TECHNICAL NOTE

Characterization of 18 polymorphic microsatellite loci for the western rock lobster *Panulirus cygnus*

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Keywords: microsatellites, western rock lobster, *Panulirus cygnus*

Abstract

We describe the isolation and development of 18 polymorphic microsatellite loci for the western rock lobster *Panulirus cygnus* (Decapoda: Palinuridae). The loci were tested in 37 individuals from a single population situated near the centre of the species distribution. No evidence of linkage disequilibrium was detected between any pair of loci. However, seven loci showed significant departures from Hardy-Weinberg expectations. The number of alleles per locus ranged from three to 31.

The western rock lobster *Panulirus cygnus* (Decapoda: Palinuridae) is confined to the west coast of Australia, from Cape Naturaliste in the south to North West Cape in the north (Phillips et al. 1979). *P. cygnus* supports Australia's most valuable wild-caught single species fishery with an average annual catch of approximately 10,000 tonnes that is worth between $250-350 million a year in export revenue (Fletcher et al. 2005). The fishery is considered to be sustainably managed (Phillips et al. 1979) and was the first in the world to be certified by the Marine Stewardship Council as being an environmentally sustainable fishery. Nevertheless, over the past 35 years the size at maturity has decreased, the abundance of undersized and legal-sized lobsters in deep water relative to shallow water has increased and there have been shifts in the catch to deep water, possibly due to rising water temperatures associated with climate change (Caputi et al. 2010).

One of the assumptions that underlie the current management system is that the breeding stock comprises of a single, demographically united population. This assumption is based on the extended pelagic larval stage of western rock lobsters, which is thought to ensure high dispersal throughout the species range (Phillips et al. 1979). The available genetic data support this idea. Investigations of allozyme variation within *P. cygnus* reveal there is no significant differences in adults or larvae caught over a wide area of the fishery (Thompson et al. 1996; Johnson & Wernham 1999). However, these analyses were based on relatively few loci (3) and may therefore lack the resolving power needed to detect subtle genetic differences. Here, we present 18 new microsatellite loci for *P. cygnus* for evaluating population structure within this commercially important species. These markers add to nine recently published microsatellite loci of *P. cygnus* that were developed for paternity testing (Groth et al. 2009), but are too few for a definitive evaluation of genetic subdivision.

Microsatellites were isolated from a *Panulirus cygnus* DNA library created by Genetic Information Services (Chatsworth, California). Methods for the DNA library construction and enrichment followed those described in Jones *et al.* (2002). Genomic DNA was partially restricted with a cocktail of seven blunt-end cutting enzymes (*Rsa* I, *Hae* III, *Bsr* B1, *Pvu* II, *Stu* I, *Sca* I, *Eco* RV). Fragments in the size range of 300 to 750 bp were adapted and subjected to magnetic bead capture (CPG, Inc., Lincoln Park, New Jersey), using biotinylated capture molecules. Four libraries were prepared in parallel using Biotin-$CA_{(15)}$, Biotin-$AAC_{(12)}$, Biotin-$AAAG_{(8)}$ and Biotin-$TAGA_{(8)}$ 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 *E. coli* DH5alpha. Recombinant clones were selected at random for sequencing, and enrichment levels were expressed as the fraction of sequences that contained a microsatellite. Sequences were obtained on an ABI 377, using ABI Prism *Taq* dye terminator cycle sequencing methodology. The CA library produced 14 microsatellites from 17 clones, the AAC library produced 24 microsatellites from 42 clones, the AAAG library produced 16 microsatellites from 39 clones and the TAGA library produced 28 microsatellites from 41 clones. PCR primers were developed for 49 of the microsatellite-containing clones using the DESIGNERPCR version 1.03 (Research Genetics, Inc.) software package.

Genomic DNA for PCR was extracted from a 5-mm × 5-mm piece of tissue from the middle lobe of the tail fan using a QIAGEN DNeasy Blood and Tissue Kit. PCR reactions of 13µL contained ~200 ng of DNA, 2.5 mM $MgCl_2$, 1× reaction buffer (Invitrogen's Platinum PCR SuperMix: 22 U/mL complexed recombinant *Taq* DNA polymerase with Platinum *Taq* antibody, 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 220 µM dGTP, 220 µM dATP, 220 µM

dTTP, 220 µM dCTP and stabilizers) and 0.3 µM of each primer (forward primer fluorescent-labelled). PCR amplifications were carried out in an Eppendorf thermal cycler and consisted of an initial denaturation at 94 ℃ for 3 min, then 35 cycles of 30 s at 94 ℃; 30 s at the annealing temperature (see Table 1) and 30 s at 72 ℃, followed with a final elongation step at 72 ℃ for 5 min. The products from each PCR (1.5 µL) were analyzed on an ABI 3730 Sequencer, sized using GeneScan-500 LIZ internal size standard and scored using GENEMAPPER version 3.7 (Applied Biosystems) software. Levels of genetic diversity were assessed by genotyping 37 adult *Panulirus cygnus* collected from Lancelin in Western Australia (30° 59.2’ S, 115° 17.2’ E). The online version of GENEPOP version 3.4 (Raymond & Rousset 1995) was used to calculate basic descriptive statistics and test for significant deviations from Hardy-Weinberg expectations (HWE) and linkage disequilibrium between all pairs of loci. Markov chain parameters for both the HWE and linkage disequilibrium exact tests were: 1000 dememorization steps, 100 batches and 1000 iterations per batch. Significance levels were adjusted to the number of simultaneous tests using sequential Bonferroni correction (Rice 1989).

Of the 24 microsatellite-containing clones for which PCR primers were developed, 18 produced consistent polymorphic genotypes within the expected size range. The number of alleles at these loci ranged from three to 31, and the observed and expected heterozygosities ranged between 0.056 and 0.972 and between 0.055 and 0.953, respectively (Table 1). Seven loci (Pcyg02, Pcyg06, Pcyg07, Pcyg10, Pcyg13, Pcyg16 and Pcyg17) showed significant deviation from Hardy-Weinberg expectations after correction for multiple tests. These loci were estimated to have null allele frequencies ranging from 0.20 (Pcyg06) to 0.49 (Pcyg13), using the CERVUS version 3.0.3 software package (Kalinowski et al. 2007). There was no evidence of linkage disequilibrium between any pair of loci.

Acknowledgements

We thank Mark Todd from Genetic Identification Services for his helpful advice. This work was funded by a grant from the Fisheries Research and Development Corporation.

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Table 1 Primer sequences, repeat motif, annealing temperature and levels of diversity at 18 microsatellite loci in the western rock lobster *Panulirus cygnus* from Lancelin, Western Australia (n = 37). T_a, annealing temperature (°C); H_O, observed heterozygosity; H_E, expected heterozygosity; P, probability value from Hardy-Weinberg test

Locus	Primer sequence (5'-3')	Repeat motif	T_a	Number of alleles	Size range (bp)	H_O	H_E	P	GenBank Accession no.
Pcyg01	F: GCTTCTTATTGCTCCGAAATG R: GGGCTCTCTATCTTCAACTCG	$(GT)_{26}$	58	15	281-330	0.917	0.876	0.803	?
Pcyg02	F: AGGTTGACAGAACACCAAGAG R: GGATAACATCAGCACCATCTC	$(GT)_{15}$	57	22	160-227	0.306	0.934	0	?
Pcyg03	F: GACCCCTTCATCACACACC R: GCAGGCAGTGGGAGAAAC	$(CA)_{10}$	58	3	280-288	0.194	0.18	1	?
Pcyg04	F: CTTTCCGATTGTTTGGTGAG R: GTGGTCTGGCGTAACCTCTA	$(TTG)_7$	58	14	279-325	0.800	0.885	0.158	?
Pcyg05	F: AACGGTTCGTATAATTTGGAC R: CCATCACTCAAACAAAGACAG	$(TTG)_{10}$	57	8	232-259	0.696	0.758	0.176	?
Pcyg06	F: GTTTTCTCTATCCGGGAACTG	$(AAC)_2AGC(AAC)_3AAT(AAC)$	57	22	176-256	0.618	0.944	0	?

	R: AGGTGGGAAAGCTGTTGTAGT	$_3$AATCAC $(AAC)_4$							
Pcyg07	F: ACCTTGAGAGAGACATGAACC	$(AAC)_9(AAT)_5$	57	8	254-281	0.318	0.856	0	?
	R: CCTTGTGTATGAGACTGGATG								
Pcyg08	F: GAGTGCGATGTGTTGATGGT	$(TTG)_7$	58	24	232-304	0.972	0.953	0.589	?
	R: GGGAGTGTTAAACGAATGAGC								
Pcyg09	F: GGACCTGTTGAGGATACTGTAG	$(AAC)_7$	56	4	155-164	0.364	0.418	0.653	?
	R: TTGTGTTGTGAGGATGTTAGTC								
Pcyg10	F: TGGGACAGATCAGGCAAAC	$(TTTC)_5$	58	31	137-346	0.471	0.971	0	?
	R: CCGAGTATCGAGGAGGGAG								
Pcyg11	F: CCGTTACGAAGGTTCACTTA	$(TTTC)_4TCC(TTTC)_5$	56	6	164-244	0.735	0.688	0.592	?
	R: AAATGCACCTAGAGTAAAGCAG								
Pcyg12	F: ATTGCTTGATGAGAATGATGTG	$(AAAG)_7$	57	6	164-244	0.905	0.931	0.307	?
	R: CATGGTTAAGGACACCTGAAA								
Pcyg13	F: TTTGTGTGTCGTGATTTCATC	$(TAGA)_4TATT(TAGA)_7$	57	17	203-290	0.310	0.929	0	?
	R: ATCCCATAAAGAGAGGGTGAC								
Pcyg14	F: ACGAAGGAATGAATAGTGAATG	$(ATCT)_2ATCC(ATCT)_5GTCT$	57	7	244-259	0.469	0.741	0.018	?

	R: TTCTGCAACATAACGAGGTC	$(ATCT)_5$							
Pcyg15	F: GTGGTTGCTTACCGCATAC	$(TAGA)_8$	56	3	112-124	0.676	0.607	0.796	?
	R: TGTTTCCTTGTTCAGTCATTG								
Pcyg16	F: GACCCCGTTCTTCGTAACT	$(ATCT)_5TT(ATCT)_5AT(ATCT)_5AT(ATCT)_7$	57	14	206-301	0.333	0.903	0	?
	R: ACCCACCTGTCTTGCTATG								
Pcyg17	F: CCCTGACGATGATACAGCC	$(TAGA)_2CAGA(TAGA)_4$	58	11	207-310	0.324	0.908	0	?
	R: TGGAGTCTTGTCTTGTCTGGTC								
Pcyg18	F: CGGGACCATCAAATTACC	$(ATCT)_5$	57	3	201-215	0.056	0.055	1	?
	R: GCAATGGCAAATAACCATAC								