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PRIMER NOTE

Polymorphic microsatellite loci from the red urchin, *Strongylocentrotus franciscanus*, with comments on heterozygote deficit

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Abstract

***Strongylocentrotus* sea urchins are common subjects for studies in developmental and cell biology, reproductive biology, ecology, and evolution. We report 14 microsatellite loci from the red urchin, *S. franciscanus*, isolated for the purpose of estimating paternal success of males in experimental group spawns. Most of these loci were found to be highly polymorphic in a population from British Columbia. A high frequency of null alleles appears responsible for heterozygote deficit at a majority of these loci, but if used with appropriate caution, these microsatellites should be effective markers for studies of *Strongylocentrotus* populations.**

Keywords: red sea urchin, microsatellite, dinucleotide repeat, parentage, heterozygote deficit, null allele

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Sea urchins in the genus *Strongylocentrotus* are intensively used for studies in developmental and cell biology, reproductive biology, ecology, and evolution. One of us (DRL) is investigating the fertilization ecology of populations of the red urchin, *S. franciscanus*, in Barkley Sound, British Columbia. We developed the microsatellite loci reported in this paper for parentage assignment of larvae harvested from experimental group spawns. These experiments are aimed at determining the effects of proximity and density of other spawning males on fertilization success of individual male urchins.

A partial genomic library in bacteriophage λ was constructed using a modification of the methods of Hughes & DeLoach (1997). Gonad tissues were dissected from six *S franciscanus* urchins, and genomic DNA was extracted using a CTAB/PCI method, precipitated with ethanol, and digested with *Sau*3A I. Fragments between 350 and 750 bp were size-selected on a 2% agarose gel, recovered from the gel using a modification of the 'freeze and squeeze' method (Tautz & Renz 1983), then cloned into *Bam*H I-digested λ-ZAP vector (Stratagene, La Jolla CA). Plaque lifts and hybridizations followed standard methods (Sambrook & Russell 2000). The library was screened with long DNA alternating copolymers [poly(dA-dC) poly(dG-dT) and poly(dT-dC) poly(dG-dA): Amersham] that were fill-in labelled with α-[^{32}P] dCTP and Klenow. Positive clones were plaque-purified and hybridization-screened again, then sequenced with the vector primers on an ABI 377 sequencer, using the ABI Big Dye kit. Polymerase chain reaction (PCR) primers flanking microsatellites were designed with the aid of OLIGO IV software.

Routine genotyping was accomplished as follows. Adults were collected from the mouth of Bamfield Inlet, Barkley Sound, British Columbia, Canada (48°50.5′ N, 125°08.5′ W) and tissue from gonad or tube feet was extracted using the CTAB/PCI extraction protocol. Diluted DNA was added to a standard PCR cocktail (5.9 μL autoclaved ddH20, 1.0 μL 10 × PCR buffer, 1.0 μL 10 mM dNTP's, 0.5 μL 10-μM flourescently labelled forward primer, 0.5 μL 10-μM reverse primer, 0.75 U *Taq* polymerase) and amplified as follows: 95 °C for 5 min; then 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min; then 75 °C for 25 min. PCR products from three compatible loci (i.e. different-sized fragments or different fluorescent labels) were mixed with HiDi Formamide (1:12) and analysed on an ABI 3100 automated sequencer equipped with GENESCAN and GENOTYPER software. Exact tests for deviation from Hardy–Weinberg

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Table 1 *Strongylocentrotus franciscanus* microsatellite loci

Locus	Repeat motif of clone	Accession number	Primer sequences (5′ to 3′)	Product size range (bp)
GTM2	$(GT)_{17}$	AY441960	**F:** ACT TTG CCA GAG TCA CTG CTT A **R:** CCC CAA ACA CAG GCT AAC AT	266–332
GTN11	$(GT)_2GAATGC(GT)_6TTGC(GT)_{13}$	AY441961	**F:** CCT GCT TTC AGT TCC GTG AT **R:** AAC AAC ATC CAG CCA CCA TT	121–185
GAP1	$AA(GA)_{13}GAGAAA(GA)_{15}GT(GA)_2$ $GATA(GA)_9(GC)_4(GA)_2GG(GA)_5$	AY441962	**F:** CTG AAA ATG TAA GAG GTA AGG G **R:** TTG TAT TGC CAT CGA ACT C	280–392
GAP2	$CA(GA)_4CAGAAA(GA)_{13}$ $GCGAA(GA)_4AA(GA)_3$	AY441963	**F:** GAT GAA ACA TAT AGA GGG ACA G **R:** CGT CTA TCC TTA ACT CTC CAT	160–180
GAP11	$(GA)_{13}GT(GA)_{14}$	AY441964	**F:** TTA AGT GGG AAT GAG ACA GAG A **R:** CCT GCA ATT ATC ACC TTT CA	458–498
GAO1	$(GA)_4GAAA(GA)_{14}$	AY441965	**F:** GAT TAG AAA GAA CGA GAG AAC G **R:** TGT AGG GTT GAT TTT TGA AAG T	104–132
GAO3	$(GA)_5GGAGGGGGGTT(GA)_{19}$	AY441966	**F:** GTC TGC GCA TTA TAC GTG TAA C **R:** CCT GTT CTT TCT CCA TCC C	368–462
GAO4	$(GA)_{21}AAGA$	AY441967	**F:** TTG ATT GAA AGA GCG CCA TCT C **R:** TCT TCC TGT CCC TCT ACC ACC C	115–193
GAO11	$(GA)_{10}$	AY441968	**F:** CCC TTT TCT CTA ATT CTT GCT T **R:** GGC TTG GAC TAT GAG TGT GA	166–236
GAO12	$(GA)_{11}GGG(GA)_7$	AY441969	**F:** TGC GAG AAT GCT AAA TAG AGA **R:** CCC AAT CTG TGT GAT ACA TAG A	187–201
GAO16	$GAGG(GA)_3AAGAGGCA(GA)_3$ $CA(GA)_{10}$	AY441970	**F:** TGC GAG AGG GTG AGT GAG **R:** AAG CCA AAG AAG AAC ACA TCA G	120–188
GAO20	$(GA)_{12}AA(GA)_3CA(GA)_2$ $CAGACA(GA)_2$	AY441971	**F:** GCA CGC AGT GTC AAA GTG ATA G **R:** CAC CCA ACT GGC TCT AAC GA	246–392
GAO21	$(GT)_{14}(GA)_{17}$	AY441972	**F:** TGA GGG GGA AGA GAC AG **R:** CTC CCA AAT ACT TAA ACT GCT C	242–342
GAO25	$(GA)_2GG(GA)_{19}$	AY441973	**F:** GAA CAT GAC GAA AAG AAT GAT A **R:** GCC TAC TTT CTC TCC CTC C	140–206

Table 2 Heterozygosity at *S. franciscanus* microsatellite loci. N = number of adults genotyped per locus; heterozygosity values are H_E (expected) and H_O (observed); test for HWE: ***$P < 0.001$, **$P < 0.01$; r = estimated frequency of null alleles; N_{00} = number of double-null homozygotes (exp = expected, obs = observed)

Locus	N	Number of alleles	H_E	H_O	r	N_{00} (exp)	N_{00} (obs)
GTM2	164	34	0.962	0.713***	0.127	2.6	6
GTN11	124	24	0.915	0.331***	0.305	11.5	0
GAP1	344	33	0.926	0.715***	0.109	4.1	3
GAP2	23	7	0.792	0.565***	0.127	0.4	0
GAP11	127	13	0.199	0.181	0.015	0.0	1
GAO1	23	11	0.853	0.217***	0.343	2.7	0
GAO3	134	26	0.947	0.410***	0.275	10.2	12
GAO4	91	24	0.897	0.385***	0.270	6.6	0
GAO11	352	32	0.918	0.804***	0.060	1.2	5
GAO12	23	8	0.835	0.826	0.005	0.0	0
GAO16	308	24	0.880	0.662***	0.116	4.1	0
GAO20	354	62	0.970	0.726**	0.124	5.4	2
GAO21	183	25	0.904	0.601***	0.159	4.6	6
GAO25	278	30	0.926	0.489***	0.227	14.3	5

expectations (HWE) were conducted using GENEPOP *3.3* (Raymond & Rousset 1995) using default Markov chain parameters.

Of the approximately 170 clones isolated, 39 were selected for sequencing, and 31 of these clones contained microsatellite repeats. Twenty of the loci were PCR-amplifiable and polymorphic, and of these, 14 of the those that amplified most consistently from adults and larvae (shown in Table 1) were selected for further genotyping. Each of the loci genotyped in more than 100 adults were highly polymorphic, yielding from 13 to 62 alleles (Table 2). Twelve of the 14 loci showed highly significant heterozygote deficits,

which in some cases were dramatic (Table 2). Since we encountered DNA extracts that failed to PCR-amplify, we suspected that a portion of the heterozygote deficiency was likely due to null alleles producing single-banded phenotypes in individuals that are genotypically heterozygous. The estimated frequency of null alleles (Brookfield 1996) was considerable, and at most loci generated an expected number of double-null homozygotes that was similar to the observed number of double nulls (the latter scored as the number of individuals that failed to produce a band upon genotyping: Table 2). A *t*-test for paired comparisons across all loci indicated no significant difference ($t = 1.605$, $df = 13$, $P = 0.132$) between expected and observed number of double-null homozygotes.

While many factors may cause deviations from HWE in marine populations (e.g. Hare *et al*. 1996; Ruzzante *et al*. 1996), and whereas a full evaluation of the contribution of null alleles requires pedigree data, nulls seem to be a parsimonious explanation for heterozygote deficit at these microsatellite loci. Nevertheless, the loci reported here substantially expand the list of microsatellite markers available for *Strongylocentrotus* sea urchins (Cameron *et al*. 1999; Addison & Hart 2002), and if used with appropriate caution, provide high allelic diversity for studies of molecular ecology and population genetics. We developed these loci for parentage studies, and hence devoted little effort to redesigning primers to eliminate null alleles. However, we would certainly recommend primer redesign prior to using these loci for population genetics. Moreover, several of the loci contain imperfect and compound repeats within the core repeat array (Table 1), and it is possible that sequence variation within the repeat generates allele-length homoplasy. For this reason, we would also recommend sequencing of a subsample of alleles from natural populations, so as to determine the degree to which size homoplasy may confound parentage and population genetic analysis.

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