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Isolation and Characterization of Polymorphic Microsatellite Loci in the Redclaw Crayfish, *Cherax Quadricarinatus*

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Abstract

Here we developed and optimized 15 polymorphic microsatellites isolated from *Cherax quadricarinatus* enriched for CA repeats. We tested variability of these microsatellites in 60 unrelated individuals cultured in China. All microsatellite loci were polymorphic. Number of alleles per locus ranged from 2-7 while observed and expected heterozygosities ranged from 0.2549 to 0.8615 and from 0.3405 to 0.8174, respectively. Fourteen of the 15 microsatellites conformed to Hardy–Weinberg Equilibrium. 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.

Keywords: Aquaculture; Microsatellites; Red claw crayfish; Polymorphism

Introduction

The redclaw crayfish *Cherax quadricarinatus* accounts for the entire commercial production of freshwater crayfish in Australia [1]. Its culture potential has also been recognized in other countries, including the USA and China, and this species was introduced to China in the early 1990s for commercial culture. Genetic markers can characterize relative levels of population variation and differentiation in both wild and cultured stocks [2]. Knowledge of populations is limited for cultured stocks in China and it is unlikely that much of the natural variation that exists across the large natural range of this species has been exploited in culture. So our work on SSR markers isolation and studies of genetic diversity and population structure will provide important information for promoting development of aquaculture of *C. quadricarinatus* in China.

Microsatellites are simple DNA sequences, repeated in tandems 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 [3]. Until now, only a limited number of microsatellite loci have been developed for *C. quadricarinatus* [4]. Thus, isolation of more microsatellite loci from *C. quadricarinatus* will be important for elucidating population structure and for monitoring relative levels of genetic diversity in sampled populations.

Materials and Methods

Sampling and DNA isolation

The cultured *C. quadricarinatus* individual samples were collected from Xia Men in China. Total genomic DNA was extracted from muscle tissues using the standard proteinase K–phenol–chloroform method [5].

The isolation of microsatellite loci

Microsatellites were isolated using a magnetic beads enrichment procedure with modifications [6]. Two genomic DNA samples from male and female individuals were first pooled. About 10 μg of DNA was then digested with *Mse* I and purified with Wizard PCR Purification kit (Promega and ligated to the adaptors (ADP1: 5'-AGATGGAATTCGTACACTCGT-3' and antisense strand ADP2: 5'-TAACGAGTGTACGAATTCCATCT-3'). Magnetic beads captured DNA containing microsatellites were eluted in 30 μL water. DNA enriched with microsatellites was amplified in a 25 μL reaction using the ADP1 as primer to know the successful ligation [7]. PCR products of 25 μL were electrophoresed on 1% agarose gel, fragments between 400-1000bp were cut from the gel and purified by Wizard PCR Purification kit (Promega). About 50ng concentrated DNA was directly ligated to 25 ng pGEM-T vector (Promega) according to the manufacturer's protocol. The ligation products were transformed into *Escherichia coli* TOP 10 competent cells (Tiangen) and plated upon LB-agar containing 50 mg/L ampicilin, 60 mg/L IPTG, and 40 mg/L X-gal. A partial genomic DNA library enriched for CA- microsatellites was established. White clonies were picked into 96-well Deep Well Plates and cultured at 37° with shaking for 4h. A PCR-based method was used to screen recombinant clones using M13 and $(CA)_{10}$ as primers. We only select the clones that have two or more amplified bands in the PCR products, which ensure these clones contain CA or GT repeats fragment [8]. 45 selected clones with repeat elements were sequenced with M13 universal primer (Invitrogen), of which 30 unique sequences were chosen for primer design using PRIMER PREMIER 5.0 (PREMIER Biosoft International).

Detection of polymorphic microsatellite loci

To characterize the isolated microsatellites, we obtained 60 cultured redclaw crayfish individuals from Xia Men in Fu Jian province. Total genomic DNA was then extracted from these individuals as templates. PCR were performed in 25 μL reactions each containing: 20 ng DNA, 0.5 U Taq Polymerase (TaKaRa), 1× PCR reaction buffer (Mg^{2+}, 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 3min followed by

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Locus name	*Repeat motif*	*Primer pair sequence (5'-3')*	*Tm (°C)*	*Size range*	*No of alleles*	*HO*	*HE*	*HEW P-value*	*PIC*	*GenBank Accession no.*
CQ1	(GT)16	F:ACCTGTCGCACTCTGTATCAA R:ACCTAGTAGCGATCAATGAAGAG	56.4	273-293	3	0.6897	0.6439	0.6878	0.5617	HM035026
CQ2	(CA)42	F:TCACCAGGCTTTGAAATAGT R:CATTTGCCGTCTTCCATACC	56.4	180-240	7	0.8167	0.8174	0.2154	0.7850	HM035027
CQ3	(GT)24	F:ACTTCTCTGTGTATATATCCTGTG R:CAGACACCAAACACCAAGAT	56.4	154-193	3	0.3103	0.3405	0.1834	0.3121	HM035028
CQ4	(TG)13	F:TAGCACCAGAGAGAGTCAGG R:TGGAGCAGGAAAAGTGAG	56.4	183-200	3	0.5000	0.5118	0.4754	0.3868	HM035029
CQ5	(AC)15	F:TAGTAGCGACCAGTGAAGAG R:GCGTTGTTTTGACACAGAAG	56.4	187-237	4	0.5714	0.6173	0.0817	0.5322	HM035030
CQ6	(CA)23	F:CTAGTCGCTACCAGCAAAGA R:CAACCTCCTGGGACTTACTG	50.4	408-418	2	0.6032	0.4246	1.0000	0.3325	HM035031
CQ7	(TG)22	F:TGTCTTCAACTTCCAACTGTG R:ATAAATGTGAGAGCCCAGGT	50.4	175-246	3	0.7600	0.6305	0.9729	0.5478	HM035032
CQ8	(TG)14	F:TCCCTGTGATTCATCTGTTT R:GTAGCGATCAGTGAAGAGGC	63.9	382-405	2	0.8000	0.4837	1.0000	0.3648	HM035033
CQ9	(TG)13	F:CTCCACTATTCTGTTTGCTTTG R:GTAGCAATAAGCGAAGAGGC	62.7	171-175	2	0.4688	0.3780	0.9944	0.3047	HM035034
CQ10	(CA)18	F:ACACACACGCACAAGGGTAGAC R:CCGATTTCCCTCAACCTTTC	62.7	261-289	4	0.8615	0.6730	0.9998	0.6101	HM035035
CQ11	(TG)11	F:CCATTGTACCTCCCTTTTTT R:CAGCCACAGTAGCAGTCAGT	50.4	332-365	2	0.2549	0.3931	0.0171*	0.3135	HM035036
CQ12	(AC)40	F:GTTCAGGGAGAGTGACCTAGTAGC R:CCTCCTCGGTTGTTATGTTATT	50.4	389-403	2	0.6000	0.4923	0.9533	0.3675	HM035037
CQ13	(GT)37	F:TGTGACCCCTTGTTTCTGTG R:GCCCGTCATTCAAGCCAAAG	63.9	239-258	3	0.3810	0.5116	0.0978	0.4551	HM035038
CQ14	(TG)29	F:TTTCCTCAGCCTTATCACCT R:GGTAATGAACTCTGGGTAGGTC	55.0	330-363	3	0.8537	0.5285	1.0000	0.4188	HM035039
CQ15	(TG)26	F:ATACTCCCTCTTGGTGTTCC R:GTAGCGGTCAGTGAAGAGGC	63.9	380-403	2	0.7846	0.4805	1.0000	0.3631	HM035040
Average					3	0.6171	0.5284	0.6453	0.4437	

H_O, observed heterozygosity; HE, expected heterozygosity; HWE, Hardy–Weinberg equilibrium; PIC, polymorphic information content of each locus; and GenBank accession numbers for DNA sequences of loci; * Locus deviating from HWE.

Table 1: Microsatellite isolated from Redclaw Crayfish (*Cherax quadricarinatus*).

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 [9]. The QIAxcel provides an automated system that increases sample throughput, reducing labour costs and decreasing sample processing time [10].

Results and Discussion

We screened 72 recombinant clones with M13 and $(CA)_{10}$ primers, 45 selected clones with repeat elements were sequenced with M13 universal primers (Invitrogen). We designed and tested a total of 30 primer pairs, 12 of them failed to amplify or showed complex amplification, three were monomorphic and 15 were polymorphic in the population screened here. The number of alleles, observed and expected heterozygosities were analyzed by Popgen32 [11]. The number of alleles per polymorphic loci varied from 2 to 7 and the values of observed heterozygosity and expected heterozygosity ranged from 0.2549 to 0.8615 and from 0.3405 to 0.8174, respectively (showed in table 1).

P value for Hardy–Weinberg equilibrium (HWE) estimates and linkage disequilibria were estimated by using Genepop [12]. Except locus *CQ11*, fourteen loci conformed to Hardy–Weinberg Equilibrium in the population (P>0.05). Observed deviations from HWE expectations may result from sampling effects, presence of null alleles or excessive heterozygosity. Linkage disequilibria (LD) was detected between *CQ3* × *CQ14, CQ3* × *CQ10, CQ3* × *CQ9, CQ7*× *CQ9, CQ7* × *CQ13, CQ8* × *CQ15* (P<0.05) and indicate that these loci may be physically linked on individual chromosomes in redclaw. 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.

Polymorphism information content (PIC) is recognized as a measure of the relative utility of molecular markers [13] and comparisons of PIC values can provide an estimate of the relative power of individual markers [14]. Per-locus PIC values here ranged from 0.3047 (CQ9) to 0.7850 (CQ2) respectively, with a mean of 0.4437. Five of the 13 polymorphic loci identified were shown to be highly informative (PIC>0.5). The remaining ten 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 polymorphisms in red claw populations.

All the results are shown in table 1. Until recently, there are only a few DNA markers available for the red claw crayfish. Therefore, these 15 novel microsatellite markers could facilitate studies on genetic diversity

and population structure of the red claw crayfish to supply useful information for conservation of cultured stocks, and even potentially used in the study of other related freshwater crayfish species.

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