PRIMER NOTE

Microsatellite loci in the eastern form of the giant freshwater prawn (Macrobrachium rosenbergii)

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Abstract

Six microsatellite loci were identified and characterized in the eastern form of the widespread and commercially important giant freshwater prawn (Macrobrachium rosenbergii). The loci were detected by randomly screening for dinucleotide and trinucleotide repeat units within a partial genomic library developed for the species. In a sample of 29 prawns, number of alleles and heterozygosity per locus ranged from 12 to 18 and from 0.66 to 0.90, respectively. These markers provide powerful tools for the conservation and management of wild stocks, the improvement of cultured stocks of M. rosenbergii, and for investigating evolutionary processes underlying genetic divergence among populations.

Keywords: Decapoda, Macrobrachium, microsatellite, Palaemonidae, primer

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The decapod crustacean, Macrobrachium rosenbergii (giant freshwater prawn), is commercially important in culture and as a wild capture-fishery species, particularly in Southeast Asia. The natural distribution of M. rosenbergii extends from Pakistan in the west to southern Vietnam in the east, across Southeast Asia, south to northern Australia, New Guinea, and some Pacific and Indian Ocean islands. Recent studies have recognized two distinct forms of M. rosenbergii, an ‘eastern’ and a ‘western’ form (these forms may receive elevation to specific taxonomic status in the near future; D. Wowor, personal communication), based on morphology (Lindenfelser 1984), allozymes (Hedgecock et al. 1979; Lindenfelser 1984; others) and mitochondrial DNA (mtDNA) (de Bruyn et al. 2004a). The western is the common form used in culture in most parts of the world, with the Philippines being a notable exception. Understanding the distribution of genetic diversity in wild and cultured stocks is important for developing sound conservation strategies aimed at preserving wild genetic diversity levels, which are believed to be declining as a result of over-exploitation. Recognition of unique genetic diversity will also allow for informed choices in breeding programs regarding the selection of genetically diverse broodstock, and the maintenance of genetic diversity in cultured stocks. Here, we report the development and characterization of microsatellite DNA markers in the eastern form of M. rosenbergii, and we test their utility in the western form.

Approximately 10 µg of M. rosenbergii genomic DNA was digested with restriction enzyme DpnII for 3 h, and then separated on a 1.5% agarose gel. DNA fragments in the 300–700 bp size range were excised, purified and ligated to an equal volume of plasmid vector pUC18 (Amersham-Pharmacia). The plasmids were digested with BamHI, and diphosphorylated to allow the overhanging ends to match those resulting from the DpnII digest. Recombinant plasmids were heatshocked into competent Eschericia coli cells (strain JM109, Promega) and incubated at 37 °C for 1 h. Cells were spread on agar plates containing LB-ampicillin/KGAC/IPTG and incubated at 37 °C overnight. A total of c. 2500 recombinant colonies were harvested from plates and incubated overnight on new LB-ampicillin agar plates in a grid formation and then stored at 4 °C. Recombinant colonies were blotted from the plates into filter membranes (Hybond-N, Amersham). DNA from this transfer was cross-linked with the membrane, denatured and probed with oligonucleotides [(ACC)$_8$(AAG)$_8$(AAC)$_8$(AGG)$_8$(ACG)$_8$(ACT)$_9$(CA)$_{15}$(AG)$_{12}$] that had been end labelled with [$^{32}$P]dATP (Perkin-Elmer). Cross-linked single-stranded DNA was hybridized with the probes overnight before being exposed to X-ray film for 12 h. Autoradiographs revealed 61 positive clones that hybridized with probed repeats. Colonies containing repeats were identified.
and harvested from the stored agar plates and were cultured overnight at 37 °C. Plasmid DNA was extracted from cultures by an alkaline-lysis miniprep and sequenced using BigDye Terminators (Perkin-Elmer) and universal plasmid primers (M13 F & R, Amersham Pharmacia Biotech). DNA sequencing was conducted on an ABI 3730 automated sequencer at the Australian Genome Research Facility, University of Queensland, Brisbane, Australia. Thirty-four clones contained recognizable microsatellite arrays. Nineteen of the candidate microsatellites had an adequate flanking region for primer design. Primers were designed using OLIGO and PRIMERS software. Polymerase chain reaction (PCR) amplification of targeted microsatellites was successful for six of the primer pairs (Table 1). PCRs contained 50–100 ng template DNA, 0.2 U Taq DNA polymerase (Biotech), 0.25 mm of dNTPs, 1 mm MgCl₂, and 0.5 μM of each primer (forward primer end-labelled with fluorescent HEX), in 10× reaction buffer (670 mm of Tris-HCl, 166 mm of [NH₄]₂SO₄, 4.5% Triton X-100, 2 mg/mL gelatin; Biotech) up to 20 μL total reaction volume. After a preliminary denaturing step at 95°C for 3 min, PCR amplification was performed for 30–35 cycles: 30 s denaturing at 94°C, 30 s at annealing temperature (Table 1) and 30 s extension at 72°C, with a final 10 min extension at 72°C. After amplification, one part of each PCR product was mixed with one part loading buffer, heated for 3 min at 95°C, and then set on ice for at least 3 min. Denatured PCR products and TAMRA (Genescan-350) size markers were separated electrophoretically on 5% denaturing acrylamide gels using a Gelscan 2000 rig (Corbett Research) and analysed for product size with one-dscan software (Scanalytics).

Microsatellite typing of a wild ‘eastern’ (n = 29) (de Bruyn et al. 2004a, b) M. rosenbergii population sampled from the Norman River (Queensland, Australia) indicated that all six loci were polymorphic. Number of alleles per locus and the observed and expected heterozygosities are presented in Table 1. Analyses of allele frequencies in GENEPOP version 3.1d (Raymond & Rousset 1995) indicated loci Mr-89 (P = 0.013) and Mr-95 (P = 0.025) deviated from Hardy–Weinberg expectations because of heterozygote deficiency, which may result from the presence of null alleles or a Wahlund effect. No evidence for linkage disequilibrium was detected in locus-pair comparisons. We tested the efficacy of the six microsatellite loci in 10 individuals sampled from across the entire distribution of the ‘western’ form of M. rosenbergii (de Bruyn et al. 2004a, c), but none amplified successfully. This result adds further support to the contention that the two forms of M. rosenbergii have been genetically isolated for a significant evolutionary time frame (de Bruyn et al. 2004a). We are presently in the process of developing microsatellites specifically for use in ‘western form’ populations.

This suite of microsatellite markers should prove useful for genetic studies of both wild and cultured stocks of the eastern form of M. rosenbergii, and provides the foundation for future nuclear DNA-based studies that will complement previous research on mitochondrial, allozyme and morphological variation in M. rosenbergii.

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References


