

## PERMANENT GENETIC RESOURCES

# Development of DNA microsatellite markers in the multiband butterflyfish (*Chaetodon multicinctus*)

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## Abstract

Twelve polymorphic microsatellite loci were developed in the multiband (pebbled) butterflyfish *Chaetodon multicinctus*. The loci were scored in 45 individuals from Hawaii. There were five to 21 alleles per locus with observed heterozygosity ranging from 0.419 to 0.883. Four of the primer sets also reliably amplified polymorphic loci in *Chaetodon quadrimaculatus*. We expect these markers to be useful for studies of genetic population structure and kinship, for example to determine whether new recruits settling onto reefs are related.

*Keywords:* butterflyfish, *Chaetodon multicinctus*, *Chaetodon quadrimaculatus*, coral reef fishes, kinship, Microsatellite

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Many butterflyfishes (family Chaetodontidae) form monogamous pairs that defend permanent feeding territories which can persist for several years (Reese 1975; Tricas 1989). All species are broadcast spawners and produce larvae that develop for several weeks in the open ocean before recruitment back to coral reef as juveniles. The site-attached behaviour and pair spawning of these fish make them an excellent group to test for the genetic relatedness among individuals on local reefs. One important question is whether cohorts remain together throughout their larval life and then recruit with siblings to the reef habitat. Polymorphic microsatellite loci could be very useful for testing genetic relationships among butterflyfish recently recruited to the reef.

Reproductively mature adult specimens were collected by divers with pole spear from a shallow coral reef area (approximately 1 km<sup>2</sup>) on the west shore of Oahu, Hawaii (see Tricas & Hiramoto 1989 for location), placed individually in plastic bags, stored on ice and transported back to the laboratory. We collected 45 fin clips from adult *Chaetodon multicinctus* and three specimens of *Chaetodon quadrimaculatus*. Fin clip tissue was stored in ethanol. We prepared an unenriched microsatellite library following the approach of Heist & Gold (2000). Briefly, we isolated genomic DNA from a single *C. multicinctus* fin clip using a QIAGEN

DNeasy spin column kit following the manufacturer's protocols. DNA was digested with *Mbo*I and resolved on a 1.4% TAE agarose gel. Fragments spanning approximately 300–800 base pairs were excised from the gel and concentrated using the QIAGEN QIAquick gel extraction kit. Fragments were ligated into a pUC-18 vector that was digested with *Bam*HI and dephosphorylated using T4 DNA ligase. Ligation mixtures were used to transform DH5 $\alpha$ -competent cells which were plated on LB-AMP plates coated with X-gal for blue–white selection. White colonies were transferred to 96-well plates, spotted onto nylon (Hybond) membranes, and probed with radiolabelled (<sup>32</sup>P) dinucleotide motifs (GT)<sub>10</sub> and (GA)<sub>10</sub>. Of 2699 clones, 106 (3.9%) were positive. We sequenced 89 clones using M13 primers on an ABI 377 automated sequencer/genotyper and used MACVECTOR software package (Oxford Molecular) to design primers for 23 loci that had sufficient flanking region sequence and a minimum of 10 uninterrupted dinucleotide repeats.

Microsatellite polymerase chain reactions contained approximately 1–10 ng genomic DNA, 0.1 U *Taq* DNA polymerase, 0.5  $\mu$ M each primer, 200  $\mu$ M each dNTP, 2 mM MgCl<sub>2</sub>, and 1 $\times$  *Taq* buffer (50 mM KCl, 10 mM Tris, 0.1% Triton X-100, pH 9.0). One primer was radiolabelled with  $\gamma$ <sup>32</sup>P using T4 polynucleotide kinase prior to amplification. Amplification consisted of a 2-min denaturing step at 94 °C, 25 cycles of 94 °C for 30 s, 56–60 °C for 30 s, and 72 °C for 30 s, followed by a single 5-min extension step at 72 °C.

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**Table 1** Details of 12 microsatellite loci developed in *Chaetodon multicinctus* including primer sequences, repeat motif, size range of alleles (bp), number of individuals scored ( $N$ ), number of alleles observed ( $N_A$ ), observed/expected heterozygosities ( $H_O/H_E$ ), and  $P$  values for tests of Hardy–Weinberg equilibrium. GenBank Accession numbers for the cloned fragments are EU188756–EU188767

Locus	Primer sequence (5'–3')	Repeat motif	°C	Range	$N$	$N_A$	$H_O/H_E$	$P$
Cm-01	CAAAGGGTTGTTTGGAGGGCTG GCCAGTGTATCAGCTTTAATTGG	(GT) <sub>17</sub>	60	130–146	43	6	0.419/0.518	0.219
Cm-08	TCCAGACCACAACACACACCATC TTTCCTCGTGACACAACG	(GT) <sub>14</sub>	60	120–134	43	5	0.419/0.602	0.009
Cm-09	ATCTGATTGGTTGTGGATGGG GGTGTGTAACGAAAACGGGACG	(CA) <sub>11</sub> TG(CA) <sub>17</sub>	60	187–215	43	9	0.767/0.703	0.058
Cm-12	AATGGGACAATCGCAGAACAAG TGGAGGAATGCATTCATATCTGG	(GT) <sub>14</sub>	60	165–207	43	15	0.605/0.645	0.037
Cm-23	TCGATTTCTTATCAGAGCTGAGTCC CCTCTTCCATTCTTCACATAATCCC	(GT) <sub>27</sub>	60	158–216	43	20	0.744/0.754	0.130
Cm-40	AAGACACACTCTGGGTGGCTG ATCATCTCTTCCAATGCTCGTC	(GT) <sub>34</sub>	60	149–195	37	21	0.757/0.868	0.086
Cm-50	TGAGCTGTGAGTGGAAACCAAG AAATGCTTCATCCCTCTTCC	(GT) <sub>19</sub>	60	158–186	43	8	0.744/0.766	0.717
Cm-54	GATTGAGAAAGGGACCAAGTGG CAACTTGAGAGATGAGTGTGCGTG	(GT) <sub>35</sub>	60	150–200	43	13	0.883/0.867	0.160
Cm-70	TGGGACACTGTGGAGGTATCAAC GCAGAAACGAGGAAGAAGGAATG	(GT) <sub>20</sub>	60	113–153	42	11	0.643/0.771	0.057
CM-74	ACCAGATGGGAGAAGAGATGGTC ACACAGTCAACATCATCACGTCC	(GT) <sub>19</sub>	64	192–240	43	8	0.488/0.497	0.218
CM-82	CAATCACTGTCCGAGACTATGAAGG TGTGTGGTTTTGCTGGAGGC	(GT) <sub>24</sub>	60	105–227	43	14	0.837/0.846	0.958
CM-86	CATGCCCTTGGAAAGTTGTATCTGC ATGCCCTGACTATTTGAGACCCATC	(CA) <sub>24</sub>	60	180–228	43	12	0.674/0.772	0.058

Alleles at individual loci were separated on denaturing polyacrylamide gels and visualized via autoradiography using the cloned fragment at each locus as a size standard. Each locus was initially amplified in 12 specimens of *C. multicinctus*. We also tried to amplify all polymorphic microsatellite loci in three specimens of *C. quadrimaculatus*. We used the MICROSAATELLITE TOOLS add-in for Microsoft Excel (Park 2001) to tabulate data (e.g. allele frequencies) and format data for GENEPOP version 3.2 (Raymond & Rousset 1995), which we used to test for deviations from Hardy–Weinberg and linkage equilibrium data from only adult *C. multicinctus*.

Twelve of 23 primer sets (Table 1) produced loci that were polymorphic and amplified reliably. The number of alleles ranged from five to 21. Two of 12 loci exhibited nominally significant deviations from Hardy–Weinberg equilibrium (Table 1), neither of which remained significant following Bonferroni correction for multiple comparisons (Rice 1989). One pair of loci, Cm-12 and Cm-70, was significantly out of linkage equilibrium even following correction for multiple tests. Four loci (Cm-08, 09, 12, and 23) reliably amplified polymorphic loci in *C. quadrimaculatus*. Another locus (Cm-70) was monomorphic and the rest either failed to amplify or produced results that we could not interpret. We expect that these loci will prove useful for future studies of butterflyfish reproductive ecology, for example investigations of relatedness among pelagic zygotes, larvae

or recruits, and determining the reproductive fitness of encroachers.

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