NEW MICROSATELLITE MARKERS FOR THE ECOSYSTEM ENGINEER *Perumytilus purpuratus*

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INTRODUCTION

The ecological dominance by filtering vertebrates is a typical feature of the Chilean intertidal rocky shore (Prado 2004). The "chorito maico" *Perumytilus purpuratus* is a dominant species of the primary space and an ecosystem engineer. Its distribution and morphometric properties (shape, width and asymmetry of valves) have been intensively described along 3,900 km of Antofagasta Bay (Guiñez et al 1999). Both, the ecological and adaptive meanings of such distributional and morphological traits are being currently investigated from ecological and genetic perspectives, through an interdisciplinary cooperation between Chilean and Spanish scientists (Guiñez et al 2005). A compulsory tool for the fine genetic analysis at intraspecific scale, is a highly polymorphic genetic marker. Such marker should be useful to inform us not only about the regional diversity of populations but also about dispersive patterns, reproductive systems, and individual gametic contributions. In this study we report the standardisation of eight microsatellite loci from the genome or *P. purpuratus*, which technical development and final achievements are briefly presented here.

MATERIALS AND METHODS

To isolate the microsatellite regions we used a modification of the enrichment technique FIASCO: Fast Isolation by AFLPs of Sequences Containing repeats (Zane et al 2002).

Genomic DNA was extracted following Pérez (2002) from muscle of a single individual of *P. purpuratus* collected in Punta Tralca (Chile). Linked genomic fragments were enriched using three biotinylated probes $(CA)_{13}$, $(GATA)_7$ y $(GACA)_7$.

PCR products larger than 300 bp were ligated into pGEM-T Vector System II (Promega) and transformed into JM 109 high efficiency competent cells (Promega).

The recombinant clones were picked up from culture plates and grown up to amount enough material for *southern* transfer.

The filters obtained were hybridised with 87,5 ng of probe per 100 cm2 of filter at 68ºC following the recommendations of the DIG-DNA Labelling and Detection Kit (Roche)

The selected clones were lisated and their plasmids purified using the GFX Micro Plasmid Prep Kit (GE Spain). Sequencing was performed on both strands in an ABI Prism 3100 Sequencer (Applied Biosystems) using the BigDye Terminator method.

PCR primers were selected in the flanking regions with the programme Oligo 4.05 (Rychlik and Rhoads 1989) and amplification conditions were tested using a Mastercycler Gradient Thermocycler (Eppendorf).

About 100 ng of total DNA from each individual was used as template in a PCR reaction of 20 µLcontaining 1 U Taq Polymerase in 1X reaction buffer (Promega), 20 pmol of each primer, 200 µM of each dNTP and $MgCl_2$ ranging from 1.0 to 2.0 mM (Table 1). The amplification reaction consisted of one cycle at 95ºC for 5 min, followed by 35 cycles at the annealing temperature (Table 1) for 40 s, 72ºC for 1 min and 94ºC for 1 min followed by a final extension step at 72ºC for 10 min.

Allelic variation was assessed in 17 (Punta Tralca) and 15 (Puerto Lobos and Comodoro Ribadavia) individuals of *P. purpuratus.*

The PCR products were visualised in 2% agarose gels and electrophoresed in 6% acrylamide:bisacrylamide gels (19:1), followed by silver staining and gel fixation (Promega). Allele sizes were characterized using a ladder of 20 bp (Takara).

RESULTS

From the recombinant clones picked up (545), seventy clones (13%) showed positive signal after filter hybridisation with synthetic oligonucleotide probes $(CA)_{13}$, $(GATA)_7$ y $(GACA)_7$, and were subsequently sequenced. Forty two clones (60%) out of 70 did not contain microsatellite-like regions, six remained unsequenceable and 19 clones (27%) had at least one repeated motif. Eight (42%) out if the 19 microsatellite-containing sequences did not allow for primer design, and 3 sequences were redundant (16%). The remaining eight sequences (representing 42% of all microsatellites; 11% of the observed positives in *southern*, and 1,5% of the initial plated cultures) allowed for primer design and further analysis of polymorphisms using PCR and acrylamide electrophoresis. All the loci selected produced readable amplifications in *Perumytilus purpuratus*, the allele sizes ranging from 73 to 230 bp. The number of alleles recorded in two initial populations ranged from 5 (locus Ppu 1) to 7 (locus Ppu 7).

Table 1. Characteristics of eight microsatellite loci from *Perumytilus purpuratus*. Ta, annealing temperature; Na, number of alleles; Ni, number of individuals successfully amplified; NT, non tested (in advancement).

Locus	Repeat motif	Primer sequences (5' - 3')	Ta (ºC)	$MgCl_2$ (mM)	Size	Na	Ni
Ppu1	$(GACA)_3(CACG)_6$	F: TAAAAATGAAGGAGGAGAT	52	1.5	109	5	29
		R: TATTTATACCCGCACTTTC					
Ppu2	$(GACA)_6$	F: ACAAGAAAGTATGATGGACG	55	1.5	73	NT	
		R: GGGTATATTGTCGTTTCG					
Ppu3	$(TATC)_7$	F: ATGATTATATGTGTTTTGTA	50	2	160	NT	
		R: TTTGATATTTCTGGTTTC					
Ppu4	$(GACG)_5$ GCTC $(GACA)_4$	F: ACCATCATAACAAACAACTG	50	1.5	230	NT	
		R: TAAACCAAACCATAAACATT					
Ppu5	$(GACA)_6N_{12}(GACA)_5$	F: GTCACAAGAAAATTGT	50	1.5	204	6	29
		R: GTTATATGCGGTAGTT					
Ppu6	$(GGAC)_7$	F: GACCATTCAACCAAACTT	55	1.5	219	6	29
		R: GTAAACATCGCTACATACG					
Ppu7	$(CGTC)_{11}$	F: TCATTGGACCTTAGATGTTTC	55	1.5	126	7	29
		R: ACGACCAGACGGATTGAC					
Ppu8	$(ACG)_2G(ACG)_2G(ACG)_3G(ACG)_3G$	F: CCAGTGGTTTCAGAGGAG	58	1.5	107	NT	
		R: AGCTATTGCCATCACTTG					

DISCUSSION

These new markers could be used as a poweful intraspecific genetic tool to undertake fine population and individual studies in *P. purpuratus* and to provide insights onto the dynamics and significance of its regional polymorphisms in the frame of ecologically bounded ongoing projects of the authors.

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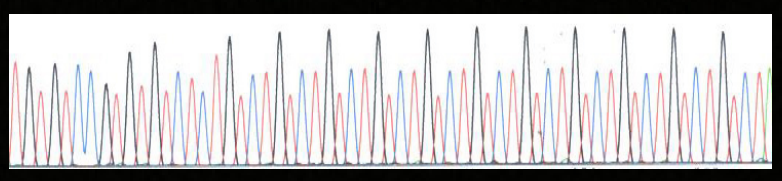

Electropherogram of a microsatellite region from *P. purpuratus* showing a DNA repeat motif of (GTC)n.

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