Isolation Via Next-Generation Sequencing of Microsatellites from the Tasmanian Macroalgae Lessonia corrugata (Lessoniaceae)

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PRIMER NOTE

ISOLATION VIA NEXT-GENERATION SEQUENCING OF MICROSATELLITES FROM THE TASMANIAN MACROALGAE *LESSONIA CORRUGATA* (LESSONIACEAE)[1]

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- *Premise of the study:* Microsatellite markers for the macroalgae *Lessonia corrugata* (Lessoniaceae) were developed, for the first time, to enable population genetic assessment of this important foundation species.
- *Methods and Results:* Ion Torrent sequencing identified 16,622 loci, 29 of which were trialed in *L. corrugata*. Seven loci were found to be polymorphic and screened for variation in 76 individuals from two populations in Tasmania, Australia. Observed heterozygosity ranged from 0.086 to 0.686 (mean 0.386) and the number of alleles per locus ranged from two to five (mean 2.57). Heterozygosity was not significantly different from expected values.
- *Conclusions:* These loci can be used to study the population genetics of *L. corrugata*, a key habitat-forming species in the Tasmanian marine ecosystem, and will help to assess gene flow among spatially discrete populations such as those in marine protected areas.

Key words: Ion Torrent; *Lessonia*; Lessoniaceae; macroalgae; microsatellites; next-generation sequencing.

Macroalgae provide habitat for many ecologically and economically important marine species, supporting highly diverse ecosystems (Wernberg et al., 2010). *Lessonia* (Lessoniaceae) is one such genus endemic to the Southern Hemisphere (Lane et al., 2006). *Lessonia corrugata* Lucas is a Tasmanian endemic, habitat-forming macroalgae found along highly exposed intertidal rocky shorelines (Edgar, 1984). Although other *Lessonia* species have been the focus of genetic studies (e.g., Tellier et al., 2011), only one of these employed microsatellite markers, and *L. corrugata* has remained relatively understudied in terms of population genetic variation.

All *Lessonia* species possess an alternation-of-generations life history, which is characterized by two alternating stages, a diploid macroscopic sporophyte stage (the stage of sample collection for this study) and a microscopic gametophyte haploid stage. Dispersal can occur via released gametophytes and by drifting fertile sporophytes that become detached from the substrate during storm events or through herbivory. Although the latter mode of dispersal has greater capacity for long-distance transport, *Lessonia* sporophytes are relatively heavy and lack flotation structures, making them unable to float for long periods of time. Their limited capacity for dispersal could mean *Lessonia* has difficulty recolonizing disturbed areas in comparison to other macroalgae species. This makes them suitable candidates for genetic studies on population genetics.

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In the age of next-generation sequencing, microsatellites remain the most polymorphic and informative genetic marker for inferring species population genetics. Technological advances have now made microsatellite development faster and cheaper, with Ion Torrent PGM platforms proving to produce substantially more data at a faster rate and cheaper cost than other sequencing platforms (Elliott et al., 2014). Here, we use this platform to develop microsatellite markers for *L. corrugata*.

METHODS AND RESULTS

Genomic DNA (1 µg) was isolated from silica gel–dried tissue following the cetyltrimethylammonium bromide (CTAB) protocol of Hoarau et al. (2007) with some modifications. Prior to pulverization, microcentrifuge tubes containing tissue and a ball bearing were placed in liquid nitrogen so as to ensure the tissue was sufficiently brittle. Instead of incubating and rotating samples at room temperature after the addition of CTAB buffer, samples were incubated at 55°C without rotation. Due to problems encountered with DNA quality, the resulting supernatant from centrifuging samples containing CTAB buffer and chloroform–isoamyl alcohol was first filtered through an Epoch spin column (Epoch Biolabs, Sugarland, Texas, USA) and an additional wash step was performed before eluting in Tris-HCl buffer. Throughout the process, Epoch spin columns were used instead of making silica fines.

DNA was submitted to the Australian Genome Research Facility (Brisbane node), purified using the Aurora Nucleic Acid Extraction System (Boreal Genomics, Vancouver, British Columbia, Canada), and sequenced on an Ion Torrent PGM platform (Life Technologies, Mulgrave, Australia) using a 318 chip with 400-bp chemistry using standard protocols. Shearing of DNA was conducted with a Covaris S2 model (Covaris, Woburn, Massachusetts, USA) with shearing parameters slightly modified (duty cycle 10%, intensity 4100 cycles per burst, 80-s duration) to provide more DNA fragments in the 350–400-bp range. These fragments were then size selected using a Pippin Prep (Sage Science, Beverly, California, USA) to ensure the majority of fragments were greater than 300 bp and did not exceed 400 bp. Fragments were tagged by ligating with standard Ion Torrent barcode adapters. Equal molar ratios of fragments

TABLE 1. Characteristics of 29 microsatellite loci developed in *Lessonia corrugata.*

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size (bp)	T_a (°C)	GenBank accession no.
LCO94*	F: ATTCGAACTCGGGACTCCA	$(AC)_{16}$	94	60	KR337302
	R: CTGGATGTCGTTGAGCAGAA				
LCO95*	F: CCGATGAGCAGAAATAACGG	$(AC)_7$	95	60	KR337303
	R: ATTTGGATAAATCGGCTCGC				
LCO96*	F: TGCTGGATGTCGACGAGTAG	$(AC)_7$	96	60	KR337304
	R: CAAGCATAACACGGGTGGAT				
LCO97*	F: CAGTATACGCCAGAGCCACC	$(AC)_8$	97	60	KR337305
	R: TGCTGGATGTCGACGAGTAG				
LCO100*	F: CTGGATGTCGATGAGCAGAA	$(AC)_{10}$	100	60	KR337306
	R: AAGGCTACCCATGCATACGA				
LCO100AG*	F: GTGAGCGCGTCTCCTATGTT	$(AG)_{15}$	100	60	KR337307
	R: TGCGAGCTGGAGATCAACTA				
LCO102	F: TTCGAGTTGCTAGGTACGGC	$(AG)_{16}$	105–107	60	KR337308
	R: CAGCATCGTGCATTGTACCT				
LCO104*	F: AACCACCCAACACGAACAGT	$(AC)_{15}$	104	60	KR337309
	R: CTTGGCTGGATGTCGAGAGT				
LCO146	F: AACACTTTGACCCGACAAGC	$(AC)_9$	149–151	60	KR337310
	R: TGTGCTGGATGTCGATGAGT				
LCO153AGC*	F: GACCAGGGACAAGAAGGGTT	$(AGC)_8$	153	60	KR337311
	R: GATGATACCGTTGCTCCACC				
LCO153AC*	F: GACGCACTGCTGTACGATGT	$(AC)_{10}$	153	60	KR337312
	R: GGGATGAAGAAATCAGCAGG				
LCO154	F: GAGTATATGCGCCAACGTGA	$(AGC)_{10}$	158–161	60	KR337313
	R: GAACACGAGCTGGACGATTC				
LCO158*	F: TTAGAAGTGCATCGTGTCGC	$(AC)_{11}$	158	60	KR337314
	R: GTGTGCTGGATGTCGATGAG				
LCO160*	F: ACTCCGCAGAGTACTGGGAA	$(AC)_{14}$	160	60	KR337315
	R: CGATGAGCAGAAGAACGGAT				
LCO197*	F: GGGTTAAGAACTCTTGCAACTTC	$(AAC)_9$	197	58	KR337316
	R: TTAGCAGCCAACACAAATACAA				
LCO198*	F: TCATCACCGTTATCGTGATATTT	$(AGC)_7$	198	59	KR337317
	R: GAGCGAGAAGTGGAGGGAC				
LCO199	F: TGGAAACCCAATCTCACAAA	$(AGC)_{10}$	201–204	59	KR337318
	R: AACGGTCTTGTGGTCTTTGC				
LCO200*	F: AATTCCCGGTCCCAAGTC	$(AC)_8$	200	60	KR337319
	R: AAGAGTTGATCCGGTGATGC				
LCO205*	F: CAACGGATTATTCTCGGAAGG	$(AG)_{10}$	205	60	KR337320
	R: ACGATAAGCTGCTGGAGGG				
LCO208	F: CTGGATGTCGATGAGCAGAA	$(AC)_{11}$	199–207	60	KR337321
	R: AAGTTGCGTTGGAACCGAT				
LCO217	F: CGAGTAGAAGACGGTTAGTGTGAA	$(AC)_{17}$	222–238	59	KR337322
	R: GCGGCCTATTGTCGTGATA				
LCO222*	F: ACGAAAGAGCGTGGAAACAC	$(AGC)_7$	222	59	KR337323
	R: CGTCTCCTTCGACAGTAGTGA				
LCO223*	F: GCGGCTTGTCTAATGTCATC	$(ACAGT)_7$	223	59	KR337324
	R: CGAGCACCGATCACAAAGTA				
LCO240*	F: GACGAATAATTGATGTTCGAAACTTG	$(AC)_8$	240	61	KR337325
	R: GAATCAGCCACGGACACTG				
LCO241AC10*	F: GTGGAGCCGAAAGGAACAAT	$(AC)_{10}$	241	60	KR337326
	R: CTTTGTGGGTGGTAGCGG				
LCO241AC17*	F: CGAGTAGAAGACGGTTAGTGTGAA	$(AC)_{17}$	241	59	KR337327
	R: ACACAAGTTAAGAAATAGGCAACTG				
LCO245*	F: GATCATCTCGAGCCTCGTCT	$(AGC)_6$	245	60	KR337328
	R: CAGATGACCGGTGCGTTC				
LCO251	F: TTTGTTTCTGCAATGCTTCG	$(AT)_9$	254–260	60	KR337329
	R: AGAAGCGCTATCATGCTGGA				
LCO252*	F: AAGAGGTCACACGGGAGATG	$(AT)_6$	252	60	KR337330
	R: CTATCGAACCTGACAAGCCG				

Note: * = monomorphic loci; T_a = annealing temperature.

were mixed prior to sequencing. Postsequencing the fragments were demultiplexed, base called, and aligned using Torrent Suite Software, which produced 285,231 total reads, with an average read length of 147 bp. We used the program QDD v. 2 (Meglécz et al., 2010) to screen the raw sequences for eight or more di-, tetra-, or pentabase repeats, remove redundant sequences, and design primers from 16,622 possible loci (automated in QDD using Primer3 [Rozen and Skaletsky, 1999]).

We followed the procedure outlined in Gardner et al. (2011) to choose 29 loci for further development (Table 1). The loci were first trialed for PCR amplification in 20 individuals of *L. corrugata* using 1× MyTaq HS Mix (Bioline, Alexandria, Australia), 1 μL (20 μM) of each forward and reverse locus-specific primer, and 1–5 ng/μL DNA in 10-μL reactions. The following PCR conditions were used: 95°C for 2 min, followed by 25 cycles at 95°C for 30 s and 57°C for 4 min. PCR products were visualized on a 1.5% agarose gel stained

Table 2. Genetic variation of the seven polymorphic loci in *Lessonia corrugata.*

	Blessington Point (n = 41)				Kingston Beach (n = 35)				Total		
Locus	A	H_o	H_e	P	A	H_o	H_e	P	A_T	PIC	F (null)
LCO102	2	0.108	0.102	0.728	2	0.457	0.496	0.640	2	0.328	0.196
LCO146	2	0.366	0.481	0.125	2	0.257	0.337	0.162	2	0.369	0.214
LCO154	2	0.268	0.232	0.550	2	0.486	0.420	0.002	2	0.336	0.368
LCO199	3	0.390	0.366	0.541	3	0.600	0.599	0.517	3	0.455	0.051
LCO208	2	0.268	0.299	0.512	2	0.500	0.479	0.046	2	0.289	0.136
LCO217	4	0.585	0.583	0.137	5	0.686	0.670	0.820	5	0.599	0.005
LCO251	3	0.350	0.434	0.396	2	0.086	0.082	0.791	3	0.274	0.113

Note: A = number of alleles; A_T = total number of alleles; F (null) = null allele frequency; H_e = expected heterozygosity; H_o = observed heterozygosity; n = number of individuals tested; P = P values obtained from Hardy–Weinberg equilibrium tests; PIC = polymorphic information content.

with Midori Green. All 29 loci amplified an unambiguous product of the expected size. These amplifiable loci were then tested for polymorphism via capillary electrophoresis. Of the 29 loci, seven (24%) were polymorphic and 22 (76%) were monomorphic in a panel of 20 individuals. Due to the possibility of self-fertilization in macroalgae species such as *L. corrugata* (Raimondi et al., 2004), we ensured testing of loci was conducted on individuals geographically separated from each other by ~8.6 km to decrease the chances of monomorphism resulting from clones. Polymorphic loci were then scored in 76 individuals from two populations from Tasmania, Australia: Blessington Point (43°02′15.08″S, 147°24′15.49″E) and Kingston Beach (42°58′29.78″S, 147°20′15.75″E) (Table 2). Voucher specimens from Blessington Point (*HO 578210*) and Kingston Beach (*HO 578209*) were deposited in the Tasmanian Herbarium.

We used MICRO-CHECKER 2.2.3 (van Oosterhout et al., 2004) to check each locus for evidence of null alleles, scoring error due to stuttering, and large allele dropout. For each locus, we calculated the number and size range of alleles, observed and expected heterozygosity, and tested conformance to Hardy–Weinberg equilibrium (HWE) using GenAlEx (Peakall and Smouse, 2006, 2012). Polymorphic information content was calculated using CERVUS 3.0.7 (Kalinowski et al., 2007). We also checked all pairs of loci for linkage disequilibrium in GENEPOP and adjusted for multiple tests of significance using the false discovery rate (FDR; Benjamini and Yekutieli, 2001).

Observed and expected heterozygosity ranged from 0.086–0.686 (mean 0.386) and 0.082–0.67 (mean 0.398), respectively, with the number of alleles per locus ranging from two to five (mean 2.57) (Table 2). Two loci (LCO154 and LCO208) were significantly different from HWE in the Kingston Beach population but not in the Blessington Point population (Table 2). No individuals were found to be genetically identical. There was no evidence for null alleles, large allele dropout, or scoring error due to stuttering ($P > 0.05$) (Table 2). There was no evidence for linkage disequilibrium after adjusting for multiple tests ($P > 0.05$, 21 tests).

CONCLUSIONS

Using the Ion Torrent platform, we identified 29 microsatellite loci, of which seven were polymorphic. Cross-amplification in 11 taxonomically accepted species in the *Lessonia* genus may be possible. In addition, the markers developed here will be used to document the population genetics of *L. corrugata* and investigate the influences of habitat patchiness on genetic structure. Understanding the processes that affect gene flow in this habitat-forming species will contribute toward conserving populations and consequently protecting the biodiversity of the marine ecosystems this macroalgal species supports.

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