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PRIMER NOTE

Isolation and characterization of microsatellite loci in *Geum urbanum* (Rosaceae) and their transferability within the genus *Geum*

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Abstract

Thirteen novel polymorphic microsatellite loci are presented for *Geum urbanum* (Rosaceae). The microsatellites will be useful tools to analyse the influence of landscape structure and land-use intensity in agricultural landscapes on genetic diversity within and among populations of *Geum urbanum*. Transferability was tested in 19 other *Geum* species and two *Waldsteinia* species. In most species polymerase chain reaction (PCR) products of the expected range were obtained, therefore the markers reported here appear to be applicable across the whole genus.

Keywords: fragmentation, *Geum urbanum*, landscape structure, microsatellite, population genetics

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Wood Avens (*Geum urbanum* L.) is a hexaploid ($2n = 6x = 42$) member of the Rosaceae, native to the Eurasian Temperate Zone. The species has a preferentially autogamous breeding system due to autodeposition of pollen (Taylor 1997). Therefore, gene flow among sites will depend mainly on seed dispersal. Seeds are hairy and have a 5–7 mm long hook which enables epizoochorous adhesive dispersal (Müller-Schneider 1977; Fischer *et al*. 1996; Kiviniemi 1996).

In agricultural landscapes, *G. urbanum* occurs in forest remnants, hedgerows and exceptionally in unshaded field edges. These landscape elements form the semi-natural network (greenveining) that serves as habitat and as corridors between larger habitat islands within a matrix of unsuitable agricultural areas. We developed polymorphic microsatellite markers for *G. urbanum* in order to investigate gene flow in European agricultural landscapes. The microsatellites will be useful tools to analyse the influence of landscape structure on gene flow and genetic diversity within and among populations of *Geum urbanum*.

Microsatellite loci were isolated using an enrichment procedure (Karagyozov *et al*. 1993). Di-, tri- and tetranucleotide repeat enriched libraries of *G. urbanum* genomic DNA were constructed as described by Arens *et al*. (2000), with the following modifications: genomic DNA of *G. urbanum* was digested with *Rsa*I, *Alu*I, *Mbo*I or *Taq*I and size-fractionated using agarose gel electrophoresis. DNA fragments between 300 and 1000 bp were recovered by electro-elution, enriched by hybridization to synthetic oligonucleotides with a di-, tri- or tetranucleotide motif [$(GA)_{12}$/$(GT)_{12}$ $(TGT)_{10}$ $(GTG)_8$ $(GAG)_8$ $(GCT)_8$ $(TCT)_{10}$ $(CGT)_8$ $(AGT)_9$ $(TGA)_9$ $(GCC)_8$, and $(TGTT)_8$/$(GATA)_8$/$(GTAT)_8$], cloned in the pGEM-T easy vector (Promega) and transformed to *Escherichia coli* XL2 Blue (Stratagene). Colonies were screened by hybridization to the appropriate oligonucleotides and positive clones were sequenced using an ABI 3700 sequencer (Applied Biosystems).

Primer pairs for 27 microsatellite loci were designed using PrimerSelect (DNAstar) and tested on a small set of individuals using silver staining detection as described by Arens *et al*. (2000). Thirteen loci were considered for further optimization and testing using fluorescent detection (Table 1). Multiplex-PCR's were performed in 10 μL reaction volumes containing 10 ng genomic DNA, 3 pmol of each forward and reverse primer, and 5 μL 2x Multiplex PCR Master Mix (QIAGEN). A fluorescent label was present on all forward primers. Loci WGU6-5 (label: FAM), WGU6-7 (JOE), WGU7-4 (JOE), WGU3-15 (TAMRA), WGU2-28 (TAMRA) and WGU2-10 (TAMRA) were amplified in one reaction, WGU2-48 (FAM), WGU1-33 (JOE) and WGU5-12

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Table 1 Microsatellite loci from *Geum urbanum* based on 688 individuals genotyped from 45 European populations. Annealing temperature for all primers sets was 57 °C

Locus	Repeat motif in sequenced clone	Primer sequence (5′–3′)	No. of alleles	Size range (bp)	H_O	H_E	A	EMBL accession no.
WGU6-5	$AGA_{28\text{-}1}$	F: AACCCCTGACTAAAGAGAAAAA R: AGAAGAGGGTATCAAAAAGATGACT	12	203–258	0.022	0.355	2.4	AJ606056
WGU6-7	TCT_{24}	F: CCTTGCACTTGCCCTTGATA R: AGTGGCCTCCTCTTCATCTGTT	14	158–203	0.022	0.352	2.4	AJ606057
WGU7-4	$(CTG)_{12\text{-}1}(CTA)_{41\text{-}7}$	F: CCATAGTCTTCGACCGAAATCCATA R: CGCGAGGTAGAGTAGGGCAGAG	5	236–248	0.016	0.277	1.9	AJ606059
WGU2-10	AAC_{20}	F: TGGGCATTGCCATTGTACATACTTT R: GAACAGTCTGCTATAGTTGGAGAAA	5	230–242	0.018	0.280	2.0	AJ606048
WGU2-28	AAC_{7}	F: AAGTAACTGCCCTGTGAAAAAGAG R: TATGCTGAGGGTGAGTATCTAATGG	4	165–186	0.017	0.231	1.7	AJ606049
WGU3-15	CCA_{6}	F: TGTGCTCTACCACCCCCACCACCTG R: AGCAGCACCATTTGCAAGCCTCGTG	2	136–139	0.002	0.062	1.2	AJ606052
WGU2-48	GTT_{12}, GTT_{7}	F: TAAAAATTAGGGCATACGGAGAAAT R: TTAGACAAAACAATAACCCACAGT	6	191–206	0.020	0.271	1.9	AJ606050
WGU1-33	CT_{14}	F: TTTTATGGCTGAAGCAGTTTATTTT R: ATGGAGTAGTTGGGTCTCTTCTTTT	3	225–229	0.016	0.149	1.5	AJ606060
WGU8-1	GAA_{4}, GAA_{6}	F: AAGACCCTCCAGCCGCTTCATC R: CAGATCCGTCGTCGTCTTCGTCATC	9	230–269*	0.020	0.170	1.7	AJ606051
WGU6-1	GAA_{15}	F: CCGCCGATATAAAAGGCACACCAA R: GAGAGCCGCCGTGAGGGAATGA	9	164–194	0.025	0.312	2.2	AJ606055
WGU6-23	AAG_{18}	F: CATGGCCATGTTGGTTAATGTAATC R: GTCTCAACAAAATGTCCCAAAGTGA	12†	220–254	—	—	—	AJ606058
WGU5-11	$(CAA)(CAG)_{28}$	F: TTCGCGGAGTCAGGTTCAACAGGTT R: AGTAAGGCGACATTGGCACTTGACC	8†	227–258	—	—	—	AJ606053
WGU5-12	GTT_5(GCA TAA CAA)GTT_3	F: TTGCACTGGATATGGTTGCTGTTTT R: ATGGGCAGTATCCGGTGCAGAGTAG	5†	250–268	—	—	—	AJ606054

F, forward primer; R, reverse primer; H_O, population mean of observed heterozygosity; H_E, population mean of expected heterozygosity; A, mean no. of alleles per population; '—'denotes the number of mismatches from a perfect repeat.
*in WGU8-1 additional monomorphic bands where detected in all samples at 224 and 228 bp.
†Loci with 2–4 alleles found; only interpretable as multiallelic phenotype.

(TAMRA) in a second reaction and WGU6-1 (FAM), WGU6-23 (FAM), WGU8-1 (JOE) and WGU5-11 (TAMRA) in a third reaction, using a Primus-96 thermocycler (MWG, Germany) with the following PCR program: 95 °C for 15 min, followed by 35 cycles of 94 °C for 30 s, 57 °C for 90 s, 72 °C for 90 s, followed by 72 °C for 10 min. Fragments were separated on an ABI 310. Since single populations harboured only a small amount of total allelic richness, we report allelic variability based on 688 individuals sampled from 45 populations across Germany, Switzerland and Estonia (Durka *et al*. in prep).

All 13 primer pairs gave reproducible PCR products (Table 1). Ten of these appear as codominant genetic markers with disomic inheritance. The remaining three microsatellites were interpretable only as multiallelic phenotypes, most likely related to the polyploidy of *Geum urbanum*. All loci showed significant shortage of heterozygotes, consistent with the species' selfing nature.

The microsatellites were tested for *trans*-species amplification in 19 other *Geum* species from several subgenera and in two species from the closely related genus *Waldsteinia* (Table 2). In most species, products of the expected range where obtained with standard PCR conditions, but sometimes fixed combinations of bands or multibanded patterns were obtained, probably related to the general polyploidy of the genus. Given that the primers amplify in *Waldsteinia*, which is basal to a clade including *Geum* and a number of other polyphyletic genera (Smedmark & Eriksson 2002), the markers presented here might be useful in these genera (e.g. *Coluria*, *Acomastylis*) as well.

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Table 2 Trans-species amplification (number of alleles, size range) of selected microsatellite loci from *Geum urbanum*. Loci that were multiplexed with the same fluorescent dyes are not reported here because of potential allele size overlap

			Number of bands, size(s)					
Taxon	Ploidy†	*n*	WGU6-5	WGU2-10	WGU2-48	WGU1-33	WGU8-1	WGU6-1
subgenus Geum								
Geum aleppicum Jacq.	$2n = 6x = 42$	4 s	2, 192–214	2, 219–233	2, 191–203	3, 219–277	3, 224–254	2, 149–182
Geum canadense Jacq.	$2n = 6x = 42$	4 s	1, 194	1, 243	1, 199	1, 216	3, 215–239	2, 149–179
Geum coccineum Sibth. & Sm.	$2n = 6x = 42$	4 s	3, 203–249	3, 230–239	2, 185–202	3, 221–242	3, 226–246	3, 125–167
Geum fauriei Levl.	$2n = 12x = 84$	4 s	3, 194–252	3, 227–242	1, 176	1, 226	5, 207–251	4, 149–176
Geum hispidum Fr.	$2n = 6x = 42$	4 s	2, 194–282	1, 231	1, 182	2, 219–239	3, 221–274	3, 144–185
Geum macrophyllum Willd.	$2n = 6x = 42$	4 s	1, 194	1, 220	—	2, 217–224	1, 245	2, 158–179
Geum molle Vis. et Pancic	$2n = 6x = 42$	4 s	2, 194–298	1, 231	1, 184	4, 225–244	3, 221–257	3, 131–179
Geum quellyon Sweet	$2n = 6x = 42$; $10x = 70$	4 s	1, 298	2, 234–242	1, 179	1, 244	3, 203–238	3, 149–179
Geum rhodopaeum Stoj & Stefanov	—	4 s	1, 217	1, 234	1, 203	1, 227	3, 224–254	1, 187
Geum rivale L.	$2n = 6x = 42$	25 l	8, 194–247	3*, 234–244	6, 176–206	6, 218–248	5, 219–247	8, 138–191
Geum sylvaticum Pourret	$2n = 6x = 42$	4 s	3, 223–255	2, 228–237	4, 193–224	1, 226	4, 218–251	4, 137–176
Geum virginianum L.	$2n = 6x = 42$	4 s	1, 194	1, 240	1, 197	2, 216–235	3, 215–233	3, 132–170
Geum × *intermedium* L.	$2n = 6x = 42$	4 s	2, 194–203	1, 234	1, 203	1, 227	3, 224–254	1, 187
subgenus Oreogeum								
Geum bulgaricum Panc.	$2n = 8x = 56$; $10x = 70$	4 s	1, 122	2, 234–237	1, 179	1, 218	4, 220–236	3, 148–188
Geum montanum L.	$2n = 6x = 42$; $4x = 28$	4 s	4, 206–244	2, 231–243	—	4, 200–236	4, 220–248	4, 156–194
Geum reptans L.	$2n = 6x = 42$	4 s	1, 197	—	2, 179–191	4, 225–234	3, 226–247	4, 146–193
subgenus Woronowia								
Geum speciosum Alboff	$2n = 10x = 70$	4 s	2, 238–298	3, 220–246	4, 175–212	4, 204–250	4, 219–241	4, 149–193
subgenus Erythrocoma								
Geum triflorum Pursh.	$2n = 6x = 42$	4 s	—	1, 234	1, 189	—	1, 230	2, 167–170
subgenus Orthostylus								
Geum heterocarpum Boiss.	$2n = 4x = 28$	4 s	—	—	—	1*, 232	2, 239–259	1, 163
Waldsteinia ternata (Stephan) Fritsch	$2n = 6x = 42$	1 l	—	1, 219	1, 175	1, 216	1, 266	2, 179–193
Waldsteinia geoides Willd.	$2n = 2x = 14$	1 l	1, 192	1, 236	2, 175–178	2, 216–218	1, 263	1, 172

(*n*) number and type of samples used: s = single seed obtained from Botanical Gardens, l = leaves from (potentially) different plants.
*in some plants no amplification, may indicate null-alleles.
†chromosome numbers from Gajewski (1957).

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