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Note

Isolation and characterization of fifteen microsatellite loci from the redclaw crayfish, *Cherax quadricarinatus*

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Abstract – The redclaw crayfish (*Cherax quadricarinatus*) is a freshwater crayfish species endemic to northern Australia and Papua New Guinea that is the focus of a growing culture industry in number of regions around the world. Here we isolated and characterized 15 microsatellite loci from *Cherax quadricarinatus* and tested variability of the loci in 66 unrelated cultured individuals. Thirteen of 15 microsatellite loci were polymorphic. Number of alleles per locus ranged from two to seven while observed and expected heterozygosities ranged from 0.172 to 0.985 and from 0.373 to 0.778, respectively. Eleven loci conformed to Hardy–Weinberg equilibrium in the sampled population. These microsatellite loci developed here provide an important resource for studying genetic diversity and population structure in redclaw crayfish and potentially in other related species.

Key words: Aquaculture / Microsatellites / DNA sequences / Genetic diversity / Population structure / Decapod Crustacea / *Cherax quadricarinatus*

1 Introduction

The redclaw crayfish (*Cherax quadricarinatus*) is a tropical freshwater crayfish native to northern Australia and southern Papua New Guinea. Today, it is cultured in many countries around the world, including the USA, Central America, South Africa, New Zealand, China, Israel and Ecuador and this species was introduced to China in the early 1990s for commercial culture. Although currently, aquaculture scale production of *C. quadricarinatus* is limited in China, redclaw crayfish possesses large market potential. Information on genetic diversity and population structure in this species will be important for assessing, managing, utilizing and conserving the genetic resources in both wild and cultured stocks (Niu et al. 2008). Studies of genetic diversity and population structure can provide important information for promoting development of aquaculture of the species in China. However until now, this knowledge has been limited.

Microsatellites are simple DNA sequences, repeated in tandem that are widely dispersed across the genomes of eukaryotic and prokaryotic organisms. Most loci are highly variable and considered to be selectively neutral, making them amenable to population genetic analysis (Tautz et al. 1986; Liu et al. 2006). Decapod crustaceans in general, have commonly displayed only low levels of allozyme variation (Busack 1988) and the redclaw crayfish is no exception (Macaranas et al. 1995; Austin 1996). Microsatellite loci from the nuclear genome have proven very useful for documenting genetic diversity in many species where allozyme variation was found to be low (Hughes and Queller 1993). To date however, only a limited number of microsatellite loci have been developed for *C. quadricarinatus* (Baker et al. 2000; Baker et al. 2008). Thus, isolation of microsatellite loci from *C. quadricarinatus* will be important for elucidating population structure and for monitoring relative levels of genetic diversity in sampled populations.

2 Materials and methods

2.1 Sampling and DNA isolation

Cultured *C. quadricarinatus* individuals were obtained from Chongming in Shanghai, China. Total genomic DNA was extracted from muscle tissues using a proteinase K/phenol extraction protocol (Sambrook and Russell 2001).

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2.2 The isolation of microsatellite loci

Isolation of microsatellite loci used a modified enrichment protocol (Gardner et al. 1999). Two genomic DNA samples from male and female individuals were first pooled. About 10 μg of DNA was then digested with *Mse* I restriction enzyme (New England Biolabs, Beijing, China). The reaction mixture contained 10 × NEB buffer, 30 U *Mse* I, 10 μg DNA and ddH_2O in a final volume of 100 μl. The reaction was incubated for 2 h at 37 °C. Resulting fragments were separated on a 1% agarose gel without further size selection. Fragments were purified using wizard SV Gel and PCR Clean-Up System (Promega, Beijing, China), then ligated to adaptors (oligoA: 5′-AGATGGAATTCGTACACTCGT-3′and oligoB: 5′-TAACGAGTGTACGAATTCCATCT-3′). The digestion-ligation mixture was directly amplified in a total volume of 25 μl with oligoA primers. Products showing a visible smear were selected for further use. DNA fragments were enriched by hybridization with the 5′-biotinylated $(CA)_{15}$ probe (Invitrogen, Shanghai, China) at 68 °C for 1h, which were then isolated using streptavidin-coated magnetic beads (Invitrogen, USA). Non-specific binding and unbound DNA was removed by several non-stringent and stringent washes. DNA enriched with microsatellites was amplified in a 25 μl reaction using the oligoA as the primer for polymerase chain reaction (PCR) and amplified DNA products were purified using wizard SV Gel and PCR Clean-Up System (Promega, Beijing, China). Cleaned DNA products were subsequently cloned into the pGEM-T Easy Vector (Promega) according to the manufacturer's protocol, and then transformed into *Escherichia coli* TOP 10 competent cells (Tiangen, Beijing, China). Transformed cells grew at 37 °C for 16 h on an LB agar plate containing ampicillin, X-gal and IPTG for blue/white selection. White colonies were picked into 96-well PCR plates containing 600 μL LB buffer/well, and cultured at 37 °C for 4 h in an incubator. Colony PCR was conducted using SP6, T7 and $(CA)_{10}$ as primers to select clones containing microsatellites. 70 positive clones were screened and sequenced (Invitrogen). 32 unique sequences were chosen for primer design using PRIMER PREMIER 5.0 software (PREMIER Biosoft International) and synthesized (Invitrogen, Shanghai, China).

2.3 Detection of polymorphic microsatellite loci

To characterize isolated microsatellites, we obtained 66 cultured "redclaw" individuals from Chongming in Shanghai, China. Total genomic DNA was then extracted from these individuals as templates. PCR amplifications were performed in 25 μl reactions each containing: 20 ng DNA, 0.5 U Taq Polymerase (TaKaRa, Dalian, China), 1× PCR reaction buffer (Mg^{2+} Plus, 0.2 mM of each primer, 0.2 mM of each dNTP and ddH_2O in a final volume of 25 μl. PCR conditions were as follows: 94 °C for 3 min followed by 35 cycles of 94 °C for 30 s, annealing temperature for 30 s and 72 °C for 30 s with a final extension for 10 min at 72 °C. Amplified products were separated on QIAxcel DNA High Resolution Cartridge (QIAGEN, Shenzhen, China) using the QIAxcel System (QIAGEN, Germany) and the resulting data were analysed with BioCalculator software (QIAGEN). The QIAxcel provides an automated system that increases sample throughput, reducing labour costs and decreasing sample processing time (McMurray 2010).

3 Results and discussion

We designed and tested a total of 32 primer pairs. 17 of which failed to amplify or showed complex amplification profiles, two were monomorphic and 13 were polymorphic in the population screened here.

The13 polymorphic microsatellite loci isolated here displayed considerable allelic polymorphism. Polymorphism information content (PIC) is recognized as a measure of the relative utility of molecular markers (Botstein et al. 1980) and comparisons of PIC values can provide an estimate of the relative power of individual markers (Liu et al. 2004). Per-locus PIC values here ranged from 0.346 (CQ18) to 0.738 (CQ17) respectively, with a mean of 0.519. Seven of the 13 polymorphic loci identified were shown to be highly informative (PIC > 0.5). The remaining six loci were moderately informative (0.25 < PIC < 0.5), and none was only slightly informative (PIC < 0.25). This indicates that the set of microsatellites developed here show considerable potential for analyzing genetic polymorphism in redclaw populations.

PopGene software (Yeh et al. 1999) was used to test for levels of observed and expected heterozygosities in the loci. Values of observed heterozygosity and expected heterozygosity ranged from 0.172 to 0.985 (average 0.726) and from 0.373 to 0.778 respectively, with a mean estimate of 0.590.

Number of alleles per locus, *p*-value for Hardy–Weinberg equilibrium (HWE) estimates and linkage disequilibria were assessed using GENEPOP (Raymond and Rousset 2004). Number of alleles per polymorphic locus varied from 2 to 7. In this study, except for two loci (CQ18 and CQ24), eleven loci conformed to HWE in the sampled population ($p > 0.05$). Observed deviations from HWE expectations may result from sampling effects, presence of null alleles or excessive heterozygosity. Our analysis suggested that four out of 78 pairwise tests for genotypic disequilibrium (CQ17 × CQ20, CQ17 × CQ23, CQ19 × CQ23, CQ22 × CQ25) were significant and could indicate that these loci may be physically linked on individual chromosomes in redlcaw. We tested for linkage disequilibrium to avoid potential for pseudo-replication errors in our analyses by searching for correlations between alleles at different loci. Where loci are in close physical proximity on a chromosome, they may not assort independently and hence can be transmitted to offspring as a pair; consequently. Where linkage is detected, one locus in the pair should be discarded (Selkoe et al. 2006). We suggest that potential linked loci identified here should be used with caution, and perhaps only one from each set should be used in order to avoid pseudo-replication in population genetic analyses.

Results are shown in Table 1. The microsatellite primer pairs developed here will provide a powerful genetic tool for management, conservation and culture applications in *C. quadricarinatus* and potentially in other related freshwater crayfish species.

Table 1. Fifteen microsatellite loci from the redclaw crayfish (*Cherax quadricarinatus) Ta*, annealing temperature; N_A, allele number; H_o, observed heterozygosity; H_E, expected heterozygosity and *PIC*, polymorphic information content of each loci. * Significant deviations from Hardy-Weinberg equilibrium (HWE); ** These loci did not show polymorphism, and were not included in the calculus of the mean.

Locus	Sequenced repeat motif	Primer sequences (5'-3')	*Ta* (°C)	Size range (bp)	N_A	H_O	H_E	*p*-values HWE	PIC	Genbank Accession numbers
CQ16	$(AC)_{35}(CA)_5$	GATAAATTTTCTTTGCGCGCTG GTCTTTCCTGAGGATTTGAT	56.4	174–228	4	0.778	0.739	0.655	0.685	HM035041
CQ17	$(AC)_{28}$	TCGCAGGCTGAGTTTCTATC TAGATGTAAATTTTGTGCAC	56.4	291–366	7	0.785	0.778	0.061	0.738	HM035042
CQ18	$(AC)_{10}(AC)_{10}(AC)_6(AC)_6(AC)_{22}$	ATTTCCTCCCTGGATGTTAC TATTCCACTTCCTGACGACT	56.4	122–202	4	0.406	0.373	0.043*	0.346	HM035043
CQ19	$(TG)_{16}$	GATAAGCAGTGGCTACAAAA TAGTAGCGACCAGTGAAGAG	56.4	238–366	3	0.523	0.482	0.226	0.418	HM035044
CQ20	$(GT)_{25}(GT)_5(CT)_6$	CATAGCTGAAACTAGGCACG CCTAGTAGCAATCAGTGAAGAG	50.4	276–312	3	0.885	0.584	1.000	0.503	HM035045
CQ21	$(TG)_{28}$	CATTTGCCATTTTCCATACC GTAGCGACCAGTGAAGAGGC	63.9	380–403	2	0.892	0.498	1.000	0.372	HM035046
CQ22	$(CA)_{14}$	TAGTAACGACCAGTGAAGAGGC AATCAAGAGCCCCTCACCAG	63.9	152–182	3	0.723	0.537	0.999	0.463	HM035047
CQ23	$(GT)_9(GT)_{10}(TG)_{21}$	CGTCTGCGTGACTGACTCGT GCAGATAGAGGACCTAGTAGTGA	64.7	240–270	3	0.726	0.593	0.447	0.514	HM035048
CQ24	$(TG)_{39}$	GACCTCCAGAGTGAAGCGTT CAGAATCAACCCAAACCACG	64.7	137–184	3	0.172	0.618	0.000*	0.539	HM035049
CQ25	$(GT)_{11}(GT)_{12}$	ATACAGCAGTTTCGGGTCAA GGTTTGATAAAGCTCATGGA	58.9	202–258	4	0.985	0.728	1.000	0.671	HM035050
CQ26	$(AC)_9(CA)_{21}(CA)_{12}(CA)_{63}(AC)_{18}$ $(AC)_{19}(AC)_{20}(AC)_{22}(GT)_5$	GACCCCTGCAACCACAAATA TCTGCATTCTCTCAGCGAC	58.9	416–483	4	0.762	0.553	0.688	0.479	HM035051
CQ27	$(AC)_{73}$	TAGTGGCGACCAGTGAAGAG AGGTTACCATTCATTCGTGT	65	371–434	3	0.846	0.560	0.980	0.460	HM035052
CQ28	$(GTA)_{13}$	ATACTGTCGGAAGGAGAGGTGC TTCTACGACTACAAGGATGATGG	66.8	314–362	4	0.954	0.628	1.000	0.560	HM035053
CQ29**	$(GC)_6(GT)_6(TG)_5(GT)_{14}$	CTTCCTCGGTTGTATGTTTT TGACCTAGTAGCGACCAGTG	50.4	200	1	0.000	0.000	-	-	HM035054
CQ30**	$(AC)_{35}$	AGAGGACCTAGTAGCGATCAGTG ATAGTGGATGAGTTTGTGTGTGTGT	50	125	1	0.000	0.000	-	-	HM035055
mean					3.6	0.726	0.590		0.519	

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