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Evaluation of introgression of Asian germplasm into natural populations of *Castanea sativa* Mill. from southern Europe

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Abstract:

Introgression refers to the incorporation (*via* hybridization and backcrossing) of genetic material from one species into the gene pool of another species. Introgression usually facilitates the rapid adaptation of tree populations to new environments, but can also have negative effects on tree species, such as genetic erosion of native populations and limitations to conservation efforts. Evidence of chestnut introgression, i.e., the transfer of genetic material between Asian *Castanea* crenata and *C. mollissima* and the native *C. sativa* through spontaneous hybridisation and later backcrossing, has been documented in wild chestnut populations in northwest Spain. However, the extent of this introgression has not been monitored in other natural *C. sativa* populations in southern Europe. We examined a natural *C. sativa* forest in Bergondo (Galicia, Spain), where ink-disease-resistant *C.*

crenata and *C. mollissima* seedlings had been planted in the 1940s. Adult trees and open pollinated saplings were genotyped by 13 SSRs, and six new alleles that were private to *C. crenata* were identified. Additional five natural *C. sativa* populations in southern Europe are being studied using this approach. We discuss why chestnut populations may benefit from the transfer of alleles involved in resistance to ink disease, and why introgression could be detrimental to drought tolerance. The study provides a reliable method for detecting the presence of Asian germplasm of *Castanea* spp. in *C. sativa* forests, which will allow for better certification of *C. sativa* germplasm. Moreover, it allows monitoring the evolutionary and ecological processes of the species in areas where ink disease resistant interspecific chestnut hybrids have been planted.

Keywords: chestnut, interspecific hybridization, genetic differentiation, microsatellite markers, monitoring system, *Phytophthora cinnamomi*

Ascertainment of ancestry and parentage analysis of the parents and progeny of elite grower selections in the Eastern United States

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Abstract:

The grafted research orchards at the University of Missouri Horticulture and Agroforestry Research Center (HARC) are the main seed source (the founding population) for the eastern US chestnut nursery industry. In 2020, over 7,000 known offspring of bearing age from more than 20 half-sibling families of *C. mollissima* or complex hybrid cultivars from HARC research orchards were in cultivation throughout the eastern US. Interested growers identified 471 elite selections, which were evaluated from 2020-2022 for 35 traits relevant to commercial production. Our strategy for ascertainment of the interspecific ancestry of the trees in the founding population included the establishment of a core collection of DNA from 430 trees representing all of the *Castanea* species, but focusing on *C. mollissima, C. crenata, C. sativa,* and *C. dentata*. We then included all of the trees in the founding population and the 471 elite selections from the progeny of these trees. We used multiplex PCR to produce amplicons from 43 EST-SSR markers developed in *C. mollissima* and *C. dentata*, then sequenced the amplicons to identify the number of repeats in each sequence. The set of 43 EST-SSR was chosen from an initial set of over 800 *Castanea* EST-SSR based on the criteria of amplification across all species, high information content, genome distribution, and amplification in PCR multiplexes. Analysis of the results revealed two major ethnic groups in our *C. mollissima* collection, both of which are represented in the founding population. Admixture was evident in over 30% of the "pure species" collection. Admixture in the founding parents was less evident, although 13 of the 151 trees genotyped are interspecific hybrids, including four trihybrids. Genotyping of the 471 elite selections is ongoing and will include verification of parentage, identification of pollen parents, and detection of close relatives. This project will provide a first look at the relationship of "eliteness" as evaluated by the growers, specific phenotypes, and actual pedigree, early in the process of elite germplasm development for the rapidly growing US chestnut nursery industry

Keywords: Germplasm development, ascertainment of the interspecific ancestry

Best adapted chestnut trees to new environmental scenario for timber production at the Montseny Natural Park

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Abstract:

Castanea sativa Mill., one of the most durable wood species in Europe, was managed for timber very intensively during the 1950 decade in north-east of Catalonia-Spain (Montseny Natural Park and Guilleries). Chestnut wood production was mainly focused on poles, stakes and barrels. However, these uses were replaced for emergent and cheap new products, causing a species decline in the area with the resulting of forest abandonment and the increase of damages caused by the Chestnut Blight (*Cryphonectria parasitica* (Murrill)). Currently, the potential use of chestnut wood for structures is presented as a revulsive for this sector. But, considering current climatic scenario it is necessary to develop Forest Reproductive Materials (FRM) from trees well adapted to: i) stressful abiotic conditions (long summer drought and crushing high temperatures), and to ii) biotic agents, in particular tolerance to canker. In 2013 a total of 9 outstanding chestnut coppice stands were located in different edaphoclimatic areas of Montseny, and seeds were collected from some 'plus trees' belonging of those identified plots. In 2014 a field test (243 trees) were planted in a representative area of the Montseny called 'Fontmartina'. Data of vegetative development, annual growth, leaf phenology, damages caused by canker and the presence of gall wasp recorded during 8 consecutive years (2014-2022) were collected in that field test. In addition, the susceptibility of the canker was evaluated for each genotype under controlled conditions. At age nine, to capture environmental adaptation during wood formation, early wood vessels of tree ring-porous chestnut trees were studied and bark lenticels density were also quantified. All of this information will lead to install a clonal seed orchard in 2023 with 45 components to get FRM potentially better adapted to the future environmental scenario of Montseny.

Keywords: *Castanea sativa*, progeny test, Chestnut, wood, biotic agents, gall wasp, Forest Reproductive Materials

Contribution of ancient rootstocks to the genetic diversity of chestnut orchards in southern Spain

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Abstract:

The Mediterranean basin harbors traditional agroecosystems of particular importance for preserving biodiversity, with the widely cultivated sweet chestnut being a good example. Domestication of chestnut has led to clonal propagation of the best genotypes, resulting in a complex genetic structure, far from it would be expected in a purely natural situation. Depending on the management practices, orchards and natural/naturalised populations show high levels of genetic variation. Several studies have characterized the genetic diversity of traditional varieties, but until now, there is no genetic data on the ancient rootstocks where varieties are grafted, or their possible relationship with natural populations. In this study, we conducted a systematic survey of the amount of genetic variation, genetic composition and spatial genetic structure of one hundred rootstocks from four stands located in southern Spain using genomic and genic microsatellite markers. The results revealed considerable genetic diversity that had not previously described, and confirmed the findings of previous studies on chestnut systems in Spain. This information provides a valuable baseline data for understanding the genetic relationships between wild and cultivated chestnuts, contributing to the expansion of genetic resources management capacities.

Keywords: Genetic diversity, molecular markers, rootsocks, traditional varieties

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Genetic diversity of chestnut to water stress and tolerance to Phytophthora cinnamomi based on EST-SSR markers

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Abstract:

Castanea sativa is threatened by the frequent extreme climatic event drought, particularly in Mediterranean countries, and by 'ink disease', a severe root rot caused by the destructive and invasive soil-borne oomycete *Phytophthora cinnamomi* (*Pc*). The current global change scenario makes it urgent to assess functional genetic diversity associated with drought and resistance to *Pc* in natural chestnut populations from southern Europe. Expressed sequence tag-simple sequence repeat (EST-SSR) makers have become one of the most widely used markers for studying the adaptive genetic diversity of populations. To this end, eight functional EST-SSRs associated with water stress and nine EST-SSRs associated with chestnut resistance to *Pc* were used to genotype 136 individuals belonging to four natural Spanish chestnut populations from areas with different climatic conditions and levels of exposure to *Pc*. The genetic variability observed within and between populations showed the potential for chestnut populations to undergo fast adaptive evolution. Four markers involved in the differentiation of individuals with different drought tolerance (*FIR059*, *FIR080*, *VIT057* and *GOT045*) and three involved in the differentiation of individuals with different *Pc* resistance (*CsPT_0005*, *CcPT_0009* and *CcPT_0035*) have been described. The identification of *FIR059* and *CsPT_0005* as markers under positive selection indicates that they could be used in marker-assisted selection to predict drought and *Pc* tolerance in unstressed and uninoculated *C. sativa* trees, respectively. The results obtained in the Spanish populations for water stress were congruent with those obtained in ten different European populations distributed in contrasting climatic sites, with *FIR059* being involved in abiotic stress response and with alleles associated with climatic variables.

Keywords: chestnut tree, climate change, ink disease, genetic differentiation, local adaptation, landscape genomics, environmental association analyses

DISTRIBUTION OF SWEET CHESTNUT (*CASTANEA SATIVA* MILL.) ÎN THE OLTENIA REGION, ROMÂNIA

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Abstract:

Castanea sativa Mill. is a Mediterranean taxon originating in western Asia and southeastern Europe. It is the only species of *Castanea* genus with an areal on the territory of Europe. The other taxa are only cultivated (eg *C. crenata* Siebold et Zucc., *C. dentata* (Marsh) Borkh. or *C. pumila* Mill.). This paper presents data on the ecology, chorology and phytocenology of *C. sativa* species from Oltenia region (Romania) (data from the literature, herbariums and the field). In the subspontaneous flora of Oltenia, the *C. sativa* species is found in sheltered resorts in the counties of Gorj (Glogova, Valea Perilor, Tismana, Pocruia, Polovragi, etc.) and Mehedinți (Comăneşti, Baia de Aramă, etc.), on mesobasic soils, balanced from a hydraulic point of view. It was identified in a cultivated state, in few specimens, in the previously mentioned counties and in Vâlcea county, near the monasteries (eg Horezu Monastery). The phytocoenoses where this species grows are rich in southern elements (eg *Cornus mas* L., *Cerasus avium* (L.) Moench, *Quercus dalechampii* Ten., *Tilia tomentosa* Moench, etc.). They are included in the association Castaneo-Quercetum Horvat 1938. If we refer to the habitats in Romania, the areas occupied by the edible chestnut are included in the R4141 habitat - Daco-Balkan forests of sessile oak (*Quercus petraea*) and chestnut (*Castanea sativa*) with *Genista tinctoria* - a habitat with a very high conservation value, and according to Natura 2000, forests of *Castanea sativa* are included in the 9260 habitat.

Keywords: chestnut, distribution, variability, diversity