

## PRIMER NOTE

# Isolation and characterization of highly polymorphic microsatellite markers in *Hypochaeris radicata* (Asteraceae)

C. MIX,\* P. F. P. ARENS,† N. J. OUBORG\* and M. J. M. SMULDERS†

\*Department of Ecology, Radboud University Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, the Netherlands and †Plant Research International, Wageningen UR, PO Box 16, 6700 AA Wageningen, the Netherlands

## Abstract

We developed five highly polymorphic dinucleotide microsatellite loci for the grassland species *Hypochaeris radicata* (Asteraceae). Polymorphism of these markers was examined in six populations in the Netherlands. All loci were polymorphic in all populations. The number of alleles per locus varied between 18 and 43. Expected heterozygosity was between 0.86 and 0.91. Cross-species amplification was tested in six *Hypochaeris* species and was successful for three different loci in four species. These microsatellites are a useful tool in population genetic, dispersal and metapopulation studies or in testing levels of inbreeding.

*Keywords:* dispersal, grassland species, habitat fragmentation, microsatellites, population genetics

Received 25 June 2004; revision accepted 23 July 2004

*Hypochaeris radicata* L. ssp. *radicata* is a widespread rosette herb occurring in sandy grasslands (Grime *et al.* 1988). The species has no seed bank, is short-lived and mainly outcrossed; however, some levels of selfing have been reported (Picó *et al.* 2004). Fruits are equipped with a plumose pappus that is mostly attached to a beak, enabling long-distance seed dispersal by wind (Soons *et al.* 2004). These characteristics make it suitable as a model species in metapopulation studies, in studies on selection on dispersal behaviour and in studies assessing levels of inbreeding. We have developed microsatellite markers for this species to be able to investigate the genetic consequences of habitat fragmentation, as fragmented habitats are a seriously threat to plant population persistence (Young *et al.* 1996).

DNA of three individuals was isolated using DNeasy Plant Mini Kit (Qiagen). Microsatellite loci were developed on *Mbo*I-digested genomic DNA of *H. radicata* using an enrichment procedure (Karagoyozov *et al.* 1993). The (GA) and (GT) repeat-enriched libraries were constructed according to Arens *et al.* (2000) with the modifications as described in Arens *et al.* (2004). *Escherichia coli* clones containing pCRII-TOPO vector with a DNA fragment that hybridized to synthetic (GA) or (GT) repeats were sequenced using

SequiTherm EXCEL™ DNA Sequencing Kit-LC and protocol of Epicentre Technologies on a Li-cor 4000 L (LI-COR). During sequencing, IRD-800 dye-labelled M13 primers were used.

Primer pairs were designed for 15 microsatellite loci using PRIMER3 (Rozen & Skaletsky 2000). Polymerase chain reactions (PCR) were performed in 20 µL containing 4 ng DNA (2 ng DNA for HrGA9 and HrGT4), 1.5–4.0 mM MgCl<sub>2</sub> (see Table 1), 1 U Red Hot *Taq* DNA polymerase (Abgene), 2 µL 10 × Reaction buffer (included in *Taq* batch), 200 µM of each dNTP, and 1 pmol of each primer (the forward primer was always labelled with 700 IR dye). Amplifications were performed on a T3 Thermocycler (Biometra). Except for HrGA10, PCR cycles followed the profile: 1 cycle of 5 min at 94 °C predenaturing, 40 cycles (in the case of HrGA9, 35 cycles) of 45 s at 94 °C denaturing, 45 s at annealing temperature (Table 1), 1 min at 72 °C extension, and a final extension of 10 min at 72 °C. For HrGA10, temperature was decreased from 60 °C to 50 °C with a 0.5-°C decrease per cycle following a Touch Down protocol. Fragments were analysed on the Li-cor 4200 IR<sup>2</sup> DNA analyser (LI-COR) using LI-COR size standard IR Dye 50–350 bp. Bands were scored using the SAGA<sup>GT</sup> software (LI-COR). Two primers [HrGA7, a (GA)<sub>28</sub> repeat, Accession no. AY650910 and HrCAGA a (CA)<sub>14</sub>(GA)<sub>9</sub> repeat, Accession no. AY650911] did not show polymorphism in a test set of 12 randomly chosen plants, and eight

Correspondence: Carolin Mix. Fax: + 31 24 365 2134;

E-mail: c.mix@science.ru.nl

**Table 1** Characteristics of five microsatellite loci in *Hypochaeris radicata* and genetic diversity in six populations. Primer sequences (F, forward; R, reverse), annealing temperature ( $T_a$ ),  $MgCl_2$  concentration, size range, mean number of alleles per locus ( $A$ ), overall means of observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity are given

Locus	Repeat motif	Primer sequence (5'–3')	$T_a$ (°C)	$MgCl_2$ (mM)	Size range (bp)	$A$	$H_O$	$H_E$	GenBank Accession no.
HrGA9	(GA) <sub>19</sub>	F: CAACCCCTCTCTTCCTTTC R: CACCACCACCAACACAAAAC	61	1.5	104–142	31	0.80	0.88	AY650905
HrGA10	(GA) <sub>61</sub>	F: CCGGAAAACAGGAGAGTCAT R: ACCACCACAGAACTCCGGTA	TD*	3.0	116–226	29	0.78	0.86	AY650906
HrGA12	(GA) <sub>26</sub>	F: GGACTCTCATCCCCATCT R: GTGTGTGGGAGGTGTGGT	52	2.0	142–240	26	0.94	0.91	AY650907
HrGA14	(GA) <sub>24</sub>	F: GATTCGAGCAGGAAGAGACG R: ACAAAAATAGGCGCGGTCTA	62	4.0	160–230	37	0.82	0.88	AY650908
HrGT4	(GT) <sub>25</sub>	F: TGTTCCTCTCTGTGTGCAT R: CCACCCTGTGGCTGAATTT	55	2.5	149–211	18	0.87	0.89	AY650909

\*Touch-Down protocol (decrease of 0.5 °C/cycle from 60 °C to 50 °C).

**Table 2** Cross-species amplification with *Hypochaeris radicata* microsatellite primers

Species	Sample origin	Locus				
		HrGA9	HrGA10	HrGA12	HrGA14	HrGT4
<i>H. salzmanniana</i>	Cádiz, near Barbate (Spain)	–	+	++	–	–
<i>H. salzmanniana</i>	Cádiz, near Palmones (Spain)	–	++	++	–	–
<i>H. salzmanniana</i>	Mamora forest (Morocco)	++	++	++	–	–
<i>H. echegarayi</i>	Cañuma (Bolivia)	–	–	†	–	–
<i>H. variegata</i>	Sierra de la Ventana (Argentina)	–	–	++	–	–
<i>H. albiflora</i>	Sarmiento (Argentina)	–	–	++	–	–
<i>H. palustris</i>	Cerro Tronador (Argentina)	–	–	–	–	–
<i>H. apargioides</i>	Yumbel (Chile)	–	–	–	–	–

Amplification tests on two individuals per species: ++, very good amplification; +, weaker amplification, – no amplification.

\*Indicates polymorphic bands between individuals.

†Indicates individuals with a homozygote band pattern.

primers produced products of unexpected sizes or many stutter bands.

Five primer pairs showed unambiguously scorable patterns and were tested on 30 plants from six populations. The five loci turned out to be highly polymorphic, having from 18 to 43 different alleles (Table 1). A significant genotypic association was found for one out of the 10 pairs of loci: HrGA9 and HrGA12 ( $P = 0.0013$ ), as tested using GENEPOP 3.4 (Raymond & Rousset 1995). The program was also used to test for Hardy–Weinberg equilibrium (HWE). Except for HrGA12 no locus was found to be in HWE. In the populations, expected and observed heterozygosities varied between 0.78 and 0.94 as estimated in FSTAT, version 2.9.3 (Goudet 1995). Results show that 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 this species.

Cross-species amplification was investigated for two samples from six different species of the genus *Hypochaeris* using the loci and PCR conditions of *H. radicata*. Results are summarized in Table 2. Successful amplification for most of the species was obtained for locus HrGA12, and *H. salzmanniana* also showed amplification for the loci HrGA9 and HrGA10 (Table 2).

### Acknowledgements

We thank Ramses Rengelink, Hans de Jong en Annemiek Wernke for their assistance in the laboratory. We are grateful to Karin Tremetsberger (University of Vienna) for samples of other *Hypochaeris* species. Vincent Castric provided helpful comments to improve the manuscript. The research was supported by the Netherlands' Organization for Scientific Research (NWO project 805-33-451) and by the European Community (TRANSPLANT project, Evk2-1999–00042).

## References

- Arens P, Van't Westende W, Bugter R, Smulders MJM, Vosman B (2000) Microsatellite markers for the European tree frog *Hyla arborea*. *Molecular Ecology*, **9**, 1944–1946.
- Arens P, Durka W, Wernke-Lenting JH, Smulders MJM (2004) Isolation and characterisation of microsatellite loci in *Geum urbanum* (Rosaceae) and their transferability within the genus *Geum*. *Molecular Ecology Notes*, **4**, 209–212.
- Goudet J (1995) FSTAT version 1.2: a computer program to calculate Fstatistics. *Journal of Heredity*, **86**, 485–486.
- Grime JP, Hodgson JG, Hunt R (1988) *Comparative Plant Ecology: a Functional Approach to Common British Species*. Unwin-Hyman, London.
- Karagyozov L, Kalcheva ID, Chapman M (1993) Construction of random small-insert genomic libraries highly enriched for simple sequence repeats. *Nucleic Acid Research*, **21**, 3911–3912.
- Picó FX, Ouborg NJ, van Groenendael JM (2004) Influence of selfing and maternal effects on life-cycle traits and dispersal ability in the herb *Hypochaeris radicata* (Asteraceae). *Botanical Journal of the Linnean Society* (in press).
- Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Rozen S, Skaletsky HJ (2000) Primer 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener), pp. 365–386. Humana Press, Totowa, NJ, USA. [http://frodo.wi.mit.edu/cgi-bin/primer3/primer3\\_http://www.cgi](http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_http://www.cgi)
- Soons MB, Heil GW, Nathan R, Katul GG (2004) Determinants of long-distance seed dispersal by wind in grasslands. *Ecology*, **85** (in press).
- Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution*, **11**, 413–418.