Reservoir of the European chestnut diversity in Switzerland

Runnig tittle: Chestnut reservoir Switzerland

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Abstract

In Switzerland, chestnut forests cover about 27100 ha, plus some 6800 ha of mixed stands. Due to environmental and historical reasons, most of these still existing forests are located in the Swiss Southern Alps, whereas in the northern parts of the country the chestnut cultivation and the related knowledge strongly regressed since the Little Ice Age period. Nevertheless, Switzerland still hosts valuable genetic resources of the sweet chestnut tree. The present genetic study bases on a nationwide inventory, identification and precise localisation of old and/or grafted chestnut trees for conservation purposes. The main objectives were 1) to evaluate the genetic diversity and the genetic structure of *Castanea sativa* Mill. in Switzerland, and 2) to define a program of conservation including the proposal of a defined core collection. We genetically analysed a pre-selection of 962 accessions (out of 14165 inventoried trees throughout Switzerland), profiling them with 24 microsatellites. We identified 675 different genotypes out of 962 accessions with a 29.8% of repetitiveness due to clonality. A structural analysis based on a Bayesian method allowed to identify two main clusters, one mostly related to the genetic group from southern Europe (Reconstructed Panmictic Population RPP1) and a second one (RPP2) which revealed to be independent and genetically different from other European groups of chestnut cultivars. The Swiss RPP2 represents a new genetic group, and consequently a complement to genetic resources of chestnut tree in Europe. Genetic analysis allowed defining a core collection of 46 genotypes, which should be used in priority for the Swiss conservation program.

Keywords: European chestnut, SSRs, genetic diversity, structure, core collection

Introduction

Paleobotanical studies on fossil data allowed to identify the main refugia of the Sweet chestnut (*Castanea sativa* Mill.) during the Last Glacial Maximum (ca. 20 ka ago) in Transcaucasia and the Italian and Iberian peninsulas, with some minor other spots in the Marmara and Levant regions, in south-eastern Balkans and Central France (Krebs, Pezzatti, Beffa, Tinner & Conedera, 2019). The post-glacial natural dispersion during the climatic warming in the first half of the Holocene (ca. 11.5-5.7 ka ago) has been totally masked by the subsequent anthropogenic diffusion and cultivation of the species (Conedera, Krebs, Tinner, Pradella & Torriani, 2004). In Western Europe, this happened during the Roman expansion in particular, when the Conqueror diffused the idea of cultivating the chestnut tree outside the Mediterranean regions. As a result, the present genetic structure of the chestnut tree in Europe displays a differentiation between the western Italian and Iberian populations and the eastern Greek and Turkish ones (Mattioni, Martín, Pollegioni, Cherubini & Villani, 2013).

Romans privileged the chestnut cultivation as coppice system for timber production, but they already knew the grafting techniques and also reported on existing chestnut varieties (Conedera et al., 2004). The use of chestnut trees for fruit production raised in the post-Roman time and during the Medieval Warm Period in particular (MWP, defined in here as AD 900–1,300, Bradley, Hughes & Díaz, 2003), especially among mountain populations in central and south-Europe, who cultivated fruit chestnut trees for staple food production as well (Pitte, 1986; Squatriti, 2013; Buonincontri, Saracino & Di Pasquale, 2015).

Switzerland followed this main stream. The chestnut tree cultivation was first introduced south of the Alps, mostly as coppice wood, concomitantly with the Roman conquest

(Conedera et al., 2004). During the Middle Ages, this cultivation turned to an autarchic *chestnut civilisation* where the chestnut fruit was the main source of food (Krebs, Tinner & Conedera, 2014). In the northern slope and the inner alpine valleys of the Swiss Alps, the sweet chestnut started to expand in the medieval warm period and was limited to spots in suitable sites in terms of both mild climate and non-calcareous bedrock (Figure 1; Tanner, 1927; Closuit 1958; Furrer, 1958, 1972; Heiniger & Conedera, 1994).

North of the Alps the chestnut culture were abandoned with the first climatic turbulences in the 17th century related to the "Little Ice Age" that may have severely restricted the chances of a normal fruit ripening (Engler 1901). Contrarily, the southern Swiss chestnut civilization survived the climatic reverses and only slowly declined as a consequence of the introduction of alternative staple food (potato and maize), the onset of old and new pathogens (*Phythophtora* spp.; *Cryphonectria parasitica*), and the post-WWII socio-economic changes, which led to an abandonment of traditional chestnut cultivation (Krebs et al., 2014). The arrival of the Asian Gall Wasp (*Dryocosmus kuriphilus*) in 2007 (Forster et al., 2009), repeated summer droughts (Conedera, Barthold, Torriani, & Pezzatti, 2010), and the recently emerged fungal pathogen *Gnomoniopsis smithogilvyi* (Pasche et al., 2016) represent additional threats to the Swiss chestnut culture and to restoration efforts.

Nowadays chestnut forests cover about 27,100 ha, to which 6,800 ha of mixed stands with at least 50% of chestnut may be added (Brändli, 1998). Due to the natural evolution following management abandonment (Conedera, Stanga, Lischer & Stöckli, 2000), the chestnut area still presenting an orchard structure dropped to 3,400 ha. Most of these chestnut orchards are located south of the Alps, where the heritage of the medieval chestnut culture is still present in terms of both living giant trees (Krebs, Koutsias, & Conedera, 2012) and knowledge about chestnut tree varieties and related names

(Conedera, Müller-Starck, & Fineschi, 1994; Gobbin et al., 2007). In the Swiss Northern Alps, on contrary, the cultural heritage including cultivar names almost completely extinguished. A few variety names are still in use on the southern bank of Lake Geneva Lake and at least one of them (the variety "Ente") can be related to accessions located in the Swiss Chablais region. Based on these observations, we can however assume that the whole Switzerland still hosts the historical botanical heritage and the related genetic resources of a medieval sweet chestnut culture.

In order to preserve this heritage, several projects have been carried out in the different cultural and linguistic regions of Switzerland in the context of the National Action Program (NAP) for the conservation and use of plant genetic resources for nutrition and agriculture, established in 1999 and coordinated by the Swiss Federal Office for Agriculture (FOA). NAP represents the Swiss implementation of the Global Plan for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture launched by the Food and Agriculture Organisation (FAO) of the United Nations.

The main objectives of the Swiss chestnut NAP projects were: 1) to localize and inventory the fruit chestnut (*Castanea sativa* Mill.) trees in Switzerland; 2) to characterize the identified accessions by a unified methodology; 3) to select the most interesting accessions in terms of phenotypical, morphological and genetic diversity, and 4) to define a core collection to be considered for a conservation program. In this paper, we report on the implementation of the objectives 3 and 4.

The aim of confirming the in situ ethnobotanical and morphological observations with genetic profiling has been implemented from 2010 on, with the additional purpose to

avoid redundancies and related costs when setting and maintaining the orchards of the conservation programs.

In this paper, we present the genetic analyses of this nation-wide chestnut accessions inventory campaign that bases on the same set of molecular markers of the EU chestnut database (Pereira-Lorenzo et al., 2017). The obtained results in terms of genetic structure and diversity are than discussed in the frame of the chestnut cultural heritage at European level.

Material and Methods

Project structure, tree identification and localization, and sampling design

Due to the heterogeneity in the history and the related still existing knowledge on the chestnut varieties, the preliminary ethnobotanical, descriptive, and tree inventorial activities were split into different subprojects for each Swiss main cultural area (i.e., the Italian-speaking, southern part, the western French-speaking part and the central-eastern German-speaking part, see Figure 1 for details).

The NAP projects in southern Switzerland lasted from 1999 to 2018, provided a list of more than one hundred historical, and introduced chestnut variety names (Conedera et al., 1994). For most of the mentioned varieties, we localized and described representative trees in situ (Rudow & Conedera, 2001), whereas for a subset of the main varieties, we provided a genotypic analysis and a conservation program in an ex-situ core collection (Gobbin et al., 2007).

Starting in 2011, the development of a comprehensive chestnut traits descriptor's catalogue (Rudow, Bischofberger, Piattini & Hatt, 2012) helped to detect, in the central and eastern parts of northern Switzerland (Figure 1), an unexpected number and an

139 astonishing diversity of morphologically different tree individuals, which potentially
140 represent ancient varieties of the former local chestnut culture. Finally, we implemented
141 the same approach between 2011 and 2015 also in the western part of northern
142 Switzerland in the frame of a separated initiative.

143 In 2015, we sampled a subset of 962 accessions (out of the 14,165 inventoried trees
144 throughout Switzerland) for genetic analysis.

145 Sample selection followed different criteria according to the chestnut areas, for both wild
146 and cultivated trees, and for these latter ones based on the interviews with growers. The
147 presence of grafted trees is confirmed by repetitiveness of the genotypes (Pereira-Lorenzo
148 et al., 2019) and the comparison with the EU database (Pereira-Lorenzo et al.,
149 2017).North to the Alps, emphasis was set on still existing grafted trees, trees based on
150 the visual identification of the grafting marks and/or the information provided by growers,
151 as well as non-grafted old specimens. In southern Switzerland on contrary, many
152 accessions carrying similar names or presenting morphological similitudes were
153 considered for the analysis in order to detect synonyms and homonyms in the inventory.
154 Therefore, several varieties have been considered with redundancies in the southern
155 samples. The final sample consisted of 375 accessions from the western part of northern
156 Switzerland (out of 2,255 identified and observed accessions, i.e. about 13%), 385 from
157 the central and eastern part of northern Switzerland (out of 11,390 identified and observed
158 accessions, i.e. about 2.8%) and 202 accessions (out of 520 accessions) from southern
159 Switzerland.

160 ***Genetic analysis***

161 *DNA extraction*

Nucleic acids were extracted from silica gel-dried and apparently healthy portions (50-70 mg) of the leaves sampled in the field using a modified CTAB method adapted from Lefort & Douglas (1999), without any additional treatment of the leaf material. DNA was resuspended in sterile ultrapure water and quality and concentration were assessed with a Nanodrop ND-1000 spectrophotometer (WITEC AG, Switzerland). All samples were diluted at a final concentration of 50 ng/µl.

PCR amplification and SSR analyses

A set of 24 SSRs (Supplementary Table S1) was used for genetic characterization. This set included ten SSR loci provided by Marinoni, Akkak, Bounous, Edwards & Botta (2003): *CsCAT1, CsCAT2, CsCAT3, CsCAT6, CsCAT8, CsCAT14, CsCAT15, CsCAT16, CsCAT17,* and *CsCAT41b*; six other loci described by Buck, Hadonou, James, Blakesley & Russell (2003): *EMCs2, EMCs14, EMCs15, EMCs22, EMCs25* and *EMCs38*; four SSR loci by Gobbin et al. (2007): *CIO, OAL, OCI,* and *RIC*; two SSR loci by Kampfer, Lexer, Glossl, & Steinkellner (199): *QrZAG96* and *QrZAG4*; and finally two loci described by Steinkellner et al. (1997); *QpZAG36* and *QpZAG110*. The loci *CsCAT16* and *EMCs2* are in the same linkage group, but at a genetic distance over 50cM, and are therefore considered as independent.

The forward SSR primers were labelled with NED, VIC, 6-FAM or PET fluorochrome, and six different multiplex PCR reactions were designed (Supplementary Table S1).

Data analysis

The presence of null alleles was determined using Micro-Checker ver. 2.2.3 (Van Oosterhout, Hutchinson, Wills & Shipley, 2004). The observed heterozygosity (Ho) and expected heterozygosity (He) (Nei, 1978) were estimated using the GenAlEx 6 (Peakall

185 & Smouse, 2006). We used the Genodive software package (Meirmans & Van Tienderen,
186 2004) to estimate the inbreeding coefficient (F_{IS}) (Weir & Cockerham, 1984).

187 In order to study population structure and assign individuals to populations based on
188 the SSR genotypes with two alleles, we used a model-based Bayesian procedure,
189 implemented using the STRUCTURE software (Pritchard et al., 2000). Using the
190 admixture model with unlinked loci and correlated allele frequencies, we computed K
191 (unknown) RPPs (reconstructed panmictic populations) of individuals testing K = 1 to
192 15, assuming that the sampled cultivars were from anonymous trees of unknown origin
193 (we used the options usepopinfo = 0, popflag = 0). This clustering approach assigns
194 individuals probabilistically to reconstructed populations (RPPs) based on genotype.
195 Assignment of a cultivar to a RPP was based on a probability of membership *qI* of 80%,
196 while a lower probability meant that this accession could have several parental RPPs.
197 Twenty replicate runs per K value were carried out (Porras-Hurtado et al., 2013), each
198 consisting of a burning period length of 30,000 steps followed by 1,000,000 MCMC
199 (Monte Carlo Markov Chain) replicates. The STRUCTURE software estimates the most
200 likely number of clusters (K) by calculating the log probability of data for each value of
201 K. According to Evanno, Regnaut & Goudet (2005), we calculated the second-order
202 change of the likelihood function divided by the standard deviation of the likelihood (ΔK)
203 in order to assess the best K-value supported by the data by using Structure Harvester
204 (Earl & Vonholdt, 2012).

205 PCoA (also known as Classical Multidimensional Scaling) was used with Bayesian-
206 based method (Structure software) to investigate the pattern of population structure.
207 PCoA is a distance-based model which uses jointly a dissimilarity matrix calculated with
208 a simple-matching index, and a factorial analysis. PCoA was performed using the
209 software DARwin 6.0.010 (Dissimilarity Analysis and Representation for Windows).

This software produces graphical representations on Euclidean plans, which preserve at best the distances between units (Perrier, Flori, & Bonnot, 2003; Perrier & Jacquemoud-Collet, 2006).

A core collection was defined using PowerCore v. 1.0, which applies the advanced M strategy using heuristic search for establishing core or allele mining sets in large databases, which could represent all alleles (Kim et al, 2007). Three core collections have been calculated, the first one without considering any preselection, the second one considering as pre-selections all those trees retained for conservation, and a third one including the minimum number of trees out of the preselected ones for its maintenance and the necessity to conserve all the allelic diversity.

Results

We identified 675 different genotypes out of 962 accessions evaluated, with 29.8% repetitiveness due to clonality. We found out 54 genotypes repeated two up to 33 times, which totally represented 341 accessions (Supplementary Table S2). All genotypes were checked with the European chestnut database (Pereira-Lorenzo et al., 2017) in the same laboratory and no matches were found.

Marker summary

This study yielded 214 alleles over 24 loci (Table 1) with an average allele number of 8.9 alleles/locus. The most polymorphic loci were *CsCAT3* with 27 alleles, *CsCAT2* with 18 alleles, *EMCs38* with 16 and *CsCAT6* with 14, respectively. The least polymorphic loci were *QrZAG4* and *EMCs25* with 2 alleles and *EMCs2* and *EMCs14* with 3, respectively.

Rare alleles (allele frequency < 0.05) numbered 117 out of 214 total alleles (54.7%, Table 1). No rare alleles were detected in *EMCs25*, *QrZAG4*, and *EMCs2*, and only one was

found for *EMCs15*, *RIC* and *CIO*. The maximum number of rare alleles was 22 in *CsCAT3*, followed by the locus *CsCAT2* with 11 rare alleles.

Observed heterozygosity (Ho) varied from 0.024 for *EMCs25* to 0.902 for *CsCAT17* with an average value of 0.578 (Table 1). The lowest expected heterozygosity (He) was 0.035 for *EMCs14* and the maximum He was 0.887 for *CsCAT2* (Table 1), with an average of 0.648.

The presence of null alleles was detected in the loci *CsCAT14*, *QrZAG4*, *CsCAT2*, *CsCAT41b*, *CIO* and *EMCs25* (Supplementary Table S1).

Genetic and geographic structure

A Bayesian analysis with the STRUCTURE software was conducted using 18 SSRs to determine the genetic structure among 675 unique genotypes. Six loci harbouring null alleles were not included in this analysis. The ln[Pr(X/K)] values increased until K = 2 (Supplementary Figure S1) estimated by using Structure Harvester (Earl & Vonholdt, 2012) in a group of 400 genotypes out of 675, with a $qI > 80\%$ (59.3% of all genotypes.). This corresponded to a strong differentiation in two main groups of genotypes (RPP, reconstructed panmictic populations), one with 206 genotypes (RPP1), including Swiss cultivars such as ‘Terematt’ (30.5% of the total number of genotypes), and a second one with 194 genotypes (RPP2, 28.7%) including the cultivar ‘Lüina’ (Supplementary Table S2). ‘Terematt’ and ‘Lüina’ have been selected as representative cultivars for RPP1 and RPP2, respectively, because of their broad regional presence in the main Swiss chestnut area in southern Alps and their charismatic role as a typical early ripening cultivar (‘Terematt’) and variety to be transformed in dry chestnuts and flower (‘Lüina’). This analysis uncovers the first structural level in these data, which corresponds to two main distinguished populations. Geographical distribution of the genotypes for each RPP

showed that the two groups were distributed in most of the areas. In many zones of northern Switzerland the two RPPs are both present too with a predominance of RPP2 in the central zone of northern Switzerland, while where RPP1 was predominant in some zones of the western and eastern parts of northern Switzerland Figure 2). Admixed genotypes were scattered everywhere, even in some valleys with no presence of RPP1 or RPP2.

RPP1 had 14 genotypes repeated two or more times (76 accessions) with a peak of 21 accessions (Figure. 3, Supplementary Table S2); some others as 'Terematt' and 'Fugascera' were found 9 times, 'Marrone dei Pirenei' 6 times, 'Maron Bregaglia', and 'Temporiva' three times each. 'Rosone' was found to be a genetic synonym of 'Belusciora', as 'Rosséra' a genetic synonym of 'Temporiva'. RPP2 had 20 repeated genotypes (177 accessions) up to 33 accessions of an unknown variety, 'Magreta Gambarogno' (putative synonym of 'Marrone di Cuneo') was found 3 times, 'Lüina' 29 times, 'Castagno Grande' 21 times, 'Buné Negro' 9 times and 'Berogna' two times. 'Marrone Gambarogno' was revealed to be a genetic synonym of the imported 'Marrone di Cuneo'. We also found repeated genotypes among the amixed genotypes with 'Moréla' repeated 14 times, 'Torcion Negro' and 'Magreta Verzasca' 11 times, 'Tenasca' 5 times and 'Negra' 4 times among others. 'Revultana' was found to be a genetic synonym of 'Moréla'. In a European context, the Swiss RPP1 genotypes grouped with most of the cultivars compiled in the European database (Pereira-Lorenzo et al., 2017), while RPP2 did not match with any of them (data not shown), making therefore RPP2 a very particular and interesting genotypic group specific to Switzerland, and possibly a remain of the medieval chestnut culture in Switzerland.

The geographical distribution of the most repeated genotypes were found mainly in three areas. The most repeated genotype in RPP1 was located only in the South and in the West, meanwhile in northern Alps were only cultivated some genotypes from RPP2 (Figure 3).

PCoA analysis utilising DARwin 6.0.010 software confirmed the results obtained with STRUCTURE (Figure 4). Chestnut cultivars grouped in two main clusters differentiated in the first axis, which was consistent with the main RPPs obtained with STRUCTURE with admixed genotypes in between. Additionally, the Neighbor-joining (NJ) tree based on the genetic distance matrix between 673 chestnut genotypes also grouped them in agreement with STRUCTURE for K = 2 (Figure 5) although without any sharp separation.

Pairwise Fst value between the two reconstructed main populations RPP1 and RPP2 was 0.096 ($P<0.001$), while it was and 0.029 ($P<0.001$) between RPP1 and admixed genotypes and 0.027 ($P<0.001$) between RPP2 and admixed genotypes, respectively (Supplementary Table S3).

When we compared the genetic diversity for each RRP (Supplementary Table S1), we found 197 alleles in RPP1 and 163 in RPP2. RPP1 displayed 91 rare alleles ($P<0.05$) while 84 rare alleles were found in RPP2. The total of exclusive alleles, meaning the alleles present in only one RPP, was 25 (11.7%). Most of the exclusive alleles were found in the 'Terematt' group (RPP1) with 12 alleles, followed by the admixed group with nine alleles and the 'Lüina' group (RPP2) with four alleles.

Among the 24 SSRs analysed in this study, 34 alleles (15.9%) were only found in Switzerland and were not present in the EU database (Pereira-Lorenzo et al., 2017). Moreover, out of these 34 alleles, 12 alleles were exclusive to RPP1, four to RPP2, nine to the admixed genotypes, whereas the remaining nine alleles were found in more than

one RPP and/or admixed genotypes. Finally, 36 alleles found in previous studies in southern Europe with the same SSRs did not appear in the Swiss samples, falling to 14 alleles if hybrids were excluded. We compared those Swiss genotypes with exclusive alleles with previous studies carried out with 10 common SSRs performed in interspecific hybrids (Pereira-Lorenzo et al., 2010, 2019). This showed that 20 Asian alleles were found in 28 Swiss genotypes, but still 14 alleles were only present in 31 Swiss genotypes (Table 1). In order to check if these introgressants could have affected the structure analysis, 28 putative hybrids genotypes were removed: 17 for RRP1, two for RPP2 and nine for the admixed group. The genetic structure without those genotypes harbouring exclusive alleles for Switzerland showed similar results with two RPPs (data not shown).

Core collection as a strategy for conservation

A total of 46 genotypes were selected (Supplementary Table S2) in the first core collection (without considering any preselection) by using heuristic search with PowerCore (v. 1.0) (Kim et al., 2007). This core collection represented 7.1% of the total number of the total 647 genotypes and 4.9% of the total accessions (out of which 28 putative hybrids were excluded). Out of this core collection, only 19 genotypes (11.1%) coincided with trees preselected by the Swiss National Action Plan for conservation (171 genotypes in total and 12 more putative hybrids). When all preselections were considered (171), 188 genotypes were selected for the core collection, which represented 29.1% of the total number of genotypes (647) and the 20.15% of the total accessions, without putative hybrids. A third core collection was calculated in order to reduce the number of genotypes to be conserved in a core collection, keeping the total allelic diversity but including as many preselections as possible. This resulted in a selection of 53 genotypes (8.5% of the total genotypes considered and 5.9% of the total accessions), of which 43 corresponded to pre-selected trees (25.14%, Supplementary Table S2).

Discussion

In comparison, clonality in the EU database was 56.5% (Pereira-Lorenzo et al., 2017), nearly double than in Switzertland because the study was mainly focused on cultivated chestnut.

SSR markers and diversity

The number of alleles and average number of alleles per locus was identical to the values referred for the EU chestnut database, even though some differences were found for some loci and the number of genotypes analysed in the present study was much higher. The total expected heterozygosity (He) was also similar in both studies, while the total observed heterozygosity (Ho) was found lower in Switzerland, which meant a higher deficit in heterozygotes. A high number of rare alleles (54.7% of all alleles) as well as a high number of exclusive alleles unique to Switzerland (15.9%) give an insight of a particular diversity of chestnut genetic resources in Switzerland.

Genetic and geographical structure and diversification process

The genetic structure revealed by this study was related to two main groups. The first one referred to as ‘Terematt’ (RPP1) belongs to the European group of cultivars listed in the European Database (Pereira-Lorenzo et al., 2017). Surprisingly, this Swiss genetic group did not match with the Italian and French groups as described in the European Database, nor with the genetics of the giant trees from southern Europe (Pereira-Lorenzo et al., 2019). Moreover, the second main cultivar group referred to as ‘Lüina ‘(RPP2), did not match with any of the European chestnut cultivars genetic profiles of the EU database, which indicates a new and very different and independent group for southern Europe. These surprising results illustrate that the nowadays remains of the abandoned Swiss

chestnut culture might provide a historical window to late medieval chestnut culture in Switzerland, which has been only partly overprinted by more recently varieties, as found in RPP1.

In addition, both Swiss RPPs were related to grafted cultivars and some many other unique putatively wild genotypes in most of the sampled places. As it happened in Spain and Italy, grafted trees in this study clustered with wild trees indicating common ancestors (Pereira-Lorenzo et al., 2019), and, in some cases with genotypes from other areas, such as cv. 'Marrone di Cuneo' from Italy, similarly to what happened with the Spanish cultivar 'Luguesa' in north-western Spain, which originated from an ancestral population of Italy.

The genetic structure was related to the geographical distribution of the genotypes in Switzerland, even though both genetic groups overlapped in many places as it happened in southern Europe with Italian genotypes, which introgressed in the Iberian Peninsula from at least the 17th century.

In chestnut trees, multilocal selection events were identified as the origin of the main cultivars (Pereira-Lorenzo et al., 2010, 2011), likewise to what was observed in olive (Claros, Crespillo, Aguilar & Canovas, 2000; Besnard, Baradat & Berville, 2001) where a high diversity of cultivars was found in the same areas of the presence of wild olive trees. As shown by this study, this also seems to be the case in Switzerland, where clonal genotypes were found in both RPPs, one of them genetically different from the European database of cultivars (Pereira-Lorenzo et al., 2017) including the main cv. 'Lüina'. This cluster also included cv. 'Magreta Gambarogno', which was found to be synonym to the putative cv. 'Marrone di Cuneo', a northwestern Italian cultivar sampled with this name in Switzerland. The genetic differentiation between Swiss RPPs was found to be even

higher, Fst 0.096 ($P<0.001$), than the one between the Iberian and Italian groups, Fst 0.068 ($P < 0.001$).

The clonality rate was 29.8%, which is much lower than the clonality rates of 56.5% reported in the EU chestnut database (Pereira Lorenzo et al., 2017) and 51.5% in the study including European giant chestnut trees (Pereira Lorenzo et al., 2019). This low clonality rate would very likely be related to the sampling approach and in particular the loss of knowledge on certified varieties and related names in the northern part of the country. In addition, the singular genetic group found in Switzerland (RPP2) showed higher clonality (44.7%) than the RPP1 (37.9%) due to clonal accessions in southern Switzerland. This is probably due to better-preserved chestnut culture and related orchards in southern Switzerland. This fact could also be an explanation for the lower allelic diversity found in this singular Swiss RPP2 group displaying 16% less alleles than the RPP1.

The set of markers used in this study has also been very effective in identifying Asian introgressants into local populations, which amounted to 4.1% in this study in comparison to 2.3% of such hybrids in the EU database (Pereira-Lorenzo et al., 2017). Introgressants were present in both RPPs, but more frequent in RPP1. As shown previously without those putative interspecific hybrids, the genetic structure was not affected, which was consistent with previous results in some other areas from Europe and related with the European origin of most of the interspecific hybrids.

Core collection for conservation

In this study, the number of genotypes kept for the core collection without considering any preselection was reduced up to the 92.9% (91.5% if preselections are considered), which was close to the 89% reduction obtained by Kim et al. (2007), and even lower at 95.1% when the total accessions (excluding putative hybrids) were considered. This is in

agreement with the values considered by van Hintum, Brown, Spillane & Hodgkin (2000), who estimated that a core collection should be no more than 10% of the genotypes, and between 5% and 20% of the accessions of the complete collections. Those obtained values are lower than the ones obtained for the EU database (Pereira-Lorenzo et al., 2017), i.e. 30.0% of the total genotypes (37 cultivars) and 13.9% of the accessions (266 accessions), respectively. These higher values could be partly due to the evaluation of a much wider area at European level.

Conclusions

A new genetic group not reported previously, genetically different from the main European group of cultivars was found in Switzerland and might represent a remain of the abandoned chestnut culture of the medieval times.

The genetic analysis carried in this study allowed defining a priority conservation program for the Swiss European chestnut trees based on a core collection of 53 genotypes. Without regard to typical regional varieties, the minimal core collection could even be reduced to 46 genotypes, corresponding to 7% of the total genotypes evaluated in this study. This core collection based on allelic diversity could be completed with some other genotypes with phenotypic traits of interest, to minimize the risk of losing relevant genetic diversity.

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Data Accessibility Statement

A comprehensive DNA samples database is stored at the Institute land Nature Environment (HES-SO University of applied sciences and arts western Switzerland)

Author Contributions

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TABLE 1 Allelic range (bp), number of alleles per locus (Na), number of rare alleles ($p < 0.05$), percentage of infrequent alleles with respect to the total number of alleles, percentage of infrequent alleles with respect to the total number in each RPP, Number of Effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), and alleles identified in the overall set.

Locus	bp	Number of alleles (Na)	Rare Alleles (p<0.05)	% of rare alleles with respect to the total number of alleles	Ne	Shannon's Information Index	Ho	Ho without hybrids	He	He without hybrids	Fixation Index	Alleles‡
CIO†	146-150	5	1	20.0	3.163	1.349	0.418	0.424	0.684	0.686	0.388	146, 147, 148, 149, 150
CsCAT1	177-225	11	6	54.5	3.974	1.638	0.757	0.756	0.748	0.747	-0.012	177, 190, 194, 206, 208, 215, 217, 219, 221, 223, **225**
CsCAT2†	196-237	18	11	61.1	8.761	2.357	0.509	0.504	0.886	0.886	0.425	196, 200 §, 205, 209, 211, 213, 215, 217, 219, 221 §, 223, 225, 227, 229, 231, 233, **235** §, 237 §
CsCAT3	190-268	27	22	81.5	7.402	2.455	0.798	0.792	0.865	0.863	0.078	190 §, 196, 208, 214 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245 §, 247, 249, 251, 253, 255, 257, 260, 262, 264, **266** §, 268
CsCAT6	157-196	14	9	64.3	5.536	1.891	0.781	0.782	0.819	0.818	0.047	157, 159, 163, 165, 171, 173, **175**, 178, 180, 182, 184, 190, 194, 196
CsCAT8	189-212	7	2	28.6	4.309	1.546	0.733	0.732	0.768	0.767	0.046	189, 199, 201, 203, 208, **210**, 212
CsCAT14†	128-161	6	3	50.0	3.294	1.308	0.688	0.690	0.696	0.696	0.012	**128** §, 133, 141, 150, 152, 161
CsCAT15	122-158	9	5	55.6	3.169	1.426	0.696	0.697	0.684	0.682	-0.017	122, 124, 126, 130, 134. 136, 140, 155, 158
CsCAT16	126-156	9	6	66.7	2.777	1.293	0.646	0.641	0.640	0.627	-0.009	126, 130, 132, 136, 141, 143, 145, 148, 156
CsCAT17	129-163	11	4	36.4	6.726	2.070	0.902	0.900	0.851	0.849	-0.059	129, 133, 139, 141, 145, 149, 155, 157, 159 §, 161 §, 163
CsCAT41b†	212-235	12	8	66.7	4.515	1.854	0.470	0.469	0.779	0.778	0.396	212, 216, 218, 220, 222, 223, 226, 228, 231, 233, 234, 235
EMCs2	160-166	3	0	0.0	2.465	0.981	0.593	0.594	0.594	0.589	0.003	160, 163, 166
EMCs14	131-144	3	2	66.7	1.036	0.092	0.033	0.028	0.035	0.027	0.067	131, 140, 144
EMCs15	82-93	5	1	20.0	2.782	1.168	0.604	0.608	0.641	0.644	0.057	82, 85, 88, 91, **93**
EMCs22	128-155	8	5	62.5	1.613	0.836	0.363	0.372	0.380	0.388	0.046	128, **130**, 132, 134, 136, 145, 147, 155 §

EMCs25†	158-160	2	0	0.0	1.486	0.509	0.024	0.025	0.327	0.330	0.927	158, 160
EMCs38	232-276	16	9	56.3	7.225	2.227	0.807	0.805	0.862	0.861	0.063	232, 238, 240, 242, 244, 246, 248, 250, 256, 258, 260, 262, 264, 272, 274, 276
OAL	297-332	9	5	55.6	2.639	1.397	0.605	0.602	0.621	0.622	0.026	297**,** 299 §, 301, 303, 305, 309, 322, 330, 332
OCI	146-161	5	2	40.0	2.567	1.104	0.592	0.588	0.610	0.608	0.030	146, 149, 157, 159, 161
QpZAG36	211-225	6	2	33.3	3.649	1.400	0.693	0.694	0.726	0.723	0.045	211, 217, 219, 221, 223, 225
QpZAG110	206-235	13	9	69.2	4.052	1.572	0.662	0.663	0.753	0.752	0.121	206, 210, 213, 215, 219, 220, 222, 223, 225, 227, 229, 233, **235** §
QrZAG4†	110-114	2	0	0.0	1.253	0.354	0.195	0.189	0.202	0.198	0.035	110, 114
QrZAG96	137-169	9	4	44.4	3.791	1.535	0.675	0.668	0.736	0.733	0.084	137, 145, 153, 155, 159, 161, 165, 167, 169 §
RIC	119-127	4	1	25.0	2.768	1.093	0.631	0.630	0.639	0.638	0.013	119, 121, 123, 127
Total		214	117	54.7								
Mean		**8.9**			3.790	1.394	0.578	0.577	0.648	0.646	0.117	
SE					0.415	0.121	0.047	0.047	0.044	0.044	0.044	

†Locus with null alleles detected with Micro-Checker (van Oosterhout et al. 2004)

‡Alleles identified. §, alleles present in Switzerland not detected in previous work in Europe (Pereira-Lorenzo et al., 2010, 2017, 2019); simple underlined, exclusive alleles of the RPP1; double underlined, exclusive alleles of the RPP2; in bold, alleles exclusives of admixed.

FIGURE 1 NAP projects to inventory chestnut trees throughout Switzerland.

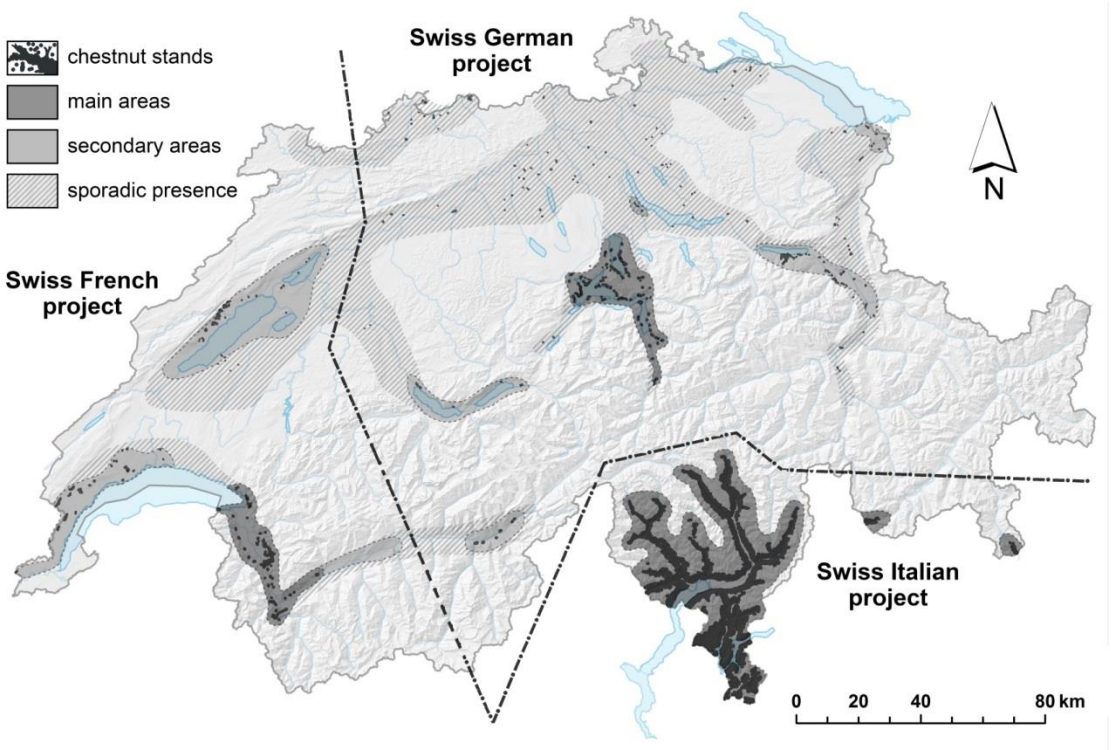

FIGURE 2 Distribution of 675 Swiss chestnut unique genotypes in reconstructed populations (RPPs) when K =2 based on data of 18 SSR loci.

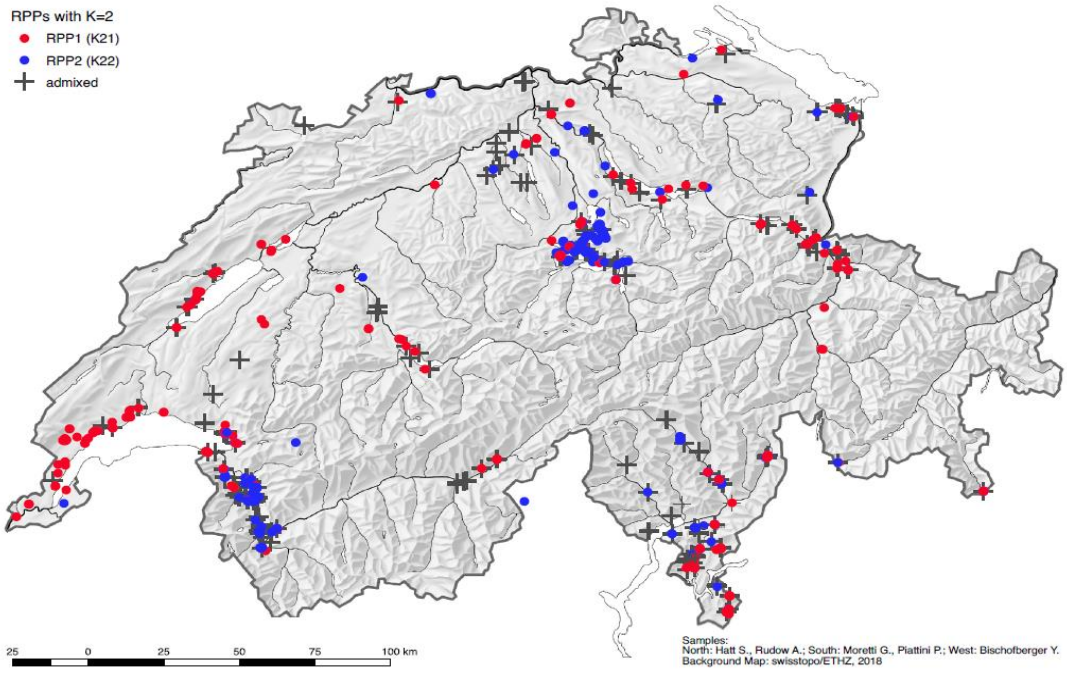

FIGURE 3 Location of the main genotypes clonally propagated by RPP when K = 2.

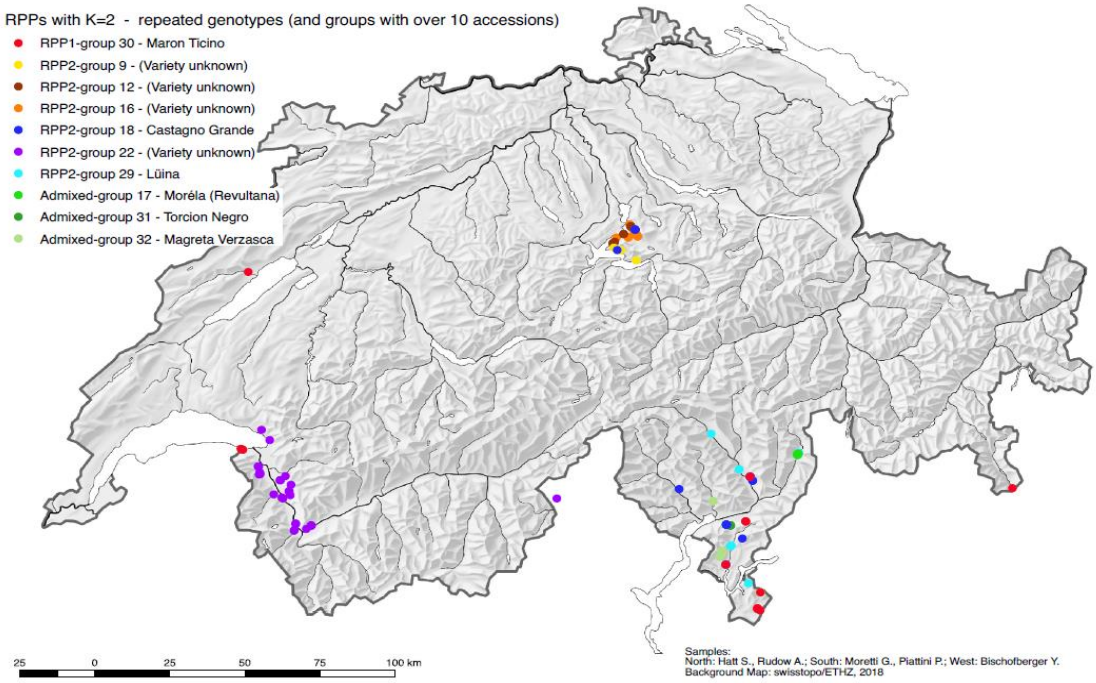

FIGURE 4 Principal coordinates analysis (PCoA). PCoA using 18 SSRs in the set of 675 Swiss chestnut genotypes showing structure (K = 2): in red RPP1 with a qI > 80%; in blue, RPP2 with a qI > 80%; in black admixed

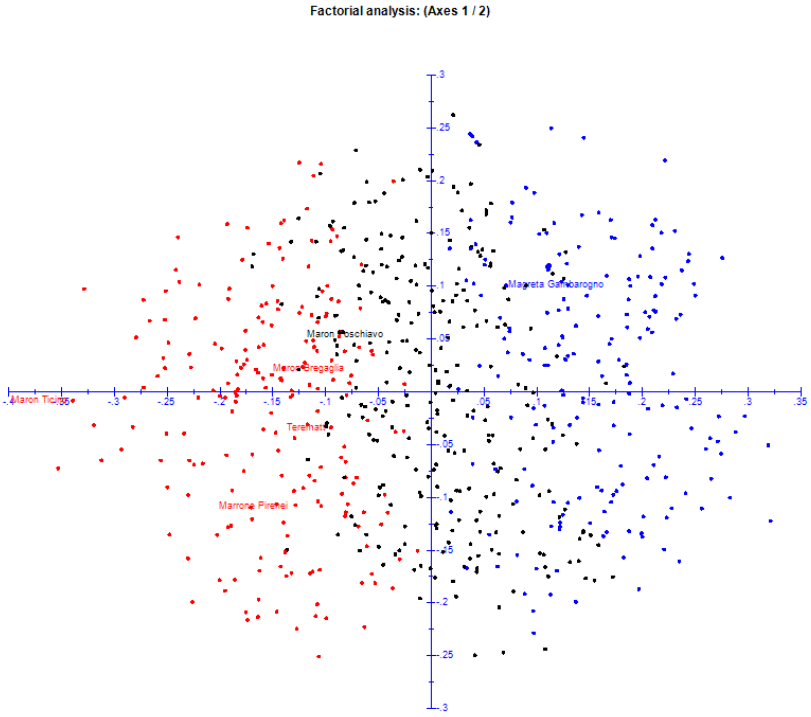

FIGURE 5 Neighbor-Joining Trees PCoA using 18 SSRs in the set of 675 Swiss chestnut genotypes showing structure (K = 2): in red RPP1 with a qI > 80%; in blue, RPP2 with a qI > 80%; in black admixed