Microsatellite Letter for Conservation Genetic Resources

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Characterisation of microsatellite DNA markers for *Grevillea paradoxa* (F. Muell)

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Abstract

A genomic library was constructed and 14 novel polymorphic di- and tri-nucleotide nuclear microsatellite markers were developed for *Grevillea paradoxa*, the Bottlebrush Grevillea, a common shrub in southwest Western Australia. Populations are patchily distributed in a highly fragmented landscape due to extensive vegetation clearing for agriculture and population genetic structure is being investigated to inform appropriate seed collection and restoration strategies. Moderate diversity was observed in two populations with the number of alleles per locus ranging from one to nine. Expected and observed heterozygosities averaged 0.434 and 0.344 respectively. All loci showed independent inheritance but there was evidence of possible null alleles at some loci in each population.

Microsatellite Letter

Microsatellite markers were developed for *Grevillea paradoxa,* the Bottlebrush Grevillea. This common prickly shrub has a widespread distribution in species-rich southwest Western Australia, although populations are patchily distributed due to extensive vegetation clearing for agriculture. DNA was extracted from 40 mg of homogenised, freeze dried material from an individual originating near the centre of the species range, following the method of Doyle and Doyle (1987), with addition of PVP-40T polyvinyl pyrrolidine to the extraction buffer and two chloroform extraction steps. 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. Loci were amplified in a total volume of 15 µl per reaction containing 5 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, 0.15 µl of *Taq* DNA polymerase (Invitrogen) and 3.0mM $MgCl_2$. Amplification was optimised for one of two PCR programs (Table 1). Program one consisted of 95°C for 4 min, 15 cycles of 30 s at 94°C, 30 s of step down from 65°C to 50°C, 45 s at 72°C followed by 30 cycles of 15 s at 94°C, 15 s at 50°C, 45 s at 72°C followed by 8 min at 72°C. Program two consisted 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 by 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).

Four primers failed to amplify or produced numerous bands that could not be resolved under high stringency conditions. Two were monomorphic. The final 14 loci were further tested in 20 individuals from each of two populations; Morowa in the north-west and Narambeen in the south east (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 an 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). 96.43% of loci were polymorphic. Number of alleles per locus averaged 4.500 and the effective number of alleles averaged 2.178. There was no evidence of significant LD ($p < 0.001$) although there was evidence of possible null alleles at some loci in each population (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 0.434 and observed heterozygosity averaged 0.344. Values of the fixation index varied over all loci (Table 1) averaging 0.213 and genetic differentiation between the two populations was high ($F_{ST} = 0.122$). These markers will be used to study genetic structure in *G. paradox*a 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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