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PRIMER NOTE

Characterization of microsatellite loci in sea urchins (*Strongylocentrotus* spp.)

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Abstract

We present six dinucleotide repeats that were developed and characterized in *Strongylocentrotus droebachiensis* and also tested in *S. purpuratus*. Four of these loci are polymorphic in *S. droebachiensis* (13–20 alleles, $N = 100$) and five are polymorphic in *S. purpuratus* (5–12 alleles, $N = 10$). We are currently using these markers to investigate the population substructure of shallow water populations of *S. droebachiensis* in the north Atlantic.

Keywords: echinoderm, microsatellite, primers, sea urchin, *Strongylocentrotus* spp.

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Sea urchins (*Strongylocentrotus* spp.) are ecologically and economically important throughout the shallow subtidal waters of the northwest Atlantic and northeast Pacific. Extreme fluctuations in the populations of *S. droebachiensis* in the northwest Atlantic may be caused by both frequent disease outbreaks (Scheibling & Hennigar 1997) and commercial harvesting practices (Hatcher & Hatcher 1997) followed by recruitment of widely dispersed planktonic larvae. Genetic structure in *S. droebachiensis* may be affected by disease epidemics, harvesting, and genetic drift associated with lottery-like reproductive variation. Microsatellite markers have been developed for similar investigations in the sea urchin *Evechinus chloroticus* (Perrin & Roy 2000), and both population genetics and genome mapping in *S. purpuratus* (Cameron *et al.* 1999). Here we describe a unique set of polymorphic microsatellite loci we use in population genetic studies of *S. droebachiensis*.

Genomic DNA was extracted from the gonad tissue of a single sea urchin (*S. droebachiensis*) using a CTAB (cetyltrimethyl ammonium bromide) buffer and phenol-chloroform extraction (Grosberg 1996). The DNA was digested to completion using *Alu*I, *Hae*III, *Hin*cII, and *Rsa*I and size fractioned in an agarose gel. Fragments (300–700 bp) were excised and purified using a standard phenol-freeze fracture protocol (Ausubel *et al.* 1999) and ligated into the *Sma*1 site of pUC19 (Pharmacia). The library was transformed into *Escheria coli* (DH5α Gibco BRL Maximum Efficiency) and screened with ^{32}P-labelled $(GT)_{15}$ and $(GA)_{15}$ oligonucleotide probes. One hundred and eighty recombinant clones were isolated and sequenced in both directions using IRD labelled universal M13 primers and resolved in 8% (25 cm, 0.25 mm thick) denaturing polyacrylamide gels using a Li-Cor 4200 automated DNA sequencer.

All clones contained microsatellite repeats, however, most were long (> 40 repeats) and interrupted in several positions. BLAST searching did not suggest homology between these sequences and those previously reported for *S. purpuratus* (Cameron *et al.* 1999). Primers were designed for 14 of the best sequences, one of which was 5′ end labelled with either an IRD700 or IRD800 dye for visualization on the Li-Cor sequencer. Primer pairs were assayed on 10 individuals each of *S. droebachiensis* (collected from Halifax Harbor, Nova Scotia) and *S. purpuratus* (collected from Friday Harbor, Washington). Amplifications were performed in 5 μL final volume containing 5–20 ng of template DNA, 10 mM Tris-HCl, 50 mM KCl, 0.01% gelatin, 0.1% Tween 20, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 1 pmol of each primer, and 0.25 μL *Taq* Polymerase (MBI). The amplifications were performed using a Stratagene Robocycler with the following polymerase chain reaction (PCR) profile: 94 °C 60 s, T_a °C (Table 1) 30 s, 72 °C 30 s for 7 cycles and then 32 cycles of 90 °C 30 s, T_a °C 30 s, 72 °C 30 s. The amplified products were resolved in 6% (25 cm, 0.2 mm thick) denaturing polyacrylamide gels on the Li-Cor sequencer. Allele sizes were estimated using pUC18 DNA sequence fragments as standards.

A total of six primer pairs yielded interpretable amplification products in either *S. droebachiensis* or *S. purpuratus* (Table 1). The eight remaining loci all amplified in *S.*

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Table 1 Characteristics of six microsatellite loci in the sea urchins *Strongylocentrotus droebachiensis* ($N = 100$) and *S. purpuratus* ($N = 10$). T_a, annealing temperature, P, optimal [primer] (pmol/5 μL reaction), M, optimal [$MgCl_2$] (mM), SR, size range in bp, A, number of alleles observed, H_E, expected heterozygosity, H_O, observed heterozygosity

						S. droebachiensis				*S. purpuratus*			
Locus	Repeat	GenBank Accession No.	Primer Sequence (5′–3′)	T_a (°C)	P, M	SR	A	H_E	H_O	SR	A	H_E	H_O
Sd52	$(GT)_{14}$	AF506800	A: CGGTATTGCAAAGCATAACAGG B: GAGTCACGTCTTTCGGATGG†	51	2, 3	MA‡	—	—	—	129–155	10	0.92	0.70
Sd63	$(GA)_{12}$	AF506801	A: CTCTATGTGTCAATGTACCC B: TGTTGCGTAAATGGTGGGC†	49	1.5, 4	140–178	13	0.83	0.71§	NA¶	—	—	—
Sd67	$(GT)_{12}$	AF506802	A: CAATCCCCAAACACCACCC* B: TTTCCCATTCTTCCTTCATCC	51	2, 2	117	1	—	—	109–117	5	0.44	0.30
Sd76	$(GT)_3GG(GT)_{14}$	AF506803	A: TAGTCATGTACATCAGTTGG B: ATTGTGATATGAAGGTGAGG†	49	1.5, 2	143–179	17	0.87	0.71§	141–159	6	0.81	0.70
Sd121	$(GA)_{26}$	AF506804	A: TTTAGGAATGGGTCAACTGG† B: CTATAGTTATCTTCTCAGTGG	49	0.5, 2	120–157	20	0.91	0.91	128–204	12	0.92	0.90
Sd156	$(GA)_{19}AG(GA)_2$	AF506805	A: TAAGTATACAGGTCGATTCG B: AGTAAAGTAAAATGCAGGCG*	49	1.5, 2.5	178–204	15	0.86	0.69§	174–198	9	0.89	0.80

*primer 5′ labelled with IRD800 dye.
†primer 5′ labelled with IRD700 dye.
‡More than 2 alleles per individual were amplified.
§Significant deviation from Hardy–Weinberg expectation ($P = < 0.000$, tested in GENEPOP 3.1a, Raymond & Rousset 1995).
¶No products amplified.

droebachiensis, however, at least one of the 10 individuals exhibited more than two alleles and these loci were deemed unsuitable for a population genetic analysis. Similar patterns were observed for two of the remaining eight loci in *S. purpuratus*, and six loci failed to amplify.

Four polymorphic loci were further characterized by scoring 100 individual *S. droebachiensis* collected from Norris Cove, Newfoundland (Table 1). Statistical analyses were performed using GENEPOP version 3.1d (Raymond & Rousset 1995). Three loci (Sd63, Sd76, Sd156) show significant deviations from Hardy–Weinberg equilibrium, suggesting the presence of null alleles. We observed segregating null alleles in laboratory reared larvae, but the frequency of such nulls in the population are unknown. Since no homozygous nulls were detected in any of the 100 individuals sampled it is possible that the departures from Hardy–Weinberg equilibrium are a result of the Wahlund effect. All polymorphic loci in *S. purpuratus* were in Hardy–Weinberg equilibrium. These markers are currently being used to investigate the population genetic structure of *S. droebachiensis* in the north Atlantic.

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