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PRIMER NOTE

Isolation and characterization of microsatellite markers in the invasive shrub *Mahonia aquifolium* (Berberidaceae) and their applicability in related species

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Abstract

Microsatellite loci were isolated from a *Mahonia aquifolium* cultivar. We describe the variability of 10 loci in invasive European and native North American *M. aquifolium* and their transspecies amplification in native *Mahonia repens* and *Mahonia pinnata* from North America and one species of the related genus *Berberis* (*Berberis vulgaris*), native to Europe. The markers should be useful to reveal the genetic origin of invasive *Mahonia* populations and differences in the genetic make up between invasive and native populations.

Keywords: biological invasions, *Mahonia aquifolium*, microsatellite, population genetics

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Mahonia aquifolium (Pursh) Nutt. (Berberidaceae) is a diploid ($2n = 28$) evergreen shrub, native to western North America. It is a successful neophyte in central Europe (Kowarik 1992) and invades a wide range of habitats from calcareous mixed forests and xerothermic shrub vegetation to pine forests on sandy soils. The invasive populations reproduce either by seedlings or by root sprouts and stem layers. *Mahonia aquifolium* was introduced in 1822 for ornamental purposes to central Europe (Hayne 1822, cited in Kowarik 1992). It was supposed that invasive *Mahonia* populations mostly originated from garden plants and consist largely of hybrids between the related species *M. aquifolium* and *Mahonia repens* (Lindl.) G. Don (Ahrendt 1961). As there are many cultivated hybrids of *M. aquifolium* with *Mahonia pinnata* (Lag.) Fedde, likewise (van de Laar 1975), we assume genetic material of all three closely related North American species to be present in invasive populations. We suppose that plant breeding has created genetic variability by multiple introductions and hybridization. Consequently, a putative genetic bottleneck during introduction may have been overcome facilitating the invasion. Using microsatellite markers, we want to look for hybridization in the invasive European populations and for genetic variability within and between the invasive populations in contrast to native populations of all putative parent species of invasive *Mahonia*. No microsatellite markers have been developed for *Mahonia* up to now. Here, we describe the development of 10 microsatellite loci for invasive *Mahonia* and the applicability of these loci for three *Mahonia* species, and for *Berberis vulgaris* L., the only species that is native to central Europe and closely related to *Mahonia* (subtribe Berberidinae).

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Development of microsatellite loci was carried out by ECOGENICS GmbH (Zürich, Switzerland) using fresh leaf material from one single cultivated *M. aquifolium* cultivar. Genomic DNA was extracted by a modified cetyltrimethyl ammonium bromide (CTAB) method. After CTAB incubation, the DNA was precipitated with potassium acetate. The supernatant was extracted with chloroform/isoamyl alcohol and an ethanol precipitation was performed. An enriched library was made from size-selected genomic DNA ligated into SAULA/SAULB-linker [5′-GCGGTAC-CCGGGAAGCTTGG/5′-GATCCCAAGCTTCCCGGGTA-CCGC (Armour *et al*. 1994)] and enriched by magnetic bead selection with biotin-labelled $(CA)_{13}$ and $(GA)_{13}$ oligonucleotide repeats (Gautschi *et al*. 2000a, b). Of 576 recombinant colonies screened, 118 gave a positive signal after hybridization. Plasmids from 72 positive clones were sequenced and primers were designed for 22 microsatellite

Table 1 Microsatellite loci from invasive *Mahonia* based on one population of 20 individuals from northeast Germany

Locus	Repeat motif in sequenced clone	Primer sequence (5′–3′)	No. of alleles	Size range (bp)	H_O	H_E	F_{IS}	*P* value	EMBL Accession no.
Mahonia CA03	$(AT)_3(GT)_{18}$	F: GGGGTGTGACCGTTTTTATG R: CAATGCCCGAAAGTTACGTC	7	130–176	0.632	0.838	0.251	0.0241	AM233740
Mahonia CA18	$(TA)_3(TG)_{27}$	F: TCAATTCTTTTGAGTTAGGGTTTTG R: CCAATGACGTTAAATCCATACG	9	162–204	0.750	0.849	0.119	0.1723	AM233741
Mahonia CA30	$(CA)_{31}$	F: TGCATTTTCGACCCATCTAC R: TCTCCTCACATGCAACAAAAG	13	92–152	0.55	0.908	0.400	0.0025	AM233742
Mahonia CA40	$(CA)_{23}$	F: CGTATCTTTACTGTGAAATGGTGAG R: AGGTTAAATAAATTTCATCAATCACTC	9	113–135	0.750	0.804	0.069	0.3478	AM233743
Mahonia CA43	$(CA)_{12}$	F: TCCGCTTTCCACTTACCATC R: GGATGAGGGAGGTGTAACAATG	5	112–128	0.421	0.451	0.068	0.4313	AM233744
Mahonia GA04	$(GA)_{18}$	F: ACCCATTGGAGCTCTCTCAG R: TTGATTTTGAAGCCGAGATG	8	106–128	0.75	0.806	0.072	0.3475	AM233745
Mahonia GA05	$(CA)_{15}$	F: AGTCATCCCTCCATCATTCG R: TGTGAGAGCTCTGTTGGACTG	3	141–151	0.632	0.482	−0.321	1.0000	AM233746
Mahonia GA31	$(GT)_{12}$	F: TCACAATAGTTTATTTGAGTTTATTTG R: CACTGTCTGGCTCAATTTTGTC	3	158–164	0.500	0.645	0.229	0.1278	AM233747
Mahonia GA33	$(CA)_{14}$	F: GATCAGGTCCATAATATCAAAGTTC R: CAGACAAGGAGAGTGCTTGTACC	4	217–225	0.316	0.324	0.027	0.4855	AM233748
Mahonia GA36	$(GT)_{16}$	F: ACGAGGGCTATACAGGAACC R: CCAAGTATGTCCAGTACCTCCAG	3	176–186	0.579	0.568	−0.021	0.6361	AM233749

F, forward primer; R, reverse primer; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; *P* value, probability that F_{IS} is not different from zero.

inserts from which 11 primers were selected after an initial screening with five invasive *Mahonia* samples from central Germany. Primers were tested for polymorphism on 20 individuals of one invasive *Mahonia* population from northeast Germany. DNA was extracted using the Plant DNA extraction mini kit (QIAGEN). Polymerase chain reaction (PCR) was performed in 96-well plates using a Primus 96 plus (MWG Biotech) or a Mastercycler gradient (Eppendorf) Thermocycler. Ten- microlitre reactions contained 1–10 ng genomic DNA, 1 pmol of each forward and reverse primer (MWG), 4 μL Multiplex PCR Kit (QIAGEN) and 3 μL H_2O. Fragments were separated on an ABI 310 Genetic Analyser (Applied Biosystems) with the size standard GeneScan ROX500 (Applied Biosystems). For visualization of fragments, each forward primer was fluorescent labelled with FAM (Mahonia CA03, CA18, CA30, GA04), JOE (CA40, GA05, GA31, GA33) or TAMRA (CA22, CA43, GA36). Optimal annealing temperature (T_a) for each primer was ascertained using a gradient from 55 °C to 65 °C. We grouped together primers with the same T_a to multiplex reactions leading to three primer combinations: (i) GA31 and CA30 (T_a = 57 °C), (ii) CA40, GA04, CA18 and CA22 (T_a = 60 °C), (iii) GA33, GA05, CA03, GA36 and CA43 (T_a = 63 °C). In these reactions, we reduced the amount for some primers (CA18, GA05, GA31) to 0.5 pmol because of unequal amounts of amplification products within multiplex reaction. The PCR programme was as following: 95 °C for 15 min, 30 cycles at 94 °C for 30 s, T_a for 90 s, 72 °C for 60 s and at the end 60 °C for 30 min. For statistical analyses, we excluded locus CA22, which gave irreproducible results. There were 10 primers left, which gave interpretable PCR products (Table 1). No homozygous null allele was observed. Expected and observed heterozygosities were calculated using MSA software (Dieringer & Schlötterer 2002) and inbreeding coefficient F_{IS} was calculated using FSTAT 2.9.3.2 software (Goudet 1995). Allele numbers, which ranged between three and 13 alleles per locus, indicated a high genetic diversity in the invasive population, which was mirrored in equally high values of observed (mean H_O = 0.573) and expected (mean H_E = 0.620) heterozygosities. Only two loci (CA03, CA30) showed a significant F_{IS} value, which may be due to non-random sampling or null alleles but probably not to inbreeding, since *M. aquifolium* is supposed to be an outbreeding species like the whole genus (Burd 1994). The test of linkage disequilibrium was performed using GENEPOP software, version 2 (Raymond & Rousset 1995). All 45 combinations of loci were tested, but only one combination of loci (GA31 and CA40) was significantly linked. In order to check the applicability of the microsatellite markers for the putative parent species of invasive *Mahonia* and the related genus *Berberis*, we tested the markers in *M. aquifolium*, *M. repens* and *M. pinnata* from North America (eight individuals from one population, each), and at nine individuals from five European populations of *Berberis vulgaris*. The results (Table 2) showed that the loci seem to be conserved

Table 2 Applicability of microsatellite loci from invasive *Mahonia* for related native *Mahonia* species and *Berberis vulgaris*

	Mahonia aquifolium (n = 8)			*Mahonia repens* (n = 8)			*Mahonia pinnata* (n = 8)			*Berberis vulgaris* (n = 9)		
Locus	No. of alleles	Null alleles	Size range (bp)	No. of alleles	Null alleles	Size range (bp)	No. of alleles	Null alleles	Size range (bp)	No. of alleles	Null alleles	Size range (bp)
Mahonia CA03	4	Yes	129–173	7	No	129–177	3	No	155–175	6	No	131–171
Mahonia CA18	8	No	164–200	9	No	168–226	3	No	184–210	—	Yes	—
Mahonia CA30	5	No	97–137	6	No	91–107	3	No	98–114	4	No	90–98
Mahonia CA40	6	Yes	113–163	4	Yes	121–139	4	No	121–143	—	Yes	—
Mahonia CA43	1	No	113	2	No	113–127	3	No	107–117	3	No	107–117
Mahonia GA04	7	No	108–126	9	No	106–122	2	No	96–110	4	Yes	88–100
Mahonia GA05	1	No	141	1	No	141	2	No	133–145	3	No	139–143
Mahonia GA31	4	No	154–178	3	No	172–180	2	No	158–160	1	Yes	138
Mahonia GA33	4	No	217–225	2	No	217–225	3	No	209–231	3	Yes	217–225
Mahonia GA36	1	No	176	4	No	176–186	2	No	188–200	—	Yes	—

within *Mahonia* and partly also in *Berberis*. However, homozygous null alleles were found in two, one and three loci, respectively, in *M. aquifolium*, *M. repens* and *B. vulgaris*. Three markers (CA18, CA40, GA36) did not reveal any fragments in *B. vulgaris*.

We will use the microsatellite markers to analyse the genetic variability in invasive and native *Mahonia* populations and to look for a hybrid origin of invasive populations.

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