MICROSATELLITE PRIMERS IDENTIFIED BY 454 SEQUENCING IN THE FLOODPLAIN TREE SPECIES Eucalyptus victrix (Myrtaceae)1

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• Premise of the study: Microsatellite primers were developed for Eucalyptus victrix (Myrtaceae) to evaluate the population and spatial genetic structure of this widespread northwestern Australian riparian tree species, which may be impacted by hydrological changes associated with mining activity.

• Methods and Results: 454 GS-FLX shotgun sequencing was used to obtain 1895 sequences containing putative microsatellite motifs. Ten polymorphic microsatellite loci were identified and screened for variation in individuals from two populations in the Pilbara region. Observed heterozygosities ranged from 0.44 to 0.91 (mean: 0.66) and the number of alleles per locus ranged from five to 25 (average: 11).

• Conclusions: These microsatellite loci will be useful in future studies of population and spatial genetic structure in E. victrix, and inform the development of seed sourcing strategies for the species.

Key words: 454 GS-FLX; Eucalyptus victrix; microsatellite primers; northwestern Australia; riparian; shotgun sequencing.

The genus Eucalyptus L’Hér. (Myrtaceae) is one of the most diverse of the temperate trees with more than 700 species recognized (Brooker, 2000), many with a high commercial or conservation significance. Eucalyptus victrix L. A. S. Johnson & K. D. Hill is a small tree (<15 m tall) that is widely but patchily distributed in northwestern Australia. The species is dependent on groundwater and is confined to floodplains and river-flats north of the Murchison River to Port Hedland and eastward into the central Northern Territory (Hill and Johnson, 1994). In the Pilbara region, mining activity and associated hydrological changes may impact some populations of this riparian tree species. To understand what impact the possible loss of populations may have on overall genetic diversity of the species and to inform seed sourcing for rehabilitation of mine sites, knowledge is needed on the level and structure of genetic diversity within the species. Here, we report the isolation and characterization of 10 polymorphic microsatellite loci that will be used to examine the spatial genetic structure and levels of gene flow within and among the E. victrix populations in riparian communities, in the Pilbara region of northwestern Western Australia.

METHODS AND RESULTS

We isolated genomic DNA (5 μg) from the leaf tissue of one individual of E. victrix following the protocol of Glaubitz et al. (2001). Shotgun sequencing was performed at the Ramaciotti Centre for Gene Function Analysis (University of New South Wales, Sydney, Australia) on a Roche 454 GS-FLX sequencer with titanium chemistry (Roche Applied Science, Indianapolis, Indiana, USA) following Gardner et al. (2011). The sample occupied 12.5% of a plate and produced 94,885 individual sequences, with an average read length of 344 bp, of which 1895 contained microsatellites. We used the program QDD version 1 (Meglécz et al., 2010) to screen the raw sequences for eight or more di-, tri-, tetra-, or pentabase repeats, remove redundant sequences, and design primers (automated in QDD using Primer3 [Rozen and Skaletsky, 2000]). Default values were used for running parameters except PCR product lengths set to 90–450 bp. Primer pairs were designed for 239 different loci. We excluded all loci that contained imperfect repeats or short repeat motifs within the flanking region or primer sequence, had a greater than 2°C difference between the forward and reverse primer annealing temperature, and polynucleotide runs of four or more in the flanking regions. We selected 30 loci for further development (GenBank accession nos.: JX423973–JX424002) and, initially, the loci were amplified using the cost-effective approach of Schuelke (2000) and a QIAGEN Multiplex PCR Kit (QIAGEN, Hilden, Germany). Loci were individually amplified in 20-μL reactions.

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Table 1. Characteristics of 10 nuclear microsatellite primers developed in Eucalyptus victrix.\(^a\)

| Locus  | Primer sequences (5′–3′) | Repeat motif | Size range (bp) | Label (Multiplex)\(^c\) | GenBank accession no. |
|--------|--------------------------|--------------|-----------------|--------------------------|----------------------|
| KPEV02 | F: CAGG/GGATTTTTACAGAAG  | (ATA)\(_4\)  | 129–205         | FAM (1)                  | JX423974             |
|        | R: TGGC/CTCTCCCTTCCAACT   |              |                 |                          |                      |
| KPEV03 | F: TCTG/TGTAGGATTTGAGAA  | (CT)\(_3\)   | 80–155          | PET (2)                  | JX423975             |
|        | R: TTGT/GCTCGGATTGAGAGA   |              |                 |                          |                      |
| KPEV04 | F: ACCG/CGGAAAGGCTTCTCA  | (CT)\(_7\)   | 154–168         | PET (1)                  | JX423976             |
|        | R: CGAGGGAAATAGAAGCACCAC |              |                 |                          |                      |
| KPEV09 | F: GCTT/TCTGCTGATCTTCTCA | (AGA)\(_4\)  | 120–138         | NED (1)                  | JX423981             |
|        | R: CGAC/GTCTGATATGCTTACA  | (AG)\(_6\)   | 154–191         | NED (2)                  | JX423982             |
| KPEV10 | F: AGTC/CCAGCAACCTCATAC  | (AGC)\(_3\)  | 120–135         | FAM (2)                  | JX423985             |
|        | R: ACCT/GTCTCTGAGAGAGAT  |              |                 |                          |                      |
| KPEV13 | F: TTTG/TCTCAGCAGCTTCTCA | (TTC)\(_6\)  | 210–216         | VIC (1)                  | JX423992             |
|        | R: TGGT/GCTCTATTGGCTGAT  |              |                 |                          |                      |
| KPEV20 | F: AGG/CTCTGCCTCGATATT  | (GA)\(_7\)   | 196–256         | FAM (S)                  | JX423994             |
|        | R: ATGC/AGGCTACCTGCTTCT  |              |                 |                          |                      |
| KPEV28 | F: CAGA/GTGGCAGACACTCGG  | (TCT)\(_12\) | 200–227         | FAM (2)                  | JX424000             |
|        | R: GTCT/GGGAGCTGGAGAGAT  |              |                 |                          |                      |
| KPEV30 | F: CCAGA/GACAGGAGCTAGAGA | (AG)\(_9\)   | 235–241         | FAM (1)                  | JX424002             |
|        | R: CTGAT/GGAGAAGCCGAAA   |              |                 |                          |                      |

\(^a\)Values are based on samples from two populations in the Pilbara region of Western Australia (WW-LP: UTM coordinates 725022E 7462878N, collection no. PN101; WW-MP: UTM coordinates 722880E 7462179N, collection no. PN102).

\(^b\)An annealing temperature of 56°C was used for all primers.

\(^c\)Multiplex marker sets are identified as (multiplex 1, 2, or single [S]).

Table 2. Results of primer screening in two populations of Eucalyptus victrix.\(^a\)

| Locus  | WW-LP | WW-MP |
|--------|-------|-------|
|        | A     | H\(_e\) | A     | H\(_e\) |
| KPEV02 | 17    | 0.91   | 18    | 0.90   |
| KPEV03 | 10    | 0.52   | 11    | 0.55   |
| KPEV04 | 6     | 0.65   | 6     | 0.50   |
| KPEV09 | 7     | 0.72   | 6     | 0.67   |
| KPEV10 | 8     | 0.44\(^*\) | 5   | 0.50\(^*\) |
| KPEV13 | 7     | 0.85   | 9     | 0.65   |
| KPEV20 | 6     | 0.62\(^*\) | 6   | 0.65\(^*\) |
| KPEV22 | 22    | 0.75   | 25    | 0.60\(^*\) |
| KPEV28 | 11    | 0.83   | 9     | 0.85   |
| KPEV30 | 5     | 0.70   | 5     | 0.80   |

\(^a\)Values are based on samples from two populations in the Pilbara region of Western Australia (WW-LP: UTM coordinates 725022E 7462878N, collection no. PN101; WW-MP: UTM coordinates 722880E 7462179N, collection no. PN102).

\(^b\)Significant deviations from Hardy–Weinberg equilibrium (\(*P < 0.01\) after correction for multiple tests (sequential Bonferroni procedure) are reported.

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CONCLUSIONS

The microsatellite loci developed for *E. victrix* in this study will be used to examine levels of past and current gene flow within and between geographically proximate riparian systems and inform seed sourcing for rehabilitation of mine sites.

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