

Isolation and characterization of new microsatellite markers in the surfclam *Mactromeris polynyma*.

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Abstract

We describe primers and amplification conditions for seven microsatellite loci that were developed and characterized in the commercially harvested surfclam *Mactromeris polynyma*. Five of these loci were polymorphic: we found 6–23 alleles per locus among 100 individuals from one population in Nova Scotia, with some large heterozygote deficits that may reflect unrecognized temporal genetic variation. We are using these markers to investigate temporal and spatial genetic structure in this species.

The surfclam *Mactromeris polynyma* is mainly harvested for global sushi and sashimi markets by towing a hydraulic dredge on the sea bottom of the northwestern Atlantic and northeastern Pacific oceans (Roddick and Smith, 1999). Like *Spisula* surfclams (to which they are closely related, and with which they were formerly classified as *Spisula*; Turgeon *et al.*, 1988), *M. polynyma* are patchily distributed, and commercially targeted populations may be widely separated from each other by hundreds of kilometers of ocean bottom in which the clams are relatively scarce. These populations also show significant age structure, with large variation in the relative abundance of age classes 50 years old or more (Roddick and Smith, 1999). Such age structure is accompanied by significant genetic variation among age classes in some *Spisula* species (David *et al.*, 1997). The combination of these spatial and temporal patterns could produce complex population genetic variation in *M. polynyma* that is directly relevant to the management of intensively harvested Atlantic populations. Here we describe polymorphic microsatellite loci that we use in population genetics studies of *M. polynyma*.

Genomic DNA was extracted from the adductor muscle and the foot of a single specimen using a high salt/chloroform extraction adapted from Simison and Lindberg (1999). The DNA was sent to Travis Glenn's group at the Savannah River Ecology Laboratory where it was enriched (Hamilton *et al.*, 1999) for di-, tri-, and tetranucleotide microsatellite repeats (e.g., Croshaw and Glenn 2003). We ligated and cloned the enrichment using protocols, reagents, and competent cells from a TOPO-TA cloning kit (Invitrogen). One hundred clones were isolated from each enrichment and sequenced in both directions using IRD-labeled universal M13 primers on a Li-Cor 4200 automated DNA sequencer. BLAST searches did not suggest any close homology between the cloned sequences and previously reported genomic DNA sequences.

Many clones contained microsatellite-like sequences that were interrupted at several positions or had a mosaic of different repeat motifs. Primers were designed for seven sequences, and the 5' end of one for each pair was labeled with either an IRD700 or IRD800 dye for visualization on the Li-Cor sequencer. Primers were assayed on 100 *M. polynyma* individuals collected from Lockeport, Nova Scotia. Amplifications were performed in 5 μ L final volume containing 10 ng of template DNA, 10 mM Tris-HCl, 50mM KCl, 0.01% gelatin, 0.1% Tween 20, 0.2 M each dNTP, *M* mM MgCl₂ (concentration varied among loci; see Table 1) *P* pmol of each primer (Table 1), and 0.5 μ L Tsg polymerase (Biobasic, Toronto, Ontario, Canada). The amplifications were

performed using a Stratagene Robocycler with the following polymerase chain reaction (PCR) profile: 95°C 180 s, 94°C 60 s, T_a °C (Table 1) 60 s, 72°C 60 s for 30 cycles. 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.

[One new change: in the paragraph above you give the primer magnesium concentrations, but in the table these varied among loci. Use some kind of convention like the one you used for annealing temperature in the following sentence to indicate that this is a variable that was manipulated to optimize each locus; I made one possible change, but you could do something different if you like; I also put these three variables in italic font for emphasis]

A total of five primer pairs yielded interpretable amplification products in *M. polynyma* (Table 1); two other loci were fixed for a single allele. Observed heterozygosity was less than the Hardy-Weinberg expectation for all loci, including the most (Mp57) and least (Mp102) polymorphic. Quantitative tests of this deviation using GDA version 1.0 (Lewis and Zarkin, 2001) were significant for four loci (Mp57, Mp90, Mp100, Mp102). Part of the shortage of heterozygotes could reflect the presence of unamplified null alleles. For example, 4% of individuals at locus Mp90 were potential null-null homozygotes. However, we found no null-null homozygotes at two other loci where the observed heterozygote deficit was similar in absolute size (Mp100) or greater as a proportion of the expected heterozygosity (Mp102) in comparison to locus Mp 90. Thus, it is possible that the departures from Hardy-Weinberg equilibrium are a result of unrecognized spatial or temporal structure within this sample (a possibility we are currently exploring across multiple populations).

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Table 1 Characteristics of five microsatellite loci in the surfclam *Mactromeris polynyma* (N = 100). T_A, annealing temperature; P, optimal [primer] (pmol/5_l reaction); M, optimal [MgCl₂] (mM); ASR, allele size range in bases; A, number of alleles observed; H_e, expected heterozygosity; H_o, observed heterozygosity; %, percent of amplified individuals.

Locus	Repeat	GenBank Assession No.	Primer Sequence (5'-3')	T _A (°C)	P,M	ASR	A	H _e	H _o	%
Mp53	(CT) ₁₁ TT(CT)TT(CT)	AY548747	A:TATGTGGCTTAATGCTTC B:ATACAATTACTACCATCTC [†]	47	1, 4.5	187 – 197	8	0.72	0.67	99
Mp57	(GATT) ₂ T(GATT) ₄ T(GATT) ₁₁ AATT(GATT) ₂	AY548748	A:GAAGAAAGAGAAATGAATG B:AACAGCATTATCATCATCTTG*	54	2, 4.5	158 – 244	23	0.91§	0.71	98
Mp90	(GA) ₁₁ AA(GA) ₄	AY548749	A:GAAATTCAACGCTCACTCG* B:AGTGACAAAGGATCGCCG	52	1, 3	76 – 118	14	0.88§	0.55	96
Mp100	(CA) ₁₁	AY548750	A:ACAAAGTAGCATTCCCGTTC* B:GCCCCGTCTGTGTGTAATAC	53	2, 4.5	90 – 110	8	0.82§	0.49	100
Mp102	GTTT(GTT) ₁₀	AY548751	A:GCCGACCATTATTTAGAAG B:ATAAACCTCTGACAAGTCG*	51	0.6, 4.5	94 – 127	6	0.33§	0.14	100

* primer 5' labeled with IRD700 dye

[†] primer 5' labeled with IRD800 dye

§ significant deviation from Hardy – Weinberg expectation (P = < 0.001)