Isolation of Polymorphic Microsatellite Loci in the New Zealand Endemic Sand-Binder, Ficinia spiralis (Cyperaceae)

Authors: Heugten, Rachel A. van, Hale, Marie L., Bryan, Stacey, Griensven, Bart van, Satter, Sophia, et al.

Source: Applications in Plant Sciences, 5(9)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1700039

Applications in Plant Sciences 2017 5(9): 1700039

PRIMER NOTE

ISOLATION OF POLYMORPHIC MICROSATELLITE LOCI IN THE NEW ZEALAND ENDEMIC SAND-BINDER, *FICINIA SPIRALIS* (CYPERACEAE)[1]

RACHEL A. VAN HEUGTEN[2], MARIE L. HALE[2], STACEY BRYAN[3], BART VAN GRIENSVEN[3,4], SOPHIA SATTER[3,4], LILY BRAILSFORD[2], AND HANNAH L. BUCKLEY[5,6]

[2]School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand; [3]Department of Ecology, Lincoln University, P.O. Box 85084, Lincoln 7647, New Zealand; [4]HAS University of Applied Sciences, P.O. Box 90108, 's-Hertogenbosch, The Netherlands; and [5]School of Science, Auckland University of Technology, Private Bag 92006, Auckland 1142, New Zealand

- *Premise of the study: Ficinia spiralis* (Cyperaceae) is a declining sand-binding sedge of ecological and cultural importance. Microsatellite primers were developed in *F. spiralis* to investigate how population genetic structure is related to the pronounced morphological, physiological, and ecological variation observed in this species.
- *Methods and Results:* A 454 shotgun-sequencing approach was used to generate 157,274 raw sequence reads, 536 of which contained microsatellites. After initial primer testing for 40 loci, 14 polymorphic loci were isolated, containing five to 27 alleles per locus. Ten of these loci also amplified in a congener, *F. nodosa*.
- *Conclusions:* These loci will enable the assessment of the population genetic structure of *F. spiralis*, improving our understanding of the population processes underlying the observed morphological, physiological, and ecological variation in this endemic species. As the first microsatellites developed in *Ficinia*, these loci are a valuable resource for population genetic studies within this genus.

Key words: Cyperaceae; *Ficinia nodosa*; *Ficinia spiralis*; pīngao; polyploidy; microsatellites.

Ficinia spiralis (A. Rich.) Muasya & de Lange (Cyperaceae), otherwise known as pīngao, pikao, or golden sand sedge, is a sand-binding sedge that occurs on the foredunes of New Zealand sandy beaches (Bergin and Herbert, 1998). Small remnants and numerous human-planted restoration populations are all that remain of this previously widespread plant (Courtney, 1983; Bergin, 2011). Despite significant effort being put into conservation and restoration activities, this species is currently ranked as "Declining" in the New Zealand Threat Classification System (de Lange et al., 2013). Pīngao is of great cultural and ecological value and significance for New Zealand because it provides habitat for other native sand dune specialists and is an important weaving fiber, being highly valued for its beautiful golden color when dry. It exhibits pronounced morphological and physiological variation throughout its geographic range (Bergin and Herbert, 1998); however, the causes and consequences of this variation are not understood.

Microsatellite markers have not previously been successfully developed for this species, but are essential for characterizing the genetic variation within *F. spiralis*. In this paper, we present the development of microsatellite markers that will enable future genotyping of individuals from *F. spiralis* populations to correlate genetic, morphological, physiological, and ecological variation within this species. Our ultimate goal is to determine (1) what genetic variation is being lost as this species continues to decline and (2) what potential there is to use knowledge of the spatial genetic variation in this species to make better-informed conservation and restoration management decisions to reverse its decline.

METHODS AND RESULTS

Whole genomic DNA was extracted from 100-mg leaf samples of two *F. spiralis* individuals (geographic coordinates: –43.435°S, 172.713°E and –43.852°S, 172.771°E) using a modified cetyltrimethylammonium bromide (CTAB) method (Weising et al., 1995). Voucher specimens could not be collected due to permit restrictions (New Zealand Department of Conservation Research and Collection Permit 47972); a photograph of *F. spiralis* is provided in Appendix S1. The samples were combined before microsatellite regions were identified using a 454 sequencing approach. The 454 sequencing was conducted by Macrogen (Seoul, Korea) as follows. One microgram of genomic DNA was run on a GS FLX Titanium platform (1/8 region) after GS FLX Rapid Library shotgun library construction (454 Life Sciences, a Roche Company, Branford, Connecticut, USA). This generated 157,274 raw reads (National Center for Biotechnology Information [NCBI] Sequence Read Archive [SRA] Bioproject number PRJNA378666), which were then screened for microsatellite regions using MSATCOMMANDER version 0.8.2 (Faircloth, 2008). The search conditions were: dinucleotide repeats >8, trinucleotide repeats >7, tetranucleotide repeats >7, pentanucleotide repeats >6, and hexanucleotide repeats >6. The number of reads containing microsatellites was 536, and Primer3 (Koressaar and Remm, 2007; Untergasser et al., 2012) was used to design primers for all reads containing microsatellites with sufficient flanking sequence for primer design. Forty loci were then chosen for testing of amplification and variation based on the number of repeats present in the raw sequence. Reads with 15 to 25 repeats were preferred as they will likely maximize

[1]Manuscript received 17 April 2017; revision accepted 18 July 2017.

Funding for this research was provided by the Brian Mason Scientific and Technical Trust.

[6]Author for correspondence: Hannah.Buckley@aut.ac.nz

doi:10.3732/apps.1700039

TABLE 1. Characteristics of 15 nuclear microsatellite loci isolated from *Ficinia spiralis*.[a,b]

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)[c]	T_a (°C)	Fluorescent dye
FSP1	F: TTGTCTCAAGGGCGGTCTAC R: ACCCTGTAAATGGCAAAGCA	$(AC)_{14}$	168–203	53	5-FAM
FSP2	F: CAAGCGACTCCTCCTCCATA R: GCATTGCTGGCTTATCGTGA	$(CT)_{12}$	234–253	TD	5-FAM
FSP4	F: GCCTCCACCCTCTCTCAG R: GTAATCGGCGACAGTGACAG	$(CATT)_{18}$	213–279	TD	VIC
FSP6	F: AGAAGCATAATGTTCATGCACA R: TGCTCTCATTTGGCGTAGTG	$(ATGT)_{18}$	220–346	TD	VIC
FSP7	F: TGCCATTCCTAGATCCACGT R: ACTTCTTGACCACCACCACA	$(ACT)_{13}$	257–279	53	VIC
FSP11	F: CCCTTTGCAAATCTGGTTGGA R: GAGTAGAGGGGCCCACTTTT	$(ATTT)_{11}$	247–293	TD	VIC
FSP16	F: TCTTCATAAGCAAGCAACTAAGC R: TCAATACTGTAACAACAAGAAGT	$(GTT)_{12}$	181–227	50	5-FAM
FSP19	F: AGCCGAACTCTGTCATCATCT R: TGCGATTTCAACCAATTCAAC	$(CT)_{13}$	219	52	VIC
FSP21	F: ACCACCACCACAACAACAAC R: TGCCATTCCTAGATCCACGT	$(AGT)_{13}$	251–269	53	5-FAM
FSP29	F: AGGCTCCCTTGGTAAAATTT R: AGGGCAGTATTCCCATGCTT	$(AGT)_{11}$	268–283	50	5-FAM
FSP30	F: TCTAGCCTACCCCAACTTGT R: TGCTGATGTCGTCTAAAGAGC	$(GTT)_{10}$	176–201	TD	VIC
FSP44	F: CGTGGTAATACTTTAAATGGACA R: GCCTAGTTTGGTTGGTTTCCT	$(AT)_{12}$	183–195	50	VIC
FSP45	F: GTATCACCGATAACATCATTG R: GTGATTCATTTAGGAGAATAAGT	$(AT)_{12}$	176–198	TD	5-FAM
FSP50	F: CACAACAACACAATGCACGC R: AGAAGACGAGAAGTGCCGAT	$(AAGACG)_7$	178–239	TD	5-FAM
FSP51	F: TGAAAGCCCAACTGATGCAT R: TGGATAGGTGTGCTACGAGT	$(ACACAT)_7$	257–285	53	VIC

Note: T_a = annealing temperature; TD = touchdown protocol (see Methods and Results).

[a]Raw reads were deposited to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (Bioproject number PRJNA378666).

[b]Values for all loci except FSP19 were based on 100 samples from four sites around New Zealand (N = 20–30 for each [Table 2]). Values for FSP19 are based on eight samples from two sites in New Zealand (N = 4 for each, geographic coordinates: −43.435°S, 172.713°E and −43.852°S, 172.771°E).

[c]Fragment sizes include the M13 tag (5′-TGTAAAACGACGGCCAGT-3′) attached to the forward primer.

the chance of polymorphism while minimizing the likelihood of excessive stutter. Fourteen di-, 15 tri-, six tetra-, two penta-, and three hexanucleotide repeat regions were chosen for testing. The forward primer for each locus was appended with an M13 sequence (5′-TGTAAAACGACGGCCAGT-3′) at the 5′ end to allow the use of universal labeled primers (Schuelke, 2000), and PIG-tails (5′-GTTTCTT-3′) were added to the 5′ end of the reverse primers to reduce incomplete A-addition during PCR (Brownstein et al., 1996).

The primers (Invitrogen, Carlsbad, California, USA) were initially tested for amplification against four *F. spiralis* individuals from two sample sites (geographic coordinates: −43.435°S, 172.713°E and −43.852°S, 172.771°E). Primers showing evidence of amplification were screened for polymorphism with a fluorescent M13 tag (Applied Biosystems, Carlsbad, California, USA) using an additional six *F. spiralis* individuals spanning the same two sample sites. PCRs used the following protocol: 15-μL reactions containing 1 μL of template DNA, 1× PCR buffer (Bioline, London, United Kingdom), 2 mM $MgCl_2$, 0.08 mM dNTPs, 0.33 μM reverse primer, 0.33 μM fluorescent M13 tag (6-FAM, VIC, NED, PET), 0.08 μM forward primer, and 0.6 unit BIOTAQ (Bioline). PCR cycles for loci FSP2, FSP4, FSP6, FSP11, FSP30, and FSP50 ran on a touchdown protocol (TD) as follows: 95°C for 15 min; 10 cycles of 94°C for 15 s, 62–53°C (decreasing 1°C per cycle) for 30 s, 72°C for 1 min; 25 cycles of 94°C for 15 s, 52°C for 30 s, 72°C for 1 min; and a final extension step of 15 min at 72°C. The remaining loci were amplified with the following PCR cycle: 95°C for 12 min; 10 cycles of 94°C for 15 s, annealing temperature (Table 1) for 30 s, 72°C for 30 s; 30 cycles of 89°C for 15 s, annealing temperature for 30 s, 72°C for 30 s; and a final extension of 72°C for 10 min. DNA fragments were detected and sized using the GeneScan 500 LIZ Internal Size Standard (Applied Biosystems) and an ABI 3130xl Genetic Analyzer (Applied Biosystems). Loci FSP19, FSP44, and FSP45 were genotyped individually. The remaining loci were combined in the following pairs: FSP6 and FSP16, FSP30 and FSP29, FSP7 and FSP2, FSP4 and FSP21, FSP51 and FSP50, and FSP11 and FSP1. Fragments were scored manually using GeneMarker software version 6.41 (SoftGenetics, State College, Pennsylvania, USA).

Of the 40 loci tested, 15 amplified cleanly. Of the 15 loci, one locus (FSP19) was monomorphic in *F. spiralis*, while 14 were polymorphic (see Appendix S2 for raw sequences for these loci). The 14 polymorphic loci were then used to genotype 100 *F. spiralis* individuals from across four sample sites (each with at least 20 samples) to gain a better understanding of the variation present at these loci (Table 2). All 15 loci were tested for cross-amplification in six specimens (from two different locations: Christchurch [−43.486°S, 172.693°E] and Auckland [−36.956°S, 174.468°E]) of *F. nodosa* (Rottb.) Goetgh., Muasya & D. A. Simpson, the only other *Ficinia* Schrad. species recorded in New Zealand (a photograph of *F. nodosa* is provided in Appendix S1). Ten of the 15 loci amplified in *F. nodosa*, and two loci (FSP19 and FSP51) were polymorphic (Table 3). Analysis with POLYSAT version 1.7-1 (Clark and Jasieniuk, 2011; Clark and Schreier, 2017) in R version 3.0.2 (R Core Team, 2013) indicated that *F. spiralis* individuals are either triploid or tetraploid. However, there is no record in the literature to indicate whether *F. spiralis* is allopolyploid or autopolyploid. Therefore, *F. spiralis* alleles were scored as either present or absent, and it was not possible to calculate Hardy–Weinberg equilibrium and linkage disequilibrium.

CONCLUSIONS

Ficinia spiralis (Cyperaceae) is a declining, endemic species of great cultural and ecological value to New Zealand. Here, we describe one monomorphic and 14 polymorphic microsatellite loci that will enable us to assess genetic variation across the geographic range of this species, and to determine whether this variation correlates with morphological and/or physiological variation. Ten of these loci also amplified in *F. nodosa*, suggesting these loci may be useful for assessing genetic variation and

Table 2. Genetic diversity statistics for 14 polymorphic microsatellite loci in four populations of *Ficinia spiralis*.

	Kaitorete (n = 20)		Matakana (n = 30)		New Brighton (n = 29)		Tumbledown Bay (n = 21)		Total (n = 100)	
Locus	A	H_o	A	H_o	A	H_o	A	H_o	A	H_o
FSP1	7	1.000	13	1.000	7	0.929	5	0.667	16	0.898
FSP2	5	0.875	9	1.000	4	0.929	3	0.950	11	0.942
FSP4	10	0.950	8	1.000	11	1.000	7	0.952	15	0.978
FSP6	11	0.950	21	0.818	14	1.000	4	0.944	27	0.933
FSP7	4	0.308	4	0.964	1	0.000	2	0.235	6	0.455
FSP11	8	0.950	12	1.000	8	0.964	7	0.952	12	0.969
FSP16	6	1.000	10	1.000	3	1.000	4	1.000	16	1.000
FSP21	2	0.235	4	0.966	1	0.000	2	0.235	5	0.439
FSP29	7	1.000	4	0.138	8	1.000	5	1.000	10	0.745
FSP30	4	0.600	4	0.793	5	0.591	2	0.095	6	0.543
FSP44	4	0.950	4	0.778	3	0.913	3	0.842	7	0.865
FSP45	7	0.842	9	0.750	7	0.862	5	0.889	12	0.833
FSP50	4	0.850	9	0.967	5	1.000	4	1.000	9	0.959
FSP51	5	0.833	5	0.929	6	0.966	4	1.000	6	0.937

Note: A = number of alleles; H_o = observed heterozygosity; n = number of individuals sampled.

[a] Geographic coordinate locations of the populations are: Kaitorete Spit (−43.827°S, 172.655°E), Matakana Island (−37.563°S, 176.081°E), New Brighton (−43.523°S, 172.739°E), Tumbledown Bay (−43.852°S, 172.771°E).

population structure in other species within this genus. Although the polyploidy of *F. spiralis* limits the range of population genetic analyses available to those that can accommodate dominant data, numerous other studies have used similar data to investigate population structure and how it relates to other factors such as the environment (i.e., Brito et al., 2016; Wu et al., 2016). The high number of alleles at many of these loci should provide sufficient information for the assessment of fine-scale genetic structure throughout the species' range.

LITERATURE CITED

Bergin, D. O. 2011. Pīngao golden sand sedge: Ecology distribution and habitat. Dune Restoration Trust of New Zealand Technical Article No. 73. Coastal Restoration Trust of New Zealand, Wellington, New Zealand.

Bergin, D. O., and J. W. Herbert. 1998. Pīngao on coastal sand dunes. Coastal Dune Vegetation Network Technical Bulletin No. 1. New Zealand Forest Research Institute, Rotorua, New Zealand.

Table 3. Cross-amplification of 15 nuclear microsatellite loci developed for *Ficinia spiralis* in *F. nodosa* (n = 6).[a]

Locus	A	Alelle size range (bp)[b]
FSP1	1	186
FSP2	1	243
FSP4	1	211
FSP6	—	—
FSP7	1	238
FSP11	1	236
FSP16	—	—
FSP19	2	219–229
FSP21	1	226
FSP29	1	256
FSP30	—	—
FSP44	1	273
FSP45	—	—
FSP50	—	—
FSP51	2	203–259

Note: A = number of alleles.

[a] Geographic coordinates for the sample sites are: −43.486°S, 172.693°E and −36.956°S, 174.468°E.

[b] Fragment sizes include the M13 tag (5′-TGTAAAACGACGGCCAGT-3′) attached to the forward primer.

Brito, V. L. G., G. M. Mori, B. B. Z. Vigna, M. Azevedo-Silva, A. P. Souza, and M. Sazima. 2016. Genetic structure and diversity of populations of polyploid *Tibouchina pulchra* Cogn. (Melastomataceae) under different environmental conditions in extremes of an elevation gradient. *Tree Genetics & Genomes* 12: 101.

Brownstein, M. J., J. D. Carpten, and J. R. Smith. 1996. Modulation of nontemplated nucleotide addition by Taq DNA polymerase: Primer modifications that facilitate genotyping. *BioTechniques* 20: 1004–1010.

Clark, L. V., and M. Jasieniuk. 2011. POLYSAT: An R package for polyploid microsatellite analysis. *Molecular Ecology Resources* 11: 562–566.

Clark, L. V., and A. D. Schreier. 2017. Resolving microsatellite genotype ambiguity in populations of allopolyploid and diploidized autopolyploid organisms using negative correlations between allelic variables. *Molecular Ecology Resources* doi:10.1111/1755-0998.12639.

Courtney, S. P. 1983. Aspects of the ecology of *Desmoschoenus spiralis* (A. Rich.) Hook. f. M.Sc. thesis, University of Canterbury, Canterbury, New Zealand.

de Lange, P., J. Rolfe, P. Champion, S. Courtney, P. Heenan, J. Barkla, E. Cameron, et al. 2013. Conservation status of New Zealand indigenous vascular plants, 2012. New Zealand Threat Classification Series 3. New Zealand Department of Conservation, Wellington, New Zealand.

Faircloth, B. C. 2008. MSATCOMMANDER: Detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources* 8: 92–94.

Koressaar, T., and M. Remm. 2007. Enhancements and modifications of primer design program Primer3. *Bioinformatics (Oxford, England)* 23: 1289–1291.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website http://www.R-project.org/ [accessed 18 August 2017].

Schuelke, M. 2000. An economic method for the fluorescent labelling of PCR fragments. *Nature Biotechnology* 18: 233–234.

Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B. C. Faircloth, M. Remm, and S. G. Rozen. 2012. Primer3—New capabilities and interfaces. *Nucleic Acids Research* 40: e115.

Weising, K., H. Nybom, K. Wolff, and W. Meyer. 1995. DNA fingerprinting in plants and fungi. CRC Press, Boca Raton, Florida, USA.

Wu, Z., D. Yu, X. Li, and X. Xu. 2016. Influence of geography and environment on patterns of genetic differentiation in a widespread submerged macrophyte, Eurasian watermilfoil (*Myriophyllum spicatum* L., Haloragaceae). *Ecology and Evolution* 6: 460–468.