	*Corylus avellana*	Spain
Jefferson	*C. avellana*	Oregon, USA
Sacajawea	*C. avellana*	Oregon, USA
Yamhill	*C. avellana*	Oregon, USA
CCOR 715.001	*C. americana*	Michigan, USA, [z]PI 617728
[y]OSU 531.016	*C. americana*	Michigan, USA
[x]NADF #10 (11-55)	*C. americana* × *C. avellana*	Nebraska, USA
Chinese Trazel #6	*C. colurna* × *C. avellana*	J.U .Gellatly, British Columbia, Canada, PI 557261
OSU 404.010	*C. heterophylla*	OSU seed selection from Suweon, South Korea
OSU 404.026	*C. heterophylla*	OSU seed selection from Suweon, South Korea
China #18	*C. heterophylla* × *C. avellana*	Dailan, China via the University of Nebraska, Lincoln, USA
Estrella #1	*C. heterophylla* × *C. avellana*	Michigan, USA, PI 557351
OSU 526.041	*C. heterophylla* 'Oygoo' × *C. avellana*	Oregon, USA

[z]PI = Plant Introduction number in the National Plant Germplasm System Germplasm Resource Information Network http://www.ars-grin.gov/.

[y] Oregon State University, Oregon, USA

[x] National Arbor Day Foundation, Nebraska, USA

Table 2. Fatty acid profiles of 13 accessions of hazelnut (Corylus sp.) recorded as the average percentage of each of the four primary components of the total fatty acid content of the oil.

Accession	Oleic Acid	Linoleic Acid	Palmitic Acid	Stearic Acid
Barcelona	79.60 I[Z]	12.99 D	5.42 DE	1.99 D
Jefferson	82.15 F	9.69 I	5.70 B	2.42 C
Sacajawea	80.18 H	11.81 F	5.57 CD	2.45 C
Yamhill	78.18 J	14.62 B	5.12 F	2.08 D
CCOR 715.001	80.64 G	12.81 E	4.02 H	2.05 D
OSU 531.016	83.73 C	10.70 H	3.74 I	1.57 F
NADF #10	82.31 E	11.13 G	4.59 G	1.96 D
Estrella #1	77.65 K	13.50 C	6.45 A	2.08 D
Chinese Trazel #6	84.69 B	5.97 I	5.66 C	3.27 A
OSU 404.010	82.74 D	12.95 D	3.02 J	1.20 G
OSU 404.026	85.20 A	7.13 K	4.68 G	2.92 B
China #18	78.18 J	20.34 A	5.25 EF	1.74 E
OSU 526.041	83.95 C	7.71 J	5.35 E	2.92 B

[Z]Means separation of the percentage of each individual fatty acid component between accessions at the p=.05 level of significance.

Figure 1. Average percentage of oil in the kernels of 13 accessions of hazelnut (*Corylus* sp.). Means with the same letter above the bars indicates that they are not significantly different at the *p*=.05 level.