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PRIMER NOTE

Isolation and characterization of polymorphic microsatellite markers from fireweed, *Senecio madagascariensis* Poir. (Asteraceae)

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Abstract

Ten polymorphic microsatellite loci were isolated and characterized from invasive fireweed (*Senecio madagascariensis*) populations in the Hawaiian archipelago. These loci provided markers with polymorphism of six to 24 alleles per locus within 96 individuals collected from two populations from the island of Maui. The expected and observed heterozygosities ranged from 0.31 to 0.91 and from 0.056 to 1. These markers should be useful to study the importance of genetic diversity in invasion success of this species.

Keywords: fireweed, invasive species, microsatellite, *Senecio madagascariensis*

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Senecio madagascariensis Poir. (Asteraceae), an Afro-Madagascan native, has been introduced outside its native range and invades regions in Kenya, Argentina, Colombia, Australia and Hawaii (Sindel *et al*. 1998; Le Roux *et al*. 2006). *Senecio madagascariensis* forms part of a species complex and its taxonomy is problematic and in need of revision (Le Roux *et al*. 2006). The population biology and genetics of this species have not been studied extensively and coupled with its invasive success and large geographical native range poses a good system to investigate the role of genetic diversity, multiple introductions and dispersal in invasion success. Here we developed 10 microsatellite markers from *S. madagascariensis* to address some of these issues.

Total DNA was extracted with the DNeasy Plant mini kit (QIAGEN) from 40 to 50 mg of fresh leaf material. Extracted genomic DNA was digested with *Rsa*I, *Hae*III, *Alu*I and *Hinc*II and the resulting fragments simultaneously ligated onto SNX oligonucleotide linkers (Hamilton *et al*. 1999). Linker-ligated digests were hybridized to biotinylated oligoprobes $(AAC)_8$ $(AAT)_8$ $(GC)_{12}$ and $(GT)_{12}$ and subsequent probe-bound DNA extracted using streptavidin-coated magnetic beads. Following polymerase chain reaction (PCR) with the SNX forward primer only, PCR products of microsatellite-enriched DNA were purified with the QIAquick PCR purification kit (QIAGEN). These purified fragments were digested with *Nhe*I, and ligated into the *Xba*I site of pUC19 followed by transformation into competent *Escherichia coli* DH5α (Invitrogen).

Colonies were transferred onto nylon membranes after transformation and screened using digoxigenin-labelled $(AAC)_8$ $(AAT)_8$ $(GC)_{12}$ and $(GT)_{12}$ probes to identify positive clones. The inserts of 162 putative microsatellite-containing colonies were amplified with universal M13 plasmid primers, purified and subjected to sequencing using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems) and an automated sequencer (ABI PRISM 377XL DNA sequencer, PE Applied Biosystems). One hundred and thirteen of the 162 sequences had limited flanking region(s) for primer design or no or imperfect microsatellite sequence repeats. PCR primers were designed from the remaining 49 sequences using the program FASTPCR (Kalendar 2004).

We tested and optimized amplification with a gradient PCR at 48–60 °C range of annealing temperatures. Each reaction contained 6 μL HotMasterMix [HotMaster *Taq* DNA polymerase, 0.3 U; 2.5× HotMaster *Taq* Buffer pH 8.5, 45 mM KCl and 2.5 mM $MgCl_2$; 200 μM of each dNTP; (Brinkman Instruments, Inc.)], 7.5 pmol of each primer and

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Table 1 Characteristics of 10 microsatellite markers isolated from *Senecio madagascariensis*. Results are given for two different populations

Locus	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	No. of alleles	Allele size range (bp)	Pop 1 H_O/H_E	Pop 2 H_O/H_E	GenBank Accession no.
Se-46*	$(GT)_{32}$	F: HEX-GGGTTAAAAGTGTAATTATGGC R: TGAAACCCGAATCGCCGTC	52	6	183–197	1/0.748	1/0.704	DQ886396
Se-76*	$(GTT)_{13}$	F: NED-GGAGGTCCAAATACGTTTGCAC R: TCGTCAAATGAAACTCACGGAC	58	8	537–615	0.47/0.841	0.3/0.82	DQ886397
Se-116	$(AAC)(AC)(AAC)_{14}$	F: NED-CCTTCTGGTTGATTTGGCTAAGC R: AGAACTGCACATTTGAAGCCTG	48	12	418–466	0.667/0.642	0.71/0.77	DQ886398
Se-136*	$(GA)_{25}$	F: HEX-CAAAGGTAGGATGATGTGAAGCTC R: TCTTGTTGGGTCAATGCTCG	51	20	315–395	0.056/0.859	0.292/0.904	DQ886399
Se-138*	$(TC)_{17}$	F: HEX-ACTTCGTGGGCCATTCCAG R: CTTCCTGCATAACATCCACCAC	58	19	180–232	0.482/0.847	0.419/0.902	DQ886400
Se-176(a)	$(GT)_{12}$	F: FAM–AGCATAGTGCAAGCATGTTCG R: CTTTGATGTTGGCTGCAATGC	60	21	232–288	0.778/0.862	0.839/0.91	DQ886401
Se-194*	$(GT)_{11}$	F: FAM–GTCGCAGTCACCGTCACTG R: GAGCAGCAGACAACGACAC	58	8	316–392	0.111/0.389	0.069/0.31	DQ886402
Se-206*	$(CT)_{43}$	F: HEX-ACGGGCGTTAAACTGCTCC R: TCCCCACCACCATCACCTC	58	18	352–404	0.333/0.82	0.581/0.616	DQ886403
Se-208*	$(TC)_{24}$	F: NED-TTTTGGGCAGGCCATATCC R: AGTGTCTCCACGGTTGTCG	55	24	301–379	0.593/0.907	0.6/0.796	DQ886404
Se-220	$(GT)_{10}$	F: NED-AACTCGACCAGTCCTCAGC	58	11	156–208	0.815/0.846	0.677/0.662	DQ886405

*Significant deficit of heterozygotes from expected under HWE ($P < 0.01$).

approximately 5 ng total genomic DNA. Thirty-six primer sets were successfully amplified. To detect polymorphism at each locus we ran PCR products from 12 different fireweed individuals on an Agilent 2100 Bioanalyser analysis LabChip (Agilent Technologies, Inc.) (Banerjea *et al*. 2003). One of the primers of loci that showed polymorphism was fluorescently labelled with either HEX, NED or 6-FAM (Integrated DNA Technologies, Inc.) and re-optimized as described above. PCR was conducted on an MJ Research PTC 100 cycler with a thermocycle of: initial denaturation of 94 °C for 2 min; 35 cycles at 94 °C for 60 s, locus-specific annealing temperature (see Table 1) for 60 s, elongation at 72 °C for 90 s; and final extension at 72 °C for 12 min. Polymorphisms were screened using an ABI PRISM 377XL DNA sequencer (PE Applied Biosystems), and PCR products were sized relative to a molecular size marker (LIZ500, PE Applied Biosystems). DNA fragments were analysed using the GENEMARKER version 1.4 program (SoftGenetics, LLC). For each locus we inferred expected and observed heterozygosities, significant deviation from Hardy–Weinberg equilibrium (HWE) and the existence of genotypic linkage disequilibrium (LD) using the GENEPOP version 3.4 program (Raymond & Rousset 1995).

Table 1 summarizes the characteristics of the 10 primer pairs developed from fireweed. Allelic variation at the 10 microsatellite loci was assessed in 96 individuals of *S. madagascariensis* from two different populations (less than 1.5 miles apart) on Maui. The number of alleles detected at the 10 loci ranged from six to 24 and expected and observed heterozygosities ranged from 0.31 to 0.91 and from 0.056 to 1. For both populations only three loci (Se-116, Se-176(a) and Se-220) had heterozygosities conforming to those expected under HWE. All other loci had heterozygosities that deviated from HWE expectations ($P < 0.01$) and could be the result of null alleles and/or inbreeding effects often experienced by introduced species originating from a single introduction event. No significant LD was found for all loci.

These 10 polymorphic loci will be utilized to assess the genetic diversity, genetic structure and dispersal patterns of invasive fireweed populations in and among the different Hawaiian Islands.

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