

# Molecular differentiation of Turkish and Common hazels (*Corylus colurna* L. and *Corylus avellana* L.) using multiplexed nuclear microsatellite markers

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**Abstract.** *Corylus colurna* is considered as important tree species under climate change for dry and warm conditions in Central Europe and was overused because of its valuable wood. Therefore Turkish hazel is now present only in small isolated populations and is protected under IUCN. Genetic conservation of this tree species plays a key role in future sustainable forest development. Turkish hazel co-occurs with Common hazel (*C. avellana*) in its whole distribution area and may form hybrids. To differentiate between the pure species and their hybrids, cross-species amplifying markers are required. In this study we have evaluated existing simple sequence repeat (SSR) markers using altogether 128 samples of *C. avellana* and *C. colurna*. Fifteen nuclear SSRs have generated easy to-score alleles in the two species and 13 of them were highly polymorphic. For all 15 markers the mean allele number, average observed heterozygosity, genetic diversity and polymorphism information index were high. The two most polymorphic SSRs were L1.10 and CaT-B501 with 19 and 16 alleles, respectively. Structure analysis proved the differentiation of the two species *C. avellana* and *C. colurna*. No hybridization was detected in the analysed populations. Results also indicated that *C. colurna* from Balkan Peninsula and Asia Minor belong to separate groups. Our study presents highly polymorphic, easy to score, ready to use SSR-multiplexes, which can be applied in population genetics and gene conservation studies.

**Keywords:** SSR, multiplex PCR, polymorphism, species differentiation, molecular markers, hazelnut

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## Introduction

Climate change affects all forest ecosystems and goods that forests provide. A suitable strat-

egy to increase the stability of forest stands is given through establishing mixed forests. By supplementing the existing spectrum of tree species in Central Europe, the risk can be min-

imized. Therefore we examine tree species from the Mediterranean region for its drought resistance and their suitability to be cultivated in Germany. Turkish hazel is considered as a valuable tree species under climate change with high adaption potential to drought and frost (Šeho et al. 2019). Turkish hazel (*C. colurna* L.) and Common hazel (*C. avellana* L.) belong to the family of birch plants (*Betulaceae*), with the subfamily *Corylaceae* which has four genera. The genus *Corylus* has 12 species distributed in the temperate zones of Eurasia and North America (Mitchell 1979, Fitschen 1994, Altheld 1996, Erdogan & Mehlenbacher 2000a). The genus is diploid ( $2n=2x=22$ ), and contains monoecious and wind-pollinated shrubs and trees (Bassil et al. 2013). Within section *Corylus*, three subsections are traditionally recognized. *C. colurna* L. and *C. avellana* belong to different subsections (Subsection *Columnae* and *Phyllochlamys*, respectively) (Erdogan & Mehlenbacher 2000).

*C. colurna* is naturally distributed on the Balkan Peninsula, Asia Minor, the Caucasus and Afghanistan (Temel et al. 2017, Šeho & Huber 2018, Šeho et al. 2019). By supplementing the existing spectrum of broad-leaved tree species with Turkish hazel in Central Europe, the risks raised by changing climate can be minimized. To recommend suitable provenances for Central Europe research on genetic and phenotypic diversity between and within *C. colurna* populations is necessary. Turkish hazel can reach an age of 400 years, DBH of 60 cm (in some cases up to 170 cm) and height of 30 m depending on the growth conditions (Alexandrov 1995, Altheld 1996, Schmidt 2003, Richter 2013, Šeho & Huber 2018, Šeho et al. 2019). In contrast, Common hazel is growing as a shrub. These two hazel species have different morphology of leaves and fruits. Turkish hazel has 17-20 mm long nuts ripening in 5 to 6 pods surrounded by deeply divided lobes. Nuts are smaller than those of *C. avellana*. Leaves of Common hazel are slightly smaller and lighter green and the bark is less grey than that of Turkish hazel. Between these

two hazelnuts (*C. colurna* L. and *C. avellana* L.) hybrids can be formed rarely (Alexandrov 1995; Erdogan & Mehlenbacher 2000, Jahn 1930). Jahn (1930) described some differences between three possible hybrids between *C. colurna* and *C. avellana* in botanical gardens in Hann. Münden, Göttingen and Jena (Germany). However, he was not absolutely certain that these trees are natural hybrids.

In Germany, Common hazel is intensively traded and planted, and thus is of central interest from a nature conservancy point of view. Leinemann et al. (2013) studied Common hazel using chloroplast markers (cpSSRs), isozymes and AFLPs with the aim to assess the within and between population variation of 20 natural populations from Central Europe. Significant differences between populations were found. Like other plant species, the distribution of Common hazel is strongly affected by post-glacial recolonization (Huntley & Birks 1983, Huntley 1990, Palmé & Vendramin 2002). A clear geographical structure was found on the European scale separating Italy and the Balkans from the rest of Europe (Palmé & Vendramin 2002). Together with pollen data the possible origin of Central European Common hazel would be France expanding into most of Europe and a local expansion in Italy and the Balkans. *C. avellana* and *C. colurna* share one haplotype indicating common ancestry or hybridization between the two species. For *C. colurna* there is a knowledge gap about potential refugia or recolonization routes after the last glacial maximum.

For further cultivation of Turkish hazel in Central Europe it is important that the invasiveness of this tree species through hybridization will be evaluated. Artificial crossing experiments between *C. colurna* x *C. avellana* L. yielded in the formation of few fruit clusters with *C. avellana* as the male parent, but failed to set full seeds. The reciprocal cross, *C. avellana* x *C. colurna*, set very few nuts only in one of three breeding years (Erdogan & Mehlenbacher 2000). Percentage of seed germination was higher when *C. avellana* was pollen

donor and *C. colurna* female crossing partner. Seedling vigor, recorded as stem diameter, was highly variable among the interspecific crosses. In general, the cultivated hazelnut *C. avellana*, and the tree hazel species *C. colurna* and *C. chinensis* yielded very vigorous interspecific hybrid seedlings (Erdogan & Mehlenbacher 2000). However, the crossability of *C. colurna* and *C. avellana* was under discussion in the 1960ies (Kasapligil 1963, 1964). Gelatly (1966) reported some hybrids and named them trazels, derived from open-pollination of *C. colurna* trees, morphologically indicating true hybrids (varieties called 'Morrisoka' and 'Faroka'). Erdogan & Mehlenbacher (2000) indicate that crossability between these two species is complicated but possible. In their study *C. colurna* x *C. avellana* resulted in cluster set every year, but with high proportion of empty seeds and only few viable seedlings. Breeders need to perform large numbers of pollinations to obtain a few hybrid seedlings (Erdogan & Mehlenbacher 2000). Bassil et al. (2013) reported about hybridization supported by nuclear and chloroplast microsatellite data, where *C. colurna* - *C. avellana* hybrids formed a distinct group, while accessions of *C. colurna* were placed in one cluster with two *C. avellana* cultivars from southern Italy ('Tonda Bianca' and 'Tonda Rossa').

Microsatellites or simple sequence repeats (SSRs) are short, tandemly repeated DNA sequences (Oliveira et al. 2006 and references therein, Wang et al. 2009). They have become valuable molecular tools for fingerprinting accessions, assessment of genetic diversity in collections and linkage mapping, due to their abundance, high degree of polymorphism, co-dominance, suitability for automation and transferability across experiments and among labs (Barbara et al. 2007, Ellis & Burke 2007, Wang et al. 2009).

For studying related species and their hybrids, markers that work in both species are needed. While development of new SSRs is costly and time-consuming the transferability

of nuclear microsatellite loci across species/taxa or genera depends on various factors such as species generation time, type of breeding system, genome size, etc. (Barbara et al. 2007, Ellis & Burke 2007). Lower success rate of SSR transferability was observed in selfing species and species with high genome size such as tree species and has therefore to be evaluated case-by-case (Barbara et al. 2007). Compared to "anonymous" SSRs EST-SSRs are easier to be transferred between species especially among plants (Ellis & Burke 2007 and references therein). The transferability of SSR markers allows their usage in related species for fingerprinting, mapping, and marker-assisted breeding (Ellis & Burke 2007). Examples include the high transferability rate of SSRs in several tree species from apple (*Malus domestica*) to European pear (*Pyrus communis*) (Pierantoni et al. 2004), from peach (*Prunus persica*) to related *Prunus* species (Cipriani et al. 1999), and within oaks (from *Quercus petraea* to *Quercus robur*, Steinkellner et al. 1997).

The goal of this study was to establish high quality sets of molecular markers (i.e. ready to use multiplexes of nSSRs) for both species *C. colurna* and *C. avellana* and use them to investigate species differentiation and genetic diversity in natural populations. Therefore we used markers developed for *C. avellana* (Bocacci et al. 2005, Gürcan et al. 2010, Gürcan & Mehlenbacher 2010b) and *Betula pendula* (Kulju et al. 2004).

## Material and methods

### Plant material

Three populations were selected for genetic analysis from their native range: two populations of *C. colurna*, one in Turkey (Bolu) and one in Bosnia and Herzegovina (Rogatica), and one population of *C. avellana* from Germany (Laubau, Bavaria) (Table 1). In order

**Table 1** Plant material and description of stands

Tree species	Location	Region	Source	Number of samples	Coordinates
<i>C. avellana</i>	Laubau, Germany	Central Europe	native	40	47°43'N 12°38'E
<i>C. colurna</i>	Rogatica, Bosnia and Herzegovina	Southeastern Europe?	native	33	43°44'N 18°57'E
<i>C. colurna</i>	Bolu, Turkey	Asia minor	native	55	40°52'N 31°48'E

to test for phylogeographic influence on marker performance the two *C. colurna* populations were chosen to represent the two major natural regions of the species. The *C. avellana* population from Central Europe was chosen in order to obtain *C. avellana* specific alleles from the tested marker set. By this procedure species specific alleles can be identified and used for species and hybrid detection in future studies. A representative sampling procedure was chosen for each stand regarding density and number of trees. In order to evaluate the markers for their degree of polymorphism between and within species and genetic diversity parameters within populations leaf material was derived from at least 30 trees per population.

### DNA isolation

Total nuclear DNA was extracted from a standardized quantity of plant tissue (four leaf discs, Ø = 6 mm). Plant material was dried in silica gel and disrupted using a Mixer Mill MM400 (Retsch, Germany). A 5 mm tungsten bead was added in each 2 ml Eppendorf tube and followed by 4-min disruption at 30 Hz. DNA was isolated following Doyle & Doyle (1990). DNA extracts were fluorometrically quantified (Gene Quant Pro, Amersham Bioscience) and adjusted to 20 ng/µl.

### Multiplex PCR optimization

Kit1 comprised four SSR loci originating from *Betula pendula* Roth (Kulju et al. 2004), Kit2 contained six SSR loci developed by Boccacci et al. (2005) and Gürcan et al. (2010), and Kit3 contained five loci developed by Gürcan et al.

(2010) and Gürcan & Mehlenbacher (2010b).

None of these markers had been multiplexed before. Criteria for SSR inclusion into the multiplexes were the number of alleles (>3), lack of evidence for null alleles ( $r < 0.05$ , as defined in Brookfield 1996), good amplification success and easy to score pattern in both species. Altogether 15 primer pairs were tested for amplification in each of the two species. Amplification success was indicated by the presence of a fragment peak in the capillary electrophoresis. Optimization was needed for concentration of the primers in the final PCR mix.

Altogether 128 individuals were genotyped at 15 highly polymorphic nuclear microsatellite loci combined into three multiplex PCRs (Table 2). Primer sequences and repeat motif with literature of original publication, fluorescent labels of primers, final concentrations in final PCRs and annealing temperature are given. Reverse primers from each primer pair were labelled with fluorescent dyes Cy5, IRD700 (both Biomers) and DY751 (Metabion).

PCR amplification was performed with Type-it Microsatellite PCR kit (Qiagen, GmbH, Hilden, Germany) in the total volume of 15 µl solution: 1 µl of 20ng/µl genomic DNA, 7.5 µl of 2X Qiagen Multiplex PCR Master mix, 5 µl of RNase free water and 1.5 µl of Primer Mix respectively.

The targeted fragments were amplified with a thermocycler (Biometra, Analytic Jena): by using the following cycling conditions: Initial denaturation (95°C for 15 min), 26 amplification cycles (94°C for 30s, T<sub>m</sub> according to multiplex for 90s, 72°C for 30 s), 1 final elon-

**Table 2** Characteristics of multiplex SSR kits for *C. colurna* and *C. avellana*: kit-1 (light shaded area), kit-2 (darker shaded area) and kit 3 (dark shaded area)

Locus	Primer sequences (5'-3')	Reference	Dye	Kit	[ $\mu$ M]	[C]	Motif
L1.10	ACGCTTCTTGATGTCAGCC TCACCAAGTTCCTGGTGGAT	Kulju et al. (2004)	Cy5	kit 1	2	57	(GA)4AA (GA)11
L7.8	GGCCAAACAGATAAACAACGACG TTTTAAATGCCACACCTTCCC	Kulju et al. (2004)	IRD700	kit 1	2	57	(CT)11 GC (AATG)2
L5.5	GAGGAAGTCTCAGCTGAGGTG TCCTTTTTCAGTTTTCTGATTTCTG	Kulju et al. (2004)	Dy751	kit 1	2	57	C12CTCC(CT)7TT(CT)5
L13.1	CACCACCACAACCAACCATTA AACACCCCTTGGCAACAATGA	Kulju et al. (2004)	IRD700	kit 1	2	57	(CA)3(GA)14
CaT-B508	GGGTCAAAGATTTGATAAAGTGGGA GCACTCCACTTGTGGGTTTTTC	Boccacci et al. (2005)	Cy5	kit 2	2	55	(GA)10
CaT-B504	CGCCATCTCCATTTCCCAAC CGGAATGGTTTTCTGCTTCAG	Boccacci et al. (2005)	Dy751	kit 2	4	55	(CT)18
CaT-B503	CTCAATTCACCTCGAACGGATAC AGCCGATACCAAGCCTCTCGC	Boccacci et al. (2005)	Dy751	kit 2	2	55	(GA)18
CaT-B501	GAAATTCAAATCACACCAATAAAGCA CCTCCCTTGTCCCTCATCACTG	Boccacci et al. (2005)	IRD700	kit 2	4	55	(GA)21
B714	CTCTCTTTCTCCGTCCTTTGA GTGGAAAGTGTGTGTCCA	Gürcan et al. (2010)	Dy751	kit 2	8	55	(TC)15
FJ98520	TGCATGAAATGGAATCACAGA AGCAAAGAGGTAAAGCAAATCA	Gürcan et al. (2010)	IRD700	kit 2	6	55	(CT)28
FJ98518	CTCTGTGTCGGTTTCTGGT ATAAACCTCACGCCACACCT	Gürcan et al. (2010)	Dy751	kit 3	2	60	(AG)14
FJ98523	CCGACTCCTTAGCATCCTTG AGCTAGTCTAAACATCAATAGAGCAA	Gürcan et al. (2010)	Cy5	kit 3	8	60	(TC)18
FJ98522	TGTGTGTGTTTCTTGGGTTTGTATGTG GGAAAGTAAACGCACTGCAACTGGT	Gürcan & Mehlenbacher (2010b)	Cy5	kit 3	0.5	60	(AT)5

**Table 2** (continuation)

KG811	GAACAACCTGAAGACAGCAAAAG AAGCGGCACTCGCTCAC	Gürcean & Mehlenbacher (2010b)	Dy751	kit 3	4	60	(GA)17
B664	CAAAAGCCGTCGACAACACAG	Gürcean et al. (2010)	IRD700	kit 3	2	60	(TC)21
FJ98510	TTTGCAITGATGCCGATAA						

gation step (60°C for 30 min). PCR amplification products were separated via capillary electrophoresis using 8-capillary DNA Analyzer automated sequencer [GenomeLab™ GeXP Genetic Analysis System (GeXP), AB Sciex]. Sizing of fragments was performed manually with Fragment Analyzer tool implemented in GenomeLab Software.

**Characterization of polymorphic markers**

Diversity values of SSRs (*nA* - total number of alleles, *A* - mean number of alleles per population, *H<sub>o</sub>* - observed heterozygosity, *H<sub>e</sub>* - expected heterozygosity, *F* - fixation index; *F<sub>st</sub>* - differentiation between sub-populations) were calculated using GenAlEx 6.5 (Peakall & Smouse 2012). The frequency of null alleles (*r*) was estimated as  $r = (He - Ho) / (1 + He)$  (Brookfield 1996) in a spreadsheet and also tested with MICRO-CHECKER 2.2.0.3 (Van Oosterhout et al. 2004). The PIC value for each locus was estimated using Cervus (Kalinowski et al. 2007).

**Genetic differentiation between populations**

The Bayesian clustering algorithm implemented in the software program STRUCTURE 2.3.4 (Pritchard et al. 2000) was used to infer population structure and assign individuals to modelled populations based on their SSR genotypes. The most likely number of clusters was assessed using the empirical statistic  $\Delta K$  calculated by STRUCTURE HARVESTER (Evanno et al. 2005; Earl & von Holdt 2012). The admix-

ture model and correlated allele frequencies between clusters with default parameter settings were used, with *K* varying from one to six. The burn-in length was set to 200,000 with runs of 100,000 steps, and each run was replicated ten times.

**Results**

**SSR amplification and polymorphism**

The variation of each nuclear microsatellite locus is given in Table 3. Fragment size ranges are given for each locus. Total number of alleles range from 6 (B714) to 19 (L1.10). The lowest mean number of alleles per population was detected for locus B714 (2.7), the highest in B719 (9.7). Observed heterozygosity was lowest in B714 (0.418) and highest in CaT-B501 (0.831). Expected heterozygosity was between 0.391 (B714) and 0.789 (CaT-B501). For one locus B719 in population Rogatica presence of null-alleles was detected by MICRO-CHECKER 2.2.0.3 (Van Oosterhout et al. 2004). No evidence of null-alleles was shown for the other two populations, therefore all 15 markers were kept for further analysis. R-value for the marker B719 was higher than 0.05, but it was kept because of its high variability. F-values ranged from -0.09 (CaT-B504) to 0.161 (B719). The mean F-value across all loci and populations was 0.001. PIC was larger than 0.5 for all marker loci, meaning all are highly polymorphic, according to Botstein et al. (1980). Six loci had a PIC value above 0.8. Combined with high



**Table 3** Genetic variability of 15 nuclear microsatellites based on 40 samples of *Corylus avellana* from southern Germany and 88 samples from *C. colurna* from Turkey and Bosnia and Herzegovina

Locus	Size	nA	A	Ho	He	F	r	PIC	Fst
L1.10	171-273	<b>19</b>	8.0	0.652	0.688	0.036	0.021	0.84	0.22
L7.8	258-264	11	7.7	0.781	0.771	-0.008	-0.006	0.80	<b>0.08</b>
L5.5	112-128	8	5.3	0.441	0.453	0.062	0.008	0.69	0.38
L13.1	100-112	7	4.0	0.544	0.528	-0.029	-0.010	<b>0.54</b>	0.11
CaT-B508	142-172	15	9.3	0.695	0.742	0.063	0.027	0.78	0.09
CaT-B504	166-184	9	6.3	0.800	0.734	<b>-0.090</b>	<b>-0.038</b>	0.81	0.12
CaT-B503	115-153	13	7.3	0.692	0.706	0.034	0.008	0.79	0.15
CaT-B501	104-136	16	7.3	<b>0.831</b>	<b>0.789</b>	-0.054	-0.024	<b>0.87</b>	0.11
B714	232-244	<b>6</b>	<b>2.7</b>	<b>0.418</b>	<b>0.391</b>	-0.034	-0.020	0.67	<b>0.44</b>
B706	170-202	11	6.3	0.659	0.640	-0.032	-0.012	0.75	0.18
B720	160-174	8	5.3	0.608	0.636	0.050	0.017	0.74	0.17
B719	278-318	14	<b>9.7</b>	0.637	0.774	<b>0.161</b>	<b>0.077</b>	0.86	0.12
KG823	151-183	11	5.0	0.719	0.675	-0.063	-0.026	0.81	0.17
KG811	240-280	12	7.3	0.710	0.684	-0.036	-0.016	0.73	0.12
B664	188-216	13	5.3	0.620	0.587	-0.052	-0.021	0.76	0.25

number of alleles, those markers are the most informative ones. Most discriminative power between the species has locus B714 with  $F_{st}$  of 0.44 followed by L5.5 ( $F_{st} = 0.38$ ). Locus L7.8 has the lowest discrimination power with  $F_{st}$  of 0.08. Overall, the allele size ranges for each locus did not vary much across the two species, except for locus L1.10. The allele size for *C. avellana* ranged from 171-191 bp and for *C. colurna* it ranged from 217-273 bp.

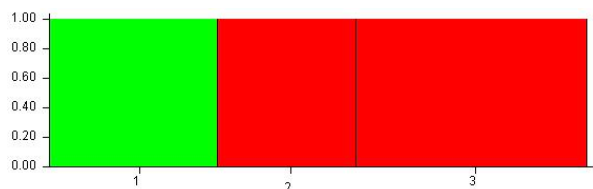
### Species and population differentiation

Based on STRUCTURE results a clear separation of the two *Corylus* species has been detected.  $K = 2$  was defined as the most likely number of clusters (Appendix 1). Figure 1 displays the separation of the *C. avellana* population (green) and the two *C. colurna* populations (red). The second most likely clustering was at  $K = 3$  pointing to the different gene pools within Turkish hazel (Figure 2).

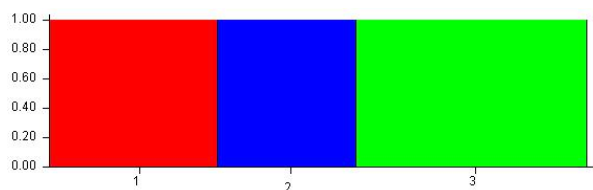
### Discussion

The development and optimization of micro-

satellites is an expensive and time-consuming process. Therefore the possibility for transferring SSRs to related species is of potential interest, particularly for *C. colurna* where only a limited number of SSRs were tested so far. Accordingly, the SSRs were tested on two populations of *C. colurna* and one *C. avellana* population. The populations were chosen from important geographic regions of *C. avellana* (Central Europe) and *C. colurna* (Balkan and Asia Minor). Cross-amplification was tested on more than 30 individuals from each population. The transferability of microsatellite loci was evaluated for the two species. All tested primer pairs amplified DNA fragments in those two species, regardless of the origin of the sample. A locus was considered conserved when one or two high peaks of the expected size were obtained from PCR. Fifteen microsatellite loci produced amplified products of the expected length in the two species tested. Therefore transferability rates for the used SSRs were 100 %. Such a high cross-amplification of hazelnut microsatellite markers agrees with previous reports in *Corylus* (74–100 % in Bassil et al. 2013, Bassil et al. 2005, Boccacci et al. 2005, Gürcan & Mehlenbacher 2010a).



**Figure 1** The plot of the inferred Structure clusters (K=2). First cluster (green) *C. avellana* population (1) and second cluster (red) *C. colurna* populations (Bolu - 2 and Rogatica - 3)



**Figure 2** The plot of the inferred Structure clusters (K=3). First cluster (red) *C. avellana* population (1), second cluster (blue) *C. colurna* population Bolu (2) and third cluster (green) *C. colurna* population Rogatica (3).

For other species (e.g. *E. globulus*) EST-SSRs transferability rates varied between 70-78% for six related Eucalyptus species (Acuña et al. 2012).

Bassil et al. (2013) reports values based on seven trinucleotide SSRs, the average heterozygosity was moderate at 0.63 while allele number, genetic diversity and PIC were high (means of 11.71, 0.79 and 0.76, respectively). Out of the 14 nSSRs used by Bassil et al. (2013) in 13 *C. colurna* accessions seven of the loci had PIC below 0.4 and/or observed heterozygosity close to zero (CaC-C003, CaC-C005, CaC-C036, CaC-C111, CaC-C112, CaC-C119, CaT-C501), therefore we decided to test other loci. Ten out of our 15 tested loci amplified more than ten alleles. The two most polymorphic markers were B719 ( $A = 9.7$ ) and CaT\_B508 ( $A = 9.3$ ). The average heterozygosity of the 15 loci was at 0.65 and average PIC was at 0.79. Thereby a set of 15 robust SSR markers was created based on low estimated percentage of null allele frequencies, high PIC values

and high cross species transferability.

The SSRs also prove to be useful for species identification and for the detection of potential hybrids in the respective populations. Bassil et al. (2013) reported on the phylogenetic organization of 11 species and 44 hybrids. Several times artificial crossing of *C. avellana* and *C. colurna* were reported, while crosses involving *C. avellana* as the male parents were more successful (Erdogan & Mehlenbacher 2000). Jahn (1930) described three trees in botanical gardens as natural hybrids. One of those trees was situated in Göttingen with only few seeds observed, which were not germinating. Another tree was placed in Hann. Münden and was defined as a natural hybrid because of morphological characteristics. Backcrossed progenies of this tree were observed due to *C. avellana* in the vicinity of the hybrid serving as pollen donor.

This is the only report on natural hybrids found in the literature. No hybrids were found within the two *C. colurna* populations analysed in this study. Furthermore also a clear separation of populations of *C. colurna* from Balkan and Turkey was found (Šeho et al. 2017) and point to differentiation of gene pools within Turkish hazel.

Altogether the newly combined marker sets can be used to genetically characterize the structure and diversity of natural forest populations of *C. colurna*. They can help to identify glacial refugia and to study hybridization in mixed stands of the two species. Combined with phenotypic characterization of forest stands the results of this study build the base of provenance research under climate change. Genetic data based on the developed markers will assist in provenance recommendation and proof of identity of forest reproductive material derived from characterized stands.



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## Supporting information

**Supp. Info. 1.** Graphic representation of the rate of change for each K value. Delta K calculated by the Evanno et al. (2005) method. The maximum value was observed at K = 2. The second most likely clustering structure was observed at K = 3.