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Genetic structure and diversity in *Juniperus communis* populations in Saxony, Germany

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Abstract: In recent years, land use changes led to a rapid decline and fragmentation of *J. communis* populations in Germany. Population isolation may lead to a restricted gene flow and, further, to negative effects on genetic variation. In this study, genetic diversity and population structure in seven fragmented *J. communis* populations in Saxony, Germany, were investigated using nuclear microsatellites (nSSR) and chloroplast single nucleotide polymorphism (cpSNP). In all Saxony *J. communis* populations, a high genetic diversity was determined but no population differentiation could be detected whatever method was applied (Bayesian cluster analysis, F-statistics, AMOVA). The same was true for three *J. communis* out-group samples originating from Italy, Slovakia and Norway, which also showed high genetic diversity and low genetic differences regarding other *J. communis* populations. Low genetic differentiation among the *J. communis* populations ascertained with nuclear and chloroplast markers indicated high levels of gene flow by pollen and also by seeds between the sampled locations. Low genetic differentiation may also provide an indicator of Juniper survival during the last glacial maximum (LGM) in Europe. The results of this study serve as a basis for the implementation of appropriate conservation measures in Saxony.

Key words: *Juniperus communis*, nSSR, cpSNP, genetic diversity, population differentiation

1. Introduction

Common Juniper (*Juniperus communis* L.) belongs to the *Juniperus* genus, in the family of Cupressaceae. It is the most widely distributed conifer in the Northern Hemisphere occurring in North America, Europe, Asia, and parts of North Africa (Thomas *et al.* 2007).

J. communis grows on a variety of soil types and tolerates nutrient-poor sites, drought and winter temperatures as low as -40°C (Thomas *et al.* 2007). However, Juniper has high light requirements and does not tolerate competitive pressure well (Ellenberg 1996). Despite this unpretentiousness, Juniper populations have increasingly declined in recent decades and have now a threatened status in several regions in Europe (Gruwez *et al.* 2012). Due to land use changes, typical Juniper habitats such as heathlands and calcareous grassland are sharply declining or have already completely disappeared in Germany. At present, a spatially inclusive distribution of *J. communis* populations covering large areas can only be found in the Calcareous Alps, Swabian and Frankish Alp and in the sandy areas of Lüneburg and Lusatia Heath. In the remaining areas of Germany, only very fragmented small groups of *J. communis* occur. Therefore, the German Red List of Endangered species categorizes *J. communis* as vulnerable (http://www.floraweb.de/pflanzenarten/rotelisten.html, state: 01.01.2016). The reduction of population numbers and spatial fragmentation reduce connectivity between populations and lead to population isolation.

Associated with the population size, the fertilization type of *J. communis* could be a further cause for endangering this species. Juniper is a dioecious species with female and male plants. It is wind-pollinated and seeds are, primarily, dispersed by birds. Compared to hermaphroditic species, the separation of sexes halves the density of potential pollen donors and seed-producing individuals. The absence of an appropriate quantity of juniper pollen has, in turn, negative effects on the amount of Juniper cones. In addition, like many other conifer species, Juniper produces a large proportion of empty seeds (McCartan & Gosling 2013). Seed viability

is subject to a remarkable variation depending on plant age, habitat, climate or seed predators (Thomas *et al.* 2007; Vanden-Broeck *et al.* 2011; Gruwez *et al.* 2012). Juniper seeds are usually deeply dormant and require a long stratification time to germinate. On average, seeds need two years and seven months for germination (Broome 2003). Limited sexual regeneration, in addition to the fragmentation of *J. communis* populations, may have impact on genetic diversity and genetic structure of the remaining populations.

Habitat fragmentation leads to a reduction of random mating and immigration rates (Michalczyk 2008; Ashley 2010). As a result, genetic diversity decreases and genetic differentiation among populations becomes more pronounced due to inbreeding and restricted gene flow (Couvet 2002). Genetic diversity limitation may lead to a decline in fitness and, further, to extinction of the species (Severns *et al.* 2003; Provan *et al.* 2009). Depending on the mode of pollination (wind or insect pollinated), the extent of negative influences – due to habitat fragmentation – is more or less distinctive (reviewed in Ashley 2010; Vranckx 2012). In general, wind-pollinators seem to be less influenced by habitat fragmentation effects because their pollen is transported over long distances (Ashley 2010). Recent studies reported high pollen immigration in wind-pollinated populations indicating a random mating over large spatial scales. For example, results from a pollen dispersal investigation in *Pinus sylvestris* L. showed pollination distances of up to 30 kilometers (Robledo-Arnuncio & Gil 2005). This suggests that even small isolated fragments are not necessarily reproductively isolated and are able to maintain pollination with more distant trees or shrubs (Ashley 2010).

In several studies, *J. communis* genetic diversity and population differentiation were investigated using different genetic marker systems (e.g.: allozymes, AFLPs, nuclear microsatellite and chloroplast single nucleotide polymorphisms). In all Juniper populations, a high level of genetic diversity was found. The degree of population differentiation varied between moderate to high (Van der Merwe *et al.* 2000; Provan *et al.* 2009; Michalczyk *et al.* 2010) and low (Oostermeijer & De Knegt 2004; Khantemirova & Semerikov 2010; Vanden-Broeck *et al.* 2011).

In this study, we used biparentally and uniparentally inherited markers to estimate the population genetics of Juniper samples in the province of Saxony in south-eastern Germany. For *J. communis*, only five specific microsatellites with scorable polymorphic bands were available (Michalczyk 2008). Therefore, we tested cross-species applicability of numerous microsatellite markers, which were developed for other *Juniperus* species (Zhang *et al.* 2008; Opgenoorth 2009) and yellow cedar (*Chamaecyparis nootkatensis*) (Berube *et al.* 2003) with the aim to select functioning primers for the application on *J. communis*. In order to improve the genetic data set, we also used single nucleotide polymorphism markers of the chloroplast genome according to Provan *et al.* (2009). Based upon the genetic data, we estimated genetic diversity within the populations and genetic structure among these populations. Consequences for the implementation of conservation measures for the Saxony *J. communis* populations are discussed.

2. Material and methods

2.1. Study site, sampling and DNA extraction

The study site was located in the federal state of Saxony in South-Eastern Germany (Fig. 1). Saxony comprises the area of 18,413 square kilometers composed, largely, of hills and mountains. Only northern and western Saxony descends into the great North European Plain. Within this area, seven different *J. communis* patches were found and, in total, 404 shrubs were sampled (Table 1).

For each shrub originating from Saxony, sex and vitality were determined. Additional 29 *J. communis* individuals from Slovakia, 27 individuals from Norway and 28 individuals from Northern Italy were included in our analysis as out-group samples. In total, 488 *J. communis* samples were genetically analyzed.

Fresh needles were collected in 2 ml reaction tubes and dried using silica gel according to a modified protocol by Chase *et al.* (1991). Dried plant material can be stored at room temperature until DNA isolation. DNA extraction and quantification was performed by the company LGC Genomics (Berlin, Germany). All samples were diluted to 10 ng/µl.

2.2. Microsatellite analysis

For nSSR analysis, 19 nSSR primer pairs developed for *J. communis* and other *Juniperus* species (Berube *et al.* 2003; Michalczyk *et al.* 2006; Zhang *et al.* 2008; Opgenoorth 2009) were tested on 16 *J. communis* genotypes (Table 2).

Eight primer pairs (Jp01, Jp05, Jt01, Jt02, Jt04, Jt06, y2c12, y2h01) showed no amplification or multibanding patterns in *J. communis*, respectively. Four primer pairs (Jp04, Jt03, Jt05, y1e10) showed only monomorphic patterns. For this reason, these twelve primer pairs were discarded. For the remaining seven primers, one to two alleles per locus and individual were amplified. In the next step, these primers were combined in different multiplex reactions. Only five primer pairs developed for *J. communis* by Michalczyk *et al.* (2006) were suited for a combination in two multiplexes with two or three primers, respectively (multiplex 1: Jc016, Jc031, Jc032; multiplex 2: Jc035, Jc037). The forward primers in one multiplex reaction were labeled with three different dyes (D2: Dye 751, absorption max. 751nm; D3: BMN-6,

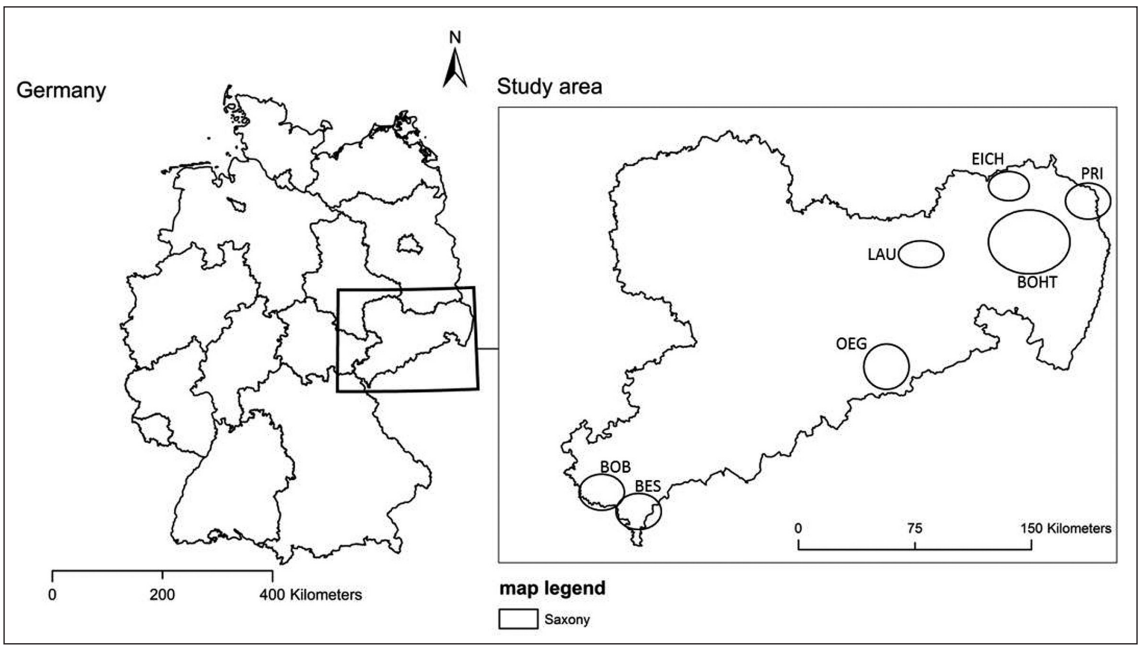

Fig. 1. Spatial distribution of seven *Juniperus communis* sampling locations within the study area in Saxony

Explanations: EICH – Lusatia, Eichbusch, PRI – Lusatia, Klein Priebus, BOHT – Upper Lusatia Heath and Pond Landscape biosphere reserve, LAU – Lusatia, Neukirch, OEG – East Ore Mountains, BES – Vogtland, Bad Elster, BOB – Vogtland, Bobenneukirch

absorption max. 681nm: D4: BMN-5, absorption max. 645nm; Biomers, Germany). Multiplex PCRs were carried out following the manufacture's guide of the type-it microsatellite kit® (Qiagen, Germany). Multiplex electrophoresis was performed on 10% polyacrylamide gel using the CEQ 8000 Genetic Analysis System. Also the obtained data were analyzed using the CEQ 8000 software (both Beckman Coulter, Germany).

2.3. cpSNP AS-PCR analysis

In order to detect cpSNP within the 488 *J. communis* genotypes, an allele-specific PCR (cpSNP AS-PCR) was performed with four cpSNP markers (IC-61, VV-435, VV-449, BD-616), which were described in Provan *et al.* (2009). After sequencing three chloroplast regions (atpI-rpoC2, trnV intron, and petB-petD) in *J. communis*, Provan *et al.* (2009) found four cpSNPs, which were suitable for differentiation of Juniper populations in Ireland. Single nucleotide mutations were detected using allele-specific primer sets (Provan *et al.* 2009) according to the nested competitive primer approach of Soleimani *et al.* (2003). The AS-PCR was carried out

Table 1. List of analysed *Juniperus communis* populations, their origin and number of sampled shrubs

Population	Country, Province	Region	Abbreviation	Latitude	Longitude	Sampled shrubs
1	Germany, Saxony	Upper Lusatia Heath and Pond Landscape biosphere reserve	BOHT	51.34269	14.51860	101
2	Germany, Saxony	Lusatia, Neukirch	LAU	51.28092	13.93839	62
3	Germany, Saxony	Vogtland, Bad Elster	BES	50.28564	12.24740	90
4	Germany, Saxony	East Ore Mountains	OEG	50.89119	13.81610	16
5	Germany, Saxony	Lusatia, river Neisse	PRI	51.44288	14.97069	49
6	Germany, Saxony	Lusatia, Eichbusch	EICH	51.49624	14.42427	49
7	Germany, Saxony	Vogtland, Bobenneukirch	BOB	50.37386	12.06986	37
8	Slovakia	Zvolen	SK	48.35190	19.04270	29
9	Norway	Setesdal	NOW	59.56020	7.35675	27
10	Italy	Lake Garda	ITA	45.63160	10.66051	28

in the total volume of 10µl following the manufacture's guide of the Phusion® High-Fidelity PCR-Kit using the annealing temperature of 52°C (Fisher Scientific, Schwerte, Germany). PCR products were resolved on 3% agarose gels.

2.4. Genetic diversity and differentiation

On the basis of the nSSR data, the following genetic parameters for each single *J. communis* harvesting plot were calculated for each locus using the GENALEX ver. 6.5 software (Peakall et al., 2012): mean number of alleles by locus (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e) (or Nei's genetic diversity, according to Nei (1973)), inbreeding coefficient F_{is} and number of private alleles (*PA*).

Furthermore, we calculated the percentage of molecular variance performing the Analysis of Molecular Variance (AMOVA) and, based on the F_{st} analogue (ϕ_{PT}),

Table 2. 19 SSR loci tested for their amplification in *Juniperus communis* individuals from Saxony

Locus		Primer sequence	Amplification
Jc016*	F	CAAAATGATGCTTATGATGA	+
	R	TGAAAATCATTGTTGTTTTCTT	
Jc031*	F	CCTAATGTTGTAATCACGTATATCT	+
	R	TGACCTTGGGCGTATAGATT	
Jc032*	F	ACATTGCAAATATGGGGTAA	+
	R	TTGATGAGTTGTTGAGTTATTAAG	
Jc035*	F	TGTGTTTATTCTCCCCATCT	+
	R	CCCCCAGTTATTCTAAACATT	
Jc037*	F	GGCAATTAGTAAGGCACAAG	+
	R	TAAGGTGGATATCACCAAGG	
Jp01#	F	AAGGCCTACCTAGCAGAATCAC	-
	R	ACTCACTATAGGGCGAATTGGG	
Jp04#	F	CTCTCAAGTTCTCTTTCTTCCTC	o
	R	TAAAACGACGGCCAGTGCC	
Jp05#	F	CTGCAGGTCGACGATTGTTTAAG	-
	R	CTCTCAAGTTCTCTTTCTTCCTC	
Jp07#	F	CATCCTCTTCAGTTAGGGTCC	+
	R	GATTTAGTGGCACCTACATGAG	
Jp08#	F	AGCAGAATCAATTCACGTTCAC	+
	R	CAATATGTGCTTAGATTTGGC	
Jt01°	F	TTGTCTCCCCTGCACACTC	-
	R	GCAGGCAGCCATGAGAAAG	
Jt02°	F	GCAATGGTAGATACTTGGGATTCAG	-
	R	TGGAATATGTATGCAGCTAGGTC	
Jt03°	F	AGTCGAGGAAACAACTACAATCC	o
	R	TTGTTGGGCGCATTTTGTC	
Jt04°	F	ATTCAAGGGATGAGCACAAAG	-
	R	CAGGCTAATCCACACACTTCAC	
Jt05°	F	ACAATGTTGGTCCCTACAACAAC	o
	R	GGAACACTTCCATTATTTGGATAGG	
Jt06°	F	CCTCCGCTCTTGTCGCAG	-
	R	GATTTGTATTGCTCAAATCCTTCAG	
y1e10+	F	GATACAACCTGACACAAGGAGG	o
	R	CCATAATCCAGAAGGTCTCACAG	
y2c12+	F	CATCTAGAAAGGCAYAGCTTGG	-
	R	ATGCCCAAACAAGACCTCTC	
y2h01+	F	TCTTAGGTGTCTCCCCTTCG	-
	R	AACTGGCCTAAAAGAATCCAGCA	

Explanations: * – Michalczyk *et al.* 2006 (SSR developed in *J. communis*), # – Zhang *et al.* 2008 (SSR developed in *J. przewalskii*), ° – Opgenoorth 2009 (SSR developed in *J. tibetica*), + – Berube *et al.* 2003 (SSR developed in *Chamaecyparis nootkatensis*), + – amplification; - – no amplification or multiband patterns, o – monomorphic bands

Table 3. Number of alleles and effective alleles, and observed and expected heterozygosity in ten different *Juniperus communis* sampling locations

Population	*N*	N_a	N_e	H_o	H_e	*Fis*	*PA*
BOHT	101	26	11	0.62	0.84	0.26	0
LAU	62	20	9	0.63	0.86	0.26	3
BES	90	25	11	0.65	0.86	0.23	0
OEG	16	12	7	0.65	0.82	0.19	1
PRI	49	17	9	0.54	0.81	0.35	3
EICH	49	17	9	0.52	0.81	0.36	1
BOB	37	17	10	0.58	0.86	0.31	0
SLO	29	16	8	0.6	0.83	0.28	5
NOW	27	16	11	0.53	0.88	0.41	8
ITA	28	16	9	0.57	0.80	0.27	3
Mean	45	18	9	0.59	0.84	0.29	2.4

Explanations: *N* – number of individuals, N_a – number of different alleles, N_e – number of effective alleles (=$1/(\sum p_i^2)$), p_i – relative frequency of the i^{th} allele, H_o – observed heterozygosity (=number of heterozygotes/N), H_e – expected heterozygosity (=$1-\sum p_i^2$), F_{is} – Fixation Index=(He-Ho)/He, *PA* – number of private alleles with a frequency > 0.03% unique to a single population (including all loci)

we calculated the migration rate (N_m) using GENALEX ver. 6.5. Genetic differentiation between single populations was measured by Wrights fixation index (F_{st}) and Nei's genetic distance. Correspondence between geographic and genetic distance was estimated only for the Saxony Juniper individuals, since single coordinates for the Slovakian, Italian and Norwegian Juniper samples were not available. The calculation was performed by Mantel test with statistical testing by 9.999 permutations using the GENALEX ver. 6.5 software.

2.5. Bayesian clustering

A model-based clustering method was applied for all 488 *J. communis* genotypes to identify specific genetic structures within individuals by using the STRUCTURE ver. 2.3.4. software (Pritchard *et al.* 2000). This analysis was performed twice: with the default mode for STRUCTURE that uses only genetic information, and with prior information on the sampling location (LOCPRIOR model). In both cases, our parameters were 50,000 burn-in periods and 50,000 Markov Chain Monte Carlo repetitions using the admixture model with correlated allele models. To estimate the optimal number of populations (*K*), we ran the program with *K* varying from 3 to 10 with 5 runs for each *K* value. The software program STRUCTURE HARVESTER (Earl *et al.* 2012) was used for detecting the most likely value for *K* based on Evanno's ΔK method (Evanno *et al.* 2005).

3. Results

3.1. Sex ratio and vitality

The sex ratio over all Saxony *J. communis* individuals was in equilibrium with 178 male and 176 female shrubs. Sex of the remaining 50 *J. communis* shrubs was not ascertainable due to the lack of flowers. The vitality of Juniper shrubs was, in most cases (90%), good to very good. Only 10% of shrubs showed moderate to serious plant damages.

3.2. Cross-species application of nSSRs and AS-PCR Analysis

In *J. communis*, only six species-specific microsatellite markers are available (Michalczyk *et al.* 2006). Therefore, we tested cross-species application of 11 microsatellite markers, which were developed in other *Juniperus* species and in yellow cedar. Cross-species amplification can be effective if the flanking regions of the primers are conserved among species. Only two nSSR markers developed in *Juniperus przewalskii* showed cross-species amplification with polymorphic bands in *J. communis* individuals (Table 2).

Furthermore, we analyzed 488 *J. communis* individuals with four allele-specific primers in order to detect cpSNP within the chloroplast sequence as described in Provan *et al.* (2009). In three chloroplast genome regions (VV-435, VV-449, BD-616), no single nucleoid polymorphism could be detected in our study. Only the IC-61 chloroplast region showed a cpSNP in 8 single individuals originating from BOHT and LAU populations.

3.3. Genetic diversity parameters

Genetic variation was determined based on the calculation of microsatellite allele frequencies of each *J. communis* sample location (BOHT, LAU, BES, OEG, PRI, EICH, BOB, SK, NOW, ITA) (Table 3).

The number of alleles varied between a maximum of N_a=26 for the BOHT sample location (101 individuals) and a minimum of N_a=12 for the OEG location (16 individuals). The average effective number of alleles (N_e)

Table 4. Pairwise population Nm values based on nSSR data (lowest and highest values in bold)

	BOHT	LAU	BES	OEG	PRI	EICH	BOB	SK	NOW	ITA
BOHT	0.00									
LAU	10.14	0.00								
BES	**25.85**	10.98	0.00							
OEG	9.57	5.50	15.50	0.00						
PRI	11.49	6.73	25.26	10.41	0.00					
EICH	9.28	7.51	12.15	6.57	9.84	0.00				
BOB	11.57	9.03	22.41	12.32	9.44	5.81	0.00			
SK	11.51	5.69	12.33	9.17	11.18	4.23	7.12	0.00		
NOW	5.04	5.66	9.02	6.39	7.85	6.63	8.97	**3.70**	0.00	
ITA	29.34	7.95	14.31	6.16	22.79	7.47	8.63	11.56	6.89	0.00

Explanation: in Bold lowest and highest values

was highest with N_e=11 in the BOHT, BES and NOW sampling location and lowest – with N_e=7 – in the OEG location. The average expected heterozygosity was very high in all sampled locations with H_e=0.84. However, the high average fixation index (F_{is}=0.29) indicated that the expected degree of heterozygosity reduction in ten *J. communis* populations was high.

The number of private alleles (with frequency > 0.03) unique to a single population ranged from 0 to 3 in Saxony populations. In the NOW and SK sampling location, the number of private alleles was remarkably higher with *PA*=8 and *PA*=5.

3.4. Genetic differentiation

The analysis of molecular variance (AMOVA) showed significant but low differences between the populations calculated on the basis of nSSR (ϕ_{PT}=0.025 p≤0.001) and on the basis of cpSNPs (ϕ_{PT}=0.109 p≤0.001). The number of effective migrants ranged from N_m=3.7 (NOW/SK) to N_m=25.85 (BES/BOHT) between pairs of populations and was N_m= 9.92 over all populations (Table 4). The pairwise population F_{st} values ranged from 0.006 (between BOHT and BES) to 0.031 (between NOW and SK) with significant levels between p≤0.001 and p≤0.05 (Table 5).

Nei's genetic distance showed similar results and indicated also low genetic differentiation between populations. The values ranged between *D*=0.075 (between BOHT and BES) and *D*=0.445 (between NOW and SK) (Table 4).

After performing the Mantel test, we found that the correlation coefficient was very low *rxy*=0.006 and not significant p=0.35, indicating that the geographic distance had no effect on genetic variance in German Juniper populations.

3.5. Population delimitation of J. communis

The Bayesian cluster analysis was performed for 488 single individuals of *J. communis* from Saxony, Slovakia, Norway and Italy using the STRUCTURE software.

The first STRUCTURE run, using the default mode, showed – as a result – a very weak population structure. This can be the case for data sets with few markers or very low structure. Therefore, we performed a second run including sampling locations as prior information in order to improve the performance of clustering. Calculating the STRUCTURE output with the downstream program STRUCTURE HARVESTER, optimal number of populations (*K*=8) based on Evanno's ΔK was estimated. However, in spite of using the LOCPRIOR model, no population structure within the *J. communis* samples was found (Fig. 2). A membership *Q*>80% of one individual for one cluster was only detected for 8.4% of *J. communis* samples. All other individuals exhibited an admixture from all gene pools, and a distinct assignment to one out of the eight clusters was not possible. Additionally, a large value of *r*=8.3 was estimated, which indicated that the content of the prior location information was low and no population structure was present.

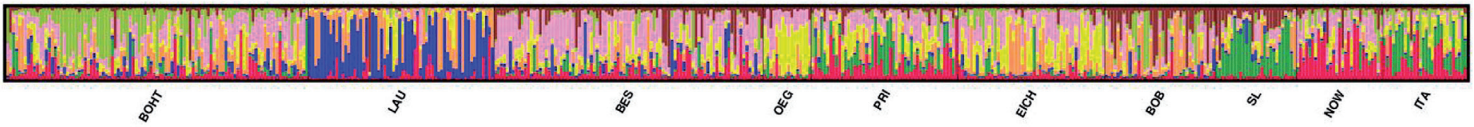

Fig. 2. STRUCTURE bar plot representing *K*= 8 for the seven studied *Juniperus communis* sample locations in Saxony and three out-group samples from Norway (NOW), Slovakia (SL) and Italy (ITA), after using prior information on the sampling location (LOCPRIOR model)

Table 5. Wright's fixation index (F_{st}) (above diagonal) and Nei's genetic distance (below diagonal) between the 10 studied *Juniperus communis* sample locations

	BOHT	LAU	BES	OEG	PRI	EICH	BOB	SK	NOW	ITA
BOHT		0.014***	**0.006****	0.019**	0.012***	0.011***	0.012***	0.014***	0.020***	0.012*
LAU	0.172		0.015***	0.028**	0.023***	0.022***	0.016***	0.022***	0.026***	0.025***
BES	**0.075**	0.206		0.016*	0.010*	0.012*	0.009*	0.013**	0.015**	0.016**
OEG	0.211	0.344	0.177		0.025*	0.021**	0.022**	0.025**	0.031**	0.033***
PRI	0.121	0.256	0.095	0.253		0.013**	0.019***	0.018**	0.021**	0.013*
EICH	0.106	0.243	0.117	0.204	0.131		0.016***	0.023***	0.026***	0.019***
BOB	0.137	0.219	0.120	0.268	0.199	0.161		0.022**	0.018**	0.024***
SK	0.161	0.282	0.149	0.277	0.180	0.240	0.276		**0.031*****	0.021**
NOW	0.283	0.415	0.218	0.415	0.229	0.320	0.270	**0.445**		0.027**
ITA	0.115	0.263	0.152	0.339	0.121	0.182	0.241	0.206	0.302	0.000

Explanations: Fixation index (F_{st}) (above diagonal), F_{st}=0.0-0.049 – low genetic differentiation, F_{st}=0.050-0.149 – moderate genetic differentiation, F_{st}=0.150-0.249 – high genetic differentiation, F_{st}>0.250 – very high genetic differentiation (Wright 1978). Nei's genetic distance (below diagonal). In bold lowest and highest values, significant levels, * –p<0.05, ** – p<0.01, *** – p<0.001

4. Discussion

4.1. Cross-species amplification of nuclear SSR marker

An interspecific amplification of microsatellite primers could be possible if microsatellite flanking regions were evolutionary conserved in related species. This approach was successful in animals (e.g.: Bezault *et al.* 2012; Marín *et al.* 2014) and plants (Robertson *et al.* 2004; Reim *et al.* 2012; Feng *et al.* 2014). However, the success of cross-species amplification within the Cupressaceae family was low in our study. Previous studies on the transferability of microsatellite markers isolated from other *Juniperus* species or *Chamaecyparis nootkatensis* also resulted in a limited application in different *Juniperus* species (Berube *et al.* 2003). The low proportion of successful cross amplification in *J. communis* suggested a high evolutionary distance between the target and the source species (Hendrix *et al.* 2010; Vinyallonga *et al.* 2011).

4.2. Genetic diversity and differentiation

In fragmented and spatially isolated populations such as *J. communis*, it is expected that the gene flow is restricted and, over time, genetic differentiation is high (Young *et al.* 1996; Leonardi *et al.* 2012; Vranckx *et al.* 2012). However, in our study, *J. communis* populations originating from Saxony, Norway, Slovakia and Italy showed a high genetic diversity and a low genetic differentiation between the populations.

This result is in keeping with several studies on *J. communis*, which also observed high genetic variation and low population differentiation in *J. communis* (Oostermeijer & De Knegt 2004; Khantemirova & Semerikov 2010; Michalczyk *et al.* 2010; Vanden-Broeck *et al.* 2011). High genetic diversity and low population genetic differences are sustained if the gene flow between populations can occur (Porth & El-Kassaby 2014). In general, gymnosperms display higher levels of gene flow than angiosperms (Govindaraju 1989). Higher levels of gene flow may be the result of a high migration rate. The number of migrants per generation was very high (N_m=9.92) indicating a continuous gene flow in *J. communis* populations in the past. A particular wind could transport pollen over long distances and, therefore, promote high N_m (Bettencourt *et al.* 2015). Slatkin (1987) and Ferreira and Eriksson (2006) determined that more than four migrants per generation prevent genetic differentiation between populations. However, indirect estimations of N_m from $??_{PT}$ must be interpreted with caution because it implies numerous unrealistic assumptions (e.g. constant population size, random migration, no selection, mutation or spatial structure), which are very likely violated in a natural population (Whitlock & McCauley 1999). Nevertheless, the N_m-value is still useful for an approximate estimation of the level of migration (Neigel 2002).

The comparative application of nuclear- and maternal-inherited chloroplast markers allows separation of the impact of pollen and seed-mediated gene flow on population differentiation (Ennos 1994). Analysis of molecular variance showed that, on the basis of nuclear markers, the variation among the *J. communis* populations in our study was only 2.5%. In a similar study of Provan *et al.* (2009) on *J. communis* populations in Ireland, the value for population differentiation based on nuclear markers was 9.6% and, thus, remarkably higher. On the basis of chloroplast markers, the differentiation between populations is often more distinct than on the basis of nuclear markers (Furnier & Stine 1995; Ribeiro *et al.* 2002; Bai *et al.* 2014), which is attributed to a lower

seed than pollen dispersal. Our value for population differentiation based on chloroplast markers was also lower (10.9%) than in the *J. communis* populations in Ireland (about 25%) estimated by Provan *et al.* (2009). The comparatively lower genetic differentiation in our study for both marker systems (nuclear and chloroplast) reflects the capability of genetic exchange by pollen and seeds between different Juniper populations in our study.

In general, the chloroplast variation in our study is with two haplotypes very low. This fact may argue for a loss of alleles due to genetic bottlenecks in the past, which was also reported in other conifers (Walter & Epperson 2001). Such historical bottlenecks are often discussed in connection with the last glacial period, in which many plant species were forced to migrate into southern refugia (Keir *et al.* 2011; Scheepens *et al.* 2013). Classic Holocene recolonizers are expected to be influenced by large-scale isolation-by-distance effects where genetic distances are positively correlated with geographic distances (Michalczyk *et al.* 2010). In our study, it was not possible to detect any genetic structure in association with the geographic distribution of the sampled *J. communis* individuals after Bayesian cluster analysis. Even after incorporating the sampling locations as prior information, no population structure was found with STRUCTURE. Also after performing the Mantel test, no correlation could be detected between the genetic and the geographic distance matrix in the Saxony *J. communis* populations. These results indicate that the loss of chloroplast variation was not caused by a historical bottleneck during the last glacial maximum (LGM). In fact, the absence of a correlation between genetic structure and geographical patterns in our study argues for a periglacial survival of *J. communis* L. as also concluded by Michalczyk *et al.* (2010) in a study on the genetic structure in *J. communis* populations in Central Europe.

The high genetic diversity and the negligible spatial structure of *J. communis* populations appear to originate from a high gene flow frequency between *J. communis* individuals. Conifer species, generally, display low population genetic differentiation due to great pollen dispersal abilities and outcrossing (Hamrick 2004; Parchman *et al.* 2011). In conifers, pollen dispersal can range from 10 to 100 kilometers (Burczyk & Chybicki 2004). However, although *J. communis* pollen is wind-dispersed, it is assumed that it is mostly locally deposited. A study on *J. communis* pollen dispersal distances showed that only 1% of *J. communis* pollen was found in a distance of 100 m to the male plant (Michalczyk 2008). On the other hand, even on spatially very isolated female *J. communis* shrubs (distance to the next male *J. communis* shrub approximately 1 km), we observed cones with full seeds, indicating that pollen flow over larger distances had occurred. High pollen dispersal distances could compensate for the spatial isolation of fragmented populations, since genetic exchange is maintained to a certain degree. Thereby, single-located individuals can act as so-called stepping stones and bridge larger distances between groups of shrubs or trees (Albaladejo *et al.* 2012). However, for better knowledge of pollen dispersal distances of *J. communis*, further investigations are necessary.

4.3. Conclusions for conservation measures

At present, genetic diversity in Saxony *J. communis* populations is high and the population differentiation is low. This indicates an unrestricted gene exchange between *J. communis* sample locations. Also most of the investigated shrubs showed high vitality and the ratio of female and male plants in the sampled locations was more or less balanced. All these factors are essential prerequisites for a long-term preservation of *J. communis* in Saxony. Nevertheless, the existing populations showed only little natural regeneration and the main parts of the populations were overaged. The poor reproductive ability of *J. communis* and unsuitable local conditions, which offer offspring low chances of survival, support the excess of aged individuals in populations. The high fixation index in the investigated *J. communis* populations makes it possible to assume that heterozygosity reduction within the populations may be expected. For this reason, the implementation of *in-situ* conservation measures is recommended in order to preserve at least the current state. Particularly, care of existing habitats and reintroduction of young *J. communis* plants for a juvenescence of existing populations would improve the long-term preservation of this species. Another suitable measure is reforestation with *J. communis* in appropriate areas such as forest and field edges, under power supply lines and along gas pipelines that are kept free of other trees.

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