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PRIMER NOTE

Microsatellite markers for the Sydney rock oyster, *Saccostrea glomerata*, a commercially important bivalve in southeastern Australia

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Abstract

The Sydney rock oyster (*Saccostrea glomerata*) is a commercially important bivalve in southeastern Australian. We describe the isolation and characterization of nine microsatellite markers for *S. glomerata*. The loci are highly polymorphic, with between five and 20 alleles identified among 30 individuals. Expected heterozygosity levels ranged from 0.608 to 0.936. The markers will be used to study natural dispersal, translocations and population structure. We will also use the microsatellites to test the genetic effects of *QX* disease on oyster populations. This infectious parasitic disease has decimated *S. glomerata* productivity in a number of areas over the past few decades.

Keywords: aquaculture, dispersal, microsatellites, oyster, Saccostrea glomerata

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Sydney rock oysters (*Saccostrea glomerata*) are an important gourmet aquaculture species along the eastern Australian coastline. *Saccostrea glomerata* is common on sheltered rocky shorelines from eastern Victoria (37°46′S, 149°25′E) to southern Queensland (27°00′S, 153°10′E). It is the main aquaculture species in the state of New South Wales where it is farmed extensively. While the industry is predominantly supported by natural breeding and recruitment of oysters, translocations of spat (recently settled young oysters) between oyster leases, often separated by several hundred kilometres, are very frequent. Like many marine invertebrates, the patterns of larval dispersal are poorly understood. This means that the origins of naturally recruited spat are unknown, as is the impact of translocations on natural population structure and genetic variability. To address these issues, we have developed nine variable microsatellite markers for *S. glomerata*. These markers will be used in population genetic studies to infer dispersal patterns and population structure among naturally occurring and farmed populations. The data will also help to identify relationships between genetic variability of oysters and the widespread mortality associated with *QX* disease, which has seriously affected oyster farming over the past three decades (Peters & Raftos 2003; Bezemer *et al*. 2006). Our work with oysters contributes to a larger ongoing study of dispersal patterns among codistributed marine species in relation to ocean currents and the geography of Australia's eastern coastline.

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We isolated microsatellites from *S. glomerata* by the enrichment technique of Fischer & Bachmann (1998) using the restriction enzymes *Rsa*I and *Hae*III (Boehringer Mannheim) to digest *S. glomerata* genomic DNA. Following ligation of the digested fragments to two oligo adaptors, microsatellite sequences were selected using dGA_{10} and dGT_{10} biotinylated probes. The annealed probes were selectively purified using streptavidin magnetic particles (Promega), and the microsatellite-enriched eluates were amplified in a polymerase chain reaction (PCR) using one of the oligo adaptors as a primer. The enrichment protocol was repeated from the probe-annealing step, and the enriched DNA was purified using an UltraClean 15 DNA purification kit (MoBio Laboratories). We ligated the DNA into the pCR 2.1-TOPO vector (Invitrogen) and transformed the vector into TOP10 cells (Invitrogen). To avoid sequencing identical inserts, we screened 120 positive clones by PCR and single-stranded conformation polymorphism (SSCP), prior to amplifying clones with different SSCP patterns for

Table 1 Primer sequences and characteristics of nine *Saccostrea glomerata* microsatellite loci. The number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosities per locus are based on 30 samples from Jervis Bay. The *P* value for the null hypothesis for the test of no deviation from Hardy–Weinberg expected genotype proportions is also presented for each locus (HWP)

Locus	Primer sequences (5′–3′)	Repeat structure	$[Mg^{2+}]$ (mM)	T_a (°C)	N_A	Size range (bp)	H_O	H_E	HWP	GenBank Accession no.
Sgo4	TTTGAGCATGACTTCTGAACC CCGTAGGCACGTTATTTTCTC	$(GA)_{10}$	2.5	55–47	19	165–227	0.701	0.832	0.244	DQ298166
Sgo6	TCTTGACACTGGTTGAATACGG GTCAGCACAAAATGCGTAGG	$(GA)_{INT}$	1.5	62	13	125–163	0.777	0.840	0.301	DQ298167
Sgo8	CGTACAAAAGCCAACTTCTGC CATCAGCATATTCTAAAAGTGGTC	$(GA)_{INT}$	2.25	62–58	20	240–305	0.885	0.936	0.412	DQ298168
Sgo9	CCTGGAATGGAATGGACTTC TTCCTCAATGGCTCCAAAAC	$(CA)_7$	1.5	62–58	5	302–328	0.571	0.608	0.323	DQ298169
Sgo13	CCATTAATTTGTCAATGCTTATCC CTCACTTAAGCCTTTGGCTCAG	$(CA)_{13}(GA)_6$	2.5	62–58	19	111–157	0.722	0.817	0.297	DQ298170
Sgo21	TTGGAGTGGGAGAACCACTG AAGCCATTAGTGATACAGGTGAAA	$(GA)_{17}$	2.5	56–50	16	175–211	0.714	0.794	0.389	DQ298171
Sgo26	CGCAATTGTTATGGGCTAGG TCTAGCCGATGTGCTCAGG	$(GA)_{15}$	2.25	55–47	7	243–263	0.642	0.714	0.328	DQ298172
Sgo28	TGGTATAGAGCACGGACACAG CTCTGGTCCTCGGAATTGTC	$(GA)_{15}$	3.5	56–50	12	225–270	0.692	0.801	0.191	DQ298173
Sgo30	AAGCTCACTTGAGCCTTCG CTGCAATGTTGCATGTTGAG	$(GA)_8$	2.5	56–50	6	177–201	0.714	0.738	0.477	DQ334275

sequencing as described in Piggott *et al*. (2006). PCR products were purified and sequenced on an ABI 377 automated DNA sequencer (PE Applied Biosystems) using dye terminator chemistry. Primers flanking 14 microsatellite loci were designed using the PRIMER 3 program (Rozen & Skaletsky 1997).

We assessed the variability of each microsatellite locus in 30 individuals sampled from a wild population at Jervis Bay (35°7′S, 150°45′E). The loci were amplified in 10 μL radiolabelled PCRs containing ~50–100 ng of template DNA, 4 pmol of each primer, 0.5 U of *Taq* DNA polymerase (Promega), 200 μM of dCTP, dGTP and dTTP, 20 μM of dATP, 1.5–2.5 mM $MgCl_2$ (see Table 1), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1% Triton X-100 and 0.04 μL [α^{33}P]-dATP at 1000 Ci/mmol. PCR amplifications were performed in an MJ Research PTC-100 Thermal Cycler and were initiated at a melting temperature of 94 °C for 5 min, followed by 35 cycles at 94 °C for 20 s, annealing for 30 s and extension at 72 °C for 45 s, with a final 10-min extension step at 72 °C. The annealing temperatures followed a 'touchdown' protocol, decreasing in 2 °C increments from the first cycle until the final annealing temperature was reached (see Table 1). PCR products were separated by 6% polyacrylamide gel electrophoresis and visualized by autoradiography.

We used GENEPOP version 3.4 (Raymond & Rousset 1995) to estimate expected and observed heterozygosities and to test for deviations from Hardy–Weinberg and linkage equilibria. Nine loci were polymorphic, incorporating between four and 23 alleles and expected heterozygosity levels ranging from 0.575 to 0.912 (Table 1). No evidence for linkage disequilibrium was detected for any locus pairs. Genotype frequencies did not differ significantly from the expectations for Hardy–Weinberg equilibrium at any locus. We believe that the high variability of these microsatellites will enable us to elucidate patterns of larval dispersal and population structure in *S. glomerata*, investigate the impacts of aquaculture activities on local genetic diversity and differentiation, and study the population genetic effects of *QX* disease.

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