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PERMANENT GENETIC RESOURCES

Ten polymorphic microsatellite loci for the trematode *Curtuteria australis* (Echinostomatidae)

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Abstract

Ten polymorphic loci were isolated and characterised from the intertidal New Zealand trematode *Curtuteria australis*. This common parasite manipulates the burrowing behaviour of its abundant bivalve host *Austrovenus stutchburyi*, with cascading impacts on the biodiversity of intertidal communities. Observed heterozygosities of the 10 loci ranged from 0.500 to 0.905, and three to 14 alleles were detected in 24 trematode metacercariae. These loci are currently being used to investigate the molecular ecology of this species within its intermediate hosts.

Keywords: bivalve, *Curtuteria australis*, microsatellites, parasite, trematode

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Introduction

The New Zealand cockle *Austrovenus stutchburyi* is host to a number of different parasites (Leung & Poulin 2007), of which the trematode *Curtuteria australis* is the most extensively studied. The life cycle of *C. australis* involves three hosts, with the mud whelk *Cominella glandiformis* serving as the first intermediate host where asexual multiplication occurs, resulting in infective cercariae which then infect the cockle *A. stutchburyi* where they encyst as metacercariae in the foot muscle, and the life cycle is completed when the infected cockle is eaten by shorebirds such as the pied oystercatcher *Haematopus ostralegus* (Allison 1979). *C. australis* has been found to impair host burying ability (Thomas & Poulin 1998) and is considered as a significant ecosystem engineer through its impact on the behaviour of cockles and associated impacts on sediment characteristics and invertebrate biodiversity (Mouritsen & Poulin 2005).

The life cycle and transmission ecology of this parasite have been well-studied over the years and due to its intensity-dependent effects on host behaviour, *C. australis* provides a good model system for investigating the determinants of different life-history strategies and intraspecific competition.

Our protocol is modified from Perrin & Roy (2000). Total genomic *C. australis* DNA was isolated from 120 rediae dissected from a single *Cominella glandiformis* collected from Lower Portobello Bay, South Island, New Zealand using QIAGEN DNeasy Tissue kit. Genomic DNA was digested using the restriction enzyme *Mbo*I (New England Biolabs) and fragments 400–1200 bp in length were ligated to a pUC19 *Bam*HI/BAP cloning vector (Qbiogene). DNA inserts were amplified using polymerase chain reaction (PCR) with M13 primers. PCR (50 µL) consisted of 5 µL of DNA ligation, 0.6 µM of each M13 primer, 1.25 U *Taq* DNA polymerase (Bioline), 200 µM of each dNTP, 1.5 mM $MgCl_2$ and 1× *Taq* buffer [16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, 0.01% Tween 20]. PCR amplification was performed on an Eppendorf Mastercycler ep gradient S thermal cycler and consisted of 5 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C and a final extension for 10 min at 72 °C. PCR products 400–1200 bp in length were annealed to biotinylated $(GA)_{12}$ and $(GT)_{12}$ probes (Invitrogen) (incubation consisted of 5 min at 94 °C, followed by 1 min at 55 °C, and 10 min at 72 °C), and microsatellite-containing DNA was retained using Streptavidin MagneSphere Paramagnetic Particles (Promega). Microsatellite-enriched DNA was purified using a High Pure PCR product purification column (Roche) and 5 µL was used in a PCR amplification with M13 primers as described previously. The microsatellite-containing fragments were digested with *Mbo*I and ligated to pUC19 *Bam*HI/BAP cloning vectors which were used to transform *Escherichia coli* XL1-Blue competent cells (Stratagene). One thousand five hundred and thirty-six colonies were picked and grown in LB-glycerol in 96-well

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Table 1 Microsatellite loci developed for *Curtuteria australis* sp. Locus name is followed by repeat motif of cloned alleles, primer sequences, primer annealing temperature (T_a), size range of alleles in base pairs (bp), number of alleles, number of amplified individuals (*n*), observed (H_O) and expected (H_E) heterozygosities, and GenBank Accession no.

Locus	Repeat motif	Primer sequences (5′–3′)	T_a (°C)	Range (bp)	No. of alleles	*n*	H_O (H_E)	GenBank Accession no.
Cau1	$(AG)_{11}$	F: GTCGGGCAAGAGTGGATAG R: CCTTCTCTTCCTGGTTCCC	64	65–73	5	40	0.600 (0.676)	EU274473
Cau2	$(CT)_7$	F: GCTTCTACTGAACGCCAGCG R: CCACCCCACCCCAACTCTCG	64	83–89	3	42	0.500 (0.482)	EU274474
Cau5	$(AC)_{10}$	F: GAATGAGGGTTGTGGTTAC R: GATAGGTAAGTGGATAGGCAG	64	76–94	9	42	0.714 (0.787)	EU274475
Cau10	$(AC)_{10}$	F: CCATTGAACAGAGCTGCAC R: CGTGCTCACTAACAACTTGG	64	79–85	5	42	0.762 (0.696)	EU274476
Cau11	$(TG)_9$	F: GGGAATAACTGTACTGTTTTCCG R: GCTCACCAACTTCCTGCAC	55	81–103	10	41	0.659 (0.789)	EU274477
Cau13	$(AC)_{11}(GA)_8$	F: GTGATACGATACAATATAATACG R: GTCTCAAGGGTGACAGTG	64	131–165	14	41	0.780 (0.890)	EU274478
Cau14	$(GT)_{10}$	F: GTGCGTGTTTTCCTCGTC R: GACACCGTCCTTTCAACC	64	69–87	7	39	0.744 (0.811)	EU274479
Cau15	$(GT)_7(GC)(GT)_4$	F: GGTGGAAAAAAGAGAAAACGAC R: CAATATAAGACCCACCCGGAG	64	87–99	9	42	0.548 (0.594)	EU274480
Cau17	$(GT)_7(CT)(GT)_6$	F: GCACATTTTCGACAAAACAGAAC R: CGGAAGGACGCCGTTATAG	64	108–130	10	42	0.905 (0.812)	EU274481
Cau19	$(GT)_7(GTAT)_5(TT)$ $(GTAT)_4(GTGC)_6$	F: CGTATTTTTATTACACCTG R: GACCTAATTTACTAATCTCG	55	155–175	8	41	0.854 (0.830)	EU274482

microtiter plates. To screen for microsatellites, colonies were transferred to Biodyne B nylon membranes (Pierce) and probed with γ-^{32}P-dATP radiolabelled $(GA)_{12}$ and $(GT)_{12}$ oligonucleotides. Positive clones were identified on exposed autorad films and amplified with M13 primers as described previously. Two hundred and nineteen colonies were positive for GA or GT microsatellites out of the 1536 colonies screened (14.3%). The PCR products from 92 of the positive colonies were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and resolved with an ABI PRISM 3730 Genetic Analyser (Applied Biosystems). A total of 19 PCR primer pairs were designed using the OLIGO software package (Molecular Biology Insights) and 10 polymorphic loci were developed.

Successful primer amplification and optimal annealing temperatures were determined using DNA from three individual *C. australis* rediae from three *C. glandiformis*. Individual redia DNA was extracted in 500 µL of 5% chelex containing 0.1 mg/mL proteinase K, incubated at 60 °C for 4 h and boiled at 100 °C for 8 min. PCRs (10 µL) consisted of 3 µL of DNA extraction (concentration approximately 3 ng/µL), 0.6 µM of each primer, 0.5 U *Taq* DNA polymerase (Bioline), 200 µM of each dNTP, 3 mM $MgCl_2$ and 1× *Taq* buffer [16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, 0.01% Tween 20]. PCR amplification was performed on an Eppendorf Mastercycler ep gradient S thermal cycler and consisted of 4 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at a gradient of 44–66 °C and 45 s at 72 °C. PCR products were resolved in 9% nondenaturing polyacrylamide gels using cloned fragments as size standards with a 10-bp DNA ladder and visualized by staining gels in 0.1X SYBR Green I (Invitrogen).

Successfully amplified loci were tested for polymorphism using the optimal annealing temperature with DNA from 42 individual *C. australis* metacercariae isolated from 18 *A. stutchburyi* collected from Lower Portobello Bay, South Island, New Zealand. DNA extractions and PCR were performed as described for primer screening. Polymorphic loci were tested for deviation from Hardy–Weinberg equilibrium (HWE). Allele frequencies and all pairs of loci were tested for genotypic disequilibrium using GENEPOP version 3.4 (Raymond & Rousset 1995). Alpha significance levels were adjusted using the sequential Bonferroni approach (Rice 1989).

Loci possessed three to 14 alleles and observed heterozygosities of 0.500–0.905 (Table 1). Nominal deviation from HWE was detected for the locus Cau14 ($P = 0.036$), but was not found to be significant after Bonferroni adjustment of alpha (initial $\alpha = 0.005$). Significant genotypic disequilibrium was found between the loci Cau13 and Cau14 ($P = 0.00012$).

The microsatellite markers are currently being used to test if there are intraspecific variations in the life-history traits of *C. australis* genetic clones and how they may determine the outcome of intraspecific clonal interactions.

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