Characterization of microsatellite loci for the Australian sea urchin *Heliocidaris erythrogramma*

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Abstract

We report 16 polymorphic microsatellite loci from *Heliocidaris erythrogramma*, a common sea urchin endemic to temperate Australian waters. These microsatellites were tested in a minimum of 30 individuals, which yielded between five and 14 alleles per locus. Expected heterozygosity ranged from 0.52 to 0.92 with four loci deviating from Hardy–Weinberg expectations. These markers are expected to be useful for experimental studies involving paternity analysis and for quantifying population structure in *H. erythrogramma* across its geographic range.

Keywords: *Heliocidaris erythrogramma*, microsatellite, sea urchin

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The sea urchin *Heliocidaris erythrogramma*, endemic to temperate Australian waters (Dix 1977), is an emerging model species in the studies of evolutionary biology (Marshall *et al.* 2004; Evans *et al.* 2007), population genetics (McMillan *et al.* 1992) and ecology (Keesing 2001; Huggett *et al.* 2006). This is, in part, due to its broadcast spawning strategy and developmental mode featuring a strikingly abbreviated larval stage (Williams & Anderson 1975; McMillan *et al.* 1992). We have developed microsatellite markers in *H. erythrogramma* to address two specific aims: to disentangle, via paternity assignment, the sources of variance in competitive fertilization success (i.e. resulting from sperm competition), and for mapping population structure and connectivity among natural populations across its geographic range.

Here we report results from the development of 16 polymorphic microsatellite loci for *H. erythrogramma*. Genomic DNA was extracted from muscle tissue using a modified salting out procedure (Gemmell & Akiyama 1996). Genetic Information Services used these samples to construct DNA libraries and isolate potential di- and tri-nucleotide microsatellites. Methods for the construction and enrichment of the libraries are as outlined in Jones *et al.* (2002). Briefly, fragments in the size range of 300–750 bp were adapted and subjected to magnetic bead capture (CPG, Inc.) to produce four libraries, and enrichment levels were expressed as the fraction of sequences that contained a microsatellite. The $CA_{(15)}$ library produced 25 microsatellites of 25 clones, the $GA_{(15)}$ library produced 23 microsatellites of 24 clones, the $AAC_{(12)}$ library produced 18 microsatellites of 24 clones and the $ATG_{(12)}$ library produced 18 microsatellites of 24 clones. Polymerase chain reaction (PCR) primers were developed for 73 of these microsatellite-containing clones using DesignerPCR v.1.03 (Research Genetics, Inc.) software.

Locus amplification in *H. erythrogramma* was tested with total genomic DNA extracted from the muscle tissue of a minimum of 30 individuals collected from Rottnest Island, Western Australia (32°01′S, 115°27′E). PCR reactions of 10 μL contained 1 μL (~10 ng) of DNA, 0.3 μM of each primer (forward primer fluorescently labelled) and 8 μL of reaction buffer [Invitrogen's Platinum PCR Supermix: 22U/mL complexed recombinant *Taq* DNA polymerase with Platinum *Taq* antibody, 22 mM Tris–HCl (pH 8.4), 2.5 μM $MgCl_2$, 55 mM KCl, 220 μM dGTP, 220 μM dATP, 220 μM dTTP, 220 μM dCTP and stabilizers]. PCR amplifications were carried out in an Eppendorf thermal cycler and consisted of an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 30 s at 94 °C; 30 s at the annealing temperature (see Table 1), 30 s at 72 °C, and a final elongation step at 72 °C for 4 min. PCR products were analysed on an ABI 3730 Sequencer and sized using GeneScan500 LIZ internal size standard. Alleles were scored using GeneMapper v.3.7 (Applied Biosystems) software.

We identified 16 polymorphic microsatellite markers from which genotypes consistently amplified within expected range sizes (Table 1). We applied MICROSATELLITE ANALYSER software (Dieringer & Schlotterer 2003) to measure summary statistics, and GENEPOP v.3.7 (Raymond

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Table 1 Primer sequences and characteristics of 16 polymorphic microsatellite loci for *Heliocidaris erythrogramma* including repeat motif, annealing temperatures (T_a), sample size (N), number of alleles (N_a) and the size range of alleles for each locus. Expected (H_E) and observed (H_O) heterozygosities are given, as well as estimated null allele frequencies, which were all nonsignificant

Locus	Primer sequences (5′–3′)	Repeat motif	T_a (°C)	N	N_a	Size range (bp)	H_E	H_O	Null	GenBank Accession no.
HerA2	F: TGTCCACATGCTACTTATCTCC R: ATAACGTAGTTCGCAGTTGATG	$(CA)_{17}$	53	43	11	169–189	0.88	0.84	0.017	FJ532225
HerA5	F: CCATTCCTTTTACCATCTTACC R: GCCTACAAACTGACTCTGACTG	$(GT)_{14}$	53	39	10	220–245	0.81	0.64	0.068	FJ532226
HerA103	F: ACCGCTTAACTTTACATACCC R: GGAGGATTATGGGATATTGAG	$(CA)_4$	55	42	9	159–200	0.60	0.74	–0.091	FJ532228
HerA106	F: AATGCGGCTCCTGTTCTC R: CGTCTTTCGGATGTGACG	$(GT)_{11}$	55	36	7	261–280	0.72	0.56	0.059	FJ532229
HerA113	F: CCGATGACGAGTAACAATAGAG R: AATCCACATGCTATCATAGACG	$(GT)_{18}$	53	30	10	135–176	0.84	0.70	0.061	FJ532230
HerB103	F: AATGAACGAATCGCAGTGA R: GCTTTGTTATTTCCCTTCCCTA	$(GA)_4AAGGGG(GA)_{14}$	55	40	14	209–237	0.92	0.88	0.018	FJ532231
HerB118	F: TAGCCAATTTTGGTCTAATGC R: TTCGGTTAGTGTGCAGTATTG	$(CT)_6CCCG(CT)_6$	53	43	11	134–166	0.77	0.79	–0.019	FJ532233
HerC10	F: ATGACTGATGGACCGACTGAC R: GCAAGAACCGATGTAGTGGAT	$(AAC)_{11}$	57	42	9	194–220	0.74	0.88*	–0.086	FJ532234
HerC101	F: CGGGATTCACAGTCTGAGC R: GCTGCCTTGAGTTGGGTC	$(AAC)_9$	53	43	8	125–146	0.79	0.79	–0.005	FJ532235
HerC102	F: TAATGGCGTCGTCTATTCTG R: CGTCTTGATGGGTTCTCAC	$(AAC)_6$	55	42	5	136–147	0.52	0.83*	–0.207	FJ532236
HerC104	F: CACCTTACGACCAATGTGA R: TCGGTTGTATGTCTATCCTTG	$(AAC)_7$	53	41	7	151–167	0.69	0.61*	0.041	FJ532237
HerC109	F: GGCTTCAAAAGACTGTATTCAC R: TAACTGGCACAAAGGACACTA	$(TTG)_7$	53	31	9	244–276	0.71	0.58*	0.062	FJ532238
HerC115	F: CGGGACTTGCATAGCAAC R: TCACGTTTCCCCATTTATG	$(AAC)_6$	55	41	6	239–253	0.72	0.66	0.033	FJ532239
HerD3	F: CACGGTCTGAAGACATTGAAAT R: AAGCAACGATTCTGATTGGTTA	$(CAT)_{15}$	55	41	9	111–158	0.76	0.83	0.051	FJ532240
HerD5	F: GCATCACCATCATTGTCAG R: AGGACCCAGATTGGATTG	$(CAT)_7$	55	38	14	141–219	0.82	0.79	–0.044	FJ532241
HerD105	F: CGGAGATAATTGTTCTATGGAG R: ATCGTAGGGCCTGTAATTTAG	$(CAT)_5$	53	43	7	175–193	0.75	0.65	0.012	FJ532243

*Significant deviation from Hardy–Weinberg expectations ($P < 0.05$) following sequential Bonferroni corrections for multiple tests.

& Rousset 1995) to test for conformity to Hardy–Weinberg expectations and linkage disequilibrium between all pairs of loci. The number of alleles per locus ranged from five to 14. Expected heterozygosity ranged from 0.51 to 0.92, with four loci significantly deviating from Hardy–Weinberg expectations after applying sequential Bonferroni corrections (Rice 1989) with a minimum critical value of 0.003. For two of these loci (HerC104 and HerC109), deviations were due to heterozygote deficits, which is a fairly common occurrence in broadcast-spawning invertebrates (Addison & Hart 2005). Additional testing with MICRO-CHECKER software (van Oosterhout *et al.* 2004) did not detect null alleles for these loci. The other two deviating loci (HerC10 and HerC102) were in excess of heterozygotes and may be linked to functional genes being acted upon by selection. Further testing with additional populations may reveal more information for these loci. Tests for linkage yielded three weakly significant *P*-values of 120 pairwise comparisons between HerC102-HerD105 ($P = 0.01$), HerA2-HerD3 ($P = 0.03$) and HerC101-HerD5 ($P = 0.03$); however, none of these pairs were significant following sequential Bonferroni corrections with a minimum critical value of 0.0004, indicating that these loci are unlikely to be physically linked. These 16 microsatellite loci will therefore be useful for experimental studies involving paternity analysis and for quantifying population structure in *H. erythrogramma* across its geographic range.

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Characterization of 10 microsatellite markers for the understorey Amazonian herb *Heliconia acuminata*

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Abstract

We characterized 10 microsatellite loci for the plant *Heliconia acuminata* from the Biological Dynamics of Forest Fragments Project (Manaus, Brazil). Markers were screened in 61 individuals from one population and were found to be polymorphic with an average of eight alleles per locus. We found moderate to high levels of polymorphic information content, and observed and expected heterozygosities. All 10 markers are suitable for spatial genetic structure and parentage analyses and will be used for understanding *H. acuminata* dynamics across a fragmented landscape.

Keywords: Amazon forest, forest fragmentation, genetic relatedness, *Heliconia acuminata*, microsatellites, parentage analysis

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Heliconia acuminata (Heliconiaceae) is a common understorey species of the nonflooded tropical forest of central Amazonia and the Guyanas (Berry & Kress 1991). *Heliconia acuminata* is a perennial, self-incompatible hermaphroditic species with limited vegetative reproduction (E. M. Bruna and W. J. Kress, unpublished data). The flowers are visited by hermit hummingbirds that 'trapline' from one plant to the next (Kress

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