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PRIMER NOTE

Characterization of 12 single nucleotide polymorphisms in weathervane scallop

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Abstract

We describe 27 single nucleotide polymorphisms (SNPs) in a commercially important bivalve, the weathervane scallop (*Patinopecten caurinus*), identified using a targeted-gene approach. We further characterize 12 of these using 5′-nuclease and allele-specific PCR assays. Polymorphisms were identified in both mitochondrial and nuclear genes. These are the first SNPs developed for delineating population structure in the weathervane scallop and will provide a useful complement to currently available genetic markers.

Keywords: *Patinopecten caurinus*, single nucleotide polymorphism, weathervane scallop

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In Alaskan waters, commercially valuable aggregations of weathervane scallops (*Patinopecten caurinus*) are found in spatially isolated beds from the northern Gulf of Alaska through the Bering Sea. Little is known about the genetic structure of weathervane scallop populations. Growth rates appear to be much greater in populations around Kodiak Island than in any other areas of the state. Preliminary analysis indicates that Bering Sea scallops mature at an older age than Gulf of Alaska scallops do. These phenomena may be signatures of genetic discreteness or they may be environmentally mediated. Improved knowledge of the genetic stock structure of weathervane scallops would lead to improved management based on stock-specific rather than species-specific criteria.

Genetic markers for population studies of scallop species have been generally limited to allozymes (e.g. Dolganov & Pudovkin 1998), mtDNA RFLPs (e.g. Orbacz *et al*. 1996) or microsatellites (e.g. Gjetvaj *et al*. 1997). In view of potential limitations of these approaches, we sought to add an additional class of markers, single nucleotide polymorphisms (SNPs), which possess a number of advantages for the study of population structure (Morin *et al*. 2004). Mitochondrial SNPs revealed intraspecific heterogeneity in the Japanese scallop *Patinopecten yessoensis* (Sato & Nagashima 2001). Here, we describe the discovery of 27 SNP markers and the development of high-throughput genotyping assays for 12 of these markers.

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DNA was extracted from 95 individual *P. caurinus* (hereafter abbreviated *Pcau*) collected from two locations in Alaska, Kamishak Bay and Umnak Island, using DNeasy 96 Tissue kits (QIAGEN). Because no DNA sequence data were available for *Pcau* in public databases, we designed primers by applying OLIGO 6.0 (Molecular Biology Insights, Inc.), to sequence data from the congeneric species *P. yessoensis*, its closest living relative (Waller 1991), to amplify and sequence several target genes. Mitochondrial targets included cytochrome oxidase subunit 1 (*COX1*), a large fragment of mostly putative noncoding region (*NCR2*) and a 1241-bp fragment encompassing the 3′ end of cytochrome oxidase subunit 3, $tRNA^{Lys}$, $tRNA^{Gln}$, $tRNA^{Glu}$ and the 5′ end of ATPase 6 (*Pcau2*). Nuclear targets included octopine dehydrogenase (*ODH*), troponin C (*TnC*), sarcoplasmic calcium-binding protein (*SPC-a*), and *18S rRNA*. A calmodulin-like fragment (*CaM*) was amplified using degenerate primers, cloned and sequenced. Amplicons were purified using magnetic beads (Ampure) and sequenced with a SCE2410 capillary sequencer (Spectrumedix) using BigDye 3.1 dye terminator chemistry (ABI). Sequence analysis revealed polymorphisms in *COX1*, *Pcau2*, *NCR2*, *ODH*, and *CaM* (Table 1); *TnC*, *SPC-a*, and *18S rRNA* were monomorphic.

Genotyping assays using 5′-nuclease were designed using Primer Express (ABI) and performed in 384-well

Table 1 Twenty-seven single nucleotide polymorphisms in weathervane scallop

GenBank Accession	Locus	SNP position: identity (most common allele first)
AY704170 (651 bp)	*COX1*	51: C/T 252: G/A
AY704168 (1251 bp)	*Pcau2*	665: T/C 995: C/T 1063: C/T 1083: G/A
AY704171 (1262 bp)	*NCR2*	56: A/G 67: A/G 71: G/C/T 104: T/C 140: A/T 193: A/T 194: A/G 197: G/T 220: G/T 347: A/G 891: G/A 999: A/G 1045: G/A
AY704172 (720 bp)	*ODH*	189: A/G 411: C/A
AY769248 (491 bp)	*CaM*	74: A/G 75: T/C 168: A/T 282: C/T 377: A/G 386: T/C

reaction plates, following the genotyping system described by Morin *et al*. (1999). The 5′-nuclease reaction relies on the exonuclease activity of DNA polymerase and cleaving of the allele-specific probe during PCR. Each reaction was conducted in a 10-μL volume consisting of 0.4 μL template DNA in 1× TaqMan PCR cocktail (ABI), 900 nM each PCR primer, and 200 nM each probe. Thermal cycling was performed on either the ABI 7900 Real Time Sequence Detection system or the DNA Engine Tetrad (MJ Research) as follows: an initial denaturation of 10 min at 95 °C, followed by 45 cycles of 92 °C for 15 s and 1 min at the annealing temperature noted in Table 2. All cycling was conducted at a ramp speed of 1 °C per second. Scoring of individual genotypes was performed using SEQUENCE DETECTION software 2.1 (ABI).

An alternative genotyping approach was used for the *NCR2* region where several polymorphisms occur at adjacent bases (positions 193, 194 and 197), precluding the use of TaqMan probes. For two of these SNPs, 193 and 197, allele-specific PCR (ASPCR) assays were designed and conducted as modified after Germer & Higuchi (2003). Each reaction was carried out in a final volume of 10 μL, consisting of 0.4 μL of template DNA; 1.2 units of Ampli*Taq* DNA polymerase, Stoffel Fragment (ABI); 10 mM Tris-HCl, pH 8.0; 40 mM KCl; 2 mM $MgCl_2$; 100 μM each dNTP; 0.2× SYBR Green I (Molecular Probes); 1× Reference Dye (Sigma); 5% DMSO; and 2.5% glycerol. Real-time PCR was performed on an ABI 7900HT as follows: an initial denaturation of 1 min at 95 °C, followed by 40 cycles of 92 °C for 15 s and 20 s at the annealing temperature noted in Table 2. Fluorescence data were analysed using SEQUENCE DETECTION 2.1.

All genotypes obtained with the SNP assays were identical to those obtained by sequencing. SNPs scored by the high-throughput methods had a negligible failure rate, 1–2 individuals per collection of 95 (Table 2). Nuclear polymorphisms all displayed Hardy–Weinberg genotypic proportions, suggesting that null (non-amplifying) alleles were not present in significant numbers.

Table 2 Characterization of 12 single nucleotide polymorphisms using high-throughput genotyping. An asterisk denotes allele specific PCR; all other assays are 5′ nuclease. *N* is the sample size, H_E and H_O are expected and observed heterozygosities for nuclear genes

			Kamishak Bay				Umnak Island			
Assay name	Oligonucleotide sequences (5′–3′)	T_a (°C)	*N*	Rare allele frequency	H_E	H_O	*N*	Rare allele frequency	H_E	H_O
Pca_COX1–51	F: GAGGGACGGGTTGAACTA R: ACCCCAGACAAATGCAA VIC: ACCCTCCTCTCTCC FAM: ACCCCCCTCTCTC	58	94	0.213	N/A	N/A	93	0.172	N/A	N/A
Pca_Pcau2–1083	F: CTAAACAGTCCTTTTGGGAA R: CTAGTTCTCGCACCCTTTAA VIC: AGAAAATTAACCCTAAAGAG FAM: CAGAAAATTAACTCTAAAGAG	60	93	0.011	N/A	N/A	95	0.011	N/A	N/A
Pca_NCR2–56	F: TGGTTAGGAAGTGATGCGTA R: ACATGTTGACTGCCTACCTTAA VIC: GTTGAACAGGGTTTTTAGAA FAM: GGTTGAACAGAGTTTTTAGAA	58	93	0.032	N/A	N/A	95	0.063	N/A	N/A

Table 2 *Continued*

Assay name	Oligonucleotide sequences (5′–3′)	T_a (°C)	Kamishak Bay				Umnak Island			
			N	Rare allele frequency	H_E	H_O	*N*	Rare allele frequency	H_E	H_O
Pca_NCR2–140	F: TAAGGTAGGCAGTCAACATGTA R: TCATACACCAACTCGTTATCAA VIC: AACGTGTTAGCTTTGGTC FAM: AACGTGTAAGCTTTGGTC	58	93	0.398	N/A	N/A	94	0.447	N/A	N/A
Pca_NCR2–193*	AS1: TGAGTATGAGGCGTTTTTGTA AS2: GAGTATGAGGCGTTTTTGTT C: CCAACTCATCGCTGAGTTGAT	60	95	0.021	N/A	N/A	95	0.032	N/A	N/A
Pca_NCR2–197*	AS1: TCATCGCTGAGTTGATGC AS2: CTCATCGCTGAGTTGATGA C: TGGTGTATGAGTATGAGGCGTT	61	95	0.000	N/A	N/A	95	0.021	N/A	N/A
Pca_NCR2–891	F: GAGAAACAGGCTCAATGGA R: ACCATGGGCAGTATTTCAA VIC: TTGTCGACAGAATCCGTA FAM: TGTCGACAGGATCCGTA	58	94	0.043	N/A	N/A	95	0.053	N/A	N/A
Pca_NCR2–999	F: GGAGCTTGGAAGAATTGTGAA R: CCCTACCTCTTTTACGTCCTA VIC: CCTAAGTCCATCTTC FAM: CCTAAGTCCACCTTCA	60	94	0.234	N/A	N/A	94	0.223	N/A	N/A
Pca_NCR2–1045	F: TTGTGAAATCTCCAGGTGAA R: CTTCACAGCGAAACCGA VIC: TGAACATGAGTCTAACTTG FAM: TGAACATGAATCTAACTTGT	58	95	0.063	N/A	N/A	95	0.053	N/A	N/A
Pca_ODH_189	F: AAAAGTTGAAGTACTAGGAACAA R: GAGCTTCTGTAGCGTTGACAA VIC: ACGGTCTCTGCCGTAC FAM: ACGGTCTCTGCTGTAC	60	95	0.058	0.104	0.074	90	0.067	0.124	0.111
Pca_CaM_282	F: GTATCGTCAAAGTAATTGTGTTAA R: TTACGAGGAATTCTGGGAA VIC: TTAATAAGTCAATTTCACTTTAA FAM: TTAATAAGTCAACTTCACTTTA	58	93	0.156	0.177	0.269	95	0.158	0.266	0.295
Pca_CaM_386	F: GTAATGATGGCACGGAAGA R: GCTAGTCATCACATGACGCA VIC: TACCCGGAACGCC FAM: ATACCCGAAACGCCT	60	95	0.110	0.162	0.179	93	0.081	0.148	0.161

F, forward primer; R, reverse primer; VIC, allele-specific probe; FAM, allele-specific probe; AS1, allele-specific primer 1; AS2, allele-specific primer 2; C, common primer.

A limitation of the targeted-gene approach is that SNP discovery is restricted to the genes for which sequence data are available from sister taxa. Examining tightly linked SNPs will result in diminishing returns for population studies which argues that it would be most efficient to collect data from only one each of the nuclear SNPs and a subset of the mtDNA SNPs presented here. However, rare compound haplotypes may become more informative as additional populations are examined. In this sample set, five (Kamishak Bay) and seven (Umnak Island) compound haplotypes were seen for locus *NCR2*. Sequencing random fragments incorporated into a genomic library would provide additional, presumably unlinked SNPs for population study.

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