Molecular Ecology Resources (2008) **8**, 1491–1493 doi: 10.1111/j.1755-0998.2008.02233.x

PERMANENT GENETIC RESOURCES

Development of microsatellite markers for the bracken fern, *Pteridium aquilinum*

XIAOQING CHEN,*† JIANXIU WANG,*† HONGLI TIAN,*† XIANCHUN ZHANG,* JUN WEN‡ and SHILIANG ZHOU*

**State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China, †Graduate School of the Chinese Academy of Sciences, Beijing 100039, China, ‡Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington D.C. 20013-7012, USA*

Abstract

We isolated eight novel polymorphic microsatellite loci from *Pteridium aquilinum*. These loci were characterized in 30 individuals, one from Bolivia, two from Peru, one from the USA, one from Japan, and 25 from Northeast China to Southwest China. The number of alleles per locus ranged from two to seven. The observed heterozygosity (H_O) ranged from 0.000 to 0.600 with an average of 0.3051, and the expected heterozygosity (H_E) ranged from 0.0966 to 0.7780 with an average of 0.4267. One locus deviated from Hardy–Weinberg equilibrium and four pairs of loci were found to be in linkage disequilibrium. These polymorphic loci will be useful in the study of the population genetic structure of *Pteridium*.

Keywords: microsatellite, polymorphic, primers, *Pteridium aquilinum*

Received 30 November 2007; revision received 23 February 2008; revision accepted 9 May 2008

Pteridium aquilinum (L.) Kuhn (Dennstaedtiaceae) is widespread throughout the world and its distribution and abundance are tending to increase with global warming (Le Duc *et al*. 2003). What seems a serious problem is that it has recently become a weed invasive to farmland and toxic to other plants. The invasion of bracken fern has caused loss of incomes of farmers and threatens the survival of other valued plants (Pakeman *et al*. 2000). Although mechanical, chemical and biological controls have been imposed on the weed, none of these methods has been effective so far (Le Duc *et al*. 2003). To control *P. aquilinum* effectively, it is necessary to understand the biology of the species, for example, the relative contribution of sexual reproduction vs. clonal propagation, mechanisms of spore dispersal, its genetic structure and geographical patterns, in addition to ecological and evolutionary strategies adopted by the species to cope with suppressions. To achieve these goals, we need molecular markers. Although some molecular markers such as isozyme (Speer *et al*. 1999), AP-PCR and ISSR (Thomson *et al*. 2005) have already been tried on *P. aquilinum*, hypervariable codominant markers seem more helpful in answering the questions mentioned above. Microsatellite markers are favoured for their high sensitivity, uniform genome coverage and neutrality (Pan *et al*. 2007). These markers have been very popular in studies of seed plants, but they have rarely been used in ferns. Here we report microsatellite loci isolated from *P. aquilinum* for the first time.

Fresh leaves of *P. aquilinum* were collected in the botanical garden of the Institute of Botany, the Chinese Academy of Sciences, Beijing, China. Genomic DNA was extracted using Plant Genomic DNA Purification Kit (Tiangen). An enriched microsatellite library was constructed following Glenn & Schable (2005) with some modifications. Total DNA was digested with *Rsa*I (New England Biolabs). The digested fragments were ligated to double-stranded SuperSNX-24 linkers (forward 5′-GTTTAAGGCCTAGCTAG-CAGAATC-3′, reverse 5′-pGATTCTGCTAGCTAGGCCT-TAAACAAA-3′). The ligated fragments were amplified through polymerase chain reaction (PCR) using the forward strand of the SuperSNX24 linker as a primer. The amplified product was hybridized to a mixture of single-stranded biotinylated microsatellite probes: $(AG)_{12}$, $(AT)_{12}$, $(CG)_{12}$, $(GT)_{12}$, $(ACG)_{12}$, $(ACT)_{12}$, $(CCA)_8$, $(AACT)_8$, $(AAGT)_8$ and $(AGAT)_8$ (Invitrogen) for enrichment. The DNA fragments hybridized to the probes were captured by

Correspondence: Shiliang Zhou, Tel. +86 (10) 62836503;
E-mail: slzhou@ibcas.ac.cn

Table 1 Characteristics of the eight microsatellite loci from *Pteridium aquilinum*

Locus name	Motif	Primer sequence (5′–3′)	Expected size (bp)	T_a (°C)	A	H_E	H_O	Brookfield 1	P	GenBank Accession no.
Pter03	$(TG)_{14}$	F: AAGATCAATCGCGGACAC	245	57	3	0.4384	0.5600	–0.0912	0.3208	EU514456
		R: CATTCTCACTAACTACCCAC								
Pter04	$(CA)_6$	F: ATCAAGCCAAGGTCAC	215	48	2	0.3429	0.2143	0.0916	0.0730	EU514457
		R: AACCCATGATTGCTAAT								
Pter06	$(AG)_{19}$	F: CCTCCATTCTTGTCTCATTTTATC	214	50	3	0.3367	0.2667	0.0484	0.0209	EU514458
		R: CTACTCATCTACCTGCTCTTGC								
Pter07	$(AG)_{27}$	F: CTCCTTCACTTATGCTCTGG	192	54	3	0.3473	0.1481	0.1437	0.0011	EU514459
		R: TACGGCTGGATTTTATTTATC								
Pter09	$(CT)_8(GTCTCT)_3(CT)_{15}$	F: GGAGGTGGCTATTATTGT	313	50	7	0.6243	0.6000	0.0086	0.0958	EU514460
		R: CTCTTCTAGCAAGCAGGT								
Pter10	$(CT)_{18}(ATCT)_5$	F: TTAGGCCACGCAGCAAAG	342	50	5	0.7780	0.5517	0.1059	0.0140	EU514461
		R: GGGTAGATGGGATCACTC								
Pter11	$(TC)_5$	F: GCCTTGCCTCGTAAGCATCTAG	286	50	2	0.0966	0.1000	–0.0046	1.0000	EU514462
		R: GAGTGAGAAAGTGCCTTTAGCC								
Pter12*	$(AT)_9$	F: TGGTGAAGTTGTGATGCCTAC	366	58	5	0.4497	0.0000			EU514463
		R: TATCGGTGGAAAGAAAGAGTG								
Mean						0.4267	0.3051			

T_a, annealing temperature; A, the number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; Brookfield 1, estimated null allele frequency; P, the P value for HWE testing for each locus; *locus from the chloroplast genome.

streptavidin-coated paramagnetic beads (Dynal Biotech Dynabeads M-280 Streptavidin) and eluted from the beads. The enriched DNA was recovered by PCR amplification and enriched again. Fragments recovered from the second enrichment were purified with PEG8000 and ligated into pGEM-T easy vectors (Promega) and transformed into competent cells of *Escherichia coli* Top10 (TransGen Biotech). Transformants were selected by blue–white colour screening followed by PCR verification. Selected inserts were sequenced with ABI 3730*xl* DNA Analyser (Applied Biosystems), using BigDye Terminator version 3.1 and T7 and SP6 primers. Sequences were analysed with SEQUENCHER 4.6 (Gene Codes Corporation). Microsatellite motifs were identified (by Qiang Li, Nanjing Agricultural University, Nanjing, China) using the program SSRHUNTER 1.3. Primers were designed according to the flanking sequences of microsatellite motif using PRIMER PREMIER 5.0 (Premier Biosoft International).

These loci were characterized in 30 individuals, one from Bolivia, two from Peru, one from the USA, one from Japan, and 25 from Northeast China to Southwest China. PCR amplifications were performed and amplified fragments were separated by 4% denatured polyacrylamide gels using silver staining and pUC18 (Tiangen) was used as size standard.

The number of alleles and the observed/expected heterozygosities were calculated with GENEPOP version 3.4 (Raymond & Rousset 2003). Estimates of linkage disequilibrium and tests for departures from Hardy–Weinberg equilibrium (HWE) were conducted using GENEPOP version 3.4 (Raymond & Rousset 2003). Genotype frequencies were corrected with MICRO-CHECKER (Van Oosterhout *et al*. 2004).

A total of 243 positive clones were screened and 122 fragments were sequenced. Twenty-two fragments contained microsatellites and 12 fragments were unique. Eight of 12 pairs of primers were polymorphic. The number of alleles per locus varied from two to seven (Table 1). The observed heterozygosity (H_O) ranged from 0.000 to 0.600 with an average of 0.3051, and the expected heterozygosity (H_E) ranged from 0.0966 to 0.7780 with an average of 0.4267 (Table 1). The locus with zero observed heterozygosity (Pter12) is located in the chloroplast genome, and consequently, HWE testing and linkage disequilibrium (LD) estimates were performed without this locus. Locus Pter07 deviated significantly from HWE ($P < 0.01$). The deviation from HWE at Pter07 is mainly due to the occurrence of null alleles, the estimated frequency of which was 0.1437 (Table 1). LD was detected between Pter11 and four loci (Pter04, Pter09, Pter10 and Pter03) ($P < 0.01$). It is mainly because that polymorphism at Pter11 exists globally, but not within either America or China. These polymorphic loci are expected to be useful in understanding the population genetic structure of *Pteridium* in the future.

Acknowledgements

The authors thank their colleagues for help during the experiment and for comments on the manuscript. This study was partly supported by the National Science Foundation of China (0121003 and 30370154).

References

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology* , **395**, 202–222.

Le Duc MG, Pakeman RJ, Marrs RH (2003) Changes in the rhizome system of bracken subjected to long-term experimental treatment. *Journal of Applied Ecology*, **40**, 508–522.

Pakeman RJ, Le Duc MG, Marrs RH (2000) Bracken distribution in Great Britain: strategies for its control and the sustainable management of marginal land. *Annals of Botany*, **85** (Supplement B), 37–46.

Pan L, Quan ZW, Li SM *et al*. (2007) Isolation and characterization of microsatellite markers in the sacred lotus (*Nelumbo nucifera* Gaertn.). *Molecular Ecology Notes*, **7**, 1054–1056.

Raymond M, Rousset F (2003) GENEPOP 3.4, an updated version of GENEPOP V.1.2 (1995): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Speer WD, Werth CR, Hilu KW (1999) Relationships between two infraspecific taxa of *Pteridium aquilinum* (Dennstaedtiaceae) II. Isozyme evidence. *Systematic Botany*, **23**, 313–325.

Thomson JA, Chikuni AC, Mcmaster CS (2005) The taxonomic status and relationships of bracken ferns (*Pteridium*, Dennstaedtiaceae) from sub-Saharan Africa. *Botanical Journal of the Linnaean Society*, **148**, 311–321.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.