Development of microsatellite markers in *Fontainea picrosperma*, isolated using 454 pyrosequencing

Gabriel Conroy, Robert Lamont, Steven Ogbourne

University of the Sunshine Coast, Maroochydore DC 4558, Qld, Australia

Introduction

The genus *Fontainea* consists of ten species, six of which occur in Australia. Five of these species are classified as threatened under the Environment Protection and Biodiversity Conservation (EPBC) Act.

While *Fontainea picrosperma* is the most common within the genus, very little is known about the species other than it is a small, dioecious understorey shrub that occurs on basalt soils at altitudes of 700–1000 m in the Atherton Tableland region of Queensland's Wet Tropics .

Aim

This research aims to ascertain whether the current distribution of genetic diversity within the species is reflective of historical, climate-related patterns of expansion and contraction during the Pleistocene.

The initial research objective towards this end goal is the isolation of informative polymorphic loci from the species and an assessment of the transferability of these loci across the genera.

Method

Leaf samples were collected from 166 *F. picrosperma* individuals from the Atherton Tableland region of Far North Queensland (Figure 1). Leaf samples were also collected from the closely related species *F. australis*, *F. fugax*, *F. oraria*, *F. rostrata* and *F. venosa*.

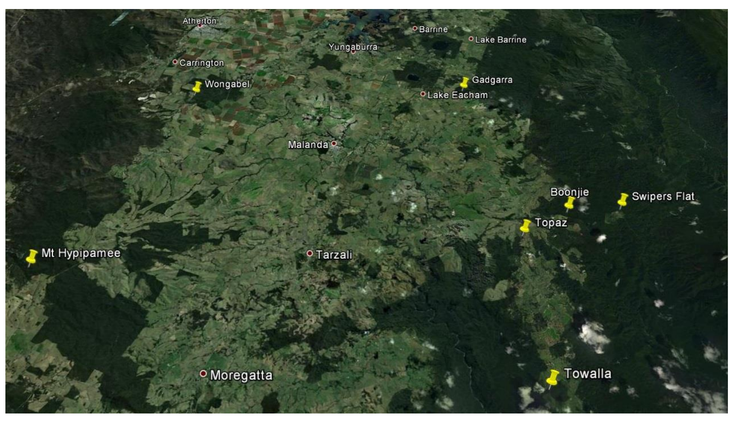

Figure 1: The Atherton Tableland region of Far North QLD, where F. picrosperma populations occur naturally .

Genomic DNA was extracted from the leaf material and submitted to the Australian Genome Research Facility (AGRF) to construct a random library that was sequenced using GS-FLX Titanium chemistry (Figure 2; Roche Applied Science).

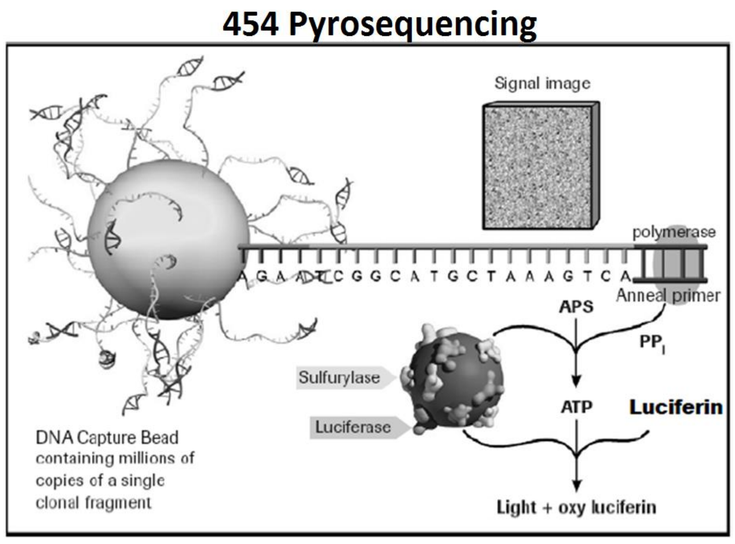

Figure 2: Phase II of the pyrosequencing process. DNA polymerase initiates incorporation of dNTPs to the template. As each correct complementary nucleotide is incorporated, pyrophosphates are released and converted to ATP. This provides fuel for the conversion of luciferin to oxyluciferin in a reaction that generates visible light in magnitudes proportional to the quantity of ATP. These chemiluminescent signals allow the determination of the sequence of the template. Image Source: NHGRI (2010).

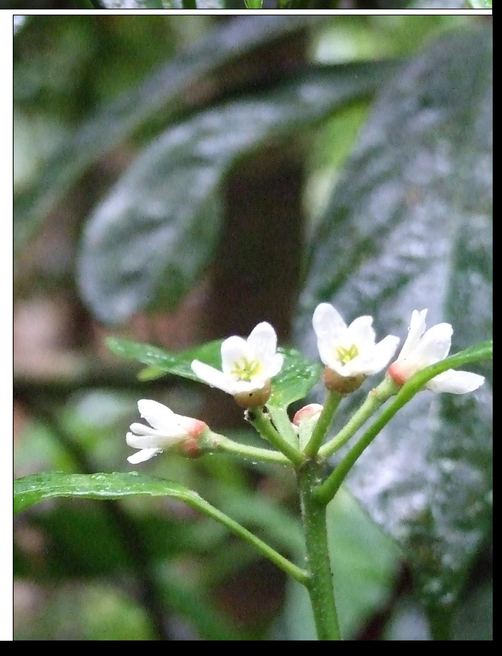

Figure 3: Male (above) and female (right) F. picrosperma plants with male and female flowers respectively .

Method

Microsatellite loci with a minimum of six repeats for di-nucleotides, and four repeats for tri- and tetra-nucleotides, were selected.

These loci were amplified and visualised on agarose gel to look for evidence of polymorphism (Figure 4).

Potentially useful microsatellite loci were forward labelled with fluorescent dyes (FAM, VIC, NED or PET).

Subsequent PCR products were separated by capillary electrophoresis.

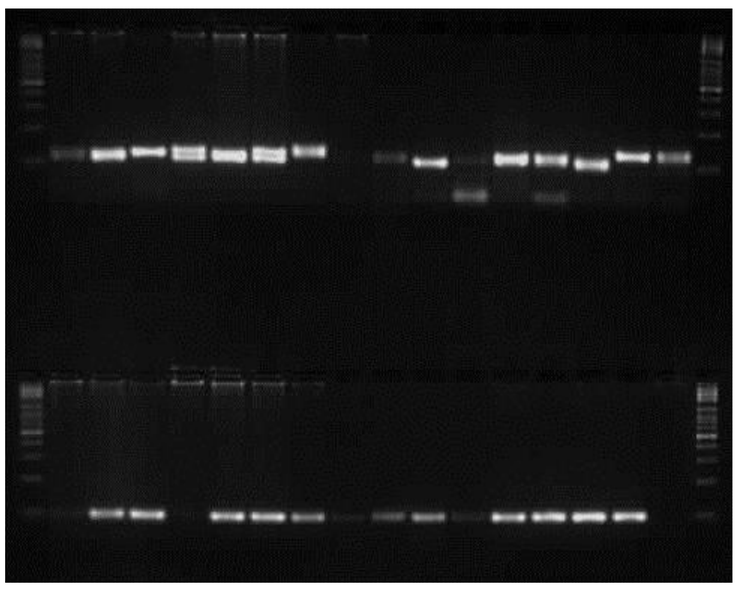

Figure 4: PCR products from four primer pairs across eight F. picrosperma individuals, visualised on 3% agarose gel.
The two primers on the top row are polymorphic, whereas the two primer pairs on the bottom row are relatively monomorphic by comparison

Fragment sizes were determined relative to internal lane standard using GENEMARKER v1.95 software (Figure 5; SoftGenetics).

Loci that exhibited polymorphism in *F. picrosperma* were tested for cross-amplification across the genera.

Population genetic diversity measures and inbreeding statistics were calculated for each loci.

Results

Eight polymorphic loci were identified in the study.

Numbers of alleles per locus (*NA*) ranged from 2 to 6 (mean 3.63; Table 1).

Three out of the eight loci deviated significantly from Hardy-Weinberg equilibrium ($p < 0.05$).

The polymorphism information content (*PIC*) of individual loci was generally low and ranged from 0.047 to 0.581 (mean 0.322; Table 1).

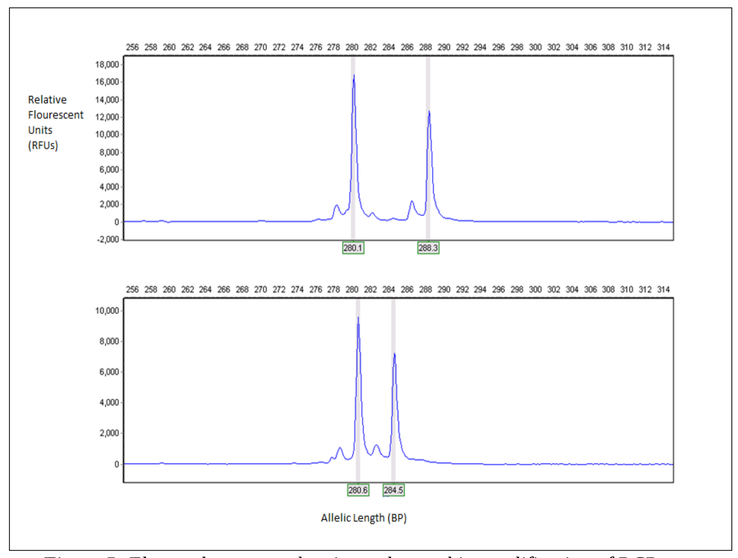

Figure 5: Electropherogram showing polymorphic amplification of PCR products for two F. picrosperma individuals within the expected size range of a microsatellite locus isolated using 454 pyrosequencing.

*Table 1: Characterization of eight microsatellite loci isolated from 166 individuals of Fontainea picrosperma collected from the Atherton Tablelands, Australia. PIC, polymorphic information content; N, number of individuals successfully amplified; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; * significant departure from HWE.*

Locus GenBank	Repeat motif	Primer sequences (5'-3')	Size range (bp)	*PIC*	*N*	N_A	H_O	H_E	F_{IS}
FP21 KC759358	$(TA)_{13}$	F: TCACTGAATTCGCTTGGTTG R: TGCAAATACCAGAAGTGCCA	194-204	0.581	166	6	0.500*	0.645	0.028
FP32 KC759359	$(GT)_8$	F: CTGGCTTGCATTTGCTTGTA R: TGCTAAACTTCAAGGGCTTAGG	190-192	0.332	166	2	0.337	0.422	-0.031
FP34 KC759360	$(AT)_6$	F: AGAGGTAGGCCGAAAGCAAT R: TGCAAACCAAAACCATTTCA	102-104	0.163	166	2	0.163	0.180	0.048
FP36 KC759361	$(AATA)_8$	F: ATTGTAGCACCCGCGAAAT R: CAGGACCATGAACGATGGAT	127-135	0.047	166	3	0.036	0.047	0.179
FP39 KC759362	$(GA)_{15}$	F: CTGCACGACAAGAAAACTCG R: TGAGTCAATATTGTAAGGGAATTATGA	203-213	0.288	166	3	0.277	0.315	-0.023
FP40 KC759363	$(TG)_{16}$	F: TTCTCGTCCTCTACTGGGCT R: CCCTACCTTTCCCACTCACA	134-152	0.449	166	6	0.512	0.550	-0.092
FP44 KC759364	$(AT)_7$	F: TGAAGCTAATTGCTTGATCTTCC R: GGGTATTTATTTTCTTGTTTGTTTCC	116-122	0.377	166	4	0.404	0.493	-0.025
FP47 KC759365	$(TC)_7$	F: CCTAAAAGTGCCCTTTGGCTA R: TGTGACTTTCCATGCTCCAG	238-242	0.340	166	3	0.331*	0.423	-0.044

These 8 loci were found to cross-amplify in 19 individuals from five congeneric taxa (*F. australis*, *F. fugax*, *F. oraria*, *F. rostrata* and *F. venosa*).

Furthermore, all 8 loci were found to be polymorphic between each of the 6 species and many loci were polymorphic within each of the 6 species.

Conclusion

These preliminary analyses indicate the utility of these markers for studies of population genetic diversity, structure, and parentage in *F. picrosperma*.

Their successful amplification in five other *Fontainea* species will facilitate valuable interspecific comparisons and efficient conservation management of these species in the future.

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