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ORIGINAL ARTICLE

Genetic relationships among three native North-American *Mahonia* species, invasive *Mahonia* populations from Europe, and commercial cultivars

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Abstract Horticulture is one of the most important pathways for plant invasion. We used microsatellite markers to reveal the impact of plant breeding on *Mahonia aquifolium*, an invasive ornamental shrub. Since it was bred by hybridization with the related species *M. repens* and *M. pinnata*, we compared populations of the three native species, various commercial cultivars and invasive populations. Invasive populations and cultivars were genetically differentiated from the native groups, but differences did not result from genetic bottlenecks. In cultivars but not in invasive populations, we proved genes from *M. pinnata*. No significant amount of *M. repens* genes were found in cultivars and invasive populations, but this result has to be viewed with caution because of the close relationship between native *M. aquifolium* and *M. repens*. We conclude that the evolution of invasive *Mahonia* populations was a result of restriction of gene pool during introduction, secondary release, and artificial selection, in combination with an increase of genetic diversity by plant breeders and by extensive gene flow.

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A major reason for changes of our floras is the spread of exotic plant species that were introduced intentionally as horticultural and agricultural plants (Preston et al. 2002; Mack 2000). In Germany cultivated plants make up 50% of all neophytes and 70% of those neophytes, which are established in natural habitats (Klotz et al. 2002). Two factors may particularly contribute to the success of cultivated plants in the invasion of new habitats. The first one is a mass effect of cultivated plants that are planted in very large numbers at various locations and are often protected by man from detrimental environmental effects. This causes a high propagule pressure at numerous sites and a high probability of invasion (Kowarik 2005; Mack 2000). Propagule pressure has generally been shown to be one of the few factors that can be identified to determine invasion success (Rejmanek 2000). Second, cultivation of plants does usually include evolutionary changes. Evolutionary changes are suggested to play a major role in plant invasion (Ellstrand and Schierenbeck 2000). Indeed, it has been repeatedly shown that invasive populations differ genetically from their ancestral populations in natural habitats (reviewed in Bossdorf et al. 2005).

Genetic differences between invasive and native populations may result from genetic bottlenecks after introduction (Barrett and Richardson 1986). This may result in reduced genetic diversity in the founder populations and a lower probability of persistence of the new invader (Allendorf and Lundquist 2003). In contrast to unintentionally introduced plant species, cultivated plants

are not introduced randomly but selective. Wild individuals are selected because of their preferred phenotype which may lead to a limited but above-average fit subsample of genotypes being introduced in a new area. In cultivation, plants are intensively selected and modified by man resulting in further changes of their genetic makeup. Artificial selection for fitness-related traits such as flower size, seed number or cold tolerance, may enhance species success not only in gardens but also in natural habitats (e.g. Kitajima et al. 2006). In addition, a common method in plant breeding is interspecific hybridization, which results in an increased genetic variability and novel genotypes that are potentially better adapted to the new environment (Ellstrand and Schierenbeck 2000). Thus, invasions may be facilitated by hybridization because of a few well adapted genotypes and/or because hybrid populations overcome genetic bottlenecks and are thus able to respond to changing environmental conditions. Several studies have shown that hybrids, representing new genetic entities, may colonize territories were the parent species do not occur (e.g. Milne and Abbott 2000; Neuffer et al. 1999; Hollingsworth et al. 1998).

Although cultivated plants are above average successful in invasion, only a small proportion of all cultivated species is likely to spread (Kowarik 2005). A proscription of all cultivated plants would be needless and inappropriate. However, this small number of invasive species may cause a ecological and economic impact (Kowarik 2005) and it is important to understand how cultivation of ornamentals facilitates plant invasion. Studies of the evolution of invasive species should contribute to our understanding of invasiveness (Ellstrand and Schierenbeck 2000). However, the role of plant breeding in invasion success has rarely been studied (but see Kitajima et al. 2006), although some of the most serious invaders are ornamentals. The fast spread of *Impatiens glandulifera* is likely to result from dispersal of garden plants by man (Perrins et al. 1993) and *Rhododendron ponticum* was hybridized by breeders with cold tolerant related species and that may have facilitated its invasion in Great Britain (Milne and Abbott 2000).

In our study we investigated the woody plant, *Mahonia aquifolium* Pursh. (Nutt.) (Berberidaceae). It was introduced from North America to Europe as an ornamental because of its evergreen leaves, yellow flowers and blue berries and is one of the most successful alien shrubs in central and eastern Germany today (Kowarik 1992). In cultivation, the related North American species, *M. repens* and *M. pinnata*, were hybridized with *M. aquifolium*. Different cultivars with various characteristics in flowering, clonal growth and resistance against parasites arose (Houtman et al. 2004) and were frequently planted in gardens, parks and along roads. *M. aquifolium* produces many fleshy fruits which are eaten by birds that disperse seeds also into adjacent habitats. Today, the species is spreading and invades anthropogenic and natural habitats (Kowarik 1992), and propagates not only by seeds but also by stolons and stem layering (Auge and Brandl 1997). This is less known from native *M. aquifolium* but commonly from *M. repens* (Ahrendt 1961) and also found in some cultivars (Houtman et al. 2004). It is assumed that invasive populations descend from cultivars, which are supposed to be hybrids (van de Laar 1975; Ahrendt 1961). Therefore it is likely that invasive populations consist of hybrids, although the invasive shrubs are referred to as *M. aquifolium*. Thus, *Mahonia* is a well-suited case study for investigating the role of plant breeding for invasion success.

In this study we explore whether invasion success of *Mahonia* populations is a result of evolution by plant breeding. We investigated the following questions. (1) Was there a genetic bottleneck after introduction of *Mahonia* to Europe? (2) Is there genetic differentiation of invasive *Mahonia* populations from the native species, and how arrange cultivated individuals to native species and invasives? (3) Do the invasive populations consist of hybrids between the three *Mahonia* species?

Materials and methods

Species

The genus *Mahonia* Nutt. (Berberidaceae) comprises fleshy-fruited evergreen shrubs with pinnate leaves. The genus is treated distinct from the genus *Berberis* (Ahrendt 1961), but inclusion into *Berberis* is also common (Kim et al. 2004; Laferriere 1997). Oregon Grape, *Mahonia aquifolium* (Pursh) Nutt., is native to western North America (Fig. 1), has a simple and erect stem that reaches 1.80 m in height with leaves that are shiny above and dull underneath (Ahrendt 1961; Piper 1922). *M. aquifolium* was introduced into Europe for ornamental purposes in 1822 (Hayne 1822, cited in Kowarik 1992) and repeatedly later on. The first spontaneous occurrence outside gardens was observed in 1860 after a time lag of 38 years (Kowarik 1992). The species was extensively hybridized by plant breeders with related, North American species, in particular with *M. repens* (Lindl.) G.Don (Ahrendt 1961) and *Mahonia pinnata* (Lag.) Fedde., as indicated by many cultivated hybrids (van de Laar 1975). *M. repens* is morphologically very similar to *M. aquifolium* and some specimens are difficult to assign to one of the two species (Ahrendt 1961). *M. repens* reaches only 90 cm in height and grows usually more stoloniferous than *M. aquifolium* (Ahrendt 1961). The leaves are mostly dull above (Piper

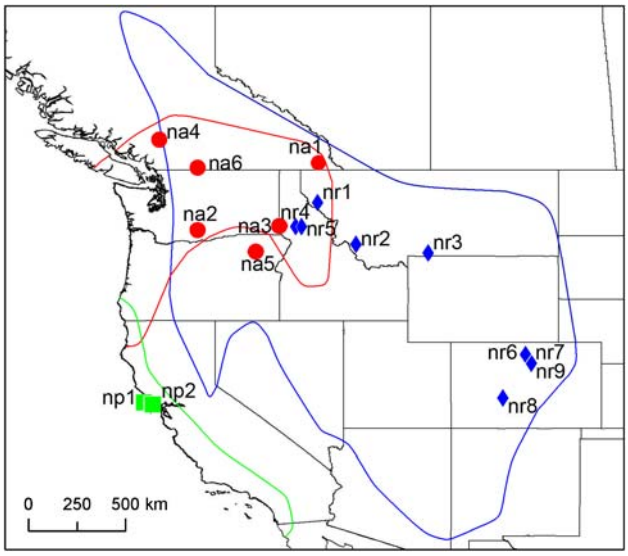

Fig. 1 Map of the distribution areas of the three native species after Whittemore (1997) and the locations were populations were sampled. red • *M. aquifolium*, blue ◆ *M. repens*, green ■ *M. pinnata*

1922). *M. pinnata* reaches 3 m height (Ahrendt 1961) and has shiny leaves above and underneath. In contrast to the other two species the first leaflets of the pinnate leaf arise near base of petiole (Munz 1959). The breeding and hybridization of the three *Mahonia* species resulted in many cultivars (van de Laar 1975) of the three species and their hybrids (*M. x decumbens* = *M. aquifolium* x *M. repens*; *M. x wagneri* = *M. aquifolium* x *M. pinnata*).

Sampling and genetic analyses

We analyzed *Mahonia* individuals of five taxa including three native species, invasive individuals and cultivars (Table 1 and Table 2). In the native range in North America, individuals of *M. aquifolium*, *M. repens* and *M. pinnata* were sampled in six ($n = 65$ samples), nine ($n = 119$) and two ($n = 34$) populations, respectively (Fig. 1). Invasive *Mahonia* were sampled from 23 invasive populations ($n = 416$ individuals) in Germany and the Czech Republic. Within populations individuals were sampled randomly and attention was paid to sample spatially separated plant individuals. However, in some dense populations, individuals were not clearly separated. Furthermore we sampled 127 individuals that belonged to 39 different cultivars from Botanical Gardens and commercial nurseries (Table 2). We either sampled leaves directly in the field which were dried and stored in silica gel (20 populations and cultivars) or we sampled seeds (20 populations). In the latter case seedlings were grown from cold stratified seeds in a climate chamber with a 14 h/10 h day/night cycle at 15/10°C. When the seedlings had secondary leaves we harvested and stored them at −80°C. From each mother plant only one seedling was analyzed. DNA was extracted from dried or frozen leaves with the Plant DNA extraction mini kit (QIAGEN). A total of 761 individuals were genotyped at eight microsatellite loci (CA03, CA18, CA40, CA43, GA05, GA31, GA33, GA36) as described previously (Roß and Durka 2006).

Data analysis

We measured a number of genetic parameters to compare genetic diversity between invasive and native populations. We analyzed the number of alleles and observed and expected heterozygosity using MSA 3.0 (Dieringer and Schlötterer 2002). Allelic richness (A_r), a measure of allelic diversity corrected for sample size, was calculated with FSTAT 2.9.3.2. software (Goudet 1995). Departure from Hardy-Weinberg-equilibrium was also tested with FSTAT. Samples with identical multilocus genotypes were regarded as clones. Genetic parameters at population level were calculated using each multilocus genotype once. Differences of genetic parameters between invasive and native populations and between the species nested within status (invasive or native) were calculated by a nested ANOVA using the GLM procedure in SAS 9.1. (SAS Institute, Cary, NC, USA).

We calculated the number of private alleles at species level. Private alleles were defined as alleles present in more than one population of a species and in no other species. Overall genetic differentiation among populations was assessed with *F*-statistics (Weir and Cockerham 1984; Wright 1951) using FSTAT. Genetic differentiation between populations within species was estimated as the pair wise F_{ST}-value and compared between the taxa using FSTAT with 1,000 permutations. We excluded *M. pinnata* populations from this analysis due to low sample size. We tested for isolation by distance for the three native species with a Mantel test with 2,000 randomizations using FSTAT. We also assessed genetic differentiation among species and invasives by a hierarchical analysis of molecular variance (AMOVA) with Arlequin 2.0 (Schneider et al. 2000) with populations nested in species.

We used a principle components analysis (PCA) and a model-based clustering method to analyze the relationship among native taxa, invasives and cultivars. PCA was carried out using PCAGEN 1.2 (Goudet 1999). Furthermore, we clustered all individuals using STRUCTURE 2.0 (Pritchard et al. 2000). This software uses a model-based Bayesian procedure to assign individuals into *K* clusters based on their multilocus genotypes. To identify the most probable number of clusters the algorithm was run with values of *K* from 1 to 14 ten times each. We used the admixture model with a length of burnin period of 10,000 and 10,000 iterations and the prior information about the

Table 1 Sampled populations of invasive *Mahonia* and native *M. aquifolium, M. repens* and *M. pinnata*

Code	Origin	Species	Site	Location	*n*
i1	Invasive		Germany; Barby	51.6N; 11.6E	20
i2	Invasive		Germany; Bocka-Neustaedtel	51.1N; 14.1E	22
i3	Invasive		Germany; Berlin	52.3N; 13.2E	19
i4	Invasive		Germany; Buckow	52.3N; 14.1E	20
i5	Invasive		Germany; Drebkau	51.4N; 14.1E	20
i6	Invasive		Germany; Drebkau1	51.4N; 14.1E	8
i7	Invasive		Germany; Duebener Heide	51.4N; 12.3E	20
i8	Invasive		Germany; Duisburg	51.2N; 06.5E	13
i9	Invasive		Germany; Herzfelde	52.3N; 13.5E	10
i10	Invasive		Germany; Hitzhausen	52.2N; 08.2E	9
i11	Invasive		Germany; Hornburg	52.0N; 10.4E	16
i12	Invasive		Germany; Jena	50.6N; 11.4E	20
i13	Invasive		Germany; Kirchbrak	51.6N; 09.4E	19
i14	Invasive		Germany; Halle-Lieskau	51.3N; 11.6E	10
i15	Invasive		Germany; Liepe	52.5N; 13.6E	22
i16	Invasive		Germany; Linz am Rhein	50.3N; 07.2E	28
i17	Invasive		Germany; Lueneburg	53.1N; 10.2E	22
i18	Invasive		Germany; Mannheim	49.3N; 08.3E	22
i19	Invasive		Germany; Neuhaus a.d. Pegnitz	49.4N; 11.3E	26
i20	Invasive		Czech Republik; Prag	50.7N; 14.3E	9
i21	Invasive		Germany; Rothenburg	51.4N; 11.5E	17
i22	Invasive		Germany; Suckow	53.3N; 12.2E	24
i23	Invasive		Germany; Zierenberg	51.2N; 09.2E	20
na1	Native	*M. aquifolium*	British Columbia; Tie Lake	49.3N; 115.2W	18
na2	Native	*M. aquifolium*	Washington; Cle Elum	46.2N; 120.8W	15
na3	Native	*M. aquifolium*	Idaho; Harvard	46.4N; 117.0W	7
na4	Native	*M. aquifolium*	Oregon; Viento	50.4N; 122.6W	9
na5	Native	*M. aquifolium*	Oregon; LaGrande	45.2N; 118.1W	6
na6	Native	*M. aquifolium*	British Columbia; Manning Park	49.1N; 120.8W	10
np1	Native	*M. pinnata*	California; Bodega Bay	38.2N; 123.3W	13
np2	Native	*M. pinnata*	California; Tomales Bay	38.1N; 122.9W	21
nr1	Native	*M. repens*	Montana; Bear Lake	47.6N; 115.3W	7
nr2	Native	*M. repens*	Montana; Blackfoot River	45.6N; 113.4W	15
nr3	Native	*M. repens*	Montana; Boulder River	45.2N; 110.1W	14
nr4	Native	*M. repens*	Idaho; Deary	46.5N; 116.3W	10
nr5	Native	*M. repens*	Idaho; Deer Road	46.5N; 116.0W	9
nr6	Native	*M. repens*	Colorado; Poudre Canyon	40.4N; 105.5W	21
nr7	Native	*M. repens*	Colorado; Big South Trailhead	40.4N; 105.5W	19
nr8	Native	*M. repens*	Colorado; Crested Butte Mountain	38.5N; 106.5W	14
nr9	Native	*M. repens*	Colorado; Middle Saint Vrain Valley	40.1N; 105.3W	10

The first letter of the site code identifies the origin (*i* invasive; *n* native), the second letter indicates the species, a = *M. aquifolium*, p = *M. pinnata* and r = *M. repens*

populations. The posterior probabilities of K, $L(K)$ and ΔK calculated according to Evanno et al. (2005) were used as indicators of the most probable K value. The whole data set including natives, invasive populations and cultivars was analyzed and visualized using the DISTRUCT program (Rosenberg 2004).

Results

Genetic variation

At eight microsatellite loci we detected 187 different alleles in a total of 761 individuals. The number of alleles per locus

Table 2 Cultivars included in the study. Selection year was taken from Houtman et al. (2004). Information about selection of c19–c21 was given by breeder himself

Code	Species	Cultivar	Year of selection	*n*
c1	*M. aquifolium*			11
c2	*M. aquifolium*	Apollo	1973	13
c3	*M. aquifolium*	Atropurpurea	1915	11
c4	*M. aquifolium*	Darthil®	2000	1
c5	*M. aquifolium*	Dart's Distinction	1970	1
c6	*M. aquifolium*	Dart's Quickstep	1987	1
c7	*M. aquifolium*	Euro	1996	1
c8	*M. aquifolium*	Golden Pride	Unknown	1
c9	*M. aquifolium*	Green Ripple	1970	3
c10	*M. aquifolium*	Hastings Elegant	Unknown	3
c11	*M. aquifolium*	Hans-Karl Möhring	1984	4
c12	*M. aquifolium*	Juglandifolium	Unknown	2
c13	*M. aquifolium*	Jupiter	1978	3
c14	*M. aquifolium*	Maqu	1970	5
c15	*M. aquifolium*	Marijke	1993	1
c16	*M. aquifolium*	Mirena	1979	10
c17	*M. aquifolium*	Orange flame	1965	2
c18	*M. aquifolium*	Smaragd	1978	9
c19	*M. aquifolium*	Typ1	1999	3
c20	*M. aquifolium*	Typ2	1999	3
c21	*M. aquifolium*	Typ3	1999	3
c22	*M. aquifolium*	Undulata	1930	7
c23	*M. aquifolium*	Versicolor	Unknown	3
c24	*M.* × *decumbens*	Bokrafood®	2001	1
c25	*M.* × *decumbens*	Bokrahawk®	Unknown	1
c26	*M.* × *decumbens*	Bokrarond®	2005	1
c27	*M.* × *decumbens*	Bokrasio®	2003	2
c28	*M.* × *decumbens*	Cosmo crawl	1992	1
c29	*M.* × *decumbens*	Nr17	Unknown	1
c30	*M.* × *decumbens*	Pixie	1994	1
c31	*M.* × *hybrida*	Hybrida	Cultural bastard	2
c32	*M. pinnata*	Ken Howard	Unknown	3
c33	*M. repens*		Unknown	4
c34	*M.* × *wagneri*	Darts flashlight	1993	1
c35	*M.* × *wagneri*	Fireflame	1965	1
c36	*M.* × *wagneri*	Moseri	1895	1
c37	*M.* × *wagneri*	Pinnacle	1930	4
c38	*M.* × *wagneri*	Sunset	1998	4
c39	*M.* × *wagneri*	Vicaryi	1931	1

ranged between ten (GA05) and 37 (CA03 and CA18). At species level we found 131 alleles in *M. aquifolium,* 144 alleles in *M. repens* and 69 alleles in *M. pinnata*. In European samples a smaller number of alleles was found with 101 alleles in invasives and 106 alleles in cultivars. The frequency of species specific alleles was low with 6 (5.1%) private alleles in *M. aquifolium* and 16 (11.6%) private alleles in *M. repens*, but no allele was specific to *M. pinnata*. However, several alleles were common in one species and rare in the others, or species were characterized by the absence of an otherwise common allele. In seven out of 40 populations (three invasive, two *M. repens* populations and two *M. pinnata* populations) several samples shared the same multilocus genotype indicating clonal propagation. Populations were highly diverse with mean $H_e = 0.60 \pm 0.06$ and 0.65 ± 0.02 and mean H_o 0.48 ± 0.04 and 0.57 ± 0.02 (means ± SE) in the native and invasive populations, respectively (Table 3). Most F_{IS}-values were significant, with mean $F_{IS} = 0.17$ and 0.12 in native and invasive taxa, indicating slight departure from Hardy–Weinberg expectations, which may be due to null-alleles (Roß and Durka 2006) but not due to inbreeding, because self pollination in *Mahonia* does rarely result in fruit production (Monzingo 1987, H. Auge unpublished data). Native and invasive taxa did not differ significantly in number of alleles per locus, allelic richness, expected and observed heterozygosity and inbreeding coefficient. Furthermore, there were no significant differences between the native species in these traits (ANOVA, *A*: $P = 0.374$, Ar: $P = 0.059$, F_{IS}: $P = 0.725$) except in H_e ($P = 0.037$) and H_o ($P = 0.029$) with lowest values in *M. pinnata* and highest values in *M. aquifolium* (Table 3).

As expected from the large proportion of shared alleles, the species where significantly but only weakly differentiated with 10.3% of genetic variation residing among species (AMOVA: $\Phi_{CT} = 0.103$) and 12.3% of variation residing among populations within species ($\Phi_{SC} = 0.137$) (Table 4). Populations were weakly but significantly structured with overall F_{ST} values of 0.074 ± 0.006 for invasive populations and 0.093 ± 0.015 for native *M. aquifolium*. *M. repens* had an overall F_{ST} value of 0.329 ± 0.058 and, thus, was significantly ($P = 0.007$) more structured than the other taxa. There was no isolation by distance in invasive *Mahonia* ($P = 0.328$) and in *M. aquifolium* ($P = 0.666$), whereas *M. repens* showed a strong correlation of genetic and geographic distance ($r^2 = 0.287$, $P = 0.002$).

Relationship of native species, cultivars and invasives

The analyzed taxa were not clearly separated by PCA (Fig. 2). While native *M. aquifolium* was separated from native *M. pinnata* along the first axis (score mean ± standard deviation: *M. aquifolium* -0.10 ± 0.08; *M. pinnata* 1.03 ± 0.04), *M. repens* widely scattered along the first (0.80 ± 0.57) and second axis (0.35 ± 0.81). *M. repens* was separated into two groups along the second axis (0.99 ± 0.34 and -0.44 ± 0.28, respectively). These two groups of *M. repens* correspond to two areas that were sampled in the south and north of the species range

Table 3 Genetic diversity at eight microsatellite loci of invasive and native *Mahonia* populations

population	*N*	N_{GT}	H_o	H_e	*A*	A_r	F_{is}
Invasive							
i1	20	20	0.59	0.67	5.3	3.8	0.119**
i2	22	16	0.63	0.52	4.0	2.8	−0.214
i3	19	19	0.55	0.67	5.4	3.7	0.183***
i4	20	20	0.58	0.62	5.5	3.7	0.063
i5	20	20	0.50	0.64	4.8	3.5	0.210***
i6	8	8	0.53	0.60	3.9	3.4	0.158*
i7	20	20	0.52	0.64	5.0	3.5	0.195***
i8	13	13	0.56	0.67	6.0	4.0	0.171**
i9	10	10	0.50	0.62	4.1	3.3	0.191**
i10	9	9	0.50	0.64	4.4	3.7	0.213**
i11	16	16	0.65	0.72	5.9	4.1	0.092*
i12	20	20	0.61	0.65	5.8	3.9	0.061
i13	19	19	0.64	0.68	5.6	4.1	0.066
i14	10	10	0.55	0.68	5.5	4.2	0.198**
i15	22	22	0.64	0.70	6.4	4.1	0.087*
i16	28	28	0.52	0.66	6.8	4.0	0.218***
i17	22	20	0.69	0.71	6.3	4.1	0.031
i18	22	22	0.59	0.72	7.4	4.4	0.176***
i19	26	26	0.62	0.65	5.9	3.7	0.053
i20	9	9	0.57	0.66	4.9	4.0	0.137*
i21	17	17	0.53	0.64	4.6	3.5	0.182***
i22	24	14	0.54	0.54	4.1	3.1	0.011
i23	20	20	0.58	0.67	6.0	4.0	0.130**
Mean invasive			**0.57**	**0.65**	**5.4**	**3.8**	**0.119**
Native							
M. aquifolium							
na1	18	18	0.39	0.73	7.6	4.7	0.362***
na2	15	15	0.52	0.75	7.1	4.7	0.234***
na3	7	7	0.61	0.58	4.5	3.9	−0.074
na4	6	6	0.48	0.77	5.0	4.7	0.380***
na5	9	9	0.56	0.71	5.8	4.6	0.173**
na6	10	10	0.55	0.67	4.4	3.6	0.100
Mean			*0.56*	*0.70*	*5.7*	*4.4*	*0.200*
M. pinnata							
np1	13	7	0.49	0.39	2.6	2.3	0.200*
np2	21	16	0.53	0.66	6.5	4.2	0.195***
Mean			*0.42*	*0.52*	*4.6*	*3.2*	*0.198*
M. repens							
nr1	7	7	0.47	0.76	4.8	4.3	0.308***
nr2	15	15	0.40	0.50	5.5	3.6	0.103*
nr3	14	14	0.43	0.66	5.5	3.9	0.306***
nr4	10	10	0.65	0.78	6.6	4.9	0.214***
nr5	9	9	0.35	0.74	6.3	4.9	0.282***
nr6	21	4	0.48	0.30	1.9	1.7	−0.697
nr7	19	19	0.45	0.62	7.0	4.1	0.253***
nr8	14	6	0.38	0.29	2.8	2.0	0.081
nr9	10	10	0.38	0.54	3.5	3.0	0.281***
Mean			*0.47*	*0.58*	*4.9*	*3.6*	*0.126*
Mean native			**0.48**	**0.60**	**5.1**	**3.7**	**0.173**

Sample size (*N*), number of multilocus genotypes (N_{GT}) Observed heterozygosity (H_o), expected heterozygosity (H_e), number of alleles per locus (*A*), allelic richness (A_r) based on five individuals and inbreeding coefficient (F_{IS}, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Genetic parameters were calculated on multilocus genotypes instead of individuals. Mean values of native species and invasive populations are least square means calculated by ANOVA

(Fig. 1). Cultivars were highly diverse (first axis: 0.07 ± 0.39; second axis: 0.08 ± 0.08) with *M. aquifolium* located within their cluster. Invasive *Mahonia* populations arranged mostly among cultivars, next to native *M. aquifolium* (fist axis: -0.31 ± 0.13; second axis: -0.17 ± 0.21).

In the STRUCTURE analyses we found a similar pattern of grouping, even though the ΔK analysis revealed no definite number of groups. The log likelihood of *K* increased monotonously with increasing *K* from 1 to 14 (Fig. 3). ΔK showed a peak at $K = 2$. However, the separation in only two groups did not allow to address the affiliation of invasive populations and cultivated plants to native species. Therefore, we plotted the results for $K = 2$ to $K = 6$ and, thus, zoomed into the genetic relationship of the analyzed individuals from coarse ($K = 2$) to fine structure ($K = 6$) (Fig. 4).

At $K = 2$, the coarse structure revealed two clusters which were built by native *M. repens* and *M. pinnata* on the one hand and invasive populations on the other hand. This separation was stronger than the separation of the native species. Native *M. aquifolium* showed admixture of both gene pools. Some cultivars clustered to *M. repens* and *M. pinnata*, but most cultivars clustered to the group of invasive *Mahonia* individuals. Within the group of native

Table 4 Hierarchical analysis of molecular variance (AMOVA) for 600 individuals of three native species and invasive *Mahonia* populations. Variance components and explained variation between taxa, among populations within taxa and within populations

Source of variation	*df*	Sum of squares	Variance components	Percentage of variation
Among taxa	3	252.1	0.317	10.28
Among populations within taxa	36	516.1	0.378	12.26
Within populations	1,228	2933.3	2.389	77.46
Total	1,267	3701.4	3.084	

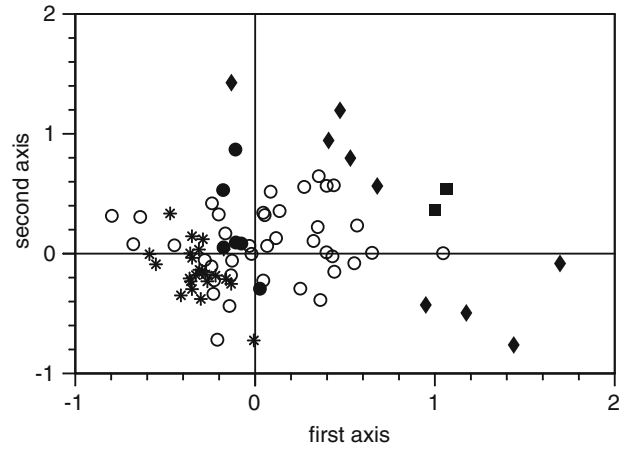

Fig. 2 Principle components analysis (PCA) of allele frequencies at eight microsatellite loci. *filled circle M. aquifolium*, *filled diamond M. repens*, *filled square M. pinnata*, *open circle* cultivars, *Asterisk* invasive populations. The first and second axis explained 19.26 and 12.37% of total variation, respectively. The analyzed taxa were not clearly separated. Nevertheless, each native species grouped apart, *M. repens* was split in two groups. Cultivars were widely scattered with invasive *Mahonia* populations arranged mostly in between cultivars

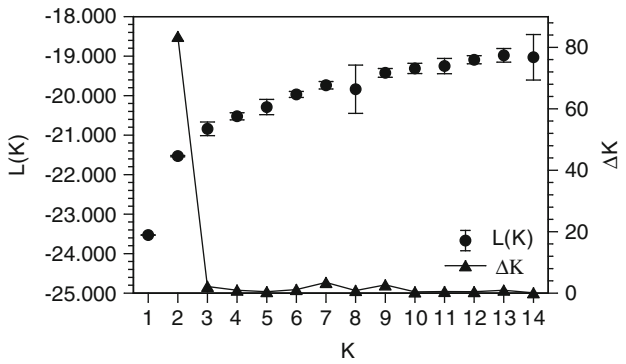

Fig. 3 Graphical method to identify the true K (Evanno et al. 2005) from STRUCTURE analyses. Mean $L(K)$ (±SD), the posterior probability of the data for a given K over ten runs of each K (*left axis*), and ΔK, the standardized second order rate of change of $L(K)$ (*right axis*). The log likelihood increased monotonously with increasing K. ΔK showed one peak at $K = 2$

taxa, at all K-values > 2, four *M. repens* populations formed a consistent cluster. This strong splitting of native *M. repens* mirrored the geographical separation of the southern populations, as indicated by PCA analysis, before. The assignment of the *M. pinnata* populations was ambiguous. They clustered either with southern *M. repens* ($K = 3$) or with northern *M. repens* ($K = 4$) or formed a gene pool of their own ($K = 5$). At each K, native *M. aquifolium* did not represent an homogenous gene pool but showed admixture of northern *M. repens*, invasives' and cultivars' gene pools. The *M. aquifolium* population na1 showed stronger admixture of northern *M. repens* than other *M. aquifolium* populations. Most cultivars shared the group with invasive individuals. However, native *M. pinnata* gene pool was also found in cultivated individuals, namely in the *M. pinnata* cultivar (c32), in *M. pinnata* hybrids (c34–c39) as well as in *M. aquifolium* cultivars (c2, c3, c5–c8, c15–c17) and in *M. repens* hybrids (c24–c30). This result indicated hybridization of *M. aquifolium* with *M. pinnata* and the presence of *M. pinnata* gene pool in hybrid cultivars and *M. aquifolium* cultivars. *M. pinnata* gene pool was not detected in invasive populations. Furthermore, we found only small proportions of northern *M. repens* in cultivars and hardly any in invasive populations. Southern *M. repens* gene pool was neither detected in cultivars nor in invasive populations. Some cultivars assigned to a group that would not be expected by there species identification.

Discussion

The main results of our study were: (1) There is no evidence for a genetic bottleneck at population level after introduction of *Mahonia* to Europe. (2) The native *Mahonia* species have largely overlapping gene pools and were significantly but weakly differentiated. The majority of cultivars and the invasive populations formed a gene pool different from the native species. (3) Hybridization of *M. aquifolium* and *M. pinnata* was displayed in cultivars but not in invasive populations. Hybridization of *M. aquifolium* and *M. repens* could neither be proved in cultivars nor in invasive populations.

Genetic diversity in invasive populations

The differences between invasive and native *Mahonia* populations were not a result of a genetic bottleneck, because the genetic diversity was not significantly reduced in invasive populations. However, at the species level more alleles were found in natives than in cultivars and invasives. Thus, our results confirm the results of Bossdorf et al. (2005) that most invasions of plant species are not associated with an overall genetic bottleneck. Many invasions come about by multiple introductions that prevent a genetic bottleneck (Durka et al. 2005; Maron et al. 2004; Neuffer et al. 1999). In addition, inter- and intraspecific hybridization can enhance genetic variation in invasive populations (Ellstrand and Schierenbeck 2000). We suppose that multiple introduction as well as hybridization of *M. aquifolium*, *M. repens* and *M. pinnata* affected the genetic makeup of invasive *Mahonia* populations.

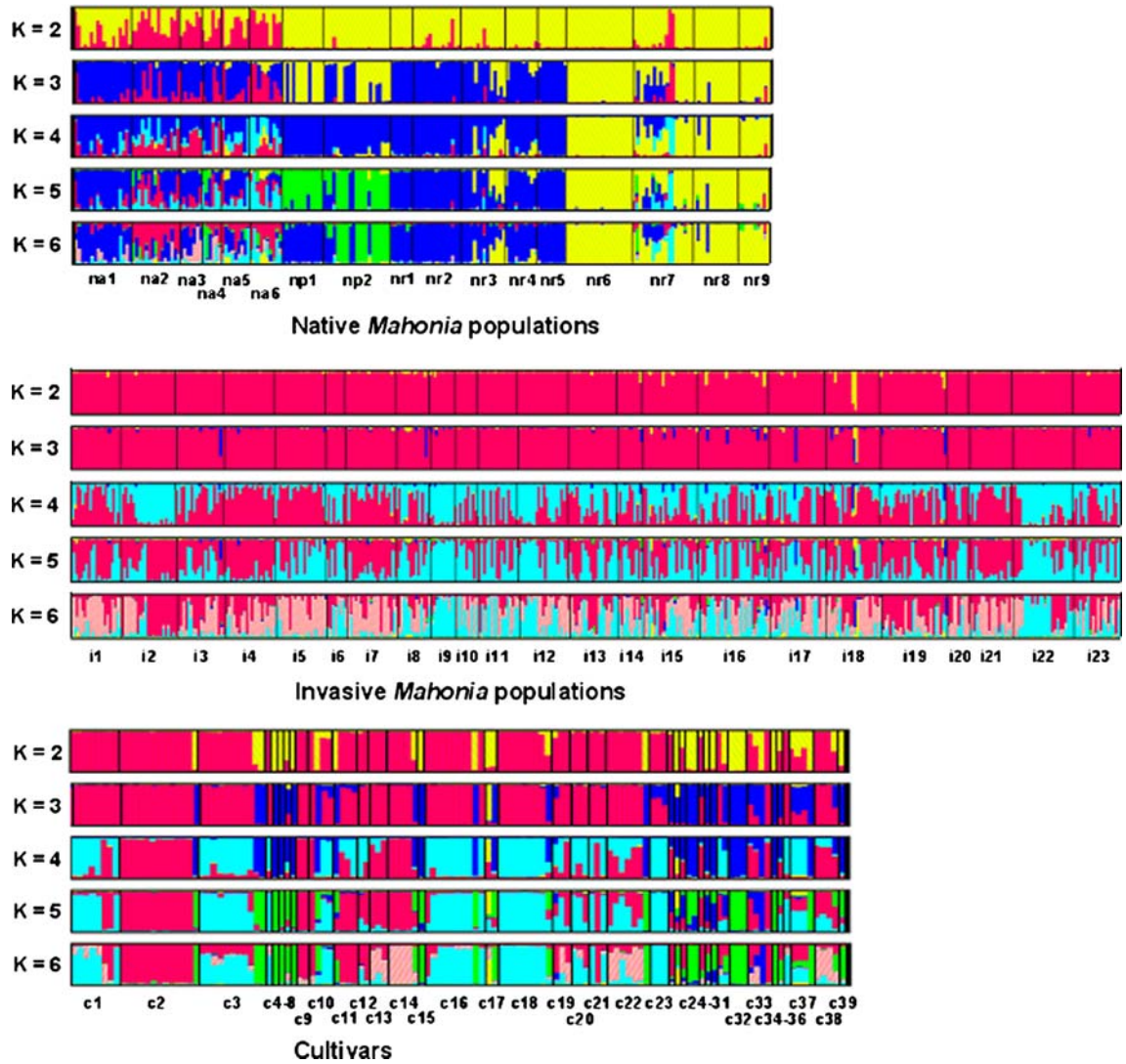

Fig. 4 Estimated membership probability for 761 *Mahonia* genotypes for K genetic clusters identified by STRUCTURE analyses. Individuals are shown by *vertical bars* representing the proportional contribution of the K clusters to their genotype. Populations are separated by *black lines*. All individuals were introduced in all analyses. The *three blocks* show native populations, invasive populations and cultivars, respectively. The *five graphs* show five representative runs for $K = 2$ to $K = 6$

Relationships among native species

We presented evidence that North American native *Mahonia* species are differentiated at microsatellite loci (Figs. 2, 4). *M. aquifolium* and *M. repens* were separated by private alleles and *M. pinnata* by the lack of certain alleles and by allele frequencies. The weak characterization of *M. pinnata* by private alleles is likely owing to the low number of investigated populations. In the STRUCTURE analysis no definite K could be detected, indicating subtle continuous structure rather than distinct gene pools. The strongest division found was that between the northern and southern subranges of *M. repens* (Fig. 4) which also corresponds to the high F_{ST}-value among *M. repens* populations compared to *M. aquifolium*. Whether there is indeed a clear cut geographical separation of distinct gene pools within *M. repens* or rather a clinal pattern, as indicated by the isolation by distance relationship, remains an open question. However, these findings are consistent with pronounced morphological variability among *M. repens* from different parts of the native areas (Houtman et al. 2004). To a great extent, *M. aquifolium* shared the group of northern *M. repens,* indicating the close relationship of these two taxa. Furthermore, our analyses indicate gene flow between the two taxa in the area of range-overlap. *M. aquifolium* and *M. repens* had been considered to be conspecific but later were accepted as two species (Piper 1906). Although both species apparently possess different morphological traits in habit, leaf color

and branching, they are sometimes difficult to distinguish and no single character can unambiguously identify either species (Piper 1922). *M. aquifolium* and *M. repens* hybridize not only in culture but also in nature (Houtman et al. 2004). This is confirmed by the admixture of the *M. repens* group in *M. aquifolium* in the STRUCTURE analysis. In particular the *M. aquifolium* population "na1" was clustered mostly with northern *M. repens*, which is based on shared alleles of this population to both groups (data not shown). In fact, the two species have overlapping ranges (Fig. 1) and all populations included in our study originated from the sympatric range where the gene pools were not well separated. This may be either due to current gene flow between the species, but may also indicate an intermediate state of ongoing speciation within the *M. aquifolium/M. repens* group. The close relationship of *M. aquifolium* and *M. repens* complicated the analysis of the relationships between invasives, cultivars and native species. In particular the role of hybridization in invasive *Mahonia* could hardly be detected because native *M. aquifolium* did not represent a well characterized uniform group. Rather, it was found to have an intermediate position between the *M. repens/M. pinnata* group and the group of invasive *Mahonia/* cultivars which were identified in the best supported STRUCTURE analysis ($K = 2$).

Cultivars as likely sources of invasive populations

We analyzed a large number of cultivars, many of which were not assigned to the native species they were labeled by breeders (Fig. 4 and Table 2). This may be due to recent gene flow. Thus, even the "pure" cultivated plants may descend from former hybridization events, either in the native area or in cultivation. In cultivars the history and identity of introduced and bred individuals is often not traceable. There are several examples in which specimens were named erroneously (Piper 1922) or in which hybrid cultivars were named after one maternal species (Houtman et al. 2004). Ahrendt (1961) noticed that plants designated as *M. aquifolium* vary in morphology and consist largely of hybrids. Cultivated *M. aquifolium* produce stolons (Günther 1979) which actually is typical for native *M. repens* (Ahrendt 1961; Piper 1922), and indicates a hybrid origin of cultivated *M. aquifolium* plants. However, we could only detect a small proportion of the *M. repens* gene pool in some cultivars and hardly any in invasive populations.

We showed that hybridization of *M. pinnata* and *M. aquifolium* seems to play a larger role for the cultivars than the hybridization of *M. repens* and *M. aquifolium*. In view of the selection times of different cultivars, hybridization with *M. pinnata* started earlier than hybridization with *M. repens.* Further on, some of the old cultivars served as basis to breeding of other cultivars. *M. x wagneri* 'Moseri', for instance, served as basis for selection of *M. x wagneri* 'Sunset' (Houtman et al. 2004). Thus, genetic traits of older *M. pinnata* cultivars could infiltrate the population of cultivars. The age of a cultivar may play a role for the distribution across tree nurseries and garden centers and is therefore important for secondary release. High presence of alien plant species in trade and in consequence high planting rates enhance the likelihood of establishment in nature by enhanced propagule pressure (Kowarik 2005). In British nurseries more established alien plants are offered than casual alien species (Dehnen-Schmutz et al. 2007). Although the *M. pinnata* gene pool in cultivars should be old, we could not prove genes from *M. pinnata* in invasive populations, indicating that not all cultivars are similarly invasive. We could not prove significant *M. repens* proportions in invasive individuals either. Nevertheless, invasive *Mahonia* are different to native *M. aquifolium*.

According to our results, we assume that the differentiation of invasive *Mahonia* and native *M. aquifolium* is a result of different stages in the invasion process where restriction of gene pools, genetic drift, artificial selection and hybridization had interacted. Thus, although multiple introductions are generally common (Bossdorf et al. 2005) and play a role also in the introduction of *Mahonia,* it is likely that genetic variability was actually reduced due to the selective import. Breeding and hybridization could have enhanced genetic variability again (Ellstrand and Schierenbeck 2000). Simultaneously, genetic makeup was likely changed directed by artificial selection, which may have caused a bias in invasive founder populations (Kitajima et al. 2006). Thus, we assume that plant breeding facilitated invasion success of *Mahonia* by enhancing genetic variability and by generating characteristics that enhance invasiveness of certain cultivars.

Hybridization may result in polyploid genotypes and fixed heterosis, that may fosters plant invasion (Ellstrand and Schierenbeck 2000). However, we did not find any evidence for polyploidy in invasive *Mahonia*, as only mono- or bi-allelic microsatellite genotypes were detected at all loci over all populations. Nevertheless, in other species polyploidy after hybridization may play a role for successful invasion, for instance in *Spartina anglica* (Gray 1986).

Plant breeding and evolution of invasive traits

Recently, some plant traits have been identified, which may contribute to the invasion success of certain species in particular environments, even though no characteristic could be found that answered the basic question for invasive characteristics satisfactorily (Pysek et al. 1995; Lodge 1993). One characteristic that is known to enhance plant invasion is high seed production (Rejmanek 1996). This

may play a role in invasive *Mahonia* populations, also. Many cultivars are praised for their large flowers, numerous fruits or the long residence time of berries at the sprout (Houtman et al. 2004). Soldaat and Auge (1998) suggested a horticultural effort for more flowers and fruits in *Mahonia*. Also other attributes, which plant breeders selected for in cultivated *Mahonia,* may be advantageous in natural habitats as well as in gardens. Invasive populations grow by vegetative below-ground stolons and stem layering (Auge and Brandl 1997), which is less known from *M. aquifolium* but from *M. repens* (Ahrendt 1961) and in some cultivars (Houtman et al. 2004). Thus, although we detected hardly any *M. repens* gene pool in invasive populations, clonal growth does obviously play an important role in these invasive populations. The cultivar *M. aquifolium* "Maqu" (c14) is characterized especially by cold resistance, and a large amount of berries that are retained during autumn (Houtman et al. 2004), thereby facilitating seed dispersal by birds. *M. aquifolium* "Maqu" is one old cultivar that shared a large proportion of genes with invasive populations and is possibly one of the successful invading cultivars.

These examples show that plant breeding may enhance invasion success by selecting for certain characteristics. Hence, there is more need to study characteristics of cultivated plant species and the relation of certain traits with invasiveness to identify general breeding efforts that go along with invasiveness. Breeders select especially for reproductive versatility, improvement in stress tolerance and pathogen resistance (Bundesverband Deutscher Pflanzenzüchter e.V. 2007), and broaden the phenotypic variation in particular by hybridization.

These attributes will enable horticultural species to invade natural habitats. There are hardly any studies that investigated characteristics of horticultural plants in relation to invasiveness. Solely, Kitajima et al. (2006) showed that invasive *Ardisia crenata* individuals that descended from cultivars produce a greater number of seeds compared to native individuals. Large inflorescences, large flowers and high fruit production increase the number of seeds and may enhance invasiveness (Rejmanek 1996). Large numbers of seeds increases propagule pressure, especially in species that are bird-dispersed like *M. aquifolium*. Moreover, the birds which feed on *M. aquifolium* are common (e.g. blackbirds) (Torrey and Gray 1838), widespread and use both natural and urban habitat. Furthermore they are comparatively large birds which may further enhance dispersal distance. However, beside special traits that are selected by breeding and may enhance invasiveness, plant breeding may enhance invasion success simply by a mass effect: horticultural non-indigenous species become very abundant in a vast array of locations. Thus, the propagule pressure to adjacent native vegetation is greatly increased (Okada et al. 2007). Also, the many locations in which the species are grown differ in ecological conditions, which will increase the probability to find suitable conditions. In general, after first introduction, non indigenous species have been shown to undergo a lag phase before becoming invasive (Kowarik 1995). This time lag is hypothesized to be related to microevolution and local adaptation (Richards et al. 2006). Plant breeding and horticultural selection may shorten this lag phase by artificial selection of highly fecund genotypes and by distributing the cultivars and enhancing the probability of an invasion.

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