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Development and characterization of microsatellite markers for *Polygonum cespitosum* (Polygonaceae)[1]

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- *Premise of the study:* We isolated and characterized microsatellite markers in *Polygonum cespitosum* Blume, an herbaceous annual plant species introduced into North America from Asia that has recently become invasive.
- *Methods and Results:* A total of 12 polymorphic and 3 monomorphic loci were screened in 1–2 individuals from each of 20 populations from the introduced and native range, for a total of 24 samples. The number of alleles per locus in the polymorphic loci ranged from 3 to 9, and expected heterozygosity ranged from 0.156 to 0.838.
- *Conclusions:* These new loci will provide tools for examining genetic relatedness among introduced and native populations of this and other related species.

Key words: microsatellite; PCR primers; *Persicaria; Polygonum*; SSR; STR.

Polygonum (s.l.) *cespitosum* Blume (*Persicaria cespitosa*, Kim and Donoghue, 2008) is an herbaceous, predominantly self-fertilizing annual introduced to North America in the early 20th century from temperate and subtropical areas of eastern Asia (Paterson, 2000). In its native range, *P. cespitosum* is largely restricted to moist, shaded habitats (Anjen et al., 2003), and initially its distribution in eastern North America was limited to disturbed shaded sites such as roadsides and forest paths. However, over the last 15–20 years, *P. cespitosum* has begun to colonize open, potentially drier sites in its introduced range (Horgan-Kobelski, Matesanz, and Sultan, in process). Coincident with this change, the species has recently been classified as invasive (Merhoff et al., 2003). This species thus provides a tractable annual system in which to investigate the dynamics of a plant invasion in progress. Here we report the development of microsatellite primers using genotypes drawn from a broad sample of introduced and native populations, as an initial step toward characterizing allelic variation and population structure in this system.

METHODS AND RESULTS

Achenes (single-seeded fruits) were collected from field parents in 20 populations of *Polygonum cespitosum,* 16 from the introduced range (northeastern North America), and 4 populations from the native range (Japan and South Korea) (Appendix 1) and grown in the greenhouse to provide leaf tissue.

Total DNA was extracted from one individual of *P. cespitosum* using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) for the construction of the microsatellite library. DNA was then serially enriched twice for microsatellites using three probe mixes following Glenn and Schable (2005) with the changes described in Lance et al. (2010). There were two primary changes to the Glenn and Schable (2005) protocol. First, a different linker was used (SimpleX-2 Forward 5′-AAAAGCTGCTGGCGAATC and SimpleX-2 Reverse 5′-pAGTTCCGCTGCTCG). Second, the enriched libraries were sequenced on a 454 using titanium chemistry following standard Roche 454 library protocols (454 Life Sciences, a Roche company, Branford, Connecticut, USA). All methods for sequencing, microsatellite identification, primer design, and primer screening are as described in Lance et al. (2010) with the exception that the sequence GTTT was added to primers without the universal CAG tag addition.

Forty-eight primer pairs were tested for amplification and polymorphism using DNA obtained from eight individuals. PCR amplifications were performed in a 12.5-μL volume (10 mM Tris pH 8.4, 50 mM KCl, 25.0 μg/ml BSA, 0.4 μM unlabeled primer, 0.04 μM tag labeled primer, 0.36 μM universal dye-labeled primer, 3.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 units AmpliTaq Gold Polymerase (Applied Biosystems, Foster City, California, USA), and 20 ng DNA template) using an Applied Biosystems GeneAmp 9700. Touchdown thermal cycling programs (Don et al., 1991) encompassing a 10°C span of annealing temperatures ranging between 65°C and 55°C (TD65) or 58–48°C (TD58) were used for all loci. Touchdown cycling parameters consisted of an initial denaturation

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TABLE 1. Characteristics of 15 microsatellite primers developed in *Polygonum cespitosum.* Shown for each primer pair are the forward and reverse sequence, repeat type, range size (bp), touchdown protocol used for PCR (TD), and the GenBank accession number. All values are based on 24 samples representing 20 North American and Asian populations located in Connecticut and Massachusetts (introduced range) and South Korea and Japan (native range). N = 1–2 individuals for each population. Note that loci Poce12, Poce22, and Poce23 are monomorphic for the screened samples.

Locus	Primer Sequence (5′–3′)	Repeat	Size range (bp)	TD	GenBank Accession No.
Poce01	F: *AACAACAACTGCACATATCC R: GTTTGGAATTATGGGCAGTCTAC	AGAT(9)	192–208	58	HQ998868
Poce02	F: *AACAACTCCACTTCGTATG R: GTTTATATTATGGGTGCGAATTTG	ACAT(8)	334–428	58	HQ998869
Poce03	F: *ACCAAAGAGCTAACCCTAG R: GTTTCCGTCTTCTTCCTTCCTAC	AG(13)	180–194	58	HQ998870
Poce11	F: *CTTTCTGCTCCCTTTATTTG R: GTTTCGGTCTAGATCTGTTCACATG	ACAT(21)	324–404	58	HQ998871
Poce12	F: *GCAAGGACCAAATGAGAG R: GTTTCCACGTTCTACACTTTCTCC	AG(14)	237	65	JF412758
Poce15	F: *GGAATCCAATAAAGCTATCC R: GTTTGTGAGATTCGTCGTCTTCAG	AGAT(10)	237–265	58	HQ998872
Poce20	F: *GTGCTTCCATTATCCATAC R: GTTTGTGGTCGGGAATCTTATAG	ACAT(16)	190–268	58	HQ998873
Poce21	F: *TAGTCATAGGTCCACGGTTC R: GTTTATAAGGACGAATGGAAGAAG	AG(14)	179–246	58	HQ998874
Poce22	F: *TCCCTCTTTAAATCCTACC R: GTTTGCTGGCGAATCACATAATC	AGAT(9)	223	65	JF412759
Poce23	F: *TCCTGTCTTGCCTATTGTTC R: GTTTCCTCGCGACTTTACTTATG	AG(16)	240	65	JF412760
Poce26	F: *TTCTTCAAGCACATTTCTG R: GTTTATGTTATCATTGACGTTTGAAC	ACAT(9)	179–205	65	JF412761
Poce28	F: *TTTATACCACTCCTCGATCC R: GTTTAGAACTTTCGGTGGTGAAC	ACAT(11)	327–393	58	HQ998875
Poce39	F: GTTTCTGGTTTGACTGGTTGTTTC R: *GGGAAATTCAAAGCAAGTAG	AAAG(8)	226–234	65	HQ998876
Poce41	F: GTTTGAATTATTGGGCAGTCTACG R: *AACTAGCTAGCGTGGTTC	AGAT(8)	117–133	65	JF412765
Poce45	F: GTTTGCCTGGATTGTTACAAATTC R: *TTCTCTCCCAAGGCTAATC	AAAC(8)	303–319	65	HQ998877

*Indicates CAG tag (5′- CAGTCGGGCGTCATCA-3′) label.

TABLE 2. Results of initial primer screening in 16 and 4 populations of *Polygonum cespitosum* in the introduced and native range, respectively. Shown for each primer pair are the number of individuals screened, number of alleles (A), and values of observed (H_o) and expected (H_e) heterozygosity, and the probability of identity for each locus (PI).

	No. of individuals	No. of alleles (A)	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Probability of identity (PI)
Poce1	22	5	0.000	0.707	0.126
Poce2	24	9	0.542	0.838	0.046
Poce3	24	7	0.000	0.788	0.075
Poce11	24	9	0.000	0.788	0.068
Poce12	24	1	–	–	–
Poce15	21	5	0.000	0.689	0.151
Poce20	22	9	0.000	0.802	0.065
Poce21	21	6	0.286	0.704	0.122
Poce22	24	1	–	–	–
Poce23	24	1	–	–	–
Poce26	20	6	0.600	0.706	0.120
Poce28	20	9	0.650	0.691	0.127
Poce39	24	3	0.000	0.156	0.718
Poce41	22	5	0.000	0.707	0.126
Poce45	24	3	0.000	0.292	0.521

step of 5 min at 95°C followed by 20 cycles of 95°C for 30 s, highest annealing temperature (decreased 0.5°C per cycle) for 30 s, and 72°C for 30 s; and 20 cycles of 95°C for 30 s, lowest annealing temperature for 30 s, and 72°C for 30 s. PCR products were run on an ABI-3130xl sequencer and sized with Naurox size standard prepared as described in DeWoody et al. (2004), except that unlabeled primers started with GTTT. Results were analyzed using GeneMapper version 3.7 (Applied Biosystems).

Fifteen of the tested primer pairs amplified high-quality PCR product and 12 of them exhibited polymorphism. We assessed the variability of the 12 polymorphic loci in 24 specimens collected from 20 populations from the introduced and native ranges. We estimated the number of alleles per locus (A), observed and expected heterozygosity (H_o and H_e), and probability of identity (PI) using GenAlEx v6.4 (Peakall and Smouse, 2006). Conditions and characteristics of all loci are provided in Tables 1 and 2 and in Appendix 1. Tests for deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted using GENEPOP v4.0 (Rousset, 2008). After Bonferroni correction for multiple comparisons, 10 loci (all but Poce 28 and Poce26) showed significant deviations from expectations under HWE, and no linkage disequilibrium was detected for any of the paired loci comparisons. The deviations from HWE are expected in this species because it is primarily self-fertilizing. In the

course of further extensive characterization of these markers, Poce2, Poce39, Poce41, and Poce45 showed low repeatability.

CONCLUSIONS

These polymorphic microsatellite loci will enable researchers to assess population structure and genetic relatedness among introduced and native populations of this newly invasive species. Additionally, the monomorphic loci may be useful in broader surveys or cross-amplification studies.

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Appendix 1. Population location and coordinates of the 16 and 4 populations from the introduced and native range, respectively, used in this study.

Population location	Geographical coordinates
Introduced Range (USA)	
Arch Road, Leeds, MA	42°21'13″N, 72°41'39″W
Black Rock State Park, Thomaston, CT	41°39'24″N, 73°06'18″W
Chester-Blandford State Forest, Chester, MA	42°14'35″N, 72°54'56″W
Devils Hopyard State Park, East Haddam, CT	41°28'42″N, 72°20'30″W
Gay City State Park, Hebron, CT	41°43'47″N, 72°26'20″W
Harvard Arnold Arboretum, Jamaica Plain, MA	42°18'08″N, 71°07'27″W
James Goodwin State Forest, Hampton, CT	41°46'40″N, 72°05'12″W
Mianus River State Park, Stamford, CT	41°04'51″N, 73°34'50″W
Naugatuck Forest, Oxford, CT	41°26'58″N, 73°05'34″W
Nye Holman State Forest, Tolland, CT	41°52'55″N, 72°18'27″W
Katherine Ordway Preserve, Weston, CT	41°12'19″N, 73°21'24″W
Rocky Woods Reservation, Medfield, MA	42°12'13″N, 71°16'49″W
Sleeping Giant State Park, Hamden, CT	41°25'15″N, 72°53'55″W
Wadsworth Estate, Middletown, CT	41°32'07″N, 72°40'33″W
Weir Farm, Wilton, CT	41°15'23″N, 73°27'22″W
Wyantenock State Forest, Bristol, CT	41°45'47″N, 73°23'52″W
Native Range	
Chiba Prefecture, Japan	35°38'08″N, 140°05'02″E
Toneunga Park, Japan	35°54'60″N, 139°54'16″E
Botanical Gardens, The University of Tokyo, Japan	35°40'30″N, 139°44'46″E
Gyeonggi-do: Namyangju-si, Byeolnae-myeon, South Korea	37°38'57″N, 127°06'10″E