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PRIMER NOTE

Isolation and characterization of microsatellite loci in the invasive *Alliaria petiolata* (Brassicaceae)

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Abstract

Eight novel polymorphic microsatellite loci are presented for garlic mustard (*Alliaria petiolata*, Brassicaceae) a European herb that is a serious invader of North American deciduous forests. The microsatellites will be useful tools to analyse pathways of introduction of garlic mustard, as well as its evolutionary potential in the invasive range.

Keywords: biological invasions, Garlic mustard, microsatellite, population genetics

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Garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara and Grande] is a hexaploid ($2n = 6x = 42$) member of the mustard family (Brassicaceae) native to the Eurasian temperate zone. In Europe, it occurs in mesic semishade habitats such as forest edges and moist woodlands. The species has been introduced to North America in the 19th century. It has continuously expanded its range and is now present in 34 US states and four Canadian provinces (Nuzzo 2000). Garlic mustard is one of the few species that invade the understory of North American deciduous forests where it is regarded a serious threat to the native flora and the integrity of native food webs (McCarthy 1997; Nuzzo 2000; Blossey *et al*. 2001). There is a great need for molecular studies that address pathways of introduction, genetic variability within and between populations, and thereby the potential for rapid evolutionary changes in garlic mustard. Routes of introduction have previously been investigated using inter simple sequence repeats (ISSR) on a limited number of populations (Meekins *et al*. 2001). No microsatellite primers have been developed yet for garlic mustard that would allow fingerprinting and the investigation of breeding systems and population genetics with a codominant maker.

Leaf samples were collected from five individuals in a native population of garlic mustard. Total genomic DNA was extracted using DNeasy kits (QIAGEN). An enriched library was made by ECOGENICS GmbH (Zurich, Switzerland) from size-selected genomic DNA ligated into TSPAD-linker (Tenzer *et al*. 1999) and enriched by magnetic bead selection with biotin-labelled $(CA)_{13}$ and $(GA)_{13}$ oligonucleotide repeats (Gautschi *et al*. 2000a; Gautschi *et al*. 2000b). Of 384 recombinant colonies screened, 88 gave a positive signal after hybridization. Plasmids from 50 positive clones were sequenced and primers were designed for 11 microsatellite inserts which were tested for polymorphism.

Variability among individuals was analysed by polymerase chain reaction (PCR) in 10 µL reaction volumes containing 10 ng genomic DNA, two pmole of each forward and reverse primers, 200 µM dNTPs, 15 mM $MgCl_2$, 0.4 units BioTherm™ DNA polymerase (DIAGONAL, Münster, Germany) and 1 µL 10x PCR buffer with $(NH_4)_2SO_4$ (MBI Fermentas). The latter contains 750 mM Tris-HCl (pH 8.8), 200 mM $(NH_4)_2SO_4$ and 0.1% Tween20. Multiplex-PCR was performed with fluorescent labelled primers Ap2 (label: FAM), Ap3 (TAMRA), Ap4 (JOE), Ap6 (JOE) and Ap7 (TAMRA) in one reaction and Ap1 (FAM), Ap5 (JOE) and Ap8 (TAMRA) in a second reaction. At all loci the forward primers were labelled. We used a Primus-96 thermocycler (MWG, Germany) and applied the following PCR program: 92 °C for 3 min, followed by 35 cycles of 92 °C for 15 s, 55 °C for 45 s, 72 °C for 60 s, followed by 72 °C for 10 min. Fragments were separated on an ABI 310 genetic analyser (Applied Biosystems) with internal size standard

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Table 1 Microsatellite loci from *Alliaria petiolata* based on 318 individuals genotyped from 27 European populations. Annealing temperature for all primers sets was 55 °C

Locus	Repeat motife based on sequence clone	Primer sequence (5′–3′)	No. of alleles	Size (bp)	H_O (SE)	H_E (SE)	Genbank accession no.
Ap1	$(CA)_{19}$	F: CACCTCAAATCTAGCAAACGAC R: AGGATGAGCTTTGTCATGTTTG	10	192–225	0.016 (0.006)	0.218 (0.046)	AY363668
Ap2	$(GA)_{29}$	F: GGCGAAGTGACCAGTAACTAAGAG R: ACCATGGTGAACTGTTGGATTC	21	118–160	0.000 (0.0)	0.327 (0.062)	AY363669
Ap3	$(GA)_{31}$	F: GGAGTAAGAAGGAAGGCTAGACG R: AAGACTTCTCCCGATATTTCTGC	24	113–159	0.028 (0.01)	0.327 (0.05)	AY363670
Ap4	$[(GA)_{10}TA(GA)_3CA](GA)_{22}$	F: ACGGGTGGCTTTTCATAAGG R: CCCACCTATAAAGACTCAGAAAGG	20	199–257	0.016 (0.009)	0.218 (0.05)	AY363671
Ap5	$(GA)_{24}$	F: TGGTGTACGTGCGTATATATGTTG R: TGCCTTTTAGATTCCCTAAACC	10	99–131	0.016 (0.006)	0.186 (0.05)	AY363675
Ap6	$(GA)_{26}$	F: GCGTCCGATCGTCCTTATAG R: TGCCTAACGTACCGCATAAC	16	114–156	0.184 (0.044)	0.021 (0.007)	AY363672
Ap7	$(GA)_{29}$	F: AAAAGTACGTGTACGCCAACC R: CCCATCTCCTCTCGTGAATC	18	208–262	0.053 (0.025)	0.170 (0.052)	AY363673
Ap8	$(CT)_7(CTCC)_3(CT)_{16}(TT)_2(CT)_{10}TT(CT_{13})$	F: TCGACTTTCACTTGGTTTGC R: TTTGCCTTAACATTCCACACTG	12	100–104, 173–205	0.019 (0.009)	0.207 (0.05)	AY363674

F, forward primer; R, reverse primer; H_O, population mean (standard error) of observed heterozygosity; H_E, population mean (standard error) of expected heterozygosity (Nei 1973).

Table 2 Presence and size range of duplicate loci and presence of null alleles based on 318 samples from 27 European populations

Locus	Duplicate loci	Size range of duplicate loci (bp)	Number of populations with null alleles
Ap1	often present	181–199	0
Ap2	often present with fragments mostly 20–40 bp longer than main product	116–164	1
Ap3	always present	87–92	6
Ap4	not detected		6
Ap5	not detected		4
Ap6	always present	106	0
Ap7	not detected		2
Ap8	always present	88–90	3

GeneScan 500. Expected and observed heterozygosity was determined using the MSA software (Dieringer & Schlötterer 2003). Since single populations harboured only a small amount of total allelic richness, we report allelic variability based on 318 samples from 27 European populations.

Eight primer pairs gave reproducible and interpretable PCR products (Table 1). Due to polyploidy of *Alliaria petiolata*, often more PCR products where detected than expected for a diploid codominant marker (Table 2). However, in most cases interpretation of banding patterns was straightforward. If present, duplicate loci where either monomorphic or if polymorphic they had very low amplification relative to the investigated locus. However, in two loci where amplification of the duplicated locus was low, interpretation problems may arise due to partial or complete overlap of allele sizes of duplicate loci with the locus of interest (Ap1 and Ap2). In some markers and some populations, fixed heterozygosity was observed. The presence of null alleles, i.e. total failure of amplification in some or all individuals of a population, was observed in six loci (Table 2). Null alleles were detected especially in populations that were geographically most distant from the location that was used for primer development.

All loci showed significant difference between expected and observed heterozygosity at the population level (data not shown). This was expected since the species has been decribed to be highly selfing (Cruden *et al*. 1996). The microsatellite markers will be used to analyse the breeding system in the native and introduced range and to infer the route of introduction from Europe into Northern America.

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