Development and Characterization of 11 Microsatellite Primers for the Sedge Trichophorum planifolium (Cyperaceae)

Authors: Nowell, Victoria J., Wang, Song, and Smith, Tyler W.

Source: Applications in Plant Sciences, 3(9)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1500050

Applications in Plant Sciences 2015 3(9): 1500050

PRIMER NOTE

DEVELOPMENT AND CHARACTERIZATION OF 11 MICROSATELLITE PRIMERS FOR THE SEDGE *TRICHOPHORUM PLANIFOLIUM* (CYPERACEAE)[1]

VICTORIA J. NOWELL[2], SONG WANG[3], AND TYLER W. SMITH[2,3,4]

[2]Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada; and [3]Agriculture and Agri-Food Canada (AAFC), 960 Carling Avenue, K.W. Neatby Building, Ottawa, Ontario K1A 0C6, Canada

- *Premise of the study:* Microsatellite loci were developed for *Trichophorum planifolium* (Cyperaceae), an endangered woodland sedge protected under federal and provincial legislation in Canada, to explore patterns of population genetic diversity and differentiation in the species.
- *Methods and Results:* Sixty-three primer pairs were evaluated for amplification consistency and screened for polymorphisms in 96 samples collected from 12 populations of *T. planifolium* distributed through the range of the species. Of these, 11 loci were shown to be polymorphic, displaying two to six alleles. Mean observed heterozygosity across loci ranged from 0.00 to 0.06 among populations tested.
- *Conclusions:* The results suggest that the 11 primer pairs developed in this study will be useful for future studies of broad-scale genetic variation in *T. planifolium* and in guiding management protocols for the species in Canada.

Key words: conservation genetics; Cyperaceae; microsatellites; *Trichophorum planifolium.*

Trichophorum planifolium (Spreng.) Palla (Cyperaceae) is a woodland sedge that occurs on dry, rocky slopes in northeastern North America. In Canada, there is only one known extant occurrence, located in a remnant natural area within one of the most highly urbanized regions in the country. Due to its limited Canadian range and an apparent decline in population size (COSEWIC, 2000), *T. planifolium* is listed as endangered under the Canadian *Species at Risk Act* (SARA). Like many species-at-risk in Canada (Yakimowski and Eckert, 2007; Gibson et al., 2009), *T. planifolium* reaches the northern limit of its range in Canada, but is relatively common farther south. Although there is some debate over whether, or when, peripheral populations merit national concern (Gibson et al., 2009), empirical evidence suggests that they may be important reserves of genetic diversity (Eckert et al., 2008), providing adaptive and evolutionary potential for the species (Lesica and Allendorf, 1995) and facilitating species' responses to climate change (Etterson and Shaw, 2001; Parmesan, 2006; Gibson et al., 2009).

An understanding of the population genetic structure of *T. planifolium* is needed to guide management strategies for this species. At present, no molecular markers appropriate for studies of intraspecific genetic variation have been developed for *T. planifolium*. To this end, we isolated and characterized 11 polymorphic microsatellite loci.

METHODS AND RESULTS

Genomic DNA (~6 μg) was extracted from the silica gel–dried leaf tissue of one individual of *T. planifolium* collected from Daniel Boone National Forest (Kentucky, USA) in 2011 using a NucleoSpin Plant II Kit (Machery-Nagel, Bethlehem, Pennsylvania, USA) following the manufacturer's protocol. The sample was submitted to the Georgia Genomics Facility at the University of Georgia (Athens, Georgia, USA) for isolation of microsatellite loci and primer development. DNA was fragmented using the Bioruptor UCD-300 sonication device (Diagenode, Denville, New Jersey, USA). Libraries compatible with Illumina TruSeq HT were prepared using the KAPA Library Preparation Kit (KR0453-v2.13; Kapa Biosystems, Wilmington, Massachusetts, USA) with custom indexes from Faircloth and Glenn (2012). Libraries were quantified with Qubit (Life Technologies, Burlington, Ontario, Canada) and sequenced using an Illumina MiSeq v3 600-cycle kit (Illumina, San Diego, California, USA). A total of 6,391,132 reads were imported and paired in Geneious 7.0.6 (Biomatters, Auckland, New Zealand). Illumina TruSeq adapters and bases with an error probability limit above 0.05 were trimmed. A de novo assembly was performed on the first 1,000,000 sequences where both reads of any pair were ≥200 bases. Consensus sequences between 200 and 420 bp were exported from Geneious as FASTA files and imported into MSATCOMMANDER 1.0.8 beta (Faircloth, 2008). A total of 721 loci with perfect di-, tri-, or tetranucleotide repeats were designed at default minimum lengths (i.e., eight repeats for di- and trinucleotide motifs, six repeats for tetranucleotide motifs) and combining loci ≤50 bp apart. Sixty-three CAG-tagged primer pairs for di- (9), tri- (47), and tetranucleotide (7) microsatellite loci with the greatest number of motif repeats were selected for further testing.

Loci were subsequently evaluated for amplification consistency and screened for polymorphisms with 96 samples of *T. planifolium* collected from 12 populations distributed through the range of the species in May 2014: (1) Tarrywile Park, Connecticut, USA; (2) Bare Mountain, Massachusetts, USA; (3) Dan's Mountain, Maryland, USA; (4) Big Spring State Park, Missouri, USA; (5) Sutton Hollow, Missouri, USA; (6) Elmer G. Raymond Park, New Hampshire, USA; (7) Mendon Ponds Park, New York, USA; (8) Strait Creek Prairie Bluffs Preserve, Ohio, USA; (9) Royal Botanical Gardens, Ontario, Canada; (10) Gifford Pinchot State Park, Pennsylvania, USA; (11) Huckleberry Trail, Virginia, USA; and (12) Fisher Mountain, West Virginia, USA (Appendix 1). These collections yielded substantially higher DNA quality than the samples obtained

---

[1]Manuscript received 29 April 2015; revision accepted 12 June 2015.

This research was supported by the Natural Sciences and Engineering Research Council of Canada (Alexander Graham Bell Canada Graduate Scholarship to V.J.N.), the Ontario Ministry of Natural Resources (Species at Risk Stewardship Fund no. 110-14-VNowell to V.J.N.), and Environment Canada (Interdepartmental Recovery Fund no. 2227 to T.W.S.).

[4]Author for correspondence: tyler.smith@agr.gc.ca

doi:10.3732/apps.1500050

Applications in Plant Sciences 2015 3(9): 1500050; http://www.bioone.org/loi/apps © 2015 Her Majesty the Queen in Right of Canada, as represented by the Minister of Agriculture and Agri-Food Canada. Published by the Botanical Society of America.

Table 1. Characterization of 11 polymorphic microsatellite loci developed in *Trichophorum planifolium.*

Genotyping group	Locus	Repeat motif	Primer sequences (5′–3′)	Allele size range (bp)	A	T_a (°C)	Fluorescent dye	GenBank accession no.
Group 1	TP142	$(AAT)_{11}$	F: CAGTCGGGCGTCATCACTTCATGTAGATCCGTCCC R: TGTCTTACTCACCTAAGCCAAG	135–145	2	53	6-FAM	KR264968
	TP174	$(AAT)_{14}$	F: CTCGAGTTCAAGTCCAAGCG R: CAGTCGGGCGTCATCATCCATCAGACCGTCACCC	167–170	2	55	VIC	KR264969
	TP341	$(AGC)_{14}$	F: CAGTCGGGCGTCATCATGGTGGGATTCTGCATGG R: GTGCTTTATCACCAACAGCAG	299–323	4	54	6-FAM	KR264970
Group 2	TP152	$(AG)_{15}$	F: CAGTCGGGCGTCATCAGTTCACAAAGGCCAAGTCG R: GTTTAATCACTCAGTCGTCCACCC	154–156	2	55	VIC	KR264971
	TP326	$(AG)_{16}$	F: GTTTGTTGCCATCAGTCAAGTGG R: CAGTCGGGCGTCATCATCTCTGCCATAGTCACTGCC	216–226	2	55	6-FAM	KR264972
	TP45	$(AAG)_{12}$	F: CAGTCGGGCGTCATCACCAAGCTAGATTCGCCAAC R: TCTCCTTGGTACCATCCTCAG	368–382	2	54	VIC	KR264973
Group 3	TP325	$(AAT)_{18}$	F: CAGTCGGGCGTCATCATTCTAAAGGTGACGTGACGG R: AAATTTCGGGAATAGCGGCG	152–171	6	54	VIC	KR264974
	TP406	$(AAT)_{15}$	F: CGGATCTCTGCTGGTGTACC R: CAGTCGGGCGTCATCAGCAGAATTTCCCTCCTAATCC	213–216	2	54	6-FAM	KR264975
Group 4	TP434	$(AAT)_{13}$	F: CAGTCGGGCGTCATCATCTGGTCGAACAATCAGAAGAC R: GCATTTGGATCAGATACCGCC	163–169	3	55	6-FAM	KR264976
	TP80B	$(AAT)_{8}$	F: ACGCCAGTGGACCTATGTG R: CAGTCGGGCGTCATCAGAGCGGTTTATGGGCCTTTC	208–220	4	55	VIC	KR264977
	TP330	$(AAT)_{11}$	F: CAGTCGGGCGTCATCATTGCCATCACAGTATTACACCG R: ACTTCCCAGAGCACCATAGC	355–358	2	55	6-FAM	KR264978

Note: A = number of alleles; T_a = theoretically optimal annealing temperature (touchdown PCR was carried out with annealing temperatures of 72–57°C for all loci).

from the Kentucky population used in microsatellite isolation. Consequently, we chose to use the more recent collections of *T. planifolium* for further testing of the microsatellite loci. Voucher specimens were obtained for each population, except when population size was estimated to be below 100 individuals, or if permits did not allow destructive sampling. For sites where a voucher was not collected, a representative voucher has been assigned if available. The deposition of vouchers is provided in Appendix 1.

Reactions were carried out in 12.5-μL reaction volumes containing 2.5 μL 5× Phusion High-Fidelity Buffer (New England Biolabs, Whitby, Ontario, Canada), 0.25 μL dNTPs (10 mM), 0.625 μL untagged primer (10 mM), 0.0625 μL tagged primer (10 mM), 0.5625 μL dye-labeled CAG Tag (10 mM), 0.375 μL DMSO, 0.125 μL Phusion High-Fidelity Polymerase (2 U/μL; New England Biolabs), 5.5 μL ddH$_2$O, and 2.5 μL DNA (10 ng/μL) using a T-100 Thermal Cycler (Bio-Rad, Hercules, California, USA). To obtain high-quality amplification product, we used the thermocycling profile of touchdown PCR (TD-PCR) (Korbie and Mattick, 2008) with some modifications. Thermal cycling began with a 5-min denaturation at 95°C; followed by the touchdown phase with 15 cycles of 30 s denaturation at 95°C, 30 s annealing from 72°C to 57°C (−1°C per cycle), and 30 s elongation at 72°C; followed by a generic amplification stage of 20 cycles of 30 s denaturation at 95°C, 30 s annealing at 57°C, and 30 s elongation at 72°C; followed by a 5-min final elongation at 72°C. Amplification products with incorporated fluorescent labels (6-FAM and VIC; Life Technologies) were pooled into groups of four and sequenced by capillary electrophoresis using a 3130xL Genetic Analyzer (Life Technologies) with the GeneScan 500 LIZ Size Standard (Life Technologies). Of the 63 primers tested, 18 exhibited consistent amplification and polymorphisms. Eleven loci that could be pooled into four genotyping runs (i.e., the fragment sizes for the primers in each run did not overlap each other) were selected (Table 1) and their utility for future studies of genetic diversity and structure in *T. planifolium* was evaluated.

Individual samples were genotyped using GeneMapper v.5 software (Life Technologies) and verified with manual scoring. Standard measures of intrapopulational genetic diversity including average number of alleles (A) and observed (H_o) and expected (H_e) heterozygosity were calculated with the R package 'adegenet' version 1.4-2 (Jombart, 2008) and 'PopGenReport' version 2.1 (Adamack and Gruber, 2014) (Table 2). Of the 96 samples initially screened, 16 failed at one or more loci and were excluded from the population genetic analysis. In total, 31 alleles were observed for 11 microsatellite loci in 80 individuals from 12 populations of *T. planifolium.* The number of alleles per locus ranged from two to six (overall mean = 2.82 alleles), with the highest number detected in the population at Fisher Mountain, West Virginia. The mean H_o per site ranged from 0.00 and 0.06, whereas the mean H_e varied between 0.00 and 0.19. Although these values are low, they are comparable to those documented in other woodland sedges (e.g., *Carex breviculmis* R. Br. and *C. hebes* Nelmes; M'Baya et al., 2013), and may be explained by limited pollen and seed dispersal among populations (Crins, 1989).

CONCLUSIONS

The primer pairs developed in this study successfully amplified 11 polymorphic microsatellite loci in populations distributed across the species' range and, as such, will be a useful tool with which to examine patterns of genetic diversity and differentiation in *T. planifolium.* An understanding of genetic variability and structure within the Canadian population, and between the Canadian and core populations in the United States, is necessary to guide the development of effective management and monitoring protocols for the species.

LITERATURE CITED

Adamack, A. T., and B. Gruber. 2014. PopGenReport: Simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution* 5: 384–387.

COSEWIC. 2000. COSEWIC assessment and status report on the bashful bulrush *Trichophorum planifolium* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, Canada.

Crins, W. J. 1989. Status of the few-flowered club-rush, *Scirpus verecundus* (Cyperaceae), in Canada. *Canadian Field Naturalist* 103: 57–60.

Eckert, C. G., E. Samis, and S. C. Lougheed. 2008. Genetic variation across species' geographical ranges: The central-marginal hypothesis and beyond. *Molecular Ecology* 17: 1170–1188.

Etterson, J. R., and R. G. Shaw. 2001. Constraint to adaptive evolution in response to global warming. *Science* 294: 151–154.

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

Faircloth, B. C., and T. C. Glenn. 2012. Not all sequence tags are created equal: Designing and validating sequence identification tags robust to indels. *PLoS One* 7: e42543.

Table 2. Results of initial primer screening and genotyping in 80 individuals from 12 populations of *Trichophorum planifolium.*

Locus	Tarrywile Park, CT (n = 8)			Bare Mountain, MA (n = 6)			Dan's Mountain, MD (n = 7)			Big Spring State Park, MO (n = 7)			Sutton Hollow, MO (n = 6)			Elmer G. Raymond Park, NH (n = 8)			Mendon Ponds Park, NY (n = 8)			Strait Creek Prairie Bluffs Preserve, OH (n = 6)			Royal Botanical Gardens, ON (n = 6)			Gifford Pinchot State Park, PA (n = 6)			Huckleberry Trail, VA (n = 5)			Fisher Mountain, WV (n = 7)		
	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o
TP142	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00
TP152	1	0.00	0.00	1	0.00	0.00	2	0.24	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	2	0.24	0.00
TP174	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	2	0.47	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	2	0.38	0.17	1	0.00	0.00	2	0.24	0.00
TP325	1	0.00	0.00	2	0.44	0.00	1	0.00	0.00	2	0.24	0.00	3	0.61	0.33	3	0.51	0.13	2	0.22	0.00	1	0.00	0.00	1	0.00	0.00	2	0.28	0.00	1	0.00	0.00	3	0.52	0.14
TP326	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	2	0.38	0.17	1	0.00	0.00	1	0.00	0.00
TP330	1	0.00	0.00	1	0.00	0.00	2	0.24	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	2	0.41	0.00
TP341	1	0.00	0.00	2	0.28	0.00	2	0.34	0.14	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	2	0.24	0.00
TP406	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	2	0.49	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00
TP434	1	0.00	0.00	1	0.00	0.00	2	0.13	0.14	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	2	0.41	0.00
TP45	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	2	0.24	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	2	0.38	0.17	1	0.00	0.00	1	0.00	0.00
TP80B	1	0.00	0.00	2	0.28	0.00	1	0.00	0.00	2	0.49	0.00	1	0.00	0.00	2	0.47	0.00	1	0.00	0.00	1	0.00	0.00	1	0.00	0.00	2	0.38	0.17	1	0.00	0.00	1	0.00	0.00
Mean	1	0.00	0.00	1.27	0.09	0.00	1.36	0.09	0.03	1.36	0.13	0.00	1.18	0.06	0.03	1.36	0.13	0.01	1.09	0.02	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.45	0.16	0.06	1.00	0.00	0.00	1.64	0.19	0.01

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

Gibson, S. Y., R. C. V. Van der Marel, and B. M. Starzomski. 2009. Climate change and conservation of leading-edge peripheral populations. *Conservation Biology* 23: 1369–1373.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics (Oxford, England)* 24: 1403–1405.

Korbie, D. J., and J. S. Mattick. 2008. Touchdown PCR for increased specificity and sensitivity in PCR amplification. *Nature Protocols* 3: 1452–1456.

Lesica, P., and F. W. Allendorf. 1995. When are peripheral populations valuable for conservation? *Conservation Biology* 9: 753–760.

M'Baya, J., M. J. Blacket, and A. A. Hoffman. 2013. Genetic structure of *Carex* species from the Australian alpine region along elevation gradients: Patterns of reproduction and gene flow. *International Journal of Plant Sciences* 174: 189–199.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* 37: 637–669.

Yakimowski, S. B., and C. G. Eckert. 2007. Threatened peripheral populations in context: Geographical variation in population frequency and size and sexual reproduction in a clonal woody shrub. *Conservation Biology* 21: 811–822.

Appendix 1. Voucher information for *Trichophorum planifolium* populations used in this study.

Locality	Geographic coordinates[a]	Collection date	Collector(s)	Deposition[b]
Tarrywile Park, CT, USA	41.37140, −73.45273	May 23, 2014	V. J. Nowell & T. W. Smith	DAO
Daniel Boone National Forest, KY, USA	37.59063, −83.78146	May 19, 2011	Tyler W. Smith	EKY
Bare Mountain, MA, USA	42.30170, −72.52946	May 26, 2014	V. J. Nowell & T. W. Smith	DAO
Dan's Mountain, MD, USA	39.56760, −78.91190	May 10, 2011	P. M. Peterson, J. M. Saarela & C. Frye	US
Big Spring State Park, MO, USA	36.94750, −90.99314	May 9, 2014	V. J. Nowell & T. W. Smith	DAO
Sutton Hollow, MO, USA	37.19962, −91.32581	May 6, 2014	V. J. Nowell & T. W. Smith	DAO
Elmer G. Raymond Park, NH, USA	—	June 12, 2011	J. Sachs, C. Kane, P. Hines & D. Allen	NHA
Mendon Ponds Park, NY, USA	43.03012, −77.57369	May 28, 2014	V. J. Nowell & T. W. Smith	DAO
Strait Creek Prairie Bluffs Preserve, OH, USA	39.05830, −83.38857	May 11, 2014	V. J. Nowell & T. W. Smith	Edge of Appalachia
Royal Botanical Gardens, ON, Canada	—	June 7, 2000	P. G. O'Hara	HAM
Gifford Pinchot State Park, PA, USA	40.07460, −76.90913	May 17, 2014	V. J. Nowell & T. W. Smith	DAO
Huckleberry Trail, VA, USA	37.18850, −80.41925	May 14, 2014	V. J. Nowell & T. W. Smith	DAO
Fisher Mountain, WV, USA	38.66417, −79.24028	—	—	—

[a] Coordinates for populations sampled in Ontario and New Hampshire have been omitted as *Trichophorum planifolium* is of conservation concern in these jurisdictions.

[b] Herbaria abbreviations are per Index Herbariorum (http://sweetgum.nybg.org/ih/), except for Edge of Appalachia, 4274 Waggoner Riffle Road, West Union, OH 45693, USA.