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Molecular Characterisation of the Swiss Fruit Genetic Resources

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Abstract Switzerland has a rich pool of fruit genetic resources as it is located in the centre of Europe where it could benefit from the intensive exchange of goods within the continent. The rich heritage of traditional fruit varieties in *Malus domestica*, *Pyrus communis*, *Prunus avium* and *Prunus domestica* was analysed with molecular markers during the last 8 years. The total genetic diversity was investigated for the accessions conserved in the National Network of Swiss Fruit Genetic Resources Collections using microsatellite markers. The obtained data set was used to detect genetic similarities between individual accessions thereby identifying duplicates within the collections. Additionally, inferences about genetic structure, relationships between genetic diversity and original geographical sampling locations and correlations between phenotypic traits and genotype were performed. The results obtained will contribute the efficient and economically feasible conservation of fruit genetic resources within Switzerland.

Keywords Fruit genetic resources · Genetic diversity · Microsatellite analysis · Fruit trees

Molekulare Charakterisierung der Schweizer Obstgenressourcen

Zusammenfassung Die Schweiz besitzt einen reichen Pool an Obstgenressourcen und, da das Land inmitten von Europa liegt, konnte es vom intensiven Austausch an Gütern innerhalb Europas profitieren. Das reiche Erbe an Obstsorten von *Malus domestica*, *Pyrus communis*, *Prunus avium* und *Prunus domestica* wurde innerhalb der letzten 8 Jahre mittels molekularen Markern charakterisiert. Die gesamte genetische Diversität konnte für alle Akzessionen der Nationalen Erhaltungssammlungen mittels Mikrosatellitenmarkern erfasst werden. Der resultierende Datensatz wurde benutzt, um genetische Ähnlichkeiten zwischen individuellen Akzessionen zu erkennen und somit Duplikate zu identifizieren. Zusätzlich konnten Rückschlüsse über die genetische Struktur, den Zusammenhang zwischen genetischer Diversität und geographischer Herkunft und über die Korrelation von Phänotyp und Genotyp gemacht werden. Die Resultate dieses Projektes können zu einer effizienten und ökonomisch sinnvollen Erhaltung von Obstgenressourcen beitragen.

Schlüsselwörter Obstgenressourcen · Genetische Diversität · Mikrosatellitenanalyse · Obstbäume

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Introduction

Based on the United Nations Convention on Biological Diversity (CBD, http://www.cbd.int) the Food and Agriculture Organization of the United Nations (FAO) developed a global plan of action (www.globalplanofaction.org) in 1996, in order to conserve plant genetic resources for food and agriculture which 150 countries have signed. In Switzerland,

the Federal office for Agriculture (FOAG) subsequently established a "National Action Plan for the conservation of Plant Genetic Resources for Food and Agriculture" (NAP-PGREL) which provides the framework to apply this global action plan. Project proposals were then submitted mainly by NGO's (Non-Governmental Organization) interested in the fruit varieties heritage. A major project within this framework was to create an inventory of the still existing traditional varieties in order to preserve existing genetic resources in decentralized collections (Kellerhals and Egger 2004). Within the NAP-PGREL, the Swiss NGO Fructus (www.fructus.ch) started in 2007 a project (BEVOG) to genotype and phenotype accessions from those collections. The aim was to characterise the pool of Swiss fruit genetic resources and to identify duplicates allowing also to simplify preservation within these collections. Additionally the project aimed at evaluating susceptibility and resistance to plant diseases (Kellerhals et al. 2012; Gassmann et al. 2014). Moreover, the traditional accessions were evaluated as to their suitability for processing. In the past, the accessions had been selected for cultivar collections according to different names and pomological characterisation, which can lead to underestimation of genetic diversity, since multiple genotypes may exhibit the same phenotype, and can produce redundancies, because growers give multiple names to the same variety within different regions. The advent of economically feasible molecular diagnostic methods such as microsatellite genotyping has facilitated the analysis of genetic diversity, thereby improving selection of cultivars of interest (Lörz and Wenzel 2007).

Biodiversity is the key to functional ecosystems. Agroecosystems provide food for mankind but the domestication of plants used for food production can lead to adverse effects on genetic diversity (Doebley et al. 2006). Prevention of excessive inbreeding and the resulting loss of fitness (Charlesworth and Willis 2009) is key for breeding of cultivars resistant to various pathogens and with favourable fruit characteristics. The preservation of existing genetic resources therefore provides an invaluable tool for the prevention of inbreeding depression and thus maintaining efficient and sustainable food production (Ramanatha Rao and Hodgkin 2002).

Here, we provide microsatellite data on the genetic diversity of the total gene pool of Swiss fruit accessions and – to a certain extent – on genetic trueness to type. In total we characterized 2495 apple accessions (*Malus domestica*), 1302 pear accessions (*Pyrus communis*), 860 cherry accessions (*Prunus avium*, *Prunus cerasus*), and 399 plum accessions (*Prunus domestica*) from 6 different cultivar collections all over Switzerland.

Materials and Methods

Sample Collection and DNA Extraction

Plant material consisted of leaves of fruit trees collected from different Swiss National cultivar collections (Figs. 1, 2). DNA was extracted from leaf tissue using the Extract-N-Amp Plant Solution (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. A small circular leaf disc was punched out (Fig. 3) using the lid of 200 µl Eppendorf tubes and added to 100 µl of extraction buffer, heated at 95 °C for 10 min and diluted with 100 µl

Fig. 1 Map of the geographical locations of the SwissFruit cultivar collections. The size of the pears corresponds to the amount of accessions present in a collection
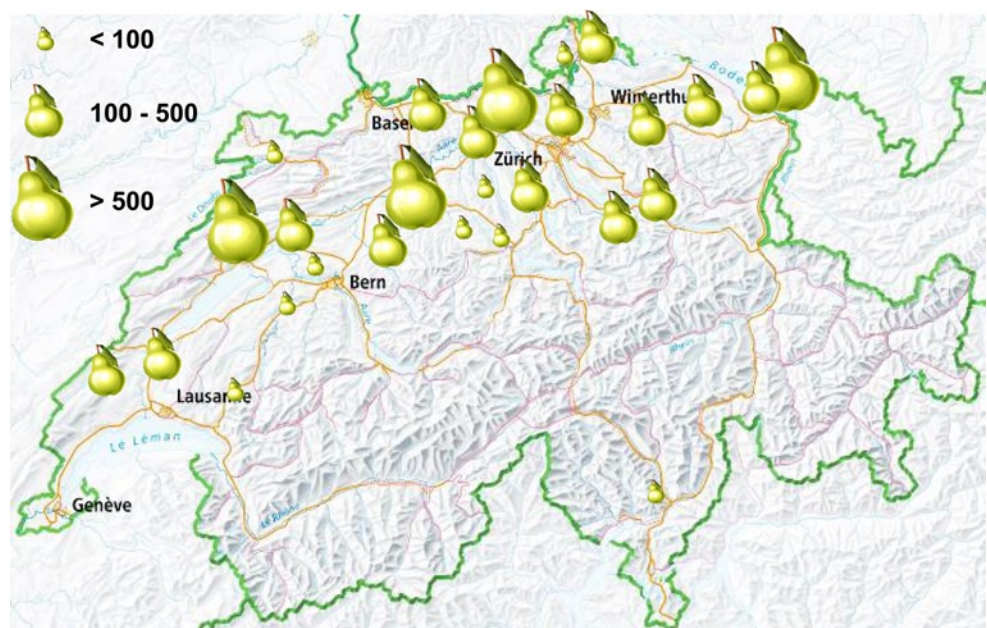
Fig. 2 Tree Genebank at Zurich University of Applied Sciences in Wädenswil, Canton Zurich

Fig. 3 Cherry leaf-sampling in the laboratory for genetic analysis at Agroscope in Wädenswil

dilution buffer. Microsatellite markers had been developed (Mnejja et al. 2005; Evans et al. 2009; Patocchi et al. 2009; Frei et al. 2010) and validated by the European Cooperative Programme for Plant Genetic Resources (ECPGR). These microsatellites were used in the multiplex primer mixes denoted in Table S 1. PCR was performed using the Multiplex Master Mix (Qiagen, Hilden, Germany) running the protocol 15 min at 95 °C initial denaturation, 40 cycles of 40 s at 94 °C, 90 s at 55 °C, 90 s at 72 °C and a final elongation of 30 min at 60 °C. For fragment analyses, PCR reactions were diluted 1:10 in PCR grade H_2O. 0.8 µl of the diluted PCR product was then mixed with 15 µl of HiDi Formamide (Life Technologies, Foster City, CA, USA) and 0.25 µl GS 500Liz3130 size standard (Applied Biosystems). Standard capillary electrophoresis was performed in an ABI PRISM 3130xl genetic analyser (Life Technologies). Results were scored and analysed using GENEMAPPER v 4.0 (Life Technologies).

Data Analysis and Genetic Diversity

Amplified fragments of each allele were scored in Gene-Mapper as length in base pairs. The resulting matrix served as input to the software Darwin v.5.0.158 (Perrier et al. 2003; Perrier and Jacquemoud-Collet 2006). For genetic similarity, hierarchical clustering was performed on dissimilarity matrices calculated in Darwin using single linkage and 0 % threshold equality options. Alternatively, grouping of accessions (i.e., accessions with identical microsatellite profiles) were performed using a custom Visual Basic script. The identified groups were numbered and only one representative from each group was included into further analysis.

Population Structure in Apple

The apple dataset was stripped of samples with triploid signals leaving 1812 samples in order to perform population genetic analysis. The used markers were checked for suitability for such analyses. Linkage disequilibrium, deviation from a neutral equilibrium model and measurements of genetic variation was tested in Genepop v. 4.0 (Raymond and Rousset 1995). Signatures for population subdivision were tested in Structure v. 2.3.4 (Pritchard et al. 2000) using 50'000 burn-in steps and 500'000 MCMC iterations for each K in triplicates. Best K was evaluated using the method of (Evanno et al. 2005). A subpopulation of the apple accessions specifically used for cider production was tested to evaluate genetic clustering explaining the given phenotypic trait. Clustering was performed in Structure v. 2.3.4 with population origin set to either cider or dessert apples. The effect of sampling location on genotype and possible isolation by distance was evaluated in a subpopulation of 217 apple samples with known sampling coordinates using Mantel tests in GenAlEx v. 6.5 (Peakall and Smouse 2012).

Results and Discussion

Identification of Duplicates

Here we provide insight into the genetic diversity of the Swiss cultivar collections (Fig. 1) using a microsatellite typing approach similar to others described previously (Garkava-Gustavsson et al. 2008; Antonius et al. 2012; Sehic et al. 2012). Based on the microsatellite profile, duplicates were identified using dissimilarity analysis. With this approach we were able to identify between 28 and 54 % duplicates depending on the crop (Table 1). The amount of accessions within groups of duplicates varies for the different fruits species analysed (Fig. 4).

Small mutations giving rise to interesting phenotypes such as a red cherry mutation leading to a phenotype with
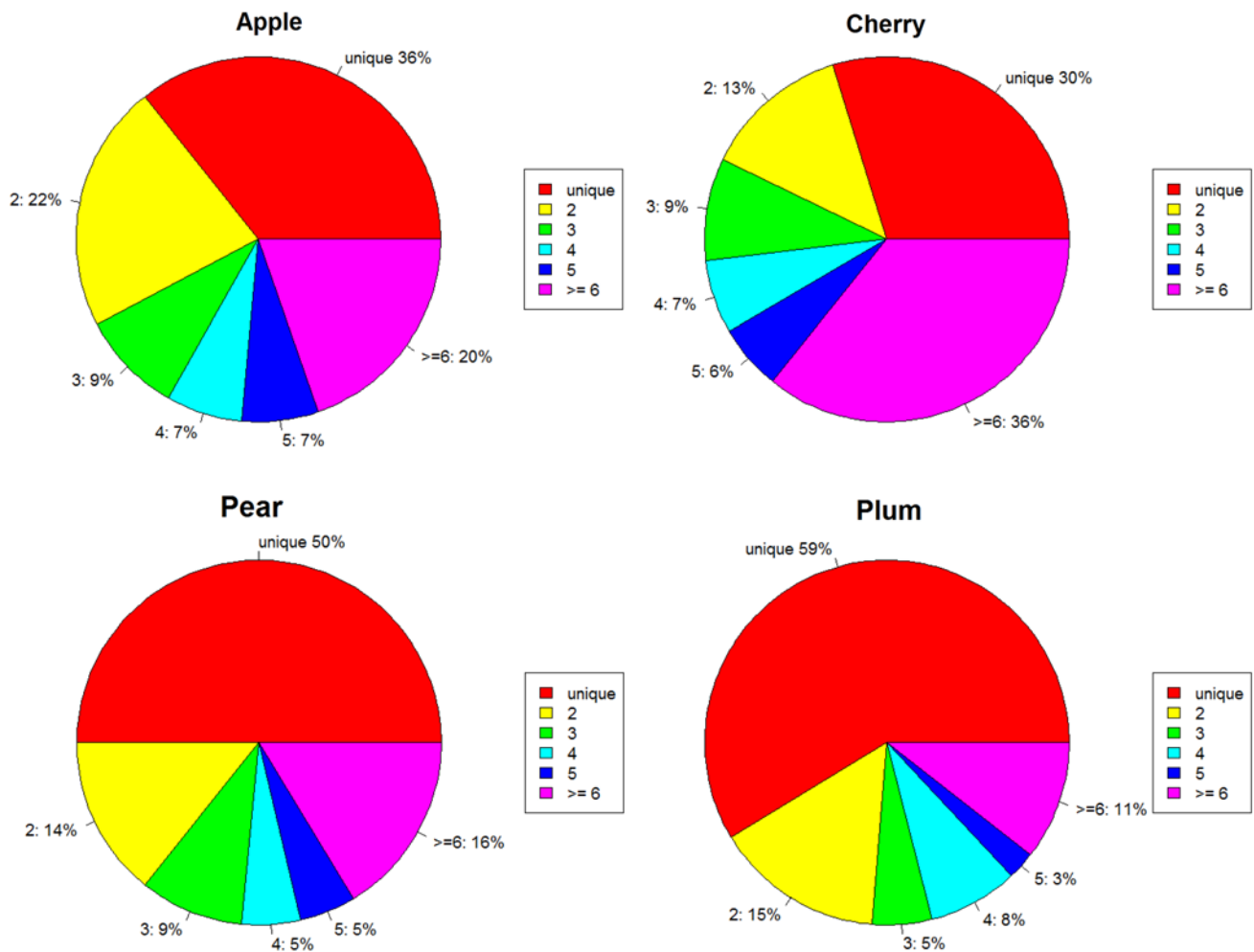
Fig. 4 Percentage of the different groups identified after molecular duplicate analysis for the four fruit species

Table 1 Duplicates identified within the different fruit species datasets as estimated using Darwin v. 5.0.158

Fruit	Samples analysed	Duplicates	% of duplicates	Nr of molecular profiles detected
Apple	2495	1108	44.41	1387
Cherry	860	465	54.07	395
Pear	1308	468	35.78	840
Plum	399	114	28.57	285

a black stripe cannot be detected with this approach as mutants cannot be distinguished (Selkoe and Toonen 2006). Thus microsatellite genotyping cannot completely substitute careful phenotypic characterisation of accessions. Nonetheless, the identified genetic profiles will allow for optimization of conservation efforts within collections.

Genetic Diversity

To identify genetic diversity within the sampled fruit accessions of each species, heterozygosity values, inbreeding coefficients and allelic richness were calculated (Table 2). All four fruit species show comparably high values of heterozygosity and low fixation indexes indicating a genetically diverse cultivar pool with a large proportion of selection induced by breeders.

Population Structure in Apple

The apple dataset was then analysed to a deeper level using clustering methods similar to those applied in (Cornille et al. 2012; Cornille et al. 2013) in order to identify possible population subdivision. A Test for Hardy-Weinberg Equilibrium resulted in significant deviations from random mating expectations. Testing for Linkage equilibrium resulted in highly non-random association of alleles at all loci indicating non-random mating and selection by breeders. These results can be expected in fruit trees of relatively small regional diversity with a high proportion of induced selection by breeders and a high percentage of asexual reproduction by cuttings. Nonetheless a bayesian cluster-

Table 2 Summary of genetic variation within the four fruit species analysed

Fruit	N_a	N_e	H_o	H_e	F
Apple	29.79	6.25	0.83	0.84	0.011904762
Cherry	12.29	3.7	0.66	0.73	0.095890411
Pear	29.06	6.25	0.76	0.84	0.095238095
Plum	2.86	10	1	0.9	−0.111111111

N_a and N_e Number of different and number of effective alleles per locus, H_o and H_e Observed and expected heterozygosity, F Fixation index

ing analysis implemented in structure was performed and yielded a highest likelihood for K = 2. Because the method cannot distinguish between K = 1 and K = 2, meaning it cannot differentiate between one or two populations, it is assumed that the cultivars were sampled from only one cluster for apple presenting no barriers for admixture within Switzerland. This confirms the previously identified weak genetic structure of apple within Europe (Cornille et al. 2012). Additionally we could not identify distinct clusters for cider or dessert apples, respectively, as weakly implied in (Cornille et al. 2012). Lastly, we tested the geographic sampling location within Switzerland for correlation with genotype on a subset of 214 apple samples from one single cultivar collection. A Mantel test in GenAlEx yielded R^2 values of < 0.004 indicating a weak to no association of genotype with geographic sampling location. Thus, within Switzerland, no geographic barriers existed in the past that could allow for population subdivision or isolation by distance within distinct geographic locations.

Conclusion

The total gene pool of the main fruit tree species in Swiss genetic resources collections was analysed using microsatellite markers. Among a total of 5062 accessions genotyped, we found 2901 different molecular profiles. The achieved identification of duplicates will assist in the efficient and sustainable management of Swiss cultivar collections. The elimination of redundancies will increase cost efficiency for existing cultivar collections thus opening opportunities for the preservation of novel accessions included in the conservation system or previously not recognized as unique.

The data provided here can be used in breeding programmes to avoid inbreeding and its unwanted effects such as pathogen susceptibility (Bannier 2011). Genotypic and phenotypic insights gained within the BEVOG projects have already contributed (Kellerhals et al. 2009) and will continue to contribute to fruit breeding programs. Rare varieties identified from and characterized within this project can contribute to the identification of genuine varieties suitable for apple juice or cider production and other uses. The

challenge here will be the evaluation of these cultivars for local commercial growing.

Our microsatellite analyses can serve as a starting point for the development of SNP typing schemes that are nowadays economically feasible and provide much deeper insight compared to microsatellite genotyping (Mammadov et al. 2012). The use of the phenotyping data of this project in combination with SNP typing genes would allow genome wide association studies that have the potential to assist in better understanding of phenotypic traits important to breeders and growers alike. Such information is essential for breeding cultivars with broad genetic basis suitable for sustainable fruit production systems.

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