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Isolation and characterisation of three microsatellite loci from the Chilean scallop *Argopecten purpuratus*

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PCR primers were developed for three microsatellite loci successfully isolated from the Chilean scallop *Argopecten purpuratus*. One locus was highly variable and therefore suitable for phylogenetic analysis. Heterozygosity was tested in four populations within the natural Chile distribution of the species. This locus will be useful for population genetic analysis if further polymorphic loci are isolated.

The Chilean scallop *Argopecten purpuratus* is a commercially important species widely cultured in Chile and Peru. Within any aquaculture industry, genetic approaches can provide valuable information upon which to base management decisions (Allendorf & Ryman 1987). Aquaculture practices aiming to obtain maximum sustainable yields need basic information on the population dynamics, the genetic status of local populations, and the recruitment rate required to replenish these populations (Beaumont 1991). Molecular information is of primary interest to aquaculture development as it reflects past genetic history and can indicate the scale of overall level of adaptive variation (Gallardo *et al*. 1998). At present no systematic studies have been carried out to investigate the population genetics of this species and therefore genetic management cannot currently be successfully practised in aquaculture of *A. purpuratus*.

The aim of the study was to improve the predictability and sustainability of scallop culture in Chile. This was to be achieved by providing baseline data for genetic improvements of scallop stocks and the conservation of genetic resources by assessing the genetic diversity in stocks using microsatellites and mitochondrial DNA genetic markers (Pickerell 2003). In this review, the isolation and characterisation of three microsatellite loci for *Argopecten purpuratus* is described.

O'Connell and Wright (1997) have shown that microsatellites have been successful at detecting population differentiation in species where mtDNA failed to do so. Microsatellites can also be used to both assess the amount of inbreeding in cultured populations (Harris *et al*. 1991, Stevens *et al*. 1993) and locate the specific chromosomal regions responsible for inbreeding depression (Ferguson & Danzmann 1998). Microsatellites have been found to be a valuable tool for aquarists for use in analysing the genetic diversity of breeding programs (Wolfus *et al*. 1997).

Total genomic DNA was extracted from 0.5 g of *A. purpuratus* abductor muscle preserved in 70% ethanol by a standard phenol-chloroform method. A size-selected library was produced by digesting total genomic *A. purpuratus* with *Sau3A* restriction enzyme and excising the 300–800 base-pair range from a low-melting point agarose gel. Size selected genomic DNA was ligated into pBluescript SK+ vector (Stratagene) and transformed with XL1-Blue electroporation-competent cells (Stratagene) using standard laboratory techniques (Sambrook *et al*. 1989). Following plating, blue/white screening enabled insert-containing colonies to be 'picked' using sterile P10 Gilson tips and replated by streaking diagonally in pre-measured 1 cm^2 squares drawn as a grid on the base of the plate. Colonies were lifted using Biodyne A membranes (1.4 μm pore-size) and probed with two radioactively-labelled dimeric repeats (GA_{10} and CA_{10}) with standard laboratory procedures (Sambrook *et al*. 1989).

Positive screen colonies were picked with sterile P10 Gilson tips and incubated overnight in 5 ml of Luria-Bertani (LB) broth containing 50 μg ml^{-1} ampicillin. The plasmid DNA was extracted from the cells using the QIAprep Spin Miniprep Kit (Qiagen). To confirm that the plasmid DNA contained an insert, an *EcoR1* digest was performed on the plasmid DNA. Insert containing, positive, plasmid DNA was sequenced by MWG-Biotech (VALUE READ® service).

From a library of approximately 7500 colonies, 35 positive colonies were isolated and sequenced and microsatellites were identified in 14 clones. Polymerase chain reaction (PCR) primers were designed in the microsatellite flanking regions for 3 clones Apur1, Apur2 and Apur3 (Table 1) using the GeneRunner program (available from www.generunner.com). Each locus was pre-screened for polymorphism by testing with 10 individuals from the broadest geographical range. For this the PCR products were subjected to single sequence conformational polymorphism (SSCP) and visualised on a non-denaturing 10% (37.5:1) TBE-polyacrylamide gel run at 10 °C using a Hoefer circulator bath at a constant voltage of 300V for 9 hours. The Apur1 primer pair detected clearly highly polymorphic PCR products, the other 2 pairs detected no variation. A repeat SSCP of PCR products from a further 20 individuals also revealed no variation and the Apur1 and Apur2 primer pairs were discarded.

The PCR reactions were carried out in a PTC-225 Peltier Thermal Cycler (MJ-Research) in a 25 μl reaction containing ~50 ng template DNA, 1 unit *Taq* polymerase (SIGMA), 1 × PCR buffer, 2.5 mM $MgCl_2$, 100 μM dNTPs and 5pmols of each primer. The PCR profile started with 3 mins of 95 °C, followed by 10 cycles of denaturing at 94 °C for 1 min, followed by locus-specific annealing (Table 1) for 30 sec, followed by 72 °C extension for 30 sec. A subsequent 22 cycles of denaturing at 90 °C for 30 sec, followed by locus-specific annealing (Table 1) for 30 sec, followed by 72 °C extension for 30 sec, followed. A final 72 °C extension was then performed.

To screen for variation, the forward primer was labelled with γ^{32}P-ATP and utilised in further PCR reactions using the same conditions as above. PCR products were run a 6% TBE-polyacrylamide sequencing gel (Sequagel, National Diagnostics) on a Gibco BRL Sequencing System vertical rig and run for 150–200 minutes at 97 V. The gel was exposed to Kodak X-Omat

Table 1. Characteristics of three microsatellite loci in *Argopecten purpuratus*.

Locus	Repeat motif	Range of PCR product (bp)	n_a	T_a	Primer sequence (5´–3´)	Accesion number
Apur1	GT_{22}	106–132	14	57	**F** ATA CAG GTG CGT TGC GTG	AY264336
					R GTG GAG CGA ACA TAC TGC	
Apur2	$CAAA_4$	81	1	60	**F** CCC TAA ATG ACC TTA GTT G	AY300025
					R GTA TTT ATA ACC AAG ATA AC	
Apur3	GT_3GTT_5	210	1	57	**F** TCC TGT GTC TAT TGG TCC	AY300024
					R AGT GAT GAC TCA TGC CTC	

n_a = number of alleles observed, T_a = annealing temperature (°C)

AR film in an autoradiography-cassette for 24 hours at –70 °C. We scored the gels by eye on a lightbox using a control DNA sequence (Sequenase 2.0).

The Apur1 locus was highly polymorphic with a range of 14 alleles (GT_{21}–GT_{34}). Observed and expected heterozygosities in each population ranged from 47%–92% and 73%–79% respectively. The Apur1 locus seems highly suitable for studying population genetics of *A. purpuratus*.

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Editorial Note

The fields of zoology, ecology and evolutionary biology are increasingly dependent on molecular tools, and as such, the *Annales Zoologici Fennici* (AnZF) welcomes papers containing information that will aid these fields. The primer note of Pickerell and colleagues published above is an honest indicator of our willingness to publish such information. However, this paper should not be taken as a template for the amount of data necessary for future submissions. In other words, while we welcome primer notes (as well as other types of technical notes), the minimum for publication of primer notes in future will be four informative (i.e. polymorphic) loci.

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