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Citation	Mehlenbacher, S. A. (2014). Geographic Distribution of Incompatibility Alleles in Cultivars and Selections of European Hazelnut. Journal of the American Society for Horticultural Science, 139(2), 191-212.
DOI	
Publisher	American Society for Horticultural Science
Version	Accepted Manuscript
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Geographic Distribution of Incompatibility Alleles in Cultivars and Selections of European Hazelnut

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Additional index words. *Corylus avellana*, filbert, sporophytic, pollenizer, nut breeding

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Received for publication 29 October 2013. Accepted for publication _____.

The OSU hazelnut breeding program is supported by State, Hatch Act and Oregon Hazelnut Commission funds. Additional support was provided by a specific cooperative agreement with the U.S. Dept. of Agriculture.

A technical paper of the Oregon Agricultural Experiment Station.

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Abstract. The european hazelnut (*Corylus avellana* L.) is native to most of Europe and nearby areas in Asia Minor and the Caucasus Mountains. Cross-pollination is enforced by sporophytic incompatibility under the control of a single locus with multiple alleles (haplotypes). Fluorescence microscopy is routinely used to determine if a pollination is compatible or incompatible, and use of an array of known testers allows identification of the alleles of cultivars and selections. Both alleles are expressed in the stigmas, but often only one is expressed in the pollen because of dominance. Cultivars are highly heterozygous diploids ($2n = 2x = 22$) and clonally propagated. Most of the world's leading cultivars were selected from local wild populations near where they are now planted on a commercial scale. Genetic improvement efforts are recent and, although tremendous genetic variability is available, such efforts have had little impact outside of Oregon (USA) and France. Studies of genetic diversity using simple sequence repeat (SSR) markers have placed most cultivars in one of the four main groups: Spanish-Italian, Central European, English or Black Sea. This study presents 17 years of data on incompatibility in hazelnut, including the discovery of six new S-alleles and determination of the dominance relationships among 105 new pairs of alleles. The total number of alleles now stands at 33. The S-alleles of 284 cultivars, 13 interspecific hybrids and 522 selections of diverse origin are presented. The S-alleles identified in hazelnut cultivars is information that should be useful to breeders in the planning of crosses, to curators of germplasm collections, and to growers and nurseries as they choose cultivars and pollinizers when designing orchards. Differences in S-allele frequency seen in the cultivars and selections are related to geographic origin. The most common alleles of cultivars in the major geographical groups are S_2 in the Spanish-Italian group, S_5 in the Central European group, S_3 in the English group and S_4 in the Black Sea group. Most

selections belonged to the Black Sea group, and S_4 was by far the most common allele. Differences in allele frequency were also observed among seed lots within a country.

Keywords *Corylus avellana* sporophytic filbert pollen stigma

Introduction

European hazelnut, *Corylus avellana* L., a member of the Betulaceae, is monoecious, dichogamous, and wind-pollinated. Hazelnuts thrive in maritime climates where temperatures are moderate in winter and summer. The major world producers of hazelnuts are Turkey and Italy, with additional production in Azerbaijan, United States of America, Georgia, Spain, Iran, China, and France (FAOSTAT, 2011). Hazelnuts were cultivated in the Roman Empire, and much earlier near the Black Sea in Turkey and the Caucasus. Most of the world's production is based on selections from local wild populations, with different cultivars grown in each zone. Most cultivars are single clones, although some, including a few leading Turkish types (e.g., Tombul), appear to be groups of clones with similar phenotypes. Cultivars are traditionally propagated from suckers or by layerage, although grafting and in vitro culture are also possible. The local origin of important cultivars, and their limited movement from one production zone to another, contrasts sharply with the situation with the major food crops.

Pollen-stigma incompatibility in hazelnut is of the sporophytic type and controlled by a single locus, designated the S-locus, with multiple alleles (haplotypes), and the stigmatic surface is the site of the incompatibility reaction (Thompson, 1979a). Thompson (1979b) listed the alleles of several cultivars. Additional early work on S-allele identification was reviewed by Germain (1994). Hampson et al. (1993) used electron microscopy to study compatible and

incompatible pollinations in detail. Hazelnuts are diploid ($2n = 2x = 22$), and most cultivars are heterozygous at the S-locus. Fluorescence microscopy is used routinely to determine if a pollination is compatible or incompatible, and to identify the S-alleles in cultivars and selections (Mehlenbacher, 1997b). Mehlenbacher (1997a) reported 25 S-alleles, and for each allele identified a tester genotype whose pollen expresses that allele. Mehlenbacher (1997a) updated the results of Mehlenbacher and Thompson (1998) and presented dominance relationships based on 233 pairs of alleles. In all pairs, both alleles were expressed in the stigmas, but often only one was expressed by the pollen because of dominance. The dominance hierarchy is linear with 8 levels (Mehlenbacher, 1997a). By convention, the allele(s) expressed in the pollen are underlined. Recently, Martins et al. (2012) investigated the S-alleles of Portuguese landraces, and Mehlenbacher (2013) listed the S-alleles of 282 cultivars. The linkage map of Mehlenbacher et al. (2006), constructed using random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers, placed the S-locus on linkage group 5 (LG5).

Self-pollination of most cultivars results in very low nut set. Mehlenbacher and Smith (1991) identified partial self-compatibility in 'Montebello', 'Tombul' and a few offspring of 'Montebello'. In this material, self-pollination unfortunately resulted in low nut set and a high frequency of blanks. More recently, Mehlenbacher and Smith (2006) identified self-compatible seedlings of the cutleaf hazelnut [*C. avellana* L. f. *heterophylla* (Loud.) Rehder]. In these seedlings, self-pollination results in good nut set and few blanks. The cutleaf hazelnut has alleles S_{20} and S_{28}. The latter gives self-compatibility when combined with a second allele low in the dominance hierarchy.

In this study, I identified six new S-alleles and determined the dominance relationships for 105 new pairs of alleles. I determined the alleles of 522 selections originating from nuts

imported from many locations, and examined the data for cultivars and selections for geographic patterns in the distribution of S-alleles.

MATERIALS AND METHODS

The hazelnut breeding program at Oregon State University (OSU), in cooperation with the United States Department of Agriculture, Agriculture Research Service, National Clonal Germplasm Repository (USDA-ARS-NCGR) in Corvallis, imported scions of cultivars from Georgia, Azerbaijan and several European countries. The scions were grafted to rooted layers, held in post-entry quarantine for two growing seasons, and then planted in the field. When the trees began to flower, generally the 4th year after planting, incompatibility testing was performed as described by Mehlenbacher (1997b). Two to four branches on each tree were marked, emasculated by clipping the catkins, and enclosed in two bags: an inner bag of Tyvek housewrap (DuPont, Wilmington, DE) and an outer bag of a cotton-polyester blend (Smith and Mehlenbacher, 1994). Pollen was collected from tester trees (Table 1) and stored in the freezer at -18 °C. From mid-January to early March, when styles had emerged and were receptive, females were collected from bagged branches using forceps, and brought to the lab in petri dishes. Pollinations were performed in the laboratory the afternoon after collection by holding the female with forceps, dipping the styles into the vial of pollen, tapping the flower on the side of the vial to remove excess pollen, and placing the pollinated flower on a double layer of moist P5 filter paper (Fisher Scientific, Pittsburgh, PA). Unpollinated flowers were held in the refrigerator for a few days in petri dishes over moist filter paper to allow repetition of the pollinations if the first results were unclear. The day following pollination, styles were detached from the buds, squashed in aniline blue dye, and examined at 100× with a fluorescence

microscope under ultraviolet light. Each pollination was scored as compatible or incompatible. In compatible pollinations, pollen germination was excellent and tubes could be seen growing parallel down the style. In incompatible pollinations, germination was often reduced, pollen tubes were short and did not penetrate the stigmatic surface, and the tubes often ended in bulbs. In most cases, the use of fresh, unpollinated female flowers and viable tester pollen made it easy and quick to distinguish the two reactions. When two of the testers gave incompatible pollinations and all others were compatible, the two alleles in the female parent had been identified. Some pollinations gave inconclusive results, and the number of flowers on young trees was limited, so testing an introduced cultivar required 1-3 years for completion.

The OSU hazelnut breeding program collected germplasm as seeds from several countries, including Turkey, Georgia, Azerbaijan, Armenia, Russia, Ukraine and Iran (Table 2). These countries represent the eastern part of the distribution of *C. avellana*. The seeds were stratified and planted in the greenhouse as root tips emerged 3-5 months later. Seedlings were grown in 3.8-L containers in a glasshouse the first summer, and transplanted into the field in October. The S-alleles in the selected selections were identified using the same procedures as for the cultivars. Their flowering generally begins in the 4^{th} year in the field. Prior to identification of their S-alleles, seedlings were selected for precocity, round nut shape, few defects, and few buds blasted by mites (primarily *Phytoptus avellanae* Nal.).

When a cultivar or selection representing a new pair of S-alleles was identified, its pollen was placed on female inflorescences of two different cultivars or selections. Each expressed one of the two alleles in the new pair, the other allele being different. If one of these pollinations was compatible and the other incompatible, the allele common to both parents in the incompatible pollination was dominant to the other allele in the cultivar or selection being tested. If both

pollinations were incompatible, the two alleles in the cultivar or selection were considered to be codominant in its pollen. Females of a third genotype expressing different alleles were pollinated to verify that the selection's pollen was viable.

If two of the testers gave incompatible pollinations and all others were compatible, the two alleles had been identified. A single incompatible reaction and 24 compatible reactions indicated either the presence of one known and one unknown allele or homozygosity. If pollen of a cultivar or selection was compatible on all known alleles, it was considered a potential tester for a novel S-allele. Testers that express the novel allele in their pollen, and produce large quantities of pollen early in the season, are preferred.

S_{28} from the cutleaf hazelnut, which gives self-compatible seedlings on some combinations (Mehlenbacher and Smith, 2006), was excluded from this study, as its presence cannot be detected by standard procedures.

Based on simple sequence repeat markers, Boccacci et al. (2006) and Gökirmak et al. (2009) assigned most hazelnut accessions to one of four groups: Spanish-Italian, Central European, English and Black Sea. SSR markers were used by Boccacci et al. (2008) to investigate cultivars in northeastern Spain, and by Gürcan et al. (2010) to investigate cultivars in the Black Sea group. Cultivars previously fingerprinted with SSR markers (Boccacci et al., 2006; Boccacci et al., 2008; Gökirmak et al., 2009; Gürcan et al., 2010; Mehlenbacher unpublished) are listed by their assigned group. Other previously fingerprinted very diverse accessions that were not placed in one of the four main groups are presented as a group labeled "other". Accessions that had not yet been fingerprinted were placed in the most suitable group based on their geographic origin, with consideration of morphological traits. Boccacci et al.

(2006), Gökirmak et al. (2009), and Gürcan et al. (2010) identified the presumed parentage of several cultivars based on microsatellite markers.

Cultivars and selections were grouped by SSR marker profiles and geographic origin, and the most common alleles in each group were identified. The data was examined for differences in S-allele frequency associated with geographic origin. Variability in S-allele frequency among seed lots from the same country was also examined.

RESULTS

New alleles and testers. Mehlenbacher (1997a) listed testers for 26 S-alleles, including S_{13} from the interspecific hybrids called "Chinese Trazels". In this study we identified six new alleles and a *C. avellana* tester for S_{13} (Table 1). S_{27} is the dominant allele in Buttner's Zellernuss (S_{11} $\underline{S_{27}}$). S_{28} is present in 'Cutleaf' ($\underline{S_{20}}$ $\underline{S_{28}}$), as mentioned above, but was excluded from this study. S_{29}, the dominant allele in tester OSU 930.081 (S_4 $\underline{S_{29}}$), was inherited from Russian selection OSU 495.049 ($\underline{S_{22}}$ $\underline{S_{29}}$). The latter originated from seeds sent from the headquarters of the N.I. Vavilov Research Institute of Plant Industry in the city then called Leningrad. Most of the hazelnuts in Russia are grown in the south, from the Black Sea coast through the Caucasus Mountains to Krasnodar. We believe that the seeds sent to us originated in this region of southern Russia. S_{30} was first detected in seedlings of 'The Shah' ($\underline{S_{14}}$ $\underline{S_{30}}$), which had been imported as scions from the United Kingdom. S_{30} was later detected in selections from Azerbaijan and Turkey. OSU 1116.049 (S_4 $\underline{S_{30}}$), a selection from Azerbaijan, is the pollen tester for S_{30}. S_{31} is the dominant allele in 'Ata Baba' (S_4 $\underline{S_{31}}$), which is the leading cultivar in Azerbaijan. S_{32} is the dominant allele in 'Reka #2' (S_2 $\underline{S_{32}}$) from Serbia. The most recent new allele, S_{33}, is a co-dominant allele in 'Ganja' ($\underline{S_4}$ $\underline{S_{33}}$) from Azerbaijan. A tester that expresses

only S_{33} in its pollen has not yet been identified. 'Ata Baba', 'Reka #2' and 'Ganja' are the pollen testers for S_{31}, S_{32} and S_{33}, respectively. S_{13}, previously identified in "Chinese Trazels", was found in 'Ashrafi' clone 1226.004 ($S_{\underline{13}}$ S_{31}) from Azerbaijan. It was also detected in selection OSU 1168.130 (S_4 $S_{\underline{13}}$), which originated from seeds purchased in the market in Holmskij, a village near Krasnodar, Russia, and in selection OSU 1090.042 which originated in a seed lot from Georgia. 'Ashrafi' and OSU 1168.130 are used as pollen testers, while the later-flowering 'Ashrafi' is also the female tester for S_{13}.

Dominance relationships. Mehlenbacher (1997a) presented dominance relationships based on 233 pairs of alleles. We identified 105 new pairs of alleles (Table 3) and in this paper show the relationships for 338 of the possible 496 pairs of alleles. The number of pairs was limited for the most recently identified alleles. In all pairs, both alleles were expressed in the stigmas, but often only one was expressed in the pollen because of dominance. A revised dominance hierarchy was drawn based on new data for pairs of alleles (Fig. 1). The dominance hierarchy is linear with 8 levels (Mehlenbacher, 1997a). The relationships among four alleles (S_{13}, S_{23}, S_{29} and S_{31}) are unknown, so they are shown as adjacent boxes. All four are dominant to S_4, and recessive to one or more of the alleles at the next higher level (S_9, S_{11}, S_{22} and S_{26}).

S-alleles in cultivars. Mehlenbacher (2013) reported the alleles of 282 unique cultivars, including 112 from previous reports (Table 3). Cultivars with different names often have identical microsatellite marker fingerprints and S-alleles (Boccacci et al., 2006; Gökirmak et al., 2009; Gürcan et al., 2010), which leads to confusion. As a result, the number of unique genotypes in collections is often less than the number of names. Mehlenbacher (2013) listed cultivars with identical fingerprints and gave the preferred name for each. The S-alleles of 13 interspecific hybrids are also listed.

Relationship of S-alleles to geographic origin in hazelnut cultivars. Boccacci et al. (2006) and Gökirmak et al. (2009) assigned most hazelnut accessions to one of four groups: Spanish-Italian, Central European, English and Black Sea. Other very diverse accessions that were not placed in one of the four main groups are presented as a group labeled "other" (Tables 4&5). Cultivars that have been fingerprinted with SSR markers (Boccacci et al., 2006; Gökirmak et al., 2009; Mehlenbacher unpublished) are listed as members of their assigned group. Accessions that have not yet been fingerprinted were placed in the most suitable group based on their geographic origin, with consideration of morphological traits. The presumed parentage of several cultivars, based on microsatellite markers (Boccacci et al., 2006; Boccacci et al., 2008; Gökirmak et al., 2009; Gürcan et al., 2010) is listed (Table 4). The most frequent alleles in the 284 hazelnut cultivars (excluding interspecific hybrids) are: S_2 (13.9%), S_1 (9.3%), S_{10} (8.5%), S_5 (7.2%), S_3 (6.2%), S_4 (6.0%) and S_6 (5.3%). Percentages for all other alleles were <5.0%. Half of the alleles had frequencies below 2%. Allele frequencies differed among the cultivar groups. In the following paragraphs, alleles with frequencies >4% are called common, alleles with frequencies between 2% and 4% are called rare, and alleles with frequencies <2% are called very rare.

The Spanish-Italian group contains 71 cultivars. The common alleles and their frequencies are: S_2 (30.3%), S_1 (14.1%), S_{10} (9.2%), S_{22} (7.8%), S_{17} (6.3%) and S_{23} (4.9%). The common alleles reflect the importance of 'Barcelona' (S_1 S_2), 'Siciliana' (syn. 'Montebello') (S_1 S_2), 'Negret' (S_{10} S_{22}), 'Segorbe' (S_9 S_{23}) and 'Tonda di Giffoni' (S_2 S_{23}) in this group.

The Central European group contains 38 cultivars. The most common alleles are S_5 (21.1%), S_{15} (14.5%), S_{25} (13.2%), S_{20} (11.8%), S_{11} (9.2%), S_6 (5.3%) and S_{10} (5.3%). The common alleles reflect the importance of 'Hall's Giant' (S_5 S_{15}) and 'Early Long Zeller' (S_{20} S_{25}) in

this group, which also includes hybrids with the English cultivars Daviana (S_3 S_{11}) and Cosford (S_3 S_{11}).

The English group contains 57 cultivars. The most common alleles are S_3 (19.2%), S_{11} (13.8%), S_{14} (12.8%), S_2 (11.7%), S_{10} (9.6%) and S_5 (5.3%). The common alleles reflect the importance of English cultivars Daviana (S_3 S_{11}), Cosford (S_3 S_{11}) and DuChilly (S_{10} S_{14}). The group includes several hybrids between these three English cultivars and Barcelona (S_1 S_2).

The Black Sea group contains 58 cultivars. The most common alleles and their frequencies are: S_4 (27.6%), S_{10} (15.5%), S_2 (8.6%), S_5 (8.6%) and S_{31} (7.8%). The first four alleles had been reported earlier in Turkish cultivars (Mehlenbacher, 1997a; Erdoğan et al., 2005), while S_{31} is common in cultivars from Azerbaijan.

To simplify discussion, all 47 other accessions were placed in a single very diverse group called "other". The most frequent alleles in this group were S_6 (16.0%), S_{20} (10.6%), S_2 (8.5%), S_1 (7.5%), S_{26} (6.4%), S_5 (5.3%) and S_9 (5.3%). This group includes the ornamentals 'Fusco Rubra', 'Aurea', 'Pendula', 'Cutleaf' and 21 selections from the Russian Research Institute of Forestry and Mechanization (Pushkino, Russian Federation). Half of the Moscow selections from Pushkino have red leaves. S_{20} is common in the cold-hardy Central European types with large nuts (e.g., 'Early Long Zeller', S_{20} S_{25}) that were probably used as parents in Moscow. The high frequency of S_{26} in this group is surprising, as its frequency is only 1.76% of the total in the 284 cultivars. S_{26} may be common in the parents of the Moscow selections. To survive in Moscow, the parents and selections must be cold-hardy.

In the 23 cultivars and pollinizers released by the OSU breeding program, the most common alleles are S_1 (28.3%), S_3 (19.6%), S_2 (10.9%), S_{15} (8.7%), S_8 (6.5%) and S_{26} (6.5%)

which reflects the contributions of Barcelona (S_1 S_2), Montebello (S_1 S_2), Daviana (S_3 S_{11}), Tonda Gentile delle Langhe (S_2 S_7), Hall's Giant (S_5 S_{15}), Tombul Ghiaghli (S_4 S_8) and Gasaway (S_3 S_{26}).

Interspecific Hybrids. The 13 interspecific hybrids include four *C. americana* Marsh. × *C. avellana* hybrids ('Bixby', 'Buchanan', 'Potomac' and 'Reed'), three *C. heterophylla* Fisch. × *C. avellana* hybrids from Dalian, China, and six hybrids with *C. colurna* L. and *C. avellana* in their pedigrees. S_{15} was present in all three Chinese Trazels.

S-alleles in selections. The S-alleles of 522 selections of diverse origin were determined, and are presented by geographic origin (Table 6). The origin of the seed lots is listed (Table 2).

Turkish selections. The S-alleles were determined for 258 selections in 8 groups of Turkish origin (Table 7). Of these, 114 originated as seeds harvested in the collection block at the Hazelnut Research Institute in Giresun, and the remainder from various orchards and markets. In the Turkish selections, S_4 was by far the most common allele, representing 30.2% of the total. S_4 was present in high frequency in all eight groups of Turkish selections. The second most common allele was S_{12} which represented 10.7% of the total and was also present in all 8 groups. The next most common alleles were S_8 (9.9%), S_{10} (9.9%), S_2 (7.8%), and S_{16} (4.3%), which were present in 8, 6, 7 and 4 groups, respectively. Rare alleles detected at frequencies of 2-4% include S_{14}, S_3, S_5, S_9 and S_{25} which were present in 4, 4, 5, 5 and 6 groups, respectively. Of the 33 alleles so far identified, only three (S_{23}, S_{29} and S_{31}) were absent in the Turkish selections; 18 others were present at frequencies <2%. The most common alleles in the selections are also present in Turkish cultivars, but the selections include a very large number of additional alleles present at low frequency.

Georgian selections. Nuts were collected in orchards in the Republic of Georgia (**Table 8**). Selections in groups 1-5 were from nuts collected in 2001 and selections in group 6 were

from nuts collected in 2003. Eight selections (Group 1) were from nuts collected in orchards in the inland area of Kakheti at the base of the Caucasus Mountains near the border with Azerbaijan. The other five groups were collected in orchards near the Black Sea coast; groups 2-5 were collected near Zugdidi. In the 43 selections, the most frequent allele was S_4 (22.1%), followed by S_2 (11.6%), S_{10} (9.3%), and S_{20} (8.1%). Four additional alleles (S_{31}, S_1, S_{19} and S_{27}) were present at frequencies of 4% - 6%. Eight alleles were rare and present at frequencies of 2-4% (S_8, S_{14}, S_3, S_5, S_6, S_{11}, S_{18}, and S_{30}). Seven alleles were present at frequencies <2% and 9 were absent. S_4 is present in 'Anakliuri', which is the most widely planted in Georgia.

Azerbaijan selections. Nuts were collected in orchards in three locations: Zaqatala, Qabala and Xaçmaz. The cultivars grown in each location are different. The most common alleles in the selections were S_4 (29.6%), S_{31} (18.4%), S_{10} (14.3%), S_{18} (7.1%), S_2 (6.1%) and S_{24} (4.1%) (Table 9). Rare alleles present in frequencies of 2-4% included S_3, S_7, S_{12}, S_{14}, and S_{19}. Eight additional alleles were present at frequencies <2% and 12 were absent. The high frequency of S_4 and S_{31} reflects the importance of 'Ata Baba' (S_4 S_{31}) in the country. Fewer alleles were detected in selections from Azerbaijan than in selections from Turkey, Russia and Georgia.

Armenian selections. The S-alleles were identified in 26 selections (Table 6) that originated from seeds purchased in markets in 2002 by J. Postman of the USDA-ARS-NCGR. The most common alleles were S_4 (17.3%), S_2 and S_{31} (11.5% each), S_3 (7.7%), S_9 and S_{26} (5.8% each). Rare alleles present at frequencies of 2-4% were S_7, S_{16}, S_{18}, S_{20}, S_{25}, S_{27} and S_{30}. Six alleles were very rare and only detected in one selection each, while 12 alleles were absent. The most common alleles (S_4 and S_{31}) are those of the most important cultivar in Azerbaijan, 'Ata Baba'.

Russian selections. The most common alleles in the 8 groups of Russian selections were S_4 (21.3%), S_{24} (10.6%), S_{10} (8.5%), S_5 (7.4%), and S_{14} (4.3%) which were found in 5, 5, 6, 3, and 2 of the 8 groups, respectively (Table 10). Groups 1 to 5 could be considered cultivated Russian types, while groups 6 and 7 originated in germplasm collection blocks, and group 8 represents selections grown from nuts collected in the wild near Moscow. S_4, S_{24} and S_{10} were very common in the cultivated Russian groups, reflecting the importance of 'Cherkesskii II' (S_4 S_{24}). Twelve rare alleles present at frequencies of 2-4% were S_2, S_6, S_{15}, S_{17}, S_{19}, S_3, S_8, S_{11}, S_{18}, S_{26}, S_{20} and S_{31}. Only S_{27} and S_{33} were absent in the Russian selections, which overall were a very diverse population.

Ukrainian selections. Nuts collected in the Crimea in 2002 gave 24 selections (Table 6). Of these, 21 were from nuts purchased from roadside vendors between Alushta and Simferopol, and three originated from seeds collected at the Nikita Botanical Garden in Yalta. Most of the nuts purchased from vendors were very small and may have been collected from the wild. The most common alleles in the Crimean selections were S_{10} (22.9%), S_2 (12.5%), S_{25} (8.3%), S_9 (8.3%), S_3 and S_{14} (6.3% each), S_{16}, S_{21}, S_{22} and S_{24} (4.2% each). Eight rare alleles were present at frequencies of 2-4%, and 13 alleles were not detected.

Iranian selections. Seeds were collected from a small orchard in a valley in the foothills of the Elburz mountains south of the Caspian Sea in 2003. Additional seeds were purchased from a roadside vendor along the main highway in the Talesh mountains west of Astara. These seed lots gave six and three selections, respectively. Only four alleles were detected in the nine selections (**Table 6**): S_2 (55.6%), S_{33} (27.8%), S_8 (11.1%) and S_4 (5.6%).

Other selections. Of the 19 selections in the miscellaneous group labeled "other" (Table 6), 13 were from seeds collected in the wild in 1990 or 1992 in northern Italy. Three were from

nuts collected in Latvia, one from Lithuania, and one from Estonia. Nuts from the University of Minnesota, likely of Scandinavian origin, gave rise to selection OSU 408.040. The most common alleles in this miscellaneous group were S_9 (15.9%), S_5 and S_6 (10.5% each), S_{10} and S_{15} (7.9% each). Rare alleles detected once or twice were: S_2, S_4, S_7, S_{12}, S_{14}, S_{16}, S_{18}, S_{19}, S_{23}, S_{24}, S_{27} and S_{30}. Twelve alleles were not detected.

Differences were noted in allele frequency between cultivars and selections (Table 11). S_4 was much more common in the selections than in the cultivars, while S_1 and S_2 were more common in the cultivars than in the selections.

Discussion

Relationship of S-alleles to geographic origin in hazelnut cultivars. Boccacci et al. (2006), Gökirmak et al. (2009) and Gürcan et al. (2010) assigned most hazelnut accessions to one of four groups based on microsatellite marker fingerprints: Spanish-Italian, Central European, English and Black Sea. We present the S-alleles of cultivars and selections by group. Accessions that had not yet been fingerprinted were placed in the most suitable group based on their geographic origin, with consideration of morphological traits. Other very diverse accessions that were not placed in one of the four main groups are presented as a group labeled "other". The most frequent alleles in the 284 hazelnut cultivars (excluding interspecific hybrids) are S_2, S_1, S_{10}, S_5, S_3 and S_4. Percentages for all other alleles were <6.0%. Many alleles were detected but at low frequency; half of the alleles had frequencies below 2%. There were striking differences in S-allele frequencies among the cultivar groups (Table 11).

In the Spanish-Italian group, the most common alleles are S_2, S_1, S_{10}, S_{22} and S_{17}. The first four reflect the importance of 'Barcelona' (S_1 S_2), 'Siciliana' (syn. 'Montebello') (S_1 S_2) and

'Negret' (S_{10} S_{22}) in this group. In the Central European group, the most common alleles are S_5, S_{15}, S_{25}, S_{20} and S_{11}. This reflects the importance of 'Hall's Giant' (S_5 S_{15}), 'Early Long Zeller' (S_{20} S_{25}) and 'Pallagrossa' (S_5 S_{25}). The German group includes several hybrids between German cultivars with the English cultivars Daviana' (S_3 S_{11}) and Cosford (S_3 S_{11}), which accounts for the high frequency of S_{11}. Three Polish cultivars in this group (Frango #2, Frango #5 and Volski Round) were determined by Gökirmak et al. (2009) to be seedlings of Cosford. In the English group, the most common alleles are S_3, S_{11}, S_1, S_{14}, S_2 and S_{10}. The common alleles reflect the importance of the English cultivars Daviana, Cosford and DuChilly (S_{10} S_{14}). Several cultivars in this group are hybrids between English cultivars and Barcelona (S_1 S_2), which accounts for the high frequencies of S_1 and S_2. Several cultivars selected by growers in the Pacific northwestern USA appear to be hybrids of Barcelona and Daviana (Butler, Ennis, Fitzgerald, Nonpareil and Woodford, and probably also Compton, Fitzgerald #20, Lansing #1 and Wallace Seedling). An additional three grower selections (Freehusker, Nixon and Royal) and the French cultivar Corabel are hybrids between Barcelona and Cosford, while three grower selections (Brixnut, Gem and Lyons) are hybrids between Barcelona and DuChilly. These grower selections were placed in the English group because of their English parents. In the Black Sea group, the most common alleles in cultivars are S_4, S_{10}, S_2, S_5 and S_{31}. The first four of these alleles had been reported earlier in Turkish cultivars (Mehlenbacher, 1997a; Erdoğan et al., 2005), including Tombul (S_4 S_{12}), Sivri (S_8 S_{10}), Mincane (S_4 S_8), Palaz (S_2 S_4), Kargalak (S_2 S_{10}) and Yassi Bademi (S_2 S_5). Erdoğan et al. (2005) reported S-alleles expressed in pollen of Turkish cultivars. S_4, which is at the bottom of the dominance hierarchy (Fig. 1), is likely the second allele in several Turkish cultivars, but is not expressed in their pollen. S_{31} is present in Ata Baba (S_4 S_{31}) and is common in other cultivars from Azerbaijan. In the other 45 accessions, the most frequent alleles

were S_6, S_{20}, S_1, S_2 and S_{26}. This group includes several ornamentals and 21 selections from a Forestry Institute near Moscow, Russia. Half of the Moscow selections have red leaves. In breeding, two sources of red leaves have been used: 'Fusco Rubra' (S_6 S_{19}) and 'Rode Zeller' (S_6 S_{11}). In *C. avellana*, red leaf color is conferred by a dominant allele at the anthocyanin locus, which is linked to the S-locus (Thompson, 1985). Given the presence of S_6 in both redleaf parents and the linkage of S_6 with the allele for leaf anthocyanin, the high frequency of S_6 is not surprising. S_{20} is common in the cold-hardy Central European types with large nuts [e.g., 'Early Long Zeller' (S_{20} S_{25})] that were probably used as parents in Moscow. The high frequency of S_{26} in this "other" group is surprising, as its overall frequency in the 284 cultivars is only 1.8%.

S-alleles in Turkish selections. Large differences in S-allele frequency were seen among the groups as well as among seed lots within a group. In the Turkish selections, S_4 was by far the most common allele, representing 30.2% of the total. It was present in high frequency in all eight groups of selections. The second most common allele, S_{12}, represented 10.7% of the total. S_{12} is present in 'Extra Ghiaghli' (S_4 S_{12}), which is a clone of the important cultivar Tombul. The next most common alleles were S_8, S_{10}, S_2 and S_{16}. Of the six most common alleles, five had been reported in Turkish cultivars, while the high frequency of S_{16} was unexpected. Four rare alleles detected at frequencies of 2-4% (S_{14}, S_3, S_9 and S_{25}) had not been previously reported for Turkish cultivars, while S_5 is present in Yassi Badem. Three alleles (S_{23}, S_{29} and S_{31}) were absent in the Turkish selections, and 18 others were present at frequencies <2%. Ata Baba (S_4 S_{31}), the leading cultivar in Azerbaijan, has more vigorous and upright growth than the leading Turkish cultivars, but its nuts, husks and kernels are very similar. Given the common ethnic origin of the people in the two countries and extensive trade over many centuries, it was surprising to find S_{31} absent in the Turkish selections. Differences in allele

frequency among the 8 Turkish seed lots were apparent. S_8 was very common in group 4 from Akçakoca and group 6 from Trabzon. S_{10} was common in group 4. S_{14} was common in group 6 but in no other Turkish group. S_{16} and S_{25} were especially common in Group 8 from the Hazelnut Research Institute. Alleles S_{27} to S_{33} are recorded as absent in Groups 1-7 of the Turkish selections (Table 7). We note, however, that testers for these alleles were not available at the time that selections in groups 1-7 were typed, and few selections from these groups have been preserved in our permanent collection. New alleles low in the dominance hierarchy (S_{27}, S_{29}, S_{31} and S_{33}) may indeed be present in selections in groups 1-7 but not detected because they were not expressed in the pollen. However, S_{30} and S_{32} are high in the dominance hierarchy. Their presence would have been detected in selections in groups 1-7 as only one other allele would have been identified, and their pollen would have been compatible on females expressing all other alleles. New testers would have been identified if S_{30} or S_{32} had been present in Turkish selections groups 1-7. In summary, the Turkish selections showed great diversity with respect to their S-alleles, and differences in S-allele frequency among groups.

Selections from the Caucasus. In the 43 selections from Georgia, the most frequent alleles were S_4, S_2, S_{10}, S_{20} and S_{31}. Three of these alleles are present in the Georgian cultivars (Table 8), but not S_2. It is likely that S_{31} was contributed by 'Ata Baba' (S_4 S_{31}) from neighboring Azerbaijan. An additional 18 alleles were detected at frequencies of 1 - 5%, indicating great diversity in the alleles present, while 9 were absent. S_4 is present in four Georgian cultivars, including Anakliuri (S_4 S_{14}), which is the most widely planted. Of three selected seedlings of 'Khachapura' (S_3 S_{18}), which has oblate nuts, two had S_{18} and the other had S_3. In selections from Azerbaijan, the most common alleles, S_4 and S_{31}, were present in all three locations (Zaqatala, Qabala and Xaçmaz). Zaqatala, located at the base of the Caucasus Mountains not far from the

Georgian border, is the most important production zone. There, 'Ata Baba' (S_4 S_{31}) is the most important cultivar. The third common allele (S_{10}) is present in several cultivars from Azerbaijan. An additional 17 alleles were present at frequencies of 1-7%, and 12 were absent. Twenty S-alleles were found in the Azeri selections, which is fewer than in selections from Turkey, Russia and Georgia. Hazelnut orchards were established during the Soviet era on state and collective farms. All aspects of production, including the choice of cultivars and pollinizers, were chosen by government officials. Most of the nuts from Azerbaijan were collected in such orchards. The most common alleles in the Armenian selections, all from nuts purchased in markets, were S_4, S_2, S_{31}, S_3, S_9 and S_{26}. The common alleles S_4 and S_{31} are those of Ata Baba, the most important cultivar in Azerbaijan. The presence of rare allele S_{31} in high frequency in all three former Soviet republics in the Caucasus indicates probable sharing of plant material. The level of diversity in Armenian selections is similar to that observed in selections from Azerbaijan.

Russia and Ukraine. Taken as a whole, the Russian selections were very diverse. The most common alleles in the eight groups of Russian selections were S_4, S_{24}, S_{10} and S_5. The first two alleles are present in Cherkesskii II (S_4 S_{24}), which is the most important cultivar in the north Caucasus. In the Crimea (Ukraine), hazelnut is cultivated to a very limited extent in the protected, mild climate on the south coast of the peninsula. Most of the nuts purchased from vendors were very small and may have been collected from the wild. The six most common alleles in the 24 Crimean selections were S_{10}, S_2, S_{25}, S_9, S_3 and S_{14}. Twelve rare alleles were present at frequencies of 2-5%, and 13 alleles were not detected. The Crimean selections showed fewer S-alleles than the Russian, Georgian and Turkish selections.

Iran. Only four alleles were detected in the Iranian selections: S_2, S_{33}, S_8 and S_4. The number of alleles was strikingly less than in selections from other countries. S_{33} is a very rare

allele present in 'Ganja' (S_4 S_{33}) from Azerbaijan and very few selections, so it was surprising that S_{33} accounted for 27.8% of the alleles in the Iranian selections. In visits by the author to orchards near the Caspian Sea, little phenotypic diversity was seen and nut yields were low, which is consistent with a narrow genetic base. It is unclear if *Corylus avellana* is native to Iran, and it seems likely that the species was introduced. In all other countries from which seeds were collected, hazelnut bushes could be seen growing in roadsides and hedgerows. Such seedlings were not seen in Iran. In fact, even a large hazelnut planting visited in the Talesh mountains had been established by planting seedlings.

Other selections. Of the selections in the miscellaneous group, 13 originated in northern Italy, three originated in Latvia and one from Lithuania. The nuts received from Latvia and Lithuania were large, and presumably from a collection of cold-hardy cultivars with large nuts. The most common alleles in this miscellaneous group were S_9, S_5, S_6, S_{10} and S_{15}.

Differences between cultivars and selections. Differences in allele frequencies (Table 11) between cultivars and selections reflect their geographic origin. The most common S-alleles in cultivars and selections in each group are presented in Table 12. Most of the cultivars were from western Europe, while most of the selections belonged to the Black Sea group and originated in the eastern part of the distribution of *C. avellana*. S_4 was very common in selections from Turkey, Georgia, Azerbaijan, Armenia and Russia. S_8 and S_{12} were common in Turkish selections. These three alleles were much more common in the selections than in the cultivars. On the other hand, some alleles were more common in the cultivars than in the selections. S_1 and S_2 were common in the Spanish-Italian cultivars and S_3 and S_{11} were common in the English cultivars. S_5 was common in the Central European cultivars, while S_6 was present

in the Spanish-Italian and other cultivars. All six of these alleles were present in the selections but at lower frequency than in the cultivars.

The spread of hazelnut and origin of cultivars. *Corylus avellana* is found throughout Europe, the Caucasus and Asia Minor where it is generally found as an understory shrub in mixed deciduous forests. Palme and Vendramin (2002) used four polymorphic chloroplast microsatellite markers to investigate diversity in 248 individuals representing 26 natural populations across Europe, but did not include samples from Turkey, the Caucasus or Iran. Boccacci and Botta (2009) used the same markers to investigate diversity in 75 cultivars, including a few from Turkey and Iran, but none from the Caucasus republics or southern Russia. Both studies give insights into the spread of the hazelnut and its domestication. The chloroplast is generally inherited maternally in angiosperms, and thus only dispersed by seeds. The present-day distribution of *C. avellana* was established about 7000 BP as a result of postglacial recolonization that had started ~11,000 years earlier (Huntley and Birks, 1983). Between 10,000 and 9000 BP there was a sharp increase in the amount of *Corylus* pollen found across Europe (Huntley and Birks, 1983). Nut dispersal during the postglacial recolonization was caused by small mammals, birds, and human migration. Archaeologists have repeatedly found nuts, kernels and shell remains from many archaeological sites all over Europe. Hazelnuts are easy to store and transport, and kernels have a high energy value, thus it is likely that Mesolithic tribes aided the spread of hazelnut and undoubtedly selected for productivity.

Palme and Vendramin (2002) found a clear geographical structure of chloroplast haplotypes that divides Europe into two parts. Chlorotype A, which represented 76% of the sampled wild individuals and chlorotype B, which represented 4%, were well-distributed across western and northern Europe. Chlorotypes C, D, E and F were restricted to southern and central

Italy, Croatia, Romania and Greece. These results indicate that recolonization of most of Europe was from one or more refugia in southwestern France by the Bay of Biscay. Expansion in Italy and the Balkans, where almost all chlorotype diversity was observed, was local.

Where and when the domestication of *C. avellana* was started is not yet clear, although it was cultivated by the Romans, especially in the southern Italian region of Campania. According to Trotter (1921), cultivars were selected from local wild populations. Many cultivars have unclear origins. Chloroplast marker data for 75 hazelnut cultivars (Boccacci and Botta, 2009) suggested considerable exchange of germplasm between Italy and Spain, probably by the Romans, and thus a common genetic base of cultivars in the two countries. Boccacci and Botta (2009) propose separate domestication of hazelnut in three areas: the Mediterranean (Spain and Italy), Turkey, and Iran. Boccacci and Botta (2009) detected little gene flow from east to west. The presence of chlorotype A in all cultivar groups may be due to spread of hazelnut throughout the empire by the Romans. Further studies of chlorotypes of germplasm from the Caucasus republics (Georgia, Armenia, and Azerbaijan) and southern Russia will be enlightening.

According to Erfatpour et al. (2011), hazelnut is native to the Talesh mountains in northwestern Iran (Tandehbin and Makesh regions of Guilan province). The nuclear microsatellite data of Erfatpour et al. (2011) and Ghanbari et al. (2005) shows considerable variation among Iranian hazelnut cultivars, in contrast to our observations during orchard visits. The people of Azerbaijan are Turkic. Treaties signed by Russia and Persia in 1813 and 1828 divided Azerbaijan. Today, the northern third is the republic of Azerbaijan and the southern two-thirds remain part of Iran. Some exchange of plant materials among farmers and gardeners would be expected. The ancient Silk Road passed through Iran, and it seems likely that hazelnut were disseminated along the route.

Although the origins of many hazelnut cultivars are unknown, humans undoubtedly played a role in their selection and spread through clonal or seed propagation. As with other fruit and nut crops, superior cultivars are propagated and sold by nurseries, and farmers and gardeners often share scions, rooted suckers and seeds. Goeschke (1887) described many old cultivars. In England, the major cultivar is DuChilly (syn. Kentish Cob). Richard Webb of Reading is credited as the source of 'Cosford', 'Daviana', 'Garibaldi', 'Empress Eugenia' and 'Princess Royal'. In Germany, at least five people are credited with developing locally-adapted types with large nuts. S.D.L. Henne of Gunsleben is the originator of 'Gunslebener Zeller'. Jacob Mackoy et Cie in Luttich is the originator of 'Berger's Zeller'. C.R. Peicker of Hertwigswalde is the originator of 'Louisen's Zeller' and 'Neue Riesen'. C. G. Buttner of Halle is the originator of 'Hall's Giant' (syn. 'Halle'sche Riesennuss') and 'Volle Zeller'. Justizrat Burchardt of Landsberg is credited as the originator of no less than 12 cultivars, including Buttner's Zeller, Gubener Zeller, Gustav's Zeller, Riekchen's Zeller and Truchsess Zeller. Some of these German cultivars were the parents of others. As noted earlier, cultivars selected by growers in the Pacific Northwestern USA and placed in the English group have been identified as hybrids between Barcelona and three other cultivars: Daviana, Cosford and DuChilly.

CONCLUSIONS

Hazelnut cultivars and selections are self-incompatible. Fluorescence microscopy is routinely used to determine if a pollination is compatible or incompatible, and use of an array of known testers allows identification of the alleles of cultivars and selections. Both alleles are expressed in the stigmas, but often only one is expressed in the pollen because of dominance. This study reports six new S-alleles, an improved tester for S_{13}, and the dominance relationships

for 105 new pairs of alleles. The S-alleles of 284 cultivars, 13 interspecific hybrids and 522 selections of diverse origin are summarized and presented. Most of the world's leading cultivars were selected from the local vegetation near where they are now planted on a commercial scale. Tremendous genetic variability is available in cultivated and wild hazelnuts, but genetic improvement efforts have only recently led to improved cultivars. Based on simple sequence repeat (SSR) markers and geographic origin, most hazelnut cultivars have been assigned to one of the four main groups (Spanish-Italian, English, Central European, or Black Sea), yet many accessions lie outside these main clusters. Differences in S-allele frequency related to geographic origin were seen in the cultivars and selections. The S-alleles identified in hazelnut cultivars and selections is information that should be useful to breeders in the planning of crosses, to germplasm curators, and to growers and nurseries as they choose cultivars and pollinizers when designing orchards.

List of Tables.

572 **List of Figures.**

573 **Figure 1.** Dominance hierarchy of S-alleles in hazelnut pollen. Alleles are dominant to alleles
574 below them, and codominant with those at the same level.

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ACKNOWLEDGEMENTS

Funding from the Oregon Hazelnut Commission, State of Oregon, and Hatch Act is acknowledged and appreciated.

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