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RESEARCH ARTICLE

Genetic variability in wild populations of *Prunus divaricata* Ledeb. in northern Iran evaluated by EST-SSR and genomic SSR marker analysis

Tina Wöhrmann · Daniela Guicking · Korous Khoshbakht · Kurt Weising

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Abstract A population genetic analysis based on eight genomic SSR markers and three EST-SSR (expressed sequence tags) markers developed in peach (*Prunus persica* (L.) Batsch) and Japanese plum (*Prunus salicina* Lindl.) was carried out in 12 wild populations of cherry plum (*Prunus divaricata* Ledeb.) sampled along the Iranian coast of the Caspian Sea. A total of 184 alleles (3–31 per locus) were detected with a mean value of 16.7 alleles per locus. None of the loci or populations showed deviation from Hardy–Weinberg equilibrium, and all markers proved to be unlinked. The mean values for the observed and the expected heterozygosity were 0.66 and 0.73, respectively. There was very little genetic differentiation among populations, as was indicated by low overall values of Wright's F_{ST} (0.03) and Nei's G_{ST} (0.08). An analysis of molecular variance (AMOVA) showed that 96.8% of the total variance was attributable to differences between individuals within populations. Genetic and geographic distances were nevertheless positively correlated, as evidenced by a Mantel test. The high level of genetic diversity and the apparent lack of genetic structure in wild *P. divaricata* may be attributed to frequent long distance gene flow through frugivorous birds and possibly humans, as has been documented for other *Prunus* species.

Keywords Conservation · Cross-species amplification · EST-SSRs · Genetic variability · Microsatellite markers · *Prunus divaricata* Ledeb.

T. Wöhrmann · D. Guicking · K. Weising (✉)
Plant Molecular Systematics, University of Kassel,
34109 Kassel, Germany
e-mail: weising@uni-kassel.de

K. Khoshbakht
Environmental Science Research Institute,
Shahid Beheshti University G. C., Tehran, Iran

Introduction

The Hyrcanian floral province stretches along the south of the Caspian Sea, ranging from northern Iran to southeastern Azerbaijan (Zohary 1973). Mean annual temperatures of about 17°C and an annual rainfall of 1,000–1,500 mm have given rise to dense deciduous forests that extend from the Caspian lowlands to about 2,500 m altitude on the slopes of the Elburs Mountains. The region exhibits an exceptional wealth of wild plant species and has been considered as an evolutionary centre for fruit trees (Khoshbakht and Hammer 2005). However, recent urbanization and human disturbance have caused increasing deforestation and degradation, with a concomitant threat to biodiversity (Scharnweber et al. 2007).

Cherry plum, *Prunus divaricata* Ledeb., is a wild growing, diploid, self-incompatible fruit tree that

belongs to section *Prunus*, subgenus *Prunus*, within the family of Rosaceae (Faust and Surányi 1999; Scholz and Scholz 1995; Reales et al. 2010). Pollination is entomophilous, and seeds are dispersed by frugivorous animals. The species is widely distributed from the Balkan Peninsula across Anatolia and the Caucasus to Central Asia, including the Hyrcanian forests of northern Iran (Browicz 1969, 1997). Individual trees have been semi-cultivated for their edible fruits, especially in home gardens along the Caspian coast (Khoshbakht et al. 2007). The fresh fruits with ~2–4 cm in diameter are part of the diet of the local people, and are either eaten raw or used to prepare a local tart candy called "Lavashak". The species is also widely used as a rootstock (Scholz and Scholz 1995) and certainly has some potential for further domestication, providing economic and livelihood benefits for subsistence farmers.

One can find two alternative names for this species, i.e., *Prunus divaricata* Ledeb. and *Prunus cerasifera* Ehrh. (Browicz 1997; Büttner 2001). The latter name is often used for cultivated forms, which are also referred to as myrobalan plums. According to Browicz (1997) wild forms (*Prunus cerasifera* ssp. *divaricata*) and cultivated forms (*Prunus cerasifera* ssp. *cerasifera*) should however, rather be distinguished at the level of subspecies. A considerable array of additional subspecies and varieties have been recognized from various regions of the natural distribution range of cherry plum, but numerous transitional characters render the distinction of these entities difficult (Browicz 1969; Büttner 2001). Eremin and Garkovenko (1989) proposed to separate the species into three subspecies, namely *P. cerasifera* ssp. *cerasifera* (syn. *Prunus divaricata* Ledeb.), *P. cerasifera* ssp. *orientalis* (Koval.) Erem. et Garkov and *P. cerasifera* ssp. *macrocarpa* (Erem. et Garkov), with all the cultivated forms included in the latter. This proposal was adopted by Büttner (2001) but is nevertheless provisional and needs further taxonomic evaluation in the light of molecular data.

Whereas the demand for cherry plums is growing steadily, their supply from the wild is threatened by deforestation. To conserve and protect such valuable plant material, optimized breeding and domestication programs are compulsory. During the last years, the cultivated form of cherry plum was in the focus of genetic investigations because several of its clones (e.g., P.2175 and P.2980) are highly resistant to root-knot nematodes of the genus *Meloidogyne* (Dirlewanger et al. 2004; Lecouls et al. 2004). The cultivated myrobalan was also suggested as a useful diploid model system for studying the molecular genetic background of self-incompatibility in plums (Sutherland et al. 2009). However, no attempts have been made so far to evaluate population genetic parameters of its wild relative *P. divaricata* and to assess the possible effects of destruction, fragmentation, and conversion of natural habitats on its genetic variability. These questions are of particular interest in a fast-developing region like northern Iran, where local people take nutritional advantage of the species.

In the last two decades, genetic information has contributed substantially to both cultivation and conservation biology of plants and animals on a worldwide scale. Molecular fingerprints based on anonymous, PCR-based molecular markers such as AFLP (amplified fragment length polymorphism, Vos et al. 1995) have proven to be efficient tools for detecting genetic relationships and genetic diversity, and were also used to elucidate population structure, gene flow and rare genotypes in plants (e.g., Panaud et al. 2002; Peters et al. 2009). However, these anonymous markers are normally inherited in a dominant fashion, which limits their applicability for population genetics. Nuclear microsatellites, also called simple sequence repeats (SSRs), are currently the genetic markers of choice in population studies and for the assessment of genetic diversity and differentiation (Balloux and Lugon-Moulin 2002; Powell et al. 1996). Microsatellites consist of tandemly repeated, short DNA sequence motifs and are frequently size-polymorphic in a population due to a variable number of tandem repeats. Moreover, they are ubiquitous components of eukaryotic genomes and can be found both in coding and non-coding regions. The popularity of nuclear microsatellites stems from a unique combination of several important advantages, namely their Mendelian and codominant inheritance, high abundance, enormous extent of allelic diversity, and the ease of assessing size variation by PCR with pairs of flanking primers. The only serious disadvantage is the necessity of sequence information for primer design. Introduction of library enrichment techniques and automatic sequencing have simplified their expensive isolation, thus allowing wide application in plant genetics (Weising et al. 2005). Moreover, flanking regions of SSRs are often

conserved in related species, which enables the use of the same primer pairs in related genomes ("cross-species amplification").

An increasingly important source for microsatellite markers are expressed sequence tag (EST) databases (Pashley et al. 2006; Ellis and Burke 2007). EST-derived SSRs combine several important advantages. First, in silico mining for EST-SSRs is fast and easy, compared to standard cloning and sequencing procedures. Second, ESTs-SSRs are physically linked to a gene, and putative gene functions can readily be identified by a BLAST comparison with protein databases (Yao et al. 2010). Third, primer target sequences that reside in transcribed regions are expected to be relatively conserved, thus enhancing the chance of marker transferability across taxa (Decrooq et al. 2003; Gasic et al. 2009; Vendramin et al. 2007). On the negative side, the association with coding regions may limit the polymorphism of EST-derived microsatellite markers (Ellis and Burke 2007).

The present study aims to investigate the population genetics of wild *Prunus divaricata* using nuclear SSRs. Whereas large numbers of SSR markers are already available from other *Prunus* species such as peach (*P. persica*; Aranzana et al. 2002; Cipriani et al. 1999; Dirlewanger et al. 2002), almond (*P. dulcis*; Mnejja et al. 2005; Xie et al. 2006), apricot (*P. armeniaca*; Messina et al. 2004) and Japanese plum (*P. salicina*; Mnejja et al. 2004), none have been developed yet for *P. divaricata*. In the first part of our study, we therefore tested the transferability of 18 anonymous and 7 EST-derived SSR markers originally developed for peach and Japanese plum to *P. divaricata.* The eleven best-performing heterologous markers were then used to assess the genetic variability and differentiation of wild *P. divaricata* within and among 12 geographically separated populations along the Iranian coast of the Caspian Sea.

Materials and methods

Plant material and DNA extraction

Leaves were collected from 117 wild *P. divaricata* individuals from 12 populations in an area between the Caspian Sea and the Elburs mountain range within the provinces of Gilan and Mazandaran (Fig. 1). The population samples were collected from natural forests with a minimum of anthropogenic influence, with only four of these populations being located close to a larger city (Table 1). Elevations ranged from −19 m up to 970 m a.s.l. Five to 15 individuals were sampled per population. The geographical distances between pairs of populations ranged from 17 km up to 430 km. Immediately after collection, leaves were quick-dried in silica gel, transported to the laboratory and stored at −80°C until use. Total genomic DNA was extracted using a variant of the CTAB method (Weising et al. 1995). Populations were named according to cities close to the collection site. Two samples each of sweet cherry (*Prunus avium* L.; diploid) and blackthorn (*P. spinosa* L.; tetraploid) and one sample of domestic plum (*P. domestica* L.; hexaploid) were used as additional reference material for the primer transferability tests.

Microsatellite analyses

Initially, 25 microsatellite-flanking primer pairs originally developed for *P. persica* (L.) Batsch and *P. salicina* Lindl. (see Table 2) were tested for

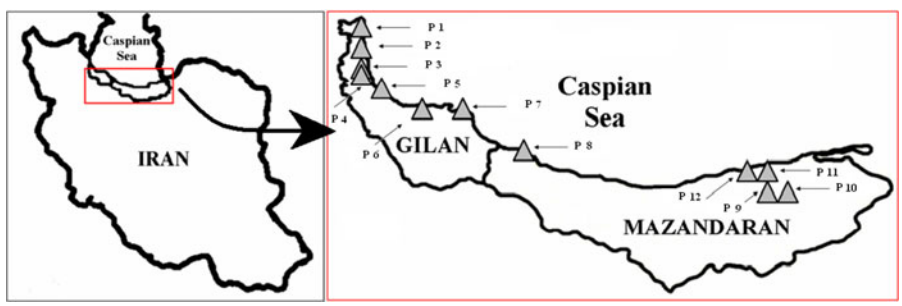

Fig. 1 Locations of *P. divaricata* populations. P1: Kashafi, P2: Astara, P3: Hashtpar, P4: Kiasar, P5: Asalem, P6: Fowman, P7: Lahijan, P8: Tonkabon, P9: Zirab, P10: Safiedkouh, P11: Amol, P12: Babol

Table 1 Sampling localities and sample sizes of *P. divaricata* populations studied

Population	Sample size (n)	Province	Averaged GPS coordinates	
P1 Kashafi	8	Gilan	38 23 N	48 37 E
P2 Astara	10	Gilan	38 18 N	48 47 E
P3 Hashtpar	10	Gilan	38 08 N	48 51 E
P4 Kiasar	8	Gilan	37 58 N	48 53 E
P5 Asalem	10	Gilan	37 41 N	48 49 E
P6 Fowman	10	Gilan	37 12 N	49 21 E
P7 Lahijan	11	Gilan	37 20 N	50 12 E
P8 Tonkabon	8	Mazandaran	36 43 N	50 50 E
P9 Zirab	12	Mazandaran	36 15 N	52 28 E
P10 Safiedkouh	15	Mazandaran	36 20 N	52 45 E
P11 Amol	10	Mazandaran	36 27 N	52 20 E
P12 Babol	5	Mazandaran	36 35 N	52 40 E

successful PCR amplification in seven randomly chosen individuals of *P. divaricata* and five samples of the reference material (see above). Fourteen primer pairs were derived from two genomic libraries of peach, enriched for AC/GT and AG/CT repeats (Cipriani et al. 1999; Testolin et al. 2000). Seven primer pairs were derived from peach EST-SSRs (Vendramin et al. 2007), and four were derived from a genomic SSR library of Japanese plum DNA enriched for CT repeats (Mnejja et al. 2004). Polymerase chain reactions (PCR) were performed in a total volume of 25 µl containing 1x PCR Mango-buffer (provided by the manufacturer, Bioline), 5 µg BSA, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 10 pmol of each primer, 10 ng of genomic DNA and 0.1 U of *Taq* DNA polymerase (Mango-*Taq*, Bioline). PCR cycling conditions consisted of an initial denaturation step of 94°C for 5 min, followed by 27 cycles of 45 s at 94°C, 45 s at 57°C and 45 s at 72°C, and a final extension step (8 min at 72°C). PCR products were separated by electrophoresis on 1.5% agarose gels (NEOO Ultra Quality, Roth) in 0.5x TBE (Sambrook and Russell 2001) at 10 V/cm, stained with ethidium bromide (1 µg/ml) and visualized under UV-light. A 100 bp DNA ladder (Roth) was used as molecular size standard. Candidate markers that passed these initial tests were used for analyzing the full sample set. The same cycling conditions were used, but one primer of each pair was 5′-labeled with IRD700 or IRD800 fluorescent dyes. Fluorescently labelled microsatellite fragments were analysed on a LiCor® IR^2 DNA Sequencer Long Readir 4200 in high resolution polyacrylamide gels (6%), and allele sizes were determined by visual comparison with a size standard and a reference sample included in all gels.

Statistical analyses

Departures from Hardy–Weinberg equilibrium (HWE) at each locus and linkage disequilibrium between individual microsatellite loci were evaluated by an exact test using a Markov chain method and Bonferroni corrections implemented in GENEPOP (Raymond and Rousset 1995). Indications for the presence of null alleles were assessed using an iterative algorithm based on the observed and expected frequencies of the various genotypes by CERVUS (version 3.0.3, Kalinowski et al. 2007). To examine the informational content of each microsatellite locus the following parameters were calculated: allele frequency, number of alleles per locus, expected (H_{exp}, Nei 1987) and observed (H_{obs}, direct count estimate) heterozygosity for each population and each locus, Wright's fixation indices (F_{IS}, F_{IT}, F_{ST}, Wright 1931, 1969, extended by Nei 1977) and Nei's coefficient of genetic differentiation (G_{ST}, Nei 1973). All of these parameters were estimated with the software programs FSTAT (version 2.9.3.2, Goudet 2002), GENEPOP and/or GENETIX (version 4.05, Belkhir et al. 2000). The total genetic variation was partitioned into a between- and

a within-population component by an analysis of molecular variance (AMOVA, Excoffier et al. 1992), using ARLEQUIN (version 3.11, Excoffier et al. 2005) with 1,000 permutations for significance testing. Genetic relationships among individuals were assessed by a multivariate principal component analysis (PCA), performed with GENETIX software using Euclidean distances between samples. The correlation between genetic distance and the corresponding geographic distances were analyzed using the Mantel test with 1,000 permutations (Mantel 1967) based on a pairwise matrix of Wright's F_{ST} (also generated in ARLEQUIN).

Results

Cross-species transferability of microsatellite markers

The success of cross-species amplification of the 25 candidate markers in *P. divaricata*, *P. avium*, *P. domestica* and *P. spinosa* was evaluated by the quality of the banding patterns on agarose gels (Table 2). PCR amplification was considered successful when the number of distinct bands was compatible with the known ploidy status, and when bands of the expected size range were present in all

Table 2 Results of cross-transferability tests of microsatellite markers from peach (UDP, EPPISF) and Japanese plum (CPSCT) amplified in cherry plum, sweet cherry, blackthorn and domestic plum

Locus code	Repeat motif in species of origin	Banding pattern	Predicted size (bp)	Reference[a]
UDP96-001	$(CA)_{17}$	Distinct PCR products in all samples	120	[1]
UDP96-003	$(CT)_{11}(CA)_{28}$	Distinct PCR products in all samples	143	[1]
UDP96-005	$(AC)_{16}TG(CT)_2CA(CT)_{11}$	One or more samples failed	155	[1]
UDP96-008	$(CA)_{23}$	Distinct PCR products in all samples	165	[1]
UDP96-013	$(AG)_{22}(TG)_8TT(TG)_{10}$	One or more samples failed	198	[1]
UDP96-015	$(CA)_{31}$	No PCR products at all	174	[1]
UDP96-018	$(AC)_{21}$	Complex	253	[1]
UDP96-019	$(TG)_{18}(AG)_7$	One or more samples failed	216	[1]
UDP97-402	$(AG)_{17}$	PCR products only in *P. avium*	136	[1]
UDP97-403	$(AG)_{22}$	Distinct PCR products in all samples	150	[1]
UDP98-407	$(CT)_{22}$	One or more samples failed	212	[1]
UDP98-409	$(AG)_{12}$	Distinct PCR products in all samples	129	[1]
UDP98-411	$(TC)_{16}$	Complex	150	[2]
UDP98-412	$(AG)_{28}$	Complex	129	[2]
EPPISF001	$(GATG)_5$	Complex	250–235	[3]
EPPISF004	$(CCA)_5$	Complex	180	[3]
EPPISF010	$(AG)_{10}$	Distinct PCR products in all samples	150–170	[3]
EPPISF014	$(CAG)CCA(CAG)_6$	Complex	245–260	[3]
EPPISF016	$(CTT)_7$	Distinct PCR products in all samples	160	[3]
EPPISF018	$(TCT)_5(TCC)_3$	Distinct PCR products in all samples	275–290	[3]
EPPISF026	$(CAG)_6(CAG)_5$	Complex	225	[3]
CPSCT 008	$(GA)_{17}$	Distinct PCR products in all samples	198	[4]
CPSCT 012	$(GA)_{16}$	Distinct PCR products in all samples	156	[4]
CPSCT 027	$(GA)_{23}$	Distinct PCR products in all samples	166	[4]
CPSCT 035	$(GA)_{23}$	Distinct PCR products in all samples	191	[4]

PCR products were evaluated by agarose gel electrophoresis. Markers selected for the population study are shown in bold

[a] [1] Cipriani et al. 1999, [2] Testolin et al. 2000, [3] Vendramin et al. 2007, [4] Mnejja et al. 2004

Prunus samples. These criteria were met by 12 candidates. Of the remaining 13 loci, seven gave complex banding patterns, and five markers failed with one or more samples of the test set. No PCR product in any sample was obtained from locus UDP96-015. Eleven of the 12 successful candidates produced well-scorable, polymorphic bands also on PAA gels, whereas UDP96-003 yielded a monomorphic banding pattern on PAA gels and was excluded from further study.

Allelic variation

Eight genomic and three EST-derived microsatellite markers were selected for the population genetic analyses (Table 2). They detected 184 alleles (3–31 depending on the locus) in 117 individuals of *P. divaricata* sampled along the southern coast of the Caspian Sea, with a mean value of 16.7 alleles per locus (Table 3). No significant deviations from HWE were detected for any of the loci or populations, and no indications for linkage disequilibrium were found. Overall numbers of alleles were very similar across populations, varying between 5.1 and 8.5 with an average of 7.0. Rare alleles with frequencies <0.05 represented between 55 and 84% of the total allele spectrum, depending on the locus. Indications for the presence of null alleles were found in three markers (UDP96-008, UDP98-409, EPPISF018), but their estimated frequencies were very low (<0.05).

Table 4 Observed (H_{obs}) and expected (H_{exp}) heterozygosities within each of 12 populations of *P. divaricata* averaged over 11 microsatellite loci

Population	H_{obs}	H_{exp}
P1 Kashafi	0.727	0.734
P2 Astara	0.682	0.697
P3 Hashtpar	0.700	0.729
P4 Kiasar	0.648	0.733
P5 Asalem	0.702	0.733
P6 Fowman	0.682	0.756
P7 Lahijan	0.669	0.708
P8 Tonkabon	0.695	0.790
P9 Zirab	0.644	0.680
P10 Safiedkouh	0.640	0.725
P11 Amol	0.618	0.658
P12 Babol	0.780	0.804
Mean	0.682	0.729

Genetic diversity

Levels of genetic diversity were generally high, with $H_{obs} = 0.664$ and $H_{exp} = 0.733$ averaged over all loci (Table 3) and $H_{obs} = 0.682$ and $H_{exp} = 0.729$ averaged over all populations (Table 4). In contrast,

Table 3 Population genetic parameters determined for each of 11 microsatellite loci averaged across 12 populations of *P. divaricata*

Locus code	NA	Mean NA	H_{obs}	H_{exp}	F_{IS}	F_{IT}	F_{ST}	G_{ST}
UDP96-001	9	4.167	0.573	0.596	0.027	0.042	0.015	0.065
UDP96-008	5	2.167	0.190	0.263	0.236	0.294	0.077	0.117
UDP97-403	24	9.917	0.904	0.930	0.002	0.032	0.030	0.074
UDP98-409	12	5.250	0.590	0.751	0.201	0.216	0.020	0.077
CPSCT008	31	11.083	0.914	0.936	0.014	0.026	0.012	0.060
CPSCT012	3	2.500	0.393	0.361	−0.096	−0.089	0.007	0.047
CPSCT027	7	4.500	0.513	0.792	0.294	0.365	0.102	0.139
CPSCT035	31	10.583	0.846	0.937	0.066	0.100	0.037	0.085
EPPISF010	21	10.167	0.913	0.922	0.013	0.008	0.000	0.049
EPPISF016	30	11.250	0.914	0.945	0.013	0.034	0.021	0.072
EPPISF018	11	5.083	0.557	0.628	0.090	0.116	0.029	0.082
Mean		16.727	0.664	0.733	0.069	0.096	0.029	0.079

NA number of alleles, H_{obs}, H_{exp} observed and expected heterozygosities, F_{IS}, F_{IT}, F_{ST} Wright's F statistics, G_{ST} Nei's coefficient of genetic differentiation

Table 5 Results of the analysis of molecular variance (AMOVA) for 117 *P. divaricata* individuals grouped in 12 populations

Source of variation	d.f.	Variance components	% total	*p*
Among populations	11	0.12872	3.2	<0.0001
Within populations	222	3.83891	96.8	<0.0001
Total	233	3.96763	100.00	<0.0001
$F_{ST} = 0.03244$				

The degrees of freedom (d.f.), variance components, the fraction of total variation contributed by each nested component (%), its associated significance (*P*; $n = 1{,}000$ permutations) and mean F_{ST} are shown

F_{ST} and G_{ST} values were low, ranging from zero (CPSCT012) to 0.102 (EPPISF018) with a mean of 0.029 over all loci for F_{ST} ($P < 0.05$) and from 0.047 (EPPISF016) to 0.139 (EPPISF018) with a mean of 0.079 over all loci for G_{ST}. The likewise low values of F_{IS} and F_{IT} calculated for each SSR locus in each population indicate that inbreeding is negligible, which is in line with the non-significant deviations from the HWE (see above). An analysis of molecular variance (AMOVA) indicated that only 3.2% of the total molecular variance was attributable to the divergence among populations. In contrast, 96.8% of the variance was found within populations (Table 5). Altogether, these results indicate a low level of genetic differentiation, and hence suggest high levels of gene flow between the studied populations of *P. divaricata*. The almost complete lack of differentiation was also reflected by a principal components analysis (PCA), where the first two axes together accounted for only 6.06% of the total genetic variability (pc 1 = 3.22%, pc 2 = 2.84%). The two-dimensional PCA diagram did not arrange the individuals into distinct geographical populations, as all 12 populations strongly overlapped (data not shown). A Mantel test nevertheless revealed a weak but significant correlation between geographic and genetic distances ($r = 0.29$, $P < 0.003$, Fig. 2).

Discussion

Cross-species transferability of microsatellite markers within *Prunus*

Whereas no microsatellite markers have been developed so far in *P. divaricata*, numerous studies reported successful cross-species transferability of SSR markers among different *Prunus* species (e.g., Cipriani et al. 1999; Dirlewanger et al. 2002; Sánchez-Pérez et al. 2006; Wünsch 2009; Mnejja et al. 2010). For example, two markers from peach, BPPCT-007 (Dirlewanger et al. 2002) and CPPCT-006 (Aranzana et al. 2002) amplified in ten different *Prunus* species from three subgenera and five sections (Wünsch 2009). Marker transportability to other genera of the Rosaceae, like apple, pear and strawberry, seems to be much less efficient (Mnejja et al. 2010).

Based upon the close taxonomic relationship of *P. divaricata* with *P. salicina* (both from section *Prunus*) we expected relatively high levels of transferability for the CPSCT primers developed in the latter (Mnejja et al. 2004). Table 2 shows that indeed four out of four (100%) CPSCT primers amplified distinct and polymorphic PCR products in cherry plum, with 3–31 alleles. Much lower success rates

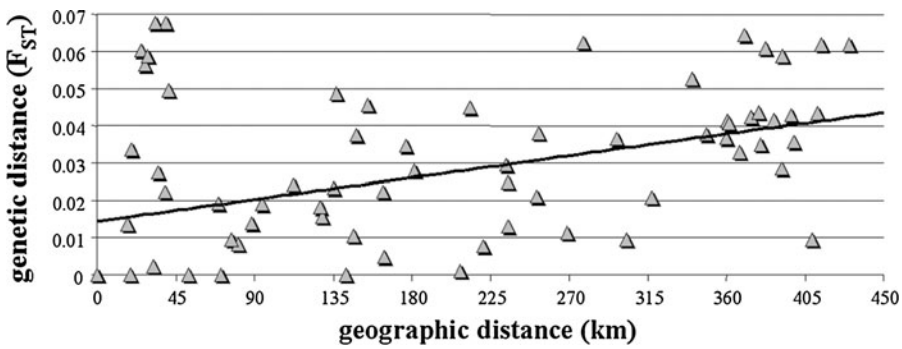

Fig. 2 Correlation of geographic distance (in kilometers) and genetic distance (pairwise F_{ST}) among 117 individuals of 12 populations of *P. divaricata*, including regression line (Mantel test, $r = 0.29$, $P < 0.003$)

were obtained with the UDP primers derived from the more distantly related peach (Cipriani et al. 1999), of which only 36% generated distinct PCR products in cherry plum. One marker, UDP96-003 only yielded a monomorphic band pattern on PAA gels. Of the EPPISF primers derived from peach ESTs (Vendramin et al. 2007), 71% gave a distinct and highly polymorphic PCR product in cherry plum, supporting the assumption that primers derived from transcribed sequences have an increased chance of cross-species functionality. With 31 alleles among 184 individuals, the EPPISF016 locus based on a trinucleotide repeat (CTT) motif in peach even turned out as one of the most polymorphic markers in cherry plum. This was quite unexpected given that trinucleotide repeats prevail in coding regions and are therefore supposed to be more conserved and therefore less polymorphic.

Genetic diversity

The 11 polymorphic microsatellite loci employed in the present study proved to be highly informative in *P. divaricata* and detected an average of 16.7 alleles per locus. This is a relatively large number compared to the average numbers of alleles per locus found in other studies dealing with different *Prunus* species, as e.g., 13.3 in *P. armeniaca* L. (Maghuly et al. 2005), 10.7 in *P. cerasus* L. (Cantini et al. 2001), 6.7 in *P. mahaleb* L. (Godoy and Jordano 2001), 8.2 in *P. cerasoides* D. Don (Pakkad et al. 2003), 7.3 in *P. persica* (L.) Batsch (Aranzana et al. 2003), and only 2.93 in *Amygdalus nana* L. syn. *Prunus tenella* Rehd. and A. (Tahan et al. 2009). The large number of alleles indicates a generally high level of genetic diversity within wild *P. divaricata*, which is supported by high values of observed and expected heterozygosity. For example, H_{obs} averaged over all loci and populations of. *P. divaricata* was 0.664, which is higher than 0.53 reported for wild *P. cerasoides* (Pakkad et al. 2003), a value that was considered by the authors as a high level of genetic variation. Mean values of H_{obs} obtained from cultivated *Prunus* species like apricot and peach ranged from 0.22 to 0.45 (Martínez-Gómez et al. 2003; Sánchez-Pérez et al. 2006), demonstrating that domesticated and cultivated species often show low levels of genetic variation as compared with their wild ancestors (Miller and Schaal 2006; Anthony et al. 2002; Cahill 2004; Panda et al. 2003).

Although the studied populations of *P. divaricata* represent only a small part of the total distribution range of this species in western Asia, the geographic distances between populations were relatively large. Nevertheless, the results of all analyses indicated that genetic differentiation between populations was very low. Both, AMOVA and PCA indicated that *P. divaricata* preserved the vast majority of its genetic variability within populations. A comparable but allozyme-based study of genetic variation in six wild *P. avium* L. populations reported by Mariette et al. (1997) likewise showed no recognizable structure and a mean G_{ST} value of 0.05, similar to our result (0.079). The authors concluded that these populations underlie both well-balanced selection and neutral genetic drift, respectively migration. Intensive research with allozyme markers has shown that seed dispersal patterns are among the main factors that determine the partitioning of genetic variation within and among populations (Hamrick et al. 1993; Hamrick and Godt 1997). The sweet and fleshy fruits of most fruit trees are dispersed by birds and mammals, leading to characteristically high levels of within-population genetic variation and low levels of among-population variation, due to large frugivorous birds acting as long-distance seed dispersers (e.g., woodpeckers, thrushes and pigeons). Long-distance flights away from the feeding trees have frequently been observed in these birds, e.g., in *Prunus mahaleb* (Jordano and Godoy 2000; Jordano and Schupp 2000). Likewise, anthropogenic influence may play a role for gene flow as the fruits of wild *P. divaricata* are part of the diet of local people in the fruiting season. Still another reason for the reduced genetic differentiation of *P. divaricata* may be associated with the geographical conditions of the area itself. The high elevations of the Elburs mountain range in the south and the Caspian Sea in the north may enhance the exchange of genetic material in east-west-direction.

Conservation aspects

The cultivated form of cherry plum, *P. cerasifera* Ehrh., is an agriculturally important species, and is distinguished from other plums through e.g. its drought tolerance, high resistance to root-knot nematodes and its suitability as a rootstock (Lecouls et al. 2004; Dirlewanger et al. 2004). Interestingly,

however, the germplasm variability of its wild ancestor, *P. divaricata*, has neither been properly assessed nor collected, and essentially nothing was known so far about the genetic variability of this *Prunus* species. The results of our study indicate high levels of genetic diversity and a lack of genetic differentiation of *P. divaricata* in northern Iran, suggesting that natural populations of cherry plum in the Caspian forests are close to Hardy–Weinberg equilibrium and have not yet experienced measurable genetic drift. However, it cannot be excluded that deforestation, habitat fragmentation and the increasing population density in the area will have a negative impact onto the population structure of the species in the near future.

At any rate, it seems reasonable to secure the genetic variability present across the gene pool of wild cherry plum both in its natural habitats in northern Iran as well as *ex situ* in germplasm collections. A focus for in situ conservation should be placed on populations with the highest level of genetic diversity, such as the populations from Kashafi (P1), Hashtpar (P3), Asalem (P5) and Babol (P12). For *ex situ* conservation, seeds of *P. divaricata* should be collected from as many populations as possible and stored in a gene bank (Li et al. 2009). Wild species can be very useful in breeding programs as sources of genetic variability that enlarge the gene pool of their cultivated relatives (Wolko et al. 2010), and the highly diverse *P. divaricata* could well turn out to be a valuable gene donor to increase variation in cultivated *Prunus* species by cross-breeding (Fritz et al. 1994; Mandegaran et al. 1999; Dirlewanger et al. 2004).

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References

Anthony F, Combes MC, Astorga C, Bertrand B, Graziosi G, Lashermes P (2002) The origin of cultivated *Coffea arabica* L. varieties revealed by AFLP and SSR markers. Theor Appl Genet 104:894–900

Aranzana MJ, Garcia-Mas J, Carbo J, Arús P (2002) Development and variability analysis of microsatellite markers in peach. Plant Breed 121:87–92

Aranzana MJ, Pineda A, Cosson P, Dirlewanger E, Ascasibar J, Cipriani G, Ryder CD, Testolin R, Abbott A, King GJ, Iezzoni AF, Arús P (2003) A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. Theor Appl Genet 106:819–825

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. Mol Ecol 11:155–165

Belkhir K, Borsa P, Chikhi L, Raufaste N (2000) GENETIX, a windowsTM based software for population genetic analysis. In: Laboratoire génome et populations, CNRS UPR 9060. Université de Montpellier II, Montpellier

Browicz K (1969) *Prunus* L. In: Rechinger KH (ed) Flora Iranica, vol 66. Akademische Druck- und Verlagsanstalt Graz, Austria, pp 162–164

Browicz K (1997) Chorology of trees and shrubs in South-West Asia and adjacent regions. Polish Academy of Sciences, Kornik

Büttner R (2001) *Prunus* sect. *Prunus*. In: Hanelt P, Institute of Plant Genetics, Crop Plant Research (eds) Mansfeld's encyclopedia of agricultural and horticultural crops, vol. 1. Springer, Berlin, pp 517–523

Cahill JP (2004) Genetic diversity among varieties of China (*Salvia hispanica* L.). Genet Resour Crop Evol 56: 947–961

Cantini C, Iezzoni AF, Lamboy WF, Boritzki M, Struss D (2001) DNA fingerprinting of tetraploid cherry germplasm using simple sequence repeats. J Am Soc Hort Sci 126:205–209

Cipriani G, Lot G, Huang WG, Marrazzo MT, Peterlunger E, Testolin R (1999) AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L.) BATSCH]: isolation, characterisation and cross-species amplification in *Prunus*. Theor Appl Genet 99:65–72

Decrooq V, Favé MG, Hagen L, Bordenave L, Decrooq S (2003) Development and transferability of apricot and grape EST microsatellite markers across taxa. Theor Appl Genet 106:912–992

Dirlewanger E, Cosson P, Tavaud M, Aranzana MJ, Poizat C, Zanetto A, Arús P, Laigret F (2002) Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor Appl Genet 105:127–138

Dirlewanger E, Posson P, Howad W, Capdeville G, Bosselut N, Claverie M, Voisin R, Poizat C, Lafargue B, Baron O, Laigret F, Kleinhentz M, Arús P, Esmenjaud D (2004) Microsatellite genetic linkage maps of myrobalan plum and an almond-peach hybrid–location of root-knot nematode resistance genes. Theor Appl Genet 109:827–838

Ellis JR, Burke JM (2007) EST-SSRs as a resource for population genetic analyses. Heredity 99:125–132

Eremin GV, Garkovenko VM (1989) Infraspecific systematics of myrobalan plum. Trudy Prikl Bot Genet Selekts 123:9–15

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction data. Genetics 131:479–491

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.11: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Faust M, Surányi D (1999) Origin and dissemination of plums. Hortic Rev (Am Soc Hortic Sci) 23:179–231

Fritz RS, Nichols-Orians CM, Brunsfeld SJ (1994) Interspecific hybridization of plants and resistance to herbivores: hypotheses, genetics, and variable responses in a diverse herbivore community. Oecologia 97:106–117

Gasic K, Han Y, Kertbundit S, Shulaev V, Iezzoni AF, Stover EW, Bell RL, Wisniewski ME, Korban SS (2009) Characteristics and transferability of new apple EST-derived SSRs to other Rosaceae species. Mol Breed 23:397–411

Godoy JA, Jordano P (2001) Seed dispersal by animals: exact identification of source trees with endocarp DNA microsatellites. Mol Ecol 10:2275–2283

Goudet J (2002) FSTAT 2.9.3.2. URL: http://www2.unil.ch/popgen/softwares/fstat.htm

Hamrick JL, Godt MJW (1997) Effects of life history traits on genetic diversity in plant species. In: Silvertown J, Franco M, Harper JL (eds) Plant life histories. Ecology, phylogeny and evolution. Cambridge University Press, Cambridge, pp 102–118

Hamrick JL, Murawski DA, Nason JD (1993) The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. In: Fleming TH, Estrada A (eds) Frugivory and seed dispersal: ecological and evolutionary aspects. Kluwer Academic Publisher, Dordrecht, pp 281–297

Jordano P, Godoy JA (2000) RAPD variation and population genetic structure in *Prunus mahaleb* (Rosaceae), an animal-dispersed tree. Mol Ecol 9:1293–1305

Jordano P, Schupp EW (2000) Seed disperser effectiveness: the quantity component and patterns of seed rain for *Prunus mahaleb*. Ecol Monogr 70(4):591–615

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error inreases success in paternity assignment. Mol Ecol 16:1099–1106

Khoshbakht K, Hammer K (2005) Savadkouh (Iran)–an evolutionary centre for fruit trees and shrubs. Genet Resour Crop Evol 00:1–11

Khoshbakht K, Reza MA, Hammer K (2007) Phenotypic diversity of fruits and kernels of *Prunus divaricata* Ledeb. in Northern Iran—implications for domestication and utilisation. For: Tropentag, October 9–11, Witzenhausen

Lecouls AC, Bergougnoux V, Rubio-Cabetas MJ, Bosselut N, Voisin R, Poëssel JL, Faurobert M, Bonnet A, Salesses G, Dirlewanger E, Esmenjaud D (2004) Marker-assisted selection for the wide-spectrum resistance to root-knot nematodes conferred by the *Ma* gene from Myrobalan plum (*Prunus cerasifera*) in interspecific *Prunus* material. Mol Breed 13:113–124

Li MM, Cai YL, Qian ZQ, Zhao GF (2009) Genetic diversity and differentiation in Chinese sour cherry *Prunus pseudocerasus* Lindl. and its implications for conservation. Genet Resour Crop Evol 56:455–464

Maghuly F, Fernandez EB, Ruthner S, Pedryc A, Laimer M (2005) Microsatellite variavility in apricots (*Prunus armeniaca* L.) reflects their geographic origin and breeding history. Tree Genetics Genomes 1:151–165

Mandegaran Z, Roberts AV, Hammatt N (1999) The ability of *Prunus avium* x *P. pseudocerasus* 'Colt' to form somatic embryos in vitro contrasts with the recalcitrance of *P. avium*. Plant cell. Plant Cell Tissue Organ Cult 59:57–63

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

Mariette S, Lefranc M, Legrand P, Taneyhill D, Frascaria-Lacoste N, Machon N (1997) Genetic variability in wild cherry populations in France. Effects of colonizing processes. Theor Appl Genet 94:904–908

Martínez-Gómez P, Sozzi GO, Sánchez-Pérez R, Rubio M, Gradziel TM (2003) New approaches to *Prunus* tree crop breeding. Food Agric Environ 1:52–63

Messina R, Lain O, Marrazzo MT, Cipriani G, Testolin R (2004) New set of microsatellite loci isolated in apricot. Mol Ecol Notes 4:432–434

Miller AJ, Schaal BA (2006) Domestication and the distribution of genetic variation in wild and cultivated populations of the mesoamerican fruit tree *Spondias purpurea* L. (Anacardiaceae). Mol Ecol 15:1467–1480

Mnejja M, Garcia-Mas J, Howad W, Badenes ML, Arús P (2004) Simple-sequence repeat (SSR) markers of Japanese plum (*Prunus salicina* Lindl.) are highly polymorphic and transferable to peach and almond. Mol Ecol Notes 4:163–166

Mnejja M, Garcia-Mas J, Howad W, Arús P (2005) Development and transportability across *Prunus* species of 42 polymorphic almond microsatellites. Mol Ecol Notes 5:531–535

Mnejja M, Garcia-Mas J, Audergon, JM, Arús P (2010) *Prunus* microsatellite marker transferability across rosaceous crops. Trees Genet Genomes 6:689–700

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Nat Acad Sci USA 70(12):3321–3323

Nei M (1977) *F*-statistics and analysis of gene diversity in subdivided populations. Ann Hum Genet 41:225–233

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Pakkad G, James C, Torre F, Elliott S, Blakesley D (2003) Genetic variation of *Prunus cerasoides* D. Don, a framework tree species in northern Thailand. New Forest 27:189–200

Panaud O, Chaib A, Sarr A (2002) Dynamic conservation of apricot Prunus armeniaca in Saharian oases: use of AFLP markers to assess genetic diversity in traditional orchards. Euphytica 128:301–305

Panda S, Martín JP, Aguinagalde I, Mohanty A (2003) Chloroplast DNA variation in cultivated and wild *Prunus avium* L: a comparative study. Plant Breed 122:92–94

Pashley CH, Ellis JR, McCauley DE, Burke JM (2006) EST databases as a source for molecular markers: lessons from *Helianthus*. Hered 97(4):381–388

Peters MD, Xiang QY, Thomas DT, Stucky J, Whiteman NK (2009) Genetic analysis of the federally endangered Echinacea laevigata using amplified fragment length polymorphisms (AFLP)–Inferences in population genetic structure and mating system. Conserv Genet 10:1–14

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci 1(7):215–222

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. J Heredity Comput Notes 86:248–249

Reales A, Sargent DJ, Tobut KR, Rivera D (2010) Phylogenetics of Eurasian plums, *Prunus* L. section *Prunus*

(Rosaceae), according to coding and non-coding chloroplast DNA sequences. Tree Genet Genomes 6:37–45

Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York

Sánchez-Pérez R, Martínez-Gómez P, Dicenta F, Egea J, Ruiz D (2006) Level and transmission of genetic heterozygosity in apricot (*Prunus armeniaca* L.) explored using simple sequence repeat markers. Genet Resour Crop Evol 53:763–770

Scharnweber T, Rietschel M, Manthey M (2007) Degradation stages of the Hyrcanian forests in southern Azerbaijan. Archiv für Naturschutz und Landschaftsforschung. 46:133–156

Scholz H, Scholz I (1995) *Prunus*. In: Hegi G, Scholz H (eds) Illustrierte Flora von Mitteleuropa, Band IV Teil 2B: Rosaceae 2. 2. neubearb. Blackwell, Berlin, pp 446–510

Sutherland BG, Cerovič R, Robbins TP, Tobutt KR (2009) The myrobalan (*Prunus cerasifera* L.): a useful diploid model for studying the molecular genetics of self-incompatibility in plums. Euphytica 166:385–398

Tahan O, Geng Y, Zeng L, Dong S, Chen F, Chen J, Song Z, Zhong Y (2009) Assessment of genetic diversity and population structure of Chinese wild almond, *Amygdalus nana*, using EST- and genomic SSRs. Biochem Syst Ecol 37:146–153

Testolin R, Marrazzo T, Cipriani G, Quarta R, Verde I, Dettori MT, Pancaldi M, Sansavini S (2000) Microsatellite DNA in peach (*Prunus persica* (L.) Batsch) and its use in fingerprinting and testing the genetic origin of cultivars. Genome 43:512–520

Vendramin E, Dettori MT, Giovinazzi J, Micali S, Quarta R, Verde I (2007) A set of EST-SSRs isolated from peach fruit transcriptome and their transferability across *Prunus* species. Mol Ecol Notes 7:307–310

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman H, Kuiper M, Zabeau M (1995) AFLP: a new technique fpr DNA fingerprinting. Nucleic Acids Res 23(21):4407–4414

Weising K, Nybom H, Wolff K, Kahl G (2005) DNA fingerprinting in plants: principles, methods, and applications, 2nd edn. Taylor & Francis, Boca Raton

Wolko L, Antkowiak W, Sips M, Slomski R (2010) Self-incompatibility alleles in Polish wild pear (*Pyrus pyraster* (L.) Burgsd.): a preliminary analysis. J Appl Genet 51(1):33–35

Wright S (1931) Evolution in mendelian populations. Genetics 16:97–159

Wright S (1969) Evolution and the genetics of populations. The theory of gene frequencies, vol. 2. University of Chicago Press, Chicago

Wünsch A (2009) Cross-transferable polymorphic SSR loci in *Prunus* species. Sci Hortic 120:348–352

Xie H, Sui Y, Chang F-Q, Xu Y, Ma R-C (2006) SSR allelic variation in almond (*Prunus dulcis* Mill.). Theor Appl Genet 112 :366–372

Yao L, Zheng X, Cai D, Gao Y, Wang K, Cao Y, Teng Y (2010) Exploitation of *Malus* EST-SSRs and the utility in evaluation of genetic diversity in *Malus* and *Pyrus*. Genet Resour Crop Evol. (Online First)

Zohary M (1973) Geobotanical foundations of the Middle East. Fischer, Stuttgart