American Journal of Botany

American Journal of Botany: e39–e40. 2011.

AJB Primer Notes & Protocols in the Plant Sciences

Polymorphic microsatellite markers in the invasive shrub *Buddleja davidii* (Scrophulariaceae)[1]

Susanne Schreiter, Susan K. Ebeling, and Walter Durka[2]

Helmholtz Centre for Environmental Research—UFZ, Department of Community Ecology, Theodor-Lieser-Strasse 4, D-06120 Halle, Germany

- *Premise of the study*: Microsatellite primers were developed for the invasive plant *Buddleja davidii,* a Chinese shrub that is an invader in most other continents.
- *Methods and Results:* An invasive population was analyzed using eight di- and tetranucleotide microsatellite loci. The number of alleles per locus ranged from 5 to 14. Due to polyploidy, exact genotypes could not be determined. Progeny arrays were used to study the outcrossing rate using presence/absence data of alleles resulting in an estimate of multilocus outcrossing rate of 93%. The markers were successfully tested in five congeneric species.
- *Conclusions:* The results indicate the utility of these loci in future studies of population genetics and breeding systems in *B. davidii* and in congeneric species.

Key words: *Buddleja davidii*; enriched library; invasion genetics; microsatellites.

A high number of invasive plant species had originally been introduced for ornamental or horticultural purposes. Ornamental plants are usually strongly affected by artificial selection. Plant breeding makes use of inter- and intraspecific crosses and often favors ecological traits that are also typical for successful invaders, e.g., high seed production or resistance to frost, pathogens, and insects. Moreover, horticultural trade may lead to colonization patterns differing from those of spontaneously spreading species. Thus, the analysis of genetic variation may help to elucidate both the invasion history and the evolutionary potential of invasive species of horticultural origin (Ross et al., 2008).

Buddleja davidii Franch. (Scrophulariaceae) is a successful alien plant in central Europe, North America, Australia, and New Zealand (Ebeling and Tallent-Halsell, 2009). The tetraploid ($2n = 4x = 76$) shrub is native to China and was introduced to Europe as an ornamental plant around 1890 and has been traded in large amounts. Quantitative genetic studies on *B. davidii* revealed variation in growth and reproduction among invasive populations (Ebeling et al., 2008). Since microsatellites are useful in tracing introduction pathways as well as analyzing the neutral genetic variation in the invasive range, we developed microsatellite loci for *B. davidii*, described the genetic variation in an invasive population, and determined the outcrossing rate.

METHODS AND RESULTS

Total genomic DNA was extracted, using DNeasy kits (QIAGEN, Hilden, Germany), from leaf samples collected from two native individuals from China (Yunnan Province) and two invasive individuals from Germany. An enriched library was built by ECOGENICS GmbH (Zurich, Switzerland). Size-selected genomic DNA ligated into TSPAD-linker (Tenzer et al., 1999) was used. Additionally, it was enriched by magnetic bead selection with biotin-labeled $(CA)_{13}$ and $(GATA)_8$ oligonucleotide repeats (Gautschi et al., 2000a; Gautschi et al., 2000b). Of 378 recombinant colonies screened, 76 (43 GA, 33 GATA) gave a positive signal after hybridization, and 45 plasmids from positive clones were sequenced. Primers were designed for 15 microsatellite inserts, which were tested for polymorphism. Eight primer pairs gave reproducible PCR products in the expected range (Table 1). Two multiplex PCR reactions were performed in 10-µl reaction volumes containing 2 ng genomic DNA, between 0.3 and 1.0 µM forward and reverse primers (Table 1), and 5 µl HotstarTaq Mastermix (QIAGEN). Multiplex-PCR was performed with loci Bud_04, Bud_09, Bud_10 and Bud_12 in the first multiplex-set, and Bud_03, Bud_06, Bud_13 and Bud_14 in a second reaction. All forward primers were labeled with different fluorescent dyes (Table 1). PCR amplifications were conducted with an Eppendorf Mastercycler using a heat-activating step at 95°C for 15 min, followed by 35 cycles at 95°C for 30 s, 50°C for 90 s and 72°C for 60 s and a final extension step of 30 min at 72°C. Fragments were separated on an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, Darmstadt, Germany) with a 50-cm capillary, POP6 polymer and internal size standard GeneScan-500 LIZ (Applied Biosystems) and genotyped manually with GeneMapper 3.7 (Applied Biosystems).

Using the microsatellites, we investigated genetic variation of one invasive population of *B. davidii* consisting of 61 individuals in Germany (Leipzig: 51°17′52″N, 12°19′37″E). Additionally, for the analysis of the mating system we sampled seeds and grew seedlings until leaves could be harvested for DNA extraction.

The population was genetically highly diverse with between 5 and 14 alleles per locus, and 79 alleles in total. Because of polyploidy, up to four bands were observed per individual, on average 2.7 alleles per locus. In genotypes with two or three alleles only the presence, but not the respective copy number, could be determined. Thus, banding patterns were interpreted as dominant markers. We used ATetra v. 1.1 (van Puyvelde et al., 2010) to calculate expected heterozygosity using 10 000 Monte Carlo simulations resulting in H_e values between 0.620 and 0.855 for the eight loci (Table 1) and mean H_e of 0.746.

With data from 205 seeds from 36 seed families, we estimated the multilocus outcrossing rate using the program MLTR v. 3.0 (Ritland, 2002). Our results demonstrate that *B. davidii* is an outcrossing species with a multilocus outcrossing rate of $92.7 \pm 0.026\%$.

Trans-species amplification of the loci developed for *B. davidii* was tested for the congeneric species *B. asiatica* Loureiro, *B. crispa* Bentham, *B. macrostachya* Wallich ex Bentham, *B. myriantha* Diels, and *B. officinalis* Maximowicz, all sampled on Cangshan Mountain, near Dalizhen, Yunnan Province, China (25°42′12″N, 100°07′20″E). Most loci could be amplified successfully in all species, in line with the known ease of interspecific hybridization in the genus (Table 2). However, complex banding patterns were found in the polyploid species.

[1] Manuscript received 21 October 2010; revision accepted 20 November 2010.

[2] Author for correspondence: walter.durka@ufz.de

doi:10.3732/ajb.1000417

TABLE 1. Characteristics of eight polymorphic microsatellite loci from *Buddleja davidii* from an invasive population in Leipzig, Germany (51°17′52″N, 12°19′37″E, $N = 61$).

Locus	Multiplex set	Primer sequence (5′–3′)	Repeat motif	Length of sequenced clone (bp)	Final primer concentration (μM)	Fluorescent dye	Allele range (bp)	Number of alleles	H_e	EMBL accession number
Bud_04	1	F: GCTATTCATGGTAATTGAGTGAGG R: ATTGACGCCTCCTCTACCTG	$(GATA)_7$	145	0.3	NED	116–166	5	0.659	FR715562
Bud_09	1	F: GCTCAACTGTCAGTACGTTGC R: CTCCTGCACTTCAGATTGTTTAC	$(CT)_{21}$	164	0.3	VIC	132–183	10	0.825	FR715566
Bud_10	1	F: TCCCTCTCATATTGGGATAACA R: GCATTTGGAACCGTTAAAGC	$(CT)_{26}$ T $(CT)_4$	183	0.9	FAM	128–184	7	0.629	FR715567
Bud_12	1	F: ACATCCCTACCCGTGATAGTAG R: TTTTCGCTGTTTGTCCACTTAC	$(GA)_{19}$	143	1.0	PET	114–150	9	0.761	FR715569
Bud_03	2	F: GCATGCGCTGACATTTTTC R: GTCTTCTCGACCCATGTGC	$(TATC)_8$ $(TA)_5$	111	1.0	PET	76–209	10	0.739	FR715564
Bud_06	2	F: CGTCACATGTCGTTCGTAGG R: TTCCGTTATTCCCATTGTCC	$(CT)_{20}$ CATA$(CA)_6$	199	0.9	NED	171–211	11	0.748	FR715563
Bud_13	2	F: CCTAACTGCGAATTGTATAGTTTCC R: TCTGATGCAGTCAGGTTTGC	$(CT)_{14}$	110	0.3	FAM	102–221	14	0.855	FR715570
Bud_14	2	F: CAAAACCAATGCCCAAAGAG R: AGCTTAGGAGTCCCCCACAC	$(GA)_{19}$	181	0.3	VIC	104–210	13	0.754	FR715571

TABLE 2. Trans-species amplification of microsatellite markers developed for *Buddleja davidii* in five congeneric *Buddleja* species of the native range sampled on Cangshan Mountain, near Dalizhen, Yunnan Province, China (25°42′12″N, 100°07′20″E). Number of alleles and allele range (bp) is given.

Species	*B. asiatica*	*B. crispa*	*B. macrostachya*	*B. myriantha*	*B. officinalis*
N	5	5	3	4	4
Ploidy level	$2N = 38$ (2*x*)	$2N = 38$ (2*x*)	$2N = 190$ (10*x*)	$2N = 76$ (4*x*)	$2N = 76/108$ (4*x*/6*x*)
Bud_04	1 (132)	3 (128–141)	8 (127–166)	6 (115–154)	3 (122–141)
Bud_09	5 (148–181)	3 (143–150)	9 (132–177)	8 (140–185)	4 (140–171)
Bud_10	3 (136–143)	2 (136–154)	6 (132–149)	5 (132–184)	3 (132–137)
Bud_12	1 (119)	3 (123–134)	6 (113–138)	8 (118–151)	5 (129–142)
Bud_03	3 (132–210)	1 (189)	–	1 (121)	1 (120)
Bud_06	3 (182–187)	6 (172–196)	9 (102–194)	8 (169–222)	5 (172–221)
Bud_13	4 (106–146)	4 (132–159)	15 (106–153)	7 (108–139)	6 (118–167)
Bud_14	2 (124–156)	3 (128–190)	9 (104–193)	8 (110–179)	5 (124–201)

CONCLUSIONS

We found that the investigated population of invasive *Buddleja davidii* is genetically highly polymorphic and has a high outcrossing rate. *Buddleja davidii* is a popular garden plant, because of both its colorful fragrant flowers and attractiveness to butterflies caused by large amounts of nectar. Foraging over large distances, butterflies are efficient pollen vectors (Cant et al., 2005), which may contribute to high gene flow within and between populations of *B. davidii*. We conclude that *B. davidii* has a high evolutionary potential and is frequently traded horticulturally, and planting may support successful invasions.

The developed microsatellite markers may be useful for tracing introduction pathways and assessing genetic variability within and among native and invasive populations to understand evolutionary changes in invasive *B. davidii*.

LITERATURE CITED

CANT, E. T., A. D. SMITH, D. R. REYNOLDS, AND J. L. OSBORNE. 2005. Tracking butterfly flight paths across the landscape with harmonic radar. *Proceedings. Biological Sciences* 272: 785–790.

EBELING, S. K., AND N. G. TALLENT-HALSELL. 2009. *Buddleja davidii in* Invasive species compendium. CABI, Wallingford, UK.

EBELING, S. K., E. WELK, H. AUGE, AND H. BRUELHEIDE. 2008. Predicting the spread of an invasive plant: Combining experiments and ecological niche model. *Ecography* 31: 709–719.

GAUTSCHI, B., I. TENZER, J. P. MULLER, AND B. SCHMID. 2000b. Isolation and characterization of microsatellite loci in the bearded vulture (*Gypaetus barbatus*) and cross-amplification in three Old World vulture species. *Molecular Ecology* 9: 2193–2195.

GAUTSCHI, B., A. WIDMER, AND J. KOELLA. 2000a. Isolation and characterization of microsatellite loci in the dice snake (*Natrix tessellata*). *Molecular Ecology* 9: 2191–2193.

PUYVELDE, K. VAN, A. V. GEERT, AND L. TRIEST. 2010. atetra, a new software program to analyse tetraploid microsatellite data: Comparison with tetra and tetrasat. *Molecular Ecology Resources* 10: 331–334.

RITLAND, K. 2002. Extensions of models for the estimation of mating systems using n independent loci. *Heredity* 88: 221–228.

ROSS, C., H. AUGE, AND W. DURKA. 2008. Genetic relationships among three native North-American *Mahonia* species, invasive *Mahonia* populations from Europe, and commercial cultivars. *Plant Systematics and Evolution* 275: 219–229.

TENZER, I., S. DEGLI IVANISSEVICH, M. MORGANTE, AND C. GESSLER. 1999. Identification of microsatellite markers and their application to population genetics of *Venturia inaequalis. Phytopathology* 89: 748–753.