Microsatellite Letter for Conservation Genetic Resources

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Characterisation of microsatellite DNA markers for *Mirbelia bursarioides* A.M.Monro & Crisp ms.

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Abstract

A genomic library was constructed and 12 novel polymorphic di- and tri-nucleotide nuclear microsatellite markers were developed for *Mirbelia bursarioides* (Fabaceae), a shrub from the semiarid zone of southwest Western Australia. Populations are patchily distributed in a highly fragmented landscape. Genetic diversity in a single population was high with the number of alleles per locus ranging from four to twelve. Expected and observed heterozygosities averaged 0.686 and 0.720 respectively. All loci showed independent inheritance. One locus showed possible evidence of null alleles. Population genetic structure is being investigated to inform appropriate seed collection and restoration strategies.

Microsatellite Letter

Microsatellite markers were developed for *Mirbelia bursarioides*. This prickly shrub is distributed in the northern parts of southwest Western Australia's semiarid rainfall zone. Populations are endemic to ironstone and granite outcrops in Acacia shrubland and mallee woodland and shrubland. Populations are patchily distributed as a result of edaphic endemism, extensive vegetation clearing for agriculture and grazing pressure. DNA was extracted from homogenised, freeze dried material from an individual originating near the centre of the species range using a NucleoSpin Plant II Maxi protocol following the manufacturers' instructions (Macherey-Nagel GmbH & Co, Düren, Germany). Genomic DNA was 454 shotgun sequenced using 1\8 of a picotiter plate on a GS-FLX Titanium machine (Roche Diagnostics Corporation, 454 Life Sciences, Branford, USA) by the Australian Genome Research Facility (Adelaide, Australia). Sequencing, microsatellite identification and primer design followed Gardner et al. (2011).

Amplification and polymorphism were evaluated in eight individuals from four populations using an M13 tailed three primer PCR system. DNA was extracted using MoBio PowerPlant Pro DNA isolation kits (MoBio, Carlsbad, USA). Loci were amplified in a total volume of 7.5 µl per reaction containing 1 ng template DNA, 50 mM KCl, 20mM Tris HCl (pH 8.4), 0.2 mM each dNTP, 0.16 µM M13 tailed fluorescently labelled forward and reverse primer, 0.032 µM M13 tailed forward primer, 0.175 µl of *Taq* DNA polymerase (Invitrogen) and 4.5 mM $MgCl_2$. Amplification reactions were performed with a program consisting of 95°C for 4 min, 25 cycles of 30 s at 94°C, 30 s of step down from 65°C to 50°C, 80 s at 72°C followed by 30 cycles of 15 s at 94°C, 15 s at 50°C, and 45 s at 72°C followed with a final step of 8 min at 72°C. Reactions were completed in an Eppendorf™ Thermal Cycler (Eppendorf, New York, USA) and PCR products visualised on 1% agarose gels stained with GelRed™ nucleic acid stain (Biotium, San Fransisco, USA).

Amplification was trialled for 50 primers. 38 failed to amplify or produced numerous bands that could not be resolved under high stringency conditions. The final 12 loci were further tested in 20 individuals from a single population located near the centre of the species distribution (Table 1). One µl of diluted PCR product was added to 12 µl of GeneScan™ LIZ®500(-250) size standard/formamide, and fragments visualised using a 3730 DNA Analyser. Genotypes were scored using Genemapper™ v3.7 (Applied Biosystems). Tests for heterozygotic deficit and excess were conducted using the Markov chain method and tests for Linkage Disequilibrium (LD) were conducted using Fisher's exact tests in GENEPOP v3.4 (Raymond & Rousset 1995).

Bonferroni corrections were applied for multiple comparisons. Evidence for large allele drop-out, scoring error due to stutter and null alleles were assessed with MICRO-CHECKER (Oosterhout et al. 2004). Genetic diversity parameters were estimated using GenAlEx v6.2 (Peakall & Smouse 2006) (Table 1).

Overall, polymorphism was moderate (Table 1). All loci were polymorphic. Number of alleles per locus averaged 7.167 and the effective number of alleles averaged 3.576. There was no evidence of significant LD ($p < 0.001$) and there was evidence of possible null alleles at one locus (Table 1). All loci were in Hardy Weinberg equilibrium and there was no evidence of large allele drop-out or scoring error due to peak stutter. Expected heterozygosity averaged 0.686 and observed heterozygosity averaged 0.720. These markers will be used to study genetic structure in *M. bursarioides* as part of a broader investigation to inform appropriate seed collection and restoration strategies for a number of keystone species in this landscape.

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References

Gardner M, Fitch A, Bertozzi T, Lowe A (2011) Rise of the machines - recommendations for ecologist when using next generation sequencing for microsatellite development. Mol Ecol Resour 11:1093-1101

Oosterhout C, Hutchinson W, Willds D, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535-538

Peakall R, Smouse P (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288-295

Raymond M, Rousset F (1995) GENEPOP (version 3.4): population genetics software for exact test and ecumenicism. Journal of Heredity 86:248-249

Table 1. Characterisation of 12 microsatellite loci for *Mirbelia bursarioides*. Details are given for locus name, primer sequence, GenBank accession number, repeat motif, program used for PCR amplification, the size range of observed alleles in base pairs (including the M13 tail), number of alleles (N), observed (H_o) and expected (H_e) heterozygosities, estimate of the fixation index (F).

Locus	Primer sequence (5′-3′)	GenBank accession no.	Repeat motif	Allele size range	N	N_e	H_o	H_e	F
Mb002	F:TTCGGTTCCGTTCAAACAGT R:GCGGAGGCAGTTATGTTGAC	KJ493766	$(AG)_{10}$	204-239	8	3.620	0.950	0.724	-0.313
Mb018	F:TCCCTCTGATAAGCCCAACA R:TCAAACTGGTCCTCCAGATCA	KJ493767	$(AGT)_9$	120-132	8	4.494	0.800	0.778	-0.029
Mb020	F: GTCCCTTATCCCACCACCTC R:AGAGGGAGACTGCAAGTGGG	KJ493768	$(AG)_{13}$	152-164	4	2.128	0.700	0.530	-0.321
Mb023	F: GGGTTCACAGATCTCCTCCA R:TCAAATTGACCAGTGAAACGG	KJ493769	$(AAC)_8$	100-108	4	2.423	0.684	0.587	-0.165
Mb025	F:TCCCTCTTTCTCTTCTCCCA R: ACCAACAAAGGTCTGCAAGC	KJ493770	$(AAT)_{12}$	169-181	5	2.974	0.550	0.664	0.171
Mb026[a]	F:GGCTTTGGCCTGTCTTGATA R:GGAAGGTGTGAGCCATAATCA	KJ493771	$(AC)_{13}$	243-331	8	4.233	0.550	0.764	0.280
Mb028	F:GCCAATGTGCTTAGTAAAGGGA R:TGAGTTCATGCAGGCGAGTA	KJ493772	$(AG)_8$	181-207	12	5.674	0.750	0.824	0.090
Mb035	F:GTGGGTGCGCATACCTTAAT R:TGCTCCATAGCACAAATCTCA	KJ493773	$(AG)_9$	116-158	11	2.000	0.450	0.500	0.100
Mb040	F:GGATCACAATAATTGATTCCGC R:CAGCTTTGGGAGAACATGAAA	KJ493774	$(AG)_9$	127-132	4	2.402	0.550	0.584	0.058
Mb046	F:TTCCAGAGGGAATAAGTCATCG R:TTCTTATTCATCGCTCCGTTTC	KJ493775	$(AAT)_{10}$	107-122	5	3.150	0.750	0.683	-0.099
Mb047	F:TGAGATCTGAATTCGGTACATGC R:CGCATCTTCCTTAGTAGCCG	KJ493776	$(AAC)_8$	99-128	8	4.651	1.000	0.785	-0.274
Mb048	F:TCTACACATTCTTGTGCGGC R:GAAAGGTGATGAAATCTTGAAGC	KJ493777	$(AAT)_{13}$	136-171	9	5.161	0.900	0.806	-0.116
Mean (standard error)					7.167 (0.796)	3.576 (0.361)	0.720 (0.050)	0.686 (0.033)	-0.051 (0.057)

[a] Evidence of null alleles.