Molecular Ecology Resources (2009) **9**, 563–565 doi: 10.1111/j.1755-0998.2008.02371.x

PERMANENT GENETIC RESOURCES NOTE

Isolation and characterization of nine polymorphic microsatellite loci of the kelp greenling, *Hexagrammos decagrammus*, a temperate reef fish

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Abstract

Nine polymorphic microsatellite loci were developed and characterized for the temperate reef fish species, *Hexagrammos decagrammus* (kelp greenling). The number of alleles varied from three to 22 in a sample of 22 individuals from one population. Expected heterozygosities ranged from 0.354 to 0.979. These microsatellites allow us to investigate reproductive success of individuals, alternative mating strategies as well as population structure and metapopulation dynamics of this species.

Keywords: *Hexagrammos decagrammus*, kelp greenling, microsatellites, paternity, population structure, temperate reef fish

Received 4 June 2008; revision accepted 21 August 2008

The Hexagrammidae consists of 12 species which are endemic to the North Pacific, ranging from Japan to Baja California, Mexico. Most species are demersal and are found on nearshore rocky reefs. One species, *Hexagrammos decagrammus* (kelp greenling), is a widely distributed temperate reef fish along the west coast of North America, ranging from southern California to the Aleutian Islands, Alaska (Miller & Lea 1972). Kelp greenling are sexually dimorphic and males guard nests that contain egg clutches from multiple females (Crow *et al*. 1997). Brood guarding behaviour of males is a common mating system in the Hexagrammidae and other benthic-spawning fishes (DeMartini & Sikkel 2006). This mating system lends itself to alternative mating strategies such as male sneaking behaviour, in which males try to fertilize eggs in other males' nests. A variety of mechanisms, including frequency-dependent evolutionary stable strategies, as well as condition-dependent fitness, have been suggested to explain this alternative mating strategy in fishes and other taxa (Sinervo & Lively 1996; Henson & Warner 1997). To investigate this behaviour, we developed nine polymorphic microsatellite loci for *Hexagrammos decagrammus*.

The microsatellites were developed by Genetic Identification Services (GIS, www.genetic-id-services.com). Libraries were built using 100 μg of genomic DNA extracted from fresh fin tissue of one individual from Monterey, California, USA following a standard phenol–chloroform procedure (Sambrook *et al*. 1989). Libraries were enriched for CA, ACC, ATG and CAGA motifs. GIS sequenced 67 microsatellite-containing clones using universal M13 primers and designed primers for 55 clones using DesignerPCR version 1.03 (Research Genetics, Inc.). DNA sequencing was carried out using Amersham's DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences P/N US81050), followed by electrophoresis on an ABI PRISM 377 DNA Sequencer (Applied Biosystems). Primers were tested by polymerase chain reaction (PCR) amplification of DNA from one individual, which were carried out in 10-μL total volume containing 1× Bio*Taq* Buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.01% gelatin), 2.0 mM $MgCl_2$, 0.2 mM dNTP, 0.6 μM of each primer, 0.025 U/μL Bio*Taq* Polymerase and 0.2 ng of template DNA. Cycling parameters were as follows: 3 min at 94 °C followed by 35 cycles of 40 s, 40 s, 30 s at 94 °C, 55–57 °C, 72 °C, respectively, and a 4-min extension at 72 °C in an Applied Biosystems GeneAmp PCR 9700 system. Alleles were visualized on a 1% agarose gel.

We tested 10 of these microsatellites, using DNA of 22 individuals from one population in Monterey, California, USA. DNA was extracted from fin tissue following a standard phenol–chloroform procedure (Sambrook *et al*. 1989). Amplification reactions were carried out in a total volume of 13 μL containing 1× PCR Mastermix (2.5 mM TAPS pH 9.5, 5.0 mM KCl, 0.2 mM $MgCl_2$, 20.0 μM each dNTP, *Taq* 0.5 U/

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Table 1 Characterization of nine polymorphic microsatellite loci for kelp greenling, *Hexagrammos decagrammus*, from a sample of 22 individuals. The GenBank Accession no. (GenBank), (F) labelled forward primer [fluorescein (FCL); Sigma-Aldrich] and (R) reverse primer (cold) sequence (primer seq.), repeat motif and number of repeats of the original clone (motif), number of alleles per locus (alleles), number of individuals that amplified (*n*), allele size range, range of numbers of repeats (repeats), and observed (H_O) and expected (H_E) heterozygosities. Asterisk (*) indicates locus is departing from Hardy–Weinberg equilibrium ($P < 0.001$)

Locus	GenBank	Primer seq.	Motif	Alleles	*N*	Size range	Repeats	H_O	H_E
HEDE_E2	EU781567	F: 5′ FCLCCAACACGAGAAGAGACTAACC 3′ R: 5′ CGTCAGCCATCAAAGTGTC 3′	$(CA)_{25}$	14	22	159–195	25–43	0.818	0.884
HEDE_E3	EU781568	F: 5′ FCLGCTTAGAGGGCAGGAAGTAG 3′ R: 5′ TACATTGTCAAGGCACACTG 3′	$(CA)_{38}$	10	18	152–208	16–44	0.944	0.946
HEDE_E4	EU781569	F: 5′ FCLCCCACCTACATTTTCTCATCC 3′ R: 5′ CTCTGCTCTCGTCTTCACTTG 3′	$(CA)_{33}$	22	20	209–279	18–53	0.650*	0.979
HEDE_E5	EU781570	F: 5′ FCLAGGCATTTTACCACCTCTCTC 3′ R: 5′ CTTTCGGAATCGTCCAGTATT 3′	$(CA)_{13}$	11	21	257–277	11–21	0.714	0.885
HEDE_E10	EU781571	F: 5′ FCLCCCTCCTCACACTGCATCT 3′ R: 5′ ACTGCACGTTACAGCGTCTC 3′	$(CA)_{13}$	15	19	176–224	7–31	0.842	0.940
HEDE_E11	EU781572	F: 5′ FCLTCAATGTGTTTTGCCTGAAC 3′ R: 5′ GAGGGCGATAAGACTGAC 3′	$(CA)_{35}$	10	20	172–216	28–50	0.850	0.849
HEDE_I1	EU781573	F: 5′ FCLGGCAACAAGCCATTCCTT 3′ R: 5′ CATCGCAGTCTCAGCCAC 3′	$(CAA)_8$	4	20	164–173	5–8	0.526	0.471
HEDE_I2	EU781574	F: 5′ FCLTGCAACAGCTTCACCTGTC 3′ R: 5′ CGTTGGGGTTGTTTCTGTC 3′	$(TCC)_6$	3	19	182–212	4–14	0.364	0.354
HEDE_I6	EU781575	F: 5′ FCLTGACTTGTGTTGCATCTCA 3′ R: 5′ TTTAGGAGCCATGTGACC 3′	$(CAA)_7$	10	21	127–163	5–17	0.762	0.860

μL, Thermo Scientific), 0.625 μL of both 20 μM primers and approximately 2 ng of DNA template. Samples were amplified with fluorescein-labelled (Sigma-Aldrich) forward and cold reverse primers (Table 1) for 35 cycles at 94 °C, 54 °C and 72 °C, for 45 s, 45 s and 60 s, respectively, in an Applied Biosystems GeneAmp PCR 9700 system. Microsatellite amplifications were mixed with Applied Biosystems GeneScan 500 ROX size standard and run on an ABI 3100 automated sequencer, and scored using the software GeneMapper 3.7 (Applied Biosystems).

Nine out of 10 loci produced polymorphic microsatellites, with ranges from three to 22 alleles per locus (Table 1). Microsatellites were analysed for Hardy–Weinberg equilibrium and linkage disequilibrium using Arlequin 2.000 (Schneider *et al*. 2000). The presence of null alleles was tested using Micro-Checker 2.2.3 (Van Oosterhout *et al*. 2004). Eight loci were in Hardy–Weinberg equilibrium ($P > 0.05$). One locus (HEDE_E4) was out of Hardy–Weinberg equilibrium ($P < 0.001$) and possibly exhibits null alleles, or more likely these results are due to our small sample size. HEDE_E4 is the most variable locus found, with an allele range of 70 bp. Therefore, it seems likely that the total number of alleles is higher than the 22 alleles that we found. There is no linkage among the nine loci described here (chi-squared tests $P >> 0.05$ for all possible pairs of loci, likelihood-ratio test; Slatkin & Excoffier 1996).

These are the first microsatellite loci described for this species. We are using these microsatellites to perform parentage analysis on eggs collected from nest-guarding males to identify alternative mating strategies in this species as well as evaluate reproductive success of individuals. They will also allow us to investigate the population structure of this and possibly closely related species within the Hexagrammidae.

Acknowledgements

We would like to thank Patrick Lane and Brennan Wenck for their help, Mark Carr, Rick Starr, Ricardo Beldade and Marloes Poortvliet for valuable comments and discussions. This research was funded by the IMS Packard Endowment for Science and Technology at UCSC, the Partnership for Interdisciplinary Studies of Coastal Oceans (PISCO), the California Department of Fish and Game, and the STEPS Institute for Innovation in Environmental Research. J.F. was supported by a National Science Foundation Graduate Research Fellowship and a University of California Marine Council's California Environmental Quality Initiative (CEQI) fellowship. This is contribution number 312 from PISCO, funded primarily by the Gordon and Betty Moore Foundation and David and Lucile Packard Foundation.

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