

PRIMER NOTE

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

C. M. ELFSTROM,* C. T. SMITH,* K. C. JONES† and J. E. SEEB*

*Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99518, USA, †Genetic Identification Services, 9552 Topanga Canyon Blvd., Chatsworth, CA, 91311, USA

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

Received 24 December 2004; revision received 5 February 2004; accepted 17 February 2005

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; Orbach *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 *Hind*III to remove the adapters. The resulting fragments were ligated into the *Hind*III 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

Correspondence: J. Seeb, Fax: + 9072672442;

E-mail: jim_seeb@fishgame.state.ak.us

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 (AGGGTTTTCCAGT-CACGACGTT) 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: MgCl₂, 2 mM; dNTPs (premixed), 0.2 mM each; primers, 0.3 µM each; BIOTAQ DNA Polymerase™ (Bioline), 0.025 U/mL; secondary primer (AGGGTTTTCCAGT-CACGACGTT, 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 ($\alpha = 0.05/\text{number of simultaneous tests}$) 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')	<i>n</i>	No. of alleles	Allele size range (bp)	H_O	H_E	GenBank Accession no.
<i>PcauA008</i>	CA	F: CCAGAGAATGTAGGAAGACAGC R: TATGGGTTTTTTGAGCTTCAAC	32	17	143–185	0.813	0.910	AY763416
<i>PcauA101</i>	CA	F: GGTGTGTGGAGTCATTGTTC R: GGCTTCACTAAAATAAACGAGT	28	16	241–285	1.000	0.914	AY763415
<i>PcauA208</i>	CA	F: TGCTACACAACACGTTTTCTA R: GAAGCAAGAAATTCGTATCG	27	10	155–179	0.963	0.847	AY841864
<i>PcauA213</i>	CA	F: GAGCCAGAGGGGTAGAGTC R: CAGGATGTCCCATCTTAGATAA	31	5	253–281	0.258	0.236	AY841865
<i>PcauA218</i>	CA	F: CGTCCATCAACAGCTAAGTTC R: AGCTTTTGTACTCCAGTAATG	32	9	281–301	0.562	0.732	AY841866
<i>PcauB110</i>	ATG	F: CGAACTGATATACCCGGATAA R: TAAACAACCCGAAAACTGAC	32	25	190–286	0.906	0.945	AY763417
<i>PcauB111</i>	ATG	F: AGGAGGGAGTGAGTGTGC R: TCCATCTTTTCACCATAATATCC	30	13	131–167	0.833	0.820	AY763418
<i>PcauB113</i>	ATG	F: GCCTAGTAACCTTTGTTCGAC R: GCCTTCACTTCTAATTGCTTTTC	32	4	199–208	0.438	0.581	AY763424
<i>PcauB127</i>	ATG	F: AAGGTATCAAAAGTCGTGTTC R: CCCTCGTCATTCATCCTAC	25	11	261–291	0.840	0.860	AY763419
<i>PcauB211</i>	ATG	F: CAAACCATCCAACAATAC R: TGGTGATGATTATGACGATAAC	32	16	194–236	0.781	0.899	AY841867
<i>PcauB216</i>	ATG	F: TATCCCACGTCAAITTTGTG R: CCAATGTTTTGTTCGTACAATC	32	6	174–189	0.281	0.254	AY841868
<i>PcauC001</i>	TACA	F: CTAAAGAGCTGCTCGAAATCTC R: AAAGCACTAAGGACGTGTGTG	32	26	151–327	0.906	0.930	AY763420
<i>PcauC205</i>	TACA	F: TGATGCTGATTTGGTGATT R: GAGCGATGACAAAGAGTG	32	5	149–173	0.625	0.594	AY841869
<i>PcauD008</i>	TAGA	F: GCAAAATGATGGTCAACTGAT R: CACAATAACAAGATCGTGACAG	31	12	205–265	0.807	0.820	AY763421
<i>PcauD011</i>	TAGA	F: GTGCATTTGCTGTTTGAATTTG R: GCGTTGGCTCTATTTTCCCTG	32	14	170–210	0.875	0.877	AY763422
<i>PcauD101</i>	TAGA	F: CGGAACACCACTGGTACTAC R: TCTGAAATCACAGGTAAGTAA	25	18	266–486	0.760	0.894	AY763423

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

Acknowledgements

This work was funded by project SP-916 Extended Jurisdiction of Alaska Groundfish by the US National Marine Fisheries Service. We thank Jeff Barnhart for his insight into the biological issues and collection of the specimens and Vincent Castric for helpful suggestions on earlier drafts of this manuscript.

References

- Boulding EG, Boom JDG, Beckenbach AT (1993) Genetic variation in one bottlenecked and two wild populations of the Japanese scallop (*Patinopecten yessoensis*): empirical parameters estimates from coding regions of mitochondrial DNA. *Canadian Journal of Fisheries and Aquatic Sciences*, **50**, 1147–1157.
- Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allelizing methods. *BioTechniques*, **31**, 26–28.
- Elfstrom CM, Gaffney PM, Smith CT, Seeb JE (2005) Characterization of 12 single nucleotide polymorphisms in weathervane scallop. *Molecular Ecology Notes*, **5**, 404–407.
- Gjetvaj B, Ball RM, Burbridge S *et al.* (1997) Amounts of polymorphism at microsatellite loci in the sea scallop *Placopecten magellanicus*. *Journal of Shellfish Research*, **16**, 547–553.
- Ignell S, Haynes E (2000) Geographic patterns in growth of the giant Pacific sea scallop, *Patinopecten caurinus*. *Fishery Bulletin*, **98**, 849–885.
- Jones KC, Levine KF, Banks JD (2002) Characterization of 11 polymorphic tetranucleotide microsatellites for forensic applications in California elk (*Cervus elaphus canadensis*). *Molecular Ecology Notes*, **2**, 425–427.
- Masuda MM, Stone RP (2003) Biological and spatial characteristics of the weathervane scallop *Patinopecten caurinus* at Chiniak Gully in the Central Gulf of Alaska. *Alaska Fishery Research Bulletin*, **10**, 104–118.
- Orbacz EA, Wilbur AE, Wakefield JR, Gaffney PM (1996) RFLP analysis of genetic diversity in a Siberian population of the Japanese scallop (*Patinopecten yessoensis*). *Journal of Shellfish Research*, **15**, 529.
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Sato M, Nagashima K (2001) Molecular characterization of a mitochondrial DNA segment from the Japanese scallop (*Patinopecten yessoensis*): demonstration of a region showing sequence polymorphism in the population. *Marine Biotechnology*, **3**, 370–379.