MOLECULAR ECOLOGY
RESOURCES

Microsatellite markers for the praying mantid *Ciulfina rentzi* (Liturgusidae)

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Abstract

Nine polymorphic microsatellite loci were characterized from an enrichment library of the Australian praying mantid *Ciulfina rentzi*, a group with a unique reproductive morphology and behaviour. The number of alleles per locus ranged from three to 16 and heterozygosity from 0.24 to 0.94. These markers are the first microsatellites developed for any praying mantid. They will be useful for paternity analysis and for population genetic studies in the Wet Tropics World Heritage Region of Australia.

Keywords: Australian Wet Tropics, Mantodea, multiple paternity, phylogeography, sexual selection

Received 17 December 2008; revision accepted 24 March 2009

One of the more intriguing genera of praying mantids is *Ciulfina* (Liturgusidae). They are not sexually cannibalistic (Holwell 2007) and do not produce airborne sex pheromones (Holwell *et al.* 2007a), and therefore represent patterns of reproductive behaviour that vary greatly from other well-studied mantid genera (see Maxwell 1999 for a review). These mantids are cryptic cursorial predators that live on tree trunks in a variety of habitats throughout northern Australia. Species do not vary in their external morphology and are distinguished based on male genital morphology (Holwell *et al.* 2007b). Three species (*C. biseriata*, *C. rentzi* and *C. baldersoni*) exhibit a remarkable genital dimorphism in orientation: the genitalia are fully asymmetrical and occur in dextral or sinistral forms (Balderson 1978). The mating behaviour of *Ciulfina* is somewhat atypical of mantids as males stealthily approach females, mount from behind and leave an external spermatophore attached to the female genital opening after copulating. Females later remove the spermatophore with their mouthparts and consume it (Holwell 2007). Both male and female *Ciulfina* are known to mate multiply in both the laboratory and the field, and females produce oothecae with eggs fertilized by multiple sires (K. D. L. Umbers, unpublished). *Ciulfina* therefore provide a very good opportunity to study patterns of multiple mating both within and between species. Microsatellite markers will be useful to assess paternity and investigate population structure in the Wet Tropics World Heritage Region of Australia. Such markers represent the first microsatellites developed for any praying mantid.

We isolated and characterized nine polymorphic di-, tri- and tetranucleotide microsatellite DNA markers for *C. rentzi*. Genomic DNA was extracted using a modified salting-out protocol (Sunnucks & Hales 1996). Markers were isolated using an enrichment technique (Fischer & Bachmann 1998) modified as in Beheregaray *et al*. (2004). Briefly, genomic DNA was digested with *Rsa*I and *Hae*III, and fragments ligated to two oligo adaptors. Two biotinylated oligo probes (dCA_{10} and dGA_{10}) were hybridized to the digested DNA and selectively retained using Streptavidin MagneSphere Paramagnetic Particles (Promega). Polymerase chain reaction (PCR) was performed on the microsatellite-enriched elutant using an oligo adaptor as a primer. The enriched library was purified using an UltraClean 15 DNA Purification Kit (MO BIO Laboratories), ligated into pCR 2.1-TOPO vector (Invitrogen) and transformed into One Shot TOP10 Chemically Competent Cells (Invitrogen). The plasmid DNA was PCR-amplified using M13(−20) forward and M13(−40) reverse primers, purified using an UltraClean 15 DNA Purification Kit (MO BIO Laboratories) and 107 clones were sequenced on an ABI 3130xl Genetic Analyzer (Applied Biosystems).

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Sequences were assembled and edited using SEQUENCHER 4.1 (Gene Codes Corporation), and screened for microsatellites manually or using IMPERFECT MICROSATELLITE EXTRACTOR 1.0 (Mudunuri & Nagarajaram 2007). Primers flanking 27 loci were designed using PRIMER 3 (Rozen & Skaletsky 2000) and a M13 universal sequence (5′-TGTAAAACGACGGCCAGT-3′) appended to the 5′ end of each forward primer.

Nine polymorphic loci were successfully amplified and characterized. Fluorescent-labelled PCR products were produced following Schuelke (2000). All amplifications were performed in a 10 μL reaction containing ~10–100 ng template DNA and 200 μM each dNTP. Reactions for C036, C051, C085, C093, C116, C106 and C120 included 2 pmol fluorescent-labelled M13(−21) primer and reverse primer, 0.4 pmol forward primer with 5′-M13(−21) tail, 0.5 μg/μL BSA, 2.5 mM $MgCl_2$, 0.5 U GoTaq Flexi DNA polymerase and its reaction buffer (Promega). PCR profile for these loci consisted of 94 °C/3 min, 30 (for C106 and C120 only) or 33 cycles 'touchdown' (94 °C/20 s; 63 °C to 53 °C until sixth cycle/45 s; 72 °C/60 s), and 72 °C/10 min. Reactions for C006 and C058 included 0.8 pmol fluorescent-labelled M13(−21) primer, 1 pmol reverse primer, 0.125 pmol forward primer with 5′-M13(−21) tail, 2 mM $MgCl_2$, 1 U GoTaq Flexi DNA polymerase and its reaction buffer (Promega). PCR profile for these two loci consisted of 90 °C/3 min, followed by a 40 cycles touchdown (94 °C/30 s [except in first cycle]; 60 °C to 50 °C until sixth cycle/30 s; 72 °C/45 s), and 72 °C/10 min.

PCR products were electrophoresed on an ABI 3130xl Genetic Analyzer with a LIZ-500 size standard and allele sizes designated with GENEMAPPER 4.0 (Applied Biosystems). We used GENEPOP 3.4 (Raymond & Rousset 1995) to estimate expected (H_E) and observed (H_O) heterozygosities per locus, deviations from Hardy–Weinberg equilibrium and linkage disequilibrium. MICRO-CHECKER 2.2.3 (van Oosterhout *et al*. 2004) was used to test for null alleles in the data. All nine loci were screened for variation in 20 to 26 *C. rentzi* collected from Cairns, Australia. Markers revealed substantial genetic variation, with the number of alleles per locus ranging from 3 to 16 and expected heterozygosities from 0.24 to 0.94 (Table 1). MICRO-CHECKER suggested the possibility of null alleles ($P < 0.05$) with frequencies between 12% and 26% for C006, C036, C106 and C120, and C036 had significant departure from Hardy–Weinberg equilibrium ($P = 0.001$). We attribute this result to Wahlund effect given the highly localized genetic substructure detected by a mtDNA phylogeographic analysis (G.I. Holwell, S. Allen, M.E. Herberstein & L.B. Beheregaray, unpublished) in this poorly dispersive species. No evidence for linkage disequilibrium was detected in locus-pair comparisons.

Table 1 Primer sequences and characteristics of nine *Ciulfina rentzi* microsatellite loci

Locus	Primer sequences (5′–3′)	Repeat motif	n_g	n_a	N_a	Size range (bp)	H_O	H_E	Estimated null allele frequency	GenBank Accession no.
C006†	AATCGGGTGGGAGAAAGG CTTCAACCTGCATGCAACC	$(TTC)_{17}$	26	26	7	287–327	0.47	0.57	0.26	FJ753581
C036*†	GCGACAGAGTGGTGACTTCC TCCGCAGCTCCTTCATTT	$(TG)_{10}$	23	23	8	170–186	0.48	0.78	0.19	FJ753582
C051	GCAGCACCCATAGTTTCACTC GTTGCCCACGGGCTCTTA	$(TGAG)_{18}$	21	23	13	163–227	0.76	0.90	—	FJ753583
C058	CTTGGAGGTTTGATCGCTTC CTTTTCCTTGTGTGCGTTTG	$(TG)_8$	26	26	3	169–173	0.42	0.54	—	FJ753584
C085	TCCCATTCGTTTACCGTCTC TGCATCCTTTAATCCCACATC	$(AC)_5$	23	23	3	217–231	0.35	0.52	—	FJ753585
C093	CTTTACTTCGCCCCTTTGC AAAAATTAGACCTAGCTGACGAC	$(GAGT)_{13}$	23	23	12	248–305	0.83	0.89	—	FJ753586
C106†	GCCAACAGCAACGTCAGTTAC GGTATGTTCCATCCGGACAC	$(GA)_{29}$	20	23	16	256–292	0.60	0.94	0.17	FJ753587
C116	GGTGAATGGGCGAGTTATTC GGAATGGAGGAAACATGCTC	$(AGTG)_{>20\ imperfect}$	23	23	4	322–350	0.26	0.24	—	FJ753588
C120†	AACCAGACGAACGAAACTCC TCTCGAATGAACCCAATCATC	$(AG)_7$	22	23	10	287–361	0.55	0.73	0.12	FJ753589

n_g, number of individuals successfully genotyped; n_a, number of individuals assayed; N_a, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; *significant departure from Hardy–Weinberg equilibrium; †significant evidence of null alleles. Forward primers were tagged with a 5′M13 universal sequence (5′-TGTAAAACGACGGCCAGT-3′), but size ranges shown excluded this portion of the sequence.

Acknowledgements

Funding was provided by Macquarie University.

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doi: 10.1111/j.1755-0998.2009.02718.x

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Isolation and characterization of microsatellite loci for mountain mullet (*Agonostomus monticola*)

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Abstract

We report on the isolation of 15 polymorphic microsatellite loci from mountain mullet (*Agonostomus monticola*). In the two populations sampled, loci exhibited two to 21 alleles and observed heterozygosity values ranged from 0.222 to 1.000. All loci conformed to Hardy–Weinberg equilibrium expectations, and none exhibited linkage disequilibrium. Although *A. monticola* is an important subsistence fishery in parts of its range, little is known about its ecology and many populations appear to be experiencing declines. These microsatellite loci should prove useful in the study of population structure of *A. monticola* and aid in other potential conservation efforts such as the management of hatchery broodstock.

Keywords: *Agonostomus monticola*, microsatellite, mountain mullet, population structure

Received 18 December 2008; revision accepted 27 February 2009

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The mountain mullet (*Agonostomus monticola*) is a diadromous fish with a range that includes the Pacific and Atlantic slopes of the Americas from the southern United