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PERMANENT GENETIC RESOURCES NOTE

Isolation and characterization of 23 microsatellite loci in the yellow tang, *Zebrasoma flavescens* (Pisces: Acanthuridae)

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Abstract

Twenty-three microsatellites were isolated from the yellow tang (*Zebrasoma flavescens*), an ecologically and commercially important reef fish. Genetic diversity was assessed in 90 adults collected from Honokohau, Hawaii. The number of alleles per locus varied from four to 29 (mean = 13.8) and observed and expected heterozygosities ranged from 0.15 to 0.94 (mean = 0.70) and from 0.29 to 0.93 (mean = 0.81), respectively. Eight loci exhibited significant departure from Hardy–Weinberg equilibrium due to the presence of null alleles. Exact tests showed no evidence of genotypic disequilibrium between loci. Overall, loci were well resolved, easy to score and highly polymorphic.

Keywords: connectivity, coral-reef fish, Hawaii, Indo-Pacific, pelagic larval dispersal, surgeonfish

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The yellow tang surgeonfish (*Zebrasoma flavescens*) occurs throughout the subtropical North Pacific from the Hawaiian archipelago to southern Japan (Randall 2007). In Hawaii, yellow tang play an important role as herbivores of filamentous algae (Walsh 1987) although aquarium fishery collections of approximately 500 000 fish per year have reduced adult abundance by 46% on unprotected reefs (Tissot & Hallacher 2003). Since the successful management of marine populations requires an understanding of metapopulation dynamics (Palumbi 2003), we developed a suite of microsatellite markers to provide insights into patterns of migration and gene flow in yellow tang at both evolutionary and ecological timescales. Allozyme and sequence-based analyses of marine fishes commonly reveal low levels of genetic differentiation within species (Grant & Bowen 1998), thus the identification of highly polymorphic microsatellite markers offers a novel opportunity to both directly and indirectly estimate patterns of larval exchange (reviewed by Hedgecock *et al*. 2007) in a commercially and ecologically important marine species.

Methods for DNA library construction, enrichment and screening were as described previously by Jones *et al*. (2002). Genomic DNA was partially restricted with a cocktail of seven blunt-end cutting enzymes (*Rsa*I, *Hae*III, *Bsr*B1, *Pvu*II, *Stu*I, *Sca*I, *Eco*RV). Fragments in the size range of 300 to 750 bp were adapted and subjected to magnetic bead capture (CPG, Inc.) using biotinylated capture molecules. Libraries were prepared in parallel using Biotin-CA(15), Biotin-GA(15), Biotin-CT(15), Biotin-AAAC(8) and Biotin-TAGA(8) as capture molecules in a protocol provided by the manufacturer. Captured molecules were amplified and restricted with *Hin*dIII to remove the adapters. The resulting fragments were ligated into the *Hin*dIII site of pUC19. Recombinant molecules were electroporated into *Escherichia coli* DH5alpha. Recombinant clones were randomly selected for sequencing, and enrichment levels were expressed as the fraction of sequences that contained a microsatellite. Sequences were visualized on an ABI PRISM 377 using ABI PRISM *Taq* dye terminator cycle sequencing methodology. The optimal amplification reaction mix for all primer pairs consisted of 1× Biolase C buffer (from 10× stock solution supplied by manufacturer), 2 mm $MgCl_2$, 0.2 mm each dNTPs, 6 m each primer (forward primer fluorescent labelled), 0.025 U/μL Biolase C *Taq* polymerase, and 0.2 ng/μL template DNA in 50-μL final reaction volume. Clones were amplified in a PerkinElmer-Cetus thermal cycler by an initial 3 min of denaturation at 94 °C, followed by 35 cycles of denaturation (94 °C, 40 s), annealing (55 °C, 40 s), and extension (72 °C, 30 s), with final extension time of 4 min at 72 °C. Sample DNA was extracted using the PureGene DNA Extraction Kit (Gentra Systems) following

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the manufacturer's instructions. All loci were amplified in 10-μL final reaction volume with the following reaction mix: $MgCl_2$, 2 mM; dNTPs (premixed), 0.2 mM each; primers, 0.3 μM each; Bio*Taq* DNA Polymerase (Bioline), 0.025 U/μL; template DNA, 0.2 ng/μL. Polymerase chain reaction (PCR) was conducted in a RoboCycler Gradient 96R thermocycler (Stratagene, Inc.) by an initial denaturation (94 °C, 3 min), followed by 35 cycles of denaturation (94 °C, 30 s), annealing (52 °C, 40 s), and extension (72 °C, 30 s), and a final extension at 72 °C for 15 min. After optimization, PCR products were labelled using the M-13 tailing technique (Schuelke 2000; Boutin-Ganache *et al*. 2001). The tail sequence (GACTATGGGCGTGAGTGCAT) was attached to one of the proprietary sequencing dyes FAM, HEX, NED or PET (Applied Biosystems, Inc.). Amplification products were separated on polyacrylamide gels in an ABI 3100 DNA sequencer and sized using Genotyper 3.7 software and LIZ 500 HD size markers (Applied Biosystems, Inc.). Markers were screened against 90 adult yellow tang collected from Honokohau, Hawaii according to loci screening procedures described by Selkoe & Toonen (2006).

Out of a total of 47 loci, six were discarded because they were found to be monomorphic and 18 were polymorphic but were not tested further. To ensure high-quality genetic data, the remaining 23 loci were screened in GenePop (http://wbiomed.curtin.edu.au/genepop/) for number of alleles, observed and expected heterozygosities and deviations from Hardy–Weinberg equilibrium and genotypic disequilibrium (Table 1). The number of alleles per locus varied from four to 29 (mean = 13.8) and observed

Table 1 Characterization of 23 microsatellite loci isolated from the yellow tang *Zebrasom flavescens* (N = 90)

Locus	Primer sequence (5′–3′) and fluorescent dye	Repeat motif	k*	Allele size range (bp)	H_O†	H_E‡	GenBank Accession no.
Zefl01	F: GGATGGGACTGATTTGAAG-PET R: GATGCTGCTGTGCTGAAT	$(TAGA)_9$	17	136–206	0.87	0.84	EU794469
Zefl02	F: ATGGCTGTTTGCCTTGTTTAG-FAM R: TGGAACGAGAGAAAAATCAGG	$(TAGA)_9$	17	173–241	0.83	0.88	EU794470
Zefl03	F: TTGTGCAATTTAGTGCTTCAG-VIC R: CTTCCAAGGTCATCTGAGTGT	$(TAGA)_{10}$	25	238–418	0.66¶	0.93	EU794471
Zefl04	F: GAAGCAGAAACACAACGATG-FAM R: AGTTCCGTAAAGGATGGTGA	$(TAGA)_8$	13	206–300	0.38¶	0.82	EU794472
Zefl05	F: CCTCACTTCACTTCACCTCT-VIC R: CACCTACTCCCCAGACTTC	$(TAGA)_9$	24	257–353	0.80¶	0.92	EU794473
Zefl06	F: CCCTGAAAATGTAAACCTTG-NED R: ATTGTTGTCTGTGTTGATGTG	$(TAGA)_8$	19	235–315	0.75§	0.83	EU794474
Zefl07	F: CGTTCTCAGTTTTCTGCTGT-PET R: CTTTCGCTCACACTTGTTG	$(TAGA)_{11}$	24	253–357	0.92	0.89	EU794475
Zefl08	F: AGGACAAGAGAAGGCAGAGAC-VIC R: CCGCACTGAGAGAGAAAAATA	$(TAGA)_9$	10	151–191	0.70	0.81	EU794476
Zefl09	F: GCATACAGAGGATTACAGATG-NED R: AGAACAGAGGCAGAAGATACT	$(TAGA)_8$	17	146–212	0.94	0.90	EU794477
Zefl10	F: TTTACCGACTGACTGTATGCT-FAM R: AGGAAAGAGACTGAGACATCTG	$(GTCA)_8$	9	198–234	0.67	0.68	EU794478
Zefl11	F: CGAACACACAATGCTCACTAA-PET R: AAACAAACCAATCACCAGTTG	$(GTCA)_9$	6	150–198	0.15¶	0.77	EU794479
Zefl12	F: GCACCGCTCAAGTGTGTT-VIC R: AGGCAAAATGGCAGCATA	$(GTCA)_{10}$	4	271–285	0.28	0.29	EU794480
Zefl13	F: GAGGGCTGAAATGAACAT-FAM R: ATCTGGCTGACTGTCTCTG	$(GTCA)_9$	8	237–355	0.66§	0.81	EU794481
Zefl14	F: TTAGGAACAGAGGAGTATTGAG-NED R: GGCTTTACCCTTCAGATAAG	$(GTCA)_{10}$	29	169–289	0.85	0.92	EU794482
Zefl15	F: TGGAACGGAAAACATTACC-FAM R: GGGTGAGAATCTGTGGTGT	$(TACA)_8$	12	308–354	0.90	0.84	EU794483
Zefl16	F: AAGATGCCATAGCGTTCAC-FAM R: GCAAAAACAAACCACAACC	$(TACA)_7$	8	132–178	0.54¶	0.84	EU794484
Zefl17	F: TGCTTGTATGGGTTCTGCAC-VIC R: CTGGCTCGCAGTTATTCTCC	$(TACA)_7$	15	276–348	0.82	0.82	EU794485
Zefl18	F: AGAAGCAAGCAGAGAAGGA-PET R: ACATCGCAGACAGTGAGTG	$(TACA)_{10}$	7	230–279	0.38	0.76	EU794486

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Table 1 *Continued*

Locus	Primer sequence (5′–3′) and fluorescent dye	Repeat motif	k*	Allele size range (bp)	H_O†	H_E‡	GenBank Accession no.
Zefl19	F: CAGCATGCAGGAATACACAG-PET R: CATTGCCAGTATAAATGAGCATC	$(CT)_{19}$	14	238–278	0.9	0.866	EU794487
Zefl20	F: CTTTCACAGGAGGTGACAGTGG-NED R: TGTTCGGCTGCACTCTGC	$(CT)_{23}$	7	146–165	0.66§	0.724	EU794488
Zefl21	F: GGTGAGATCGGGACTTTGC-FAM R: CAGTTGTGGATGCAGTGAGC	$(CA)_{14}$	8	203–231	0.82	0.858	EU794489
Zefl22	F: AGCTTCAGGCCTGTGTGG-PET R: CAAACCTCCAAACCATTTGC	$(GGAT)_{11}$	19	182–250	0.916	0.92	EU794490
Zefl23	F: GTGGGCTACTGAAGTGTTGC-NED R: GCTCTTCTCCAAATCCAAGG	$(CTT)_8$	6	172–184	0.656	0.68	EU794491

*Number of alleles; †observed heterozygosity; ‡expected heterozygosity; §denotes significant departure from Hardy–Weinberg equilibrium at 0.05 level; ¶denotes significant departure from Hardy–Weinberg equilibrium after Bonferroni correction.

and expected heterozygosities ranged from 0.15 to 0.94 (mean = 0.70) and 0.29 to 0.93 (mean = 0.81), respectively. Eight loci, Zefl03-Zefl06, Zefl11, Zefl13, Zefl16 and Zefl20 were found to have significant departure from Hardy–Weinberg equilibrium (*P* values: 0.0001, 0.0001, 0.0002, 0.0027, 0.0001, 0.0034, 0.0000, 0.0227, respectively), which were likely due to null alleles as indicated by Micro-Checker (van Oosterhout *et al*. 2004). There was no evidence for genotypic disequilibrium among any pairs of loci. These polymorphic loci will be fully utilized to determine both direct and indirect estimates of larval connectivity and self-recruitment for yellow tang.

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