

Genetic diversity of Sweet chestnut (*Castanea sativa* Mill.) populations of Central and South-eastern European origin

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Sweet chestnut (*Castanea sativa* Mill.), is a widely distributed European forest tree species of great economic importance, valued not only for its fruit and timber, but also for its contribution to the landscape and the environment. From fossil pollen data two periods have to be considered: a first one in Spain and Greece and a second one from Roman and post-Roman age, in which many human activities contributed to a spread towards central and northern Europe (FINESCHI et al., 2000). Research conducted in the Mediterranean basin and in northwestern Europe indicated five distinct *C. sativa* gene pools, three of which are located in Greece (ARAVANOPOULOS et al., 2005, MATTIONI et al., 2008). *C. sativa* has arrived in the Northern Alps around 2000 years ago. Until now, no genetic data of German *C. sativa* populations exist. Because of intensive cultivation of the species and the focus on fruit production it is assumed that German populations may consist mainly of cultivated forms. In this work we aim to detect genetic variation within and between German, Italian, Bosnian and Greek *C. sativa* populations.

Plant material

Population	Country	Number of individuals
Arnaia	Greece	24
Hortiatis	Greece	24
Walbachtal	Germany	24
Ortenau	Germany	24
Monticolo	Italy	25
Banja Luka	Bosnia	16
Bratunac	Bosnia	16
Buzim	Bosnia	16
Konjic	Bosnia	16
Kostajnica	Bosnia	16
Prijedor	Bosnia	16

Genetic analysis

Chloroplast microsatellites (cpSSRs)

Cmcs2, Cmcs7, Cmcs8, Cmcs12, Utd3, Utd5 (LIU et al. 2013)

Nuclear microsatellites (nSSRs)

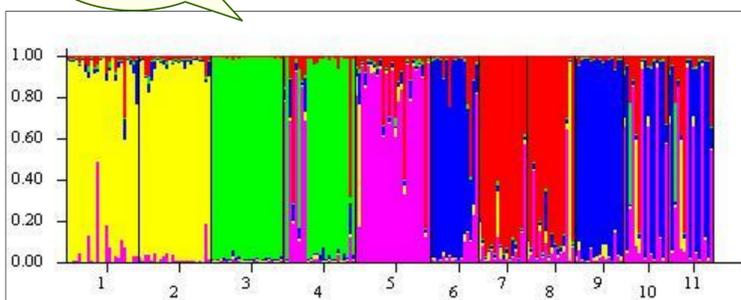
ICMA004, ICMA007, ICMA018, ICMA020, ICMA023, Qr7, Qp15 (INOUE et al. 2009)

Genetic diversity

All six tested chloroplast microsatellite markers were polymorphic. Haploid diversity varied between 0.036 (Kostajnica/ Bosnia) and 0.422 (Hortiatis/Greece). The results reveal a notable genetic differentiation of the Greek populations compared to the other studied *C. sativa* populations. All seven tested nuclear microsatellite markers were polymorphic with a higher level of polymorphism compared to the cpSSR markers. A total of 48 different alleles were identified in 215 individuals and the number of detected alleles for each locus varied between 4 and 15, with a mean of 6.85 alleles per locus. The mean number of effective alleles (N_e) varied between 2.03 (Konjic/Bosnia) and 3.39 (Kostajnica and Prijedor/Bosnia).

RESULTS

STRUCTURE
K=5

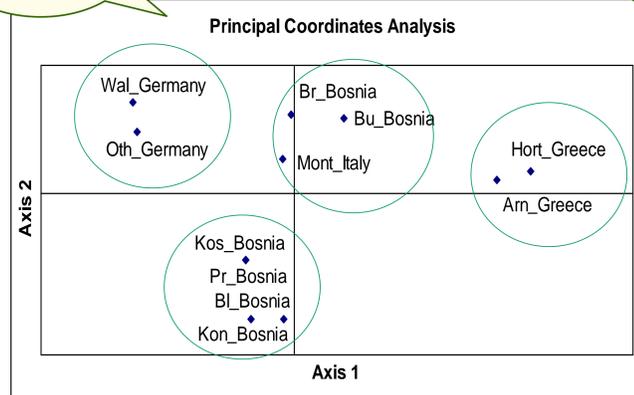


Analysis of **nSSR** data using STRUCTURE software revealed five clusters. One cluster was formed by the Greek populations (yellow), another cluster by the German populations (green).

The Bosnian populations represent two groups consisting of two (red) and four populations (mainly blue).

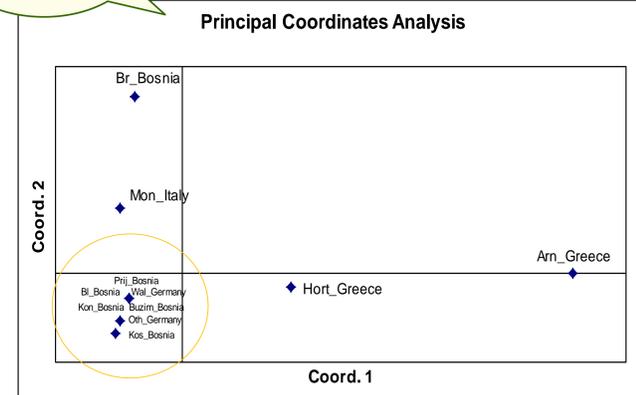
The Italian population forms a separate cluster (purple) sharing some genetic information with two Bosnian populations.

Based on Nei
genetic distance
using nSSRs



The statistical analysis of the **nSSRs** results through PCoA revealed a clear genetic differentiation of the Greek populations compared to the remaining European *C. sativa* populations. The results suggested the presence of four major groups; a first group consisted of the German populations, a second group that included the Italian and two Bosnian populations, a third group that included four Bosnian populations and a fourth group consisted of the Greek populations.

Based on Nei
genetic distance
using cpSSRs



The statistical analysis of the **cpSSRs** revealed a clear genetic differentiation of the Greek populations compared to the rest European *C. sativa* provenances. The PCoA suggested the presence of five major groups; a first group that included one Bosnian population, a second group was formed by the Italian population, a third group consisted of the remaining Bosnian and the German populations. Finally two largely distinct groups were formed, each consisted of one Greek population.

CONCLUSION

The overall results obtained from this study emphasize the distinct genetic composition of the evaluated *C. sativa* material derived from the four countries, highlighting several interesting admixtures among some provenances. According to STRUCTURE results some individuals in one German population share their genetic composition with the Italian population. Genetic distance based on nSSRs suggested that the German populations form a separate group, but with cpSSRs they appear more similar to some of the Bosnian populations. Greek populations were the most distinct ones, confirming earlier studies. Yet no clear statement about the occurrence of cultivated forms in German forests can be made.

- References:**
ARAVANOPOULOS et al., (2005): Acta Horticulturae 693, 403-412
LIU et al., (2013): Tree Genetics & Genomes, 9: 975-987
FINESCHI et al., (2000): Mol Ecol 9:1495-1503
MATTIONI et al., (2008): Tree Genetics & Genomes, 4:563-574
INOUE et al., (2009): J. Amer. Soc. Hort. Sci. 134: 610-617

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