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PRIMER NOTE

Characterization of 16 polymorphic microsatellite loci in weathervane scallop *Patinopecten caurinus*

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Abstract

Discrete and commercially important patches of weathervane scallops (*Patinopecten caurinus*) are found in the northeastern Pacific Ocean. Little is known about weathervane scallop life history and population structure, but the species is vulnerable to overexploitation because of their large size and long lifespan. Here we describe 16 polymorphic microsatellite loci developed to study the relatedness of these discrete patches. All loci were polymorphic in the 32 individuals tested; the number of alleles range from four to 26 and heterozygosities ranged from 0.437 to 1.000.

Keywords: microsatellite, *Patinopecten caurinus*, PCR, weathervane scallop

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The weathervane scallop (*Patinopecten caurinus*) is one of several species of the true scallops, family Pectinidae, found in the northeastern North Pacific Ocean. Weathervane scallops are found on sand, gravel, and rock bottoms from 2 to 300 m and are the only bivalves that are capable of swimming. This scallop supports a sporadic but important commercial fishery in the northern Gulf of Alaska and southeastern Bering Sea (Ignell & Haynes 2000). Increasing harvest and long lifespan make weathervane scallops vulnerable to overexploitation.

Relatively little is known about the early life history of weathervane scallops (see Masuda & Stone 2003). An important feature of the scallop biology, pertinent to harvest management, is that individuals occur in discrete patches. This population patchiness complicates harvest management because it is unknown whether the availability of suitable habitats, oceanographic features, or life history limits local recruitment. If scallop populations that are isolated from one another are largely self-recruiting, then emphasis must be placed on monitoring and managing individual populations.

Indirect methods must be used to measure population relatedness because it is impossible to directly measure the movement of individual larvae in ocean currents. One approach is to estimate the amount of gene flow between populations and use this measure as an indicator of the degree of isolation. Previous population studies using microsatellites or mitochondrial DNA (mtDNA) of the scallop species have been limited to the Japanese scallop *Patinopecten yessoensis* (Boulding *et al*. 1993; Orbacz *et al*. 1996; Sato & Nagashima 2001) and the sea scallop *Placopecten magellanicus* (Gjetvaj *et al*. 1997). We are developing DNA markers to study structure of weathervane scallop populations using the 5′ nuclease reaction to detect single nucleotide polymorphisms (Elfstrom *et al*. in press) and, in this study, microsatellites.

Microsatellite isolation followed the methods of Jones *et al*. (2002). Genomic DNA from a single individual was partially restricted with a cocktail of seven blunt-end cutting enzymes (*Rsa*I, *Hae*III, *Bsr* B1, *Pvu*II, *Stu*I, *Sca*I, and *Eco* RV). Fragments in the size range of 300–750 bp were adapted and subjected to magnetic bead capture (CPG) using biotinylated capture molecules. Libraries were prepared in parallel using Biotin-CA(15), Biotin-ATG(12), Biotin-TACA(8), and Biotin-TAGA(8) (Integrated DNA Technologies) as capture molecules in a protocol provided by the manufacturer. Captured molecules were amplified and restricted with *Hin*dIII to remove the adapters. The resulting fragments were ligated into the *Hin*dIII site of pUC19. Recombinant molecules were electroporated into *Escherichia coli* DH5alpha. Recombinant clones were selected at random for sequencing. Sequences were obtained on an ABI 377 DNA sequencer, using ABI Prism

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Taq dye terminator cycle sequencing methodology (Applied Biosystems).

Thirty primer pairs were designed using DESIGNERPCR version 1.03 (Research Genetics) and synthesized by Integrated DNA Technologies. Forward primers were constructed with a 5′ 23-base tail (AGGGTTTTCCCAGTCACGACGTT) to facilitate the labelling strategy described by Boutin-Ganache *et al*. (2001). DNA was extracted from 32 individual scallops collected from Yakutat Bay, Alaska, using a DNAeasy tissue kit (QIAGEN). Amplification was conducted in 10 μL volumes consisting of: $MgCl_2$, 2 mM; dNTPs (premixed), 0.2 mM each; primers, 0.3 μM each; BIOTAQ DNA Polymerase™ (Bioline), 0.025 U/mL; secondary primer (AGGGTTTTCCCAGTCACGACGTT, 5′-labelled with NED, HEX, or FAM, Applied Biosystems), 0.1 μM; and template DNA, 0.2 ng/mL. Polymerase chain reaction (PCR) was conducted in a RoboCycler Gradient 967™ thermocycler (Stratagene) using the following conditions: initial denaturation, 94 °C (3 min), followed by 35 cycles of 94 °C (40 s), 55 °C (40 s), and 72 °C (30 s), and terminated with a final extension at 72 °C (4 min). Products were separated on 5% polyacrylamide gels in an ABI 377 DNA sequencer and sized using GENOTYPER version 2.5 software™ and Rox 400 HD™ size markers (Applied Biosystems).

Of the 30 primer pairs examined, 16 produced bands that were clean enough to score and were polymorphic in the samples examined here (Table 1). The number of alleles per locus ranged from four to 26 and the expected heterozygosity across loci ranged from 0.437 to 1.000. GENEPOP version 3.7 (Raymond & Rousset 1995) was used to test for departures from Hardy–Weinberg equilibrium and genotypic disequilibrium. No significant ($\alpha = 0.05$/number of simultaneous tests) departures from either were detected; however, larger collections need to be examined before any strong inferences are made. The microsatellite markers presented

Table 1 Characterization of 16 microsatellite loci in weathervane scallop, *Pactinopecten caurinus*: repeat motif, primer sequences, number of individuals scored, number of alleles, allele size range (bp), observed and expected heterozygosities and GenBank Accession no

Locus	Repeat motif	Primer sequence (5′–3′)	*n*	No. of alleles	Allele size range (bp)	H_O	H_E	GenBank Accession no.
PcauA008	CA	F: CCAGAGAATGTAGGAAGACAGC R: TATGGGTTTTTTGAGCTTCAAC	32	17	143–185	0.813	0.910	AY763416
PcauA101	CA	F: GGTGTGTGGAGTCATTGTC R: GGCTTCACTAAAATAAACGAGT	28	16	241–285	1.000	0.914	AY763415
PcauA208	CA	F: TGCTACACAAACACGTTTTCTA R: GAAGCAAGAATTTCGTATCG	27	10	155–179	0.963	0.847	AY841864
PcauA213	CA	F: GAGCCAGAGGGGTAGAGTC R: CAGGATGTCCCATCTTAGATAA	31	5	253–281	0.258	0.236	AY841865
PcauA218	CA	F: CGTCCTATCAACAGCTAAGTTC R: AGCTTTTGTACTCCCAGTAATG	32	9	281–301	0.562	0.732	AY841866
PcauB110	ATG	F: CGAACTGATATACCCGGATAA R: TAAACAAACCGGAAAACTGAC	32	25	190–286	0.906	0.945	AY763417
PcauB111	ATG	F: AGGAGGGAGTGAGTGTGC R: TCCATCTTTCACCATAATATCC	30	13	131–167	0.833	0.820	AY763418
PcauB113	ATG	F: GCCTAGTAACCTTTTGTTCGAC R: GCCTTCACTTCTAATTGCTTTC	32	4	199–208	0.438	0.581	AY763424
PcauB127	ATG	F: AAGGTATCAAAAGTCGTTGTTC R: CCCTCGTCATTCATCCTAC	25	11	261–291	0.840	0.860	AY763419
PcauB211	ATG	F: CAAACCCATCCAAACAATAC R: TGGTGATGATTATGACGATAAC	32	16	194–236	0.781	0.899	AY841867
PcauB216	ATG	F: TATCCCACGTCAATTTGTG R: CCATGTTTTGTTCGTACAATC	32	6	174–189	0.281	0.254	AY841868
PcauC001	TACA	F: CTAAAGAGCTGCTCGAATCTC R: AAAGCACTAAGGACGTGTTGT	32	26	151–327	0.906	0.930	AY763420
PcauC205	TACA	F: TGATGCTGATTGGTGATT R: GAGCGATGACAAAGAGTG	32	5	149–173	0.625	0.594	AY841869
PcauD008	TAGA	F: GCAAATGATGGTCAACTGAT R: CACAATAACAAGATCGTGACAG	31	12	205–265	0.807	0.820	AY763421
PcauD011	TAGA	F: GTGCATTGCTGTTTGAATTTG R: GCGTTGGCTCTATTTTCCTG	32	14	170–210	0.875	0.877	AY763422
PcauD101	TAGA	F: CGGAACACCACTGGTACTAC R: TCTGAAATCACAGGTAACTGAA	25	18	266–486	0.760	0.894	AY763423

here should prove useful for estimating the degree of connectivity between populations of weathervane scallops.

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