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Small scale analysis of population structure in the woody cornelian cherry *Cornus mas* L. (Cornaceae) by AFLP accentuates the need for a population based conservation strategy

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Summary

We investigated population differentiation among and within three populations (two natural, one artificial) of the cornelian cherry (*Cornus mas* L., Cornaceae) to examine the extent of gene flow from planted cornelian cherries commonly used in planting vegetations of public parks or streets into natural stands. Additionall we assessed if natural populations show any intrapopulational and/or interpopulational differentiation pointing towards restricted gene flow with possible necessity for a population based conservation strategy rather than a taxon based strategy. Results clearly indicated within and between population structure a radius of isolation by distance for pollen and seed dispersal of about 5.0 km. Interestingly genetic distance did not support coherence of the two natural populations but mirrored the historical origin of the innertown population from diverse natural sources reflecting the traditional use and selection of edible varieties from nature. The N_em value of 1.25 implicates the prevention of population differentation. However the low level of genetic diversity and distance at all might mislead the interpretation and the degree of distance reflects more ancient similarities than actual geneflow. Given this observable isolation by distance, conservation biology of *Cornus mas* requires a population based strategy rather than a broad taxon based strategy.

Introduction

Introgression of non autochthonous genes from introduced plants of different origin into local genepools is one of the major issues in conservation biology. Here we assess the population structure of the cornelian cherry *Cornus mas* L. (Cornaceae) at two natural stands in Thuringia and compare the genetic diversity with an artifical population based on samples of garden and town origin from Jena. The primary question to which no information for *Cornus* exists is if there is gene flow between populations and to what extent introgression from planted cornelian cherries, commonly used in planting vegetations of public parks or streets, can be detected. There is no information available if natural populations show any intrapopulational and/or interpopulational differentiation pointing towards restricted gene flow with possible necessity for a population based conservation strategy rather than a taxon based strategy. Natural populations of the mainly south-east european and Asia minor distributed species (HEGI, 1927) *Cornus mas* are rare in Germany, the thuringian populations are of special interest because they might be outposts at the warm and dry limestone slopes of the Saale valleys and have been explicetly mentioned in the post-renaissance herbals e.g. by ZWINGER (1744) and since then (HEGI, 1927). As an early pasture for bees and a traditional used edible fruit *Cornus mas* is an important element of the native flora (BARTELS, 1993; FRIEDRICH and PETZOLD, 1993). Around Jena, cultural history reflects heavily on *Cornus mas*, for a long time hiking poles and batons for student leagues, the so called „Ziegenhainer" were made in the small village Ziegenhain next to Jena and distributed through Germany (TRAEGER, 1993). The populations we studied were an artificial one from plants grown in the town Jena, a population from Wöllnitz thought to be used for the production of the Ziegenhainer poles and samples from a large natural population from the conservation area of Kunitz, 5 km north of Jena. We assessed within and between population diversity and tied the results to the questions of distance of geneflow among populations, introgression of non-autochthonous gene pools into natural stands either by pollination or planting and dispersal abilities of pollen and fruits/seed. The guiding hypothesis assumes, that the two natural populations are more similar to each other than the artificial population and that the genetic diversity of the artificial town population is higher due to its diverse origin than the natural populations, reflecting the traditional use and selection of edible varieties.

Material and methods

Sampling and DNA isolation

In 2004 ninety plants were sampled from the three thuringian populations around Jena: Kunitz, Wöllnitz, and Jena town (30 plants per population). Geographic distance of Kunitz is 5.0 km north of Jena, Wöllnitz is about 2.5 km west of Jena. Plant material was dried in silica gel, ground with sterile sea sand and stored at -20 °C until DNA extraction. Total genomic DNA was isolated using the Qiagen (Hilden, Germany) plasmid mini kit following the protocol of HELLWIG et al. (1999). Extracted DNA was quantified photometric at 260 nm.

AFLP analysis

The complete AFLP procedure can be taken from VOS et al. (1995) but we used IRD- labelled primers for the selective PCR amplification and an automated DNA sequencer as described in BAUMBACH (2005).
35 primer combinations were screened and checked for band polymorphism, band brightness, band/background contrast, number of amplification products and reproducibility of band patterns. Four primer combinations have been selected for AFLP analysis: I) EcoRI-AAC/MseI-CAC (44 loci, 37-482 bp), II) EcoRI-AAC/MseI-CCT (37 loci, 36-471 bp), III) EcoRI-AAG/MseI-CGT (38 loci, 48-449 bp), IV) EcoRI-AAG/MseI-CTT (53 loci, 34-474 bp). They yielded a total of 172 well-amplified and highly reproducible polymorphic bands. It was possible to distinguish all 90 sampled plants as separate AFLP phenotypes. All selective PCR amplifications were performed twice. Only bands which could be detected both times unequivocally were used for further analyses.

Data analyses

AFLP patterns were compiled into a 0/1-matrix (state „0" for absence, „1" for presence of a band) using the „Saga generation 2" software (Li-Cor Biosciences, Bad Homburg, Germany). Bands of

equal fragment sizes were interpreted as homologous. Different staining intensities of homologous bands were not interpreted as different bands.

The partitioning of genetic variance within and among populations was assessed by an analysis of molecular variance (AMOVA, implemented in Arlequin ver. 2.000, SCHNEIDER et al. 2000). The fixation index Φ_{ST} (in analogy to F_{ST}) was calculated with the Arlequin software to estimate gene flow [(N_em=¼(1/Φ_{ST}-1)] between populations.

Principal coordinates analysis was calculated with NTSYSpc ver. 2.11a (Exeter Software, Setauket, USA) using a distance matrix based on the Band similarity coefficient (LYNCH 1990). Percentage of average heterozygosity, and percentage of polymorphic loci (calculated with TFPGA; MILLER, 1997) were used as parameters for description of genetic variability. Heterozygosity was calculated under the assumption of Hardy-Weinberg equilibrium (band state „0“ is counted as homozygous recessive genotype).

Results

Analysis of molecular variance (AMOVA)

Genetic variation within populations exceeds variation among populations clearly (17 % vs. 83 %). The corresponding Φ_{ST}-value is 0.167. Under the assumption of Wright's island model gene flow between populations is N_em=¼(1/Φ_{ST}-1)=1.25 (Tab. 1).

Tab. 1: Results of AMOVA computation: source of variation (SV), degrees of freedom (Df), sum of squares (SQ), variance components (VC), and fixation index (Φ_{ST}). Source of variation: among populations (aP), within populations (wP). Level of significance (based on 1023 iteration steps): ***p<0.001.

SV	Df	SQ	VC	Variation	Φ	N_em
aP	2	329.58	4.71 Va	16.7 %		
wP	87	2043.47	23.49 Vb	83.3 %	Φ_{ST}=0.167***	1.25
total	89	2373.05	28.20			

Parameters of genetic variability

Analysis of genetic variability revealed the highest level of variability in the artificial Jena town population, supporting the view of multiple and diverse origin. Wöllnitz again exhibits significant genetic diversity contributing to our assumption of Wöllnitz as a population with human impact by economic use and potential introduction and cultivation of useful genotypes. Kunitz shows the lowest average heterozygosity indicating a viable but isolated natural population.

Tab. 2: Genetic variability parameters of the examined *Cornus mas* L. populations.

Population	Sample Size	Average Heterozygosity	Polymorphic Loci
1 (Kunitz)	30	25.2 %	79.7 %
2 (Wöllnitz)	30	29.0 %	89.0 %
3 (Jena)	30	33.0 %	93.6 %
total	90	33.7 %	100 %

The PCO-analysis reveals a highly scattered artificial population due to the multiple and diverse origin of the samples. A similar degree is found in the Wöllnitz population with a slight substructering maybe due to planting. Kunitz is represented as an entire population (with a few exception). Due to the low level of genetic differentiation at all, we interpret the coherence of the Kunitz population as a sign of isolation by distance from the remainder populations.

Principal coordinates analysis

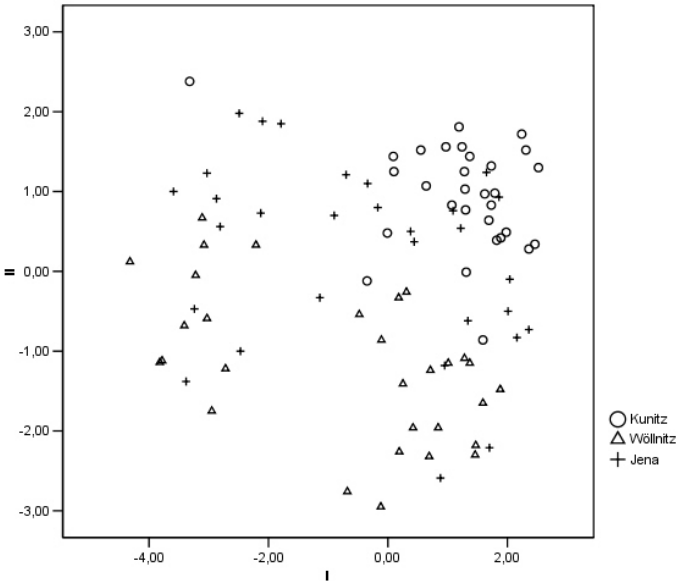

Fig. 1: Principal coordinates plot of 90 *Cornus mas* individuals for the first two principal coordinates estimated with 172 AFLP markers. Variation explained: 73.8 % (PCO I: 49.7 %, PCO II: 24.1 %).

Genetic distances

In general genetic distances (NEI, 1972) among all three populations are low. Wöllnitz (2) and Jena (3) are more similar to each other than to Kunitz (1). Kunitz (1) and Wöllnitz (2) are less similar to each other indicating isolation between the two assumed natural populations Wöllnitz and Kunitz. Genetic distances between Wöllnitz-Jena and Kunitz-Jena support the view of the town population being originated from collected samples from natural stands (more from Wöllnitz, less from Kunitz)

Tab. 3: Nei's original (1972) distance (1) Kunitz, (2) Wöllnitz, (3) Jena

Population	1	2	3
1	*****		
2	0.106	*****	
3	0.088	0.057	*****

Discussion

Our guiding hypothesis assumed, that the two natural populations are more similar to each other than the artificial population and that the genetic diversity of the artificial town population is higher due to its diverse origin than the natural populations, reflecting the traditional use and selection of edible varieties. The data corrects this hypothesis partially. Indeed, the genetic diversity of the artificial town population is higher than the within population differentiation of the naturals stands. This diversity mirrors the historical development of the town population from multiple introductions, either from commercially grown cornelian cherries or from transplantations from natural sources into the homes garden. PCO analysis indicates, that some of the genotypes can not be distinguished from the Wöllnitz population, pointing towards transplantation of tasty edible varieties from nature

into the town (preferred) or *vice versa* to produce useful wood for the production of the Ziegenhainer (less likely).

What is not supported is our expectation, that the two natural stands are more similar to each other than to the town population. Wöllnitz (2) and Jena (3) are more similar to each other than to Kunitz (1). Kunitz (1) and Wöllnitz (2) are less similar to each other indicating isolation between the two assumed natural populations Wöllnitz and Kunitz, but gene flow between town and Wöllnitz. The unity of the population in Kunitz reflects its isolated position. Obviously the distance of 5 km from Jena and Wöllnitz prevents more or less the pollen transfer by bees or seed/fruit transport although BARTELS (1993) reports endozooic seed dispersal by birds. Still, analysis of pollen fertility as a dimension of fitness does not indicate any fitness decrease within and between populations (MÜLLER, 2004; NOHLEN, 2007). The N_em value of 1.25 implicates the prevention of population differentiation. However the low level of genetic diversity and distance at all might mislead the interpretation and the degree of distance reflects more ancient similarities than actual geneflow. Given this observable isolation by distance, conservation biology of *Cornus mas* requires a population based strategy rather than a broad taxon based strategy.

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References

BARTELS, H., 1993: Gehölzkunde. Einführung in die Dendrologie. E. Ulmer, Stuttgart.

BAUMBACH, H., 2005: Genetische Differenzierung mitteleuropäischer Schwermetallsippen von *Silene vulgaris*, *Minuartia verna* und *Armeria maritima* unter Berücksichtigung biogeographischer, montanhistorischer und physiologischer Aspekte. Dissertationes Botanicae 398. Borntraeger, Stuttgart.

FRIEDRICH, G., PETZOLD, H., 1993: Obstsorten. 300 Obstsorten in Wort und Bild. Neumann, Radebeul.

HELLWIG, F., NOLTE, M., OCHSMANN, J., WISSEMANN, V., 1999: Rapid isolation of total cell DNA from milligram plant tissue. Haussknechtia 7, 29-34.

LYNCH, M., 1990: The similarity index and DNA fingerprinting. Mol. Biol. Evol. 7, 478-484.

MILLER, M., 1997: Tools for population genetic analysis (TFPGA) 1.3: A windows program for the analysis of allozyme and molecular population genetic data. Department of Biological Sciences – Northern Arizona University.

MÜLLER, S., 2004: Die Variabilität von *Cornus mas* L. im Mittleren Saaletal. Magisterarbeit, Institut für Spezielle Botanik, FSU Jena.

NEI, M., 1972: Genetic distance between populations. Amer. Naturalist 106 (949), 283-292.

NOHLEN, F., 2007: Untersuchungen zur Reproduktionsbiologie von *Cornus mas* L. und *Cornus sanguinea* L. Staatsexamensarbeit, Institut für Spezielle Botanik, FSU Jena.

TRAEGER, I., 1993: Von Lichtenhainer Kännchen und Ziegenhainer Stöcken. Burschenschaftliche Blätter 3, 136-137.

VOS, P., HOGERS, R., BLEEKER, M., REIJANS, M., VAN DE LEE, T., HORNES, M., FRITERS, A., POT, J., PALEMAN, J., KUIPER, M., ZABEAU, M., 1995: AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23, 4407-4414.

SCHNEIDER, S., ROESSLI, D., EXCOFFIER, L., 2000: Arlequin ver. 2.000. University of Geneva.

ZWINGER, T., 1744: Theatrum botanicum. J. Bischoffs, Basel.

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