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PRIMER NOTE

Polymorphic microsatellite loci in the black-and-gold chromis, *Neoglyphidodon nigroris* (Teleostei: Pomacentridae)

PHILLIP C. WATTS, PARIS VELTSOS, BENJAMIN J. SOFFA, ANDREW B. GILL* and STEPHEN J. KEMP

Animal Genomics Laboratory, School of Biological Sciences, The Biosciences Building, Crown Street, University of Liverpool, Liverpool L69 7ZB, UK

Abstract

We describe 15 polymorphic dinucleotide microsatellite loci in the black-and-gold chromis, *Neoglyphidodon nigroris* (Cuvier: Teleostei: Pomacentridae). Microsatellites were isolated from a partial genomic library that was enriched for CA repeat motifs. The 15 loci yielded between two and 23 alleles per locus in a sample of 16 fish from two forereef sites off the island of Hoga, Wakatobi National Park, Indonesia. Observed and expected heterozygosities varied from 0.13 to 1.00 and 0.24 to 0.98, respectively. These markers should allow us to discriminate between closely related individuals and also to assess the connectedness of populations of *N. nigroris* that inhabit different reefs.

Keywords: black-and-gold chromis, damselfish, microsatellite, *Neoglyphidodon nigroris*

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The black-and-gold chromis, *Neoglyphidodon nigroris*, is a highly territorial fish which is common in coral-rich lagoons and forereef habitats throughout the Indo-West Pacific (Froese & Pauly 2003). *Neoglyphidodon nigroris* deposits benthic eggs in its territory but it is not known whether the juveniles are philopatric or widely dispersed (e.g. to reduce competition between kin). Data on dispersal extent and how adjacent reefs are linked would significantly aid in understanding how management efforts are protecting the genetic integrity of these increasingly exploited ecosystems. As part of an effort to better understand the genetic relationships between *N. nigroris* individuals on reefs and also to gain an insight into the dispersal of this species between reefs we developed a panel of microsatellite loci using an enrichment procedure based on the protocol of Gardner *et al*. (1999).

We constructed a partial genomic library that was enriched for CA motifs using genomic DNA of a single adult damselfish. DNA was extracted from muscle tissue using a high-salt protocol (Aljanabi & Martinez 1997). *Sau*3AI (40 U; Boehringer-Mannheim) was used to digest approximately 10 μg of total genomic DNA which was ligated to 50 pmol of phosphorylated linkers (S61 5′-GGCCAGAGACCCCAAGCTTCG-3′ annealed to S62 5′-PO_4-GATCCGAAGCTTGGGGTCTCTGGCC-3′; Refseth *et al*. 1997). DNA fragments between 500 and 1000 bp were excised from a 2% NuSieve GTG agarose TAE gel (FMC Bioproducts) and purified using a QIAquick gel extraction kit (Qiagen). For enrichment we used 1 mg of M2-80 streptavadin-coated magnetic beads (Dynal) incubated with 200 pmol of 3′-biotin-labelled $(CA)_{12}$ oligonucleotide (MWG Biotech). After a series of differential stringency washes in 2× SSC and 1× SSC, the enriched DNA was recovered, made double stranded and amplified in a 25-μL polymerase chain reaction (PCR) [75 mM Tris-HCl, 20 mM $(NH)_4SO_4$, 0.01% (v/v) Tween 20, 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 250 pmol primer S61 and 1.25 U of *Taq* polymerase (ABgene)]. The thermal profile of the PCR was 5 min at 95 °C, 25–30 cycles of 50 s at 95 °C, 1 min at 56 °C and 2 min at 72 °C followed by 72 °C for 10 min. The DNA was purified using a QIAquick PCR purification kit (Qiagen), ligated into pGEM®-T vector (Promega) and then transformed into JM109 *Escherichia coli* competent cells (Promega). Recombinant clones were identified using black/white screening on S-gal (Sigma) agar/ampicillin plates. Plasmids containing a microsatellite insert were

Correspondence: Phill Watts. Fax: 44 (0) 151 795 4512; E-mail: p.c.watts@liv.ac.uk

*Present address: Institute of Water and Environment, Cranfield University, Silsoe, Bedford MK45 4DT, UK.

Table 1 Characterization of 15 polymorphic microsatellite loci in 16 damselfish, *Neoglyphidodon nigroris*, from off Hoga island, Indonesia

Locus	Dye	Primer sequence (5′–3′)	Repeat array	T_a	Mg	Clone size (bp) (size range)	N_a	H_O	H_E	Accession no.
LIST12-002b	NED	F: AACTTTGTTCTCTGAGCC R: TTGTGAGTTTGGCTTAGG	$(AT)_5(AC)_{29}$	55	1.5	205 (187–251)	14	0.40	0.95	BV079671
LIST12-004	NED	F: CAGTATTAGGCAGGAGGTGG R: CTGACACACAGAGAGACAGC	$(TG)_9(n)_{14}(TG)_7$ $(n)_{14}(TG)_6$	53	1.5	285 (255–307)	16	0.75	0.96	BV079672
LIST12-005	VIC	F: TCTTTGTGTCATTGGCTCCC R: TCAGTGAGGTGATAGGTTCG	$(AC)_{14}$	55	3.5	335 (327–393)	21	0.94	0.97	BV079673
LIST12-006	PET	F: CTCTACAGGTTTACTGAAGC R: GTTCTGGTTCTACAGGACG	$(AC)_{15}$	53	3.5	227 (221–241)	8	0.75	0.67	BV079674
LIST12-007	PET	F: TGGTGTTGTTGTTCTGATGG R: CTTTAGGGTTTCTTGGTAGG	$(GT)_7(n)_2(GT)_5$	53	3.5	282 (276–284)	5	0.75	0.77	BV079675
LIST12-009	VIC	F: AAAAAGGCACAAAGTTGCCC R: ATCACACAGGGGATGGTAGC	$(TG)_{26}(n)_{17}(CA)_7$	55	3.5	188 (166–220)	18	0.69	0.96	BV079676
LIST12-011	6-FAM	F: ATGTGTCACTGTAAACGCC R: TCGTATCTTCTGTGTTGCC	$(CA)_{11}(n)_2(CA)_4$	53	1.5	222 (218–270)	11	0.31	0.81	BV079677
LIST12-012	PET	F: GATTGATACATACAGCACCGC R: AAGTCTGACACTACCGCAGG	$(TG)_{34}$	57	1.5	165 (119–191)	23	0.94	0.98	BV079678
LIST12-013	NED	F: GAGCCTCTATGTACAGGAGC R: TCCTCAGACACAAAACTGAGC	$(TG)_{13}$	57	1.5	122 (110–148)	12	0.81	0.91	BV079679
LIST12-015	VIC	F: CCCATTATGTTGACAGAAGC R: TTTGGCTTCCATCCAGAGC	$(TG)_{18}$	53	1.5	99 (83–125)	13	0.75	0.91	BV079680
LIST12-016	6-FAM	F: AGACTCAAGGATTCCCAGC R: AGAAACAGCGACAGATGGC	$(AC)_{20}$	53	1.5	137 (127–201)	22	1.00	0.97	BV079681
LIST12-023	NED	F: ATATCACTGCACAGACAGCGG R: CAAAATGCTCCAACAGTTCAGG	$(AC)_{17}$	57	3.5	302 (282–330)	14	0.47	0.97	BV079683
LIST12-025	VIC	F: ATCAGAGCTCATCTGTATACGC R: ATCTCCACACCCTCACATTGC	$(CA)_{19}(n)_4(CA)_4$	57	3.5	282 (250–282)	9	0.81	0.82	BV079685
LIST12-028	6-FAM	F: GGGCTGTTTATGTCAGTTTAGC R: CTAATGCGGTGTTTCCATCCC	$(AC)_3(n)_2(AC)_2$ $(n)_4(AC)_6$	57	3.5	285 (283–285)	2	0.13	0.24	BV079687
LIST12-029	VIC	F: ATGCCGTAGCCAAAGAGAAGG R: TAAATGGGACCCTTGTCAGAGC	$(CA)_{20}(GA)_8$	57	3.5	267 (249–301)	15	0.67	0.95	BV079688

T_a, annealing temperature (°C); Mg, magnesium chloride concentration (mM); N_a, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

identified by two or more amplified products after PCR primed with 50 pmol SP1 and 25 pmol $(GT)_{12}$ oligonucleotide (see Gardner *et al*. 1999). Positive clones were cycle sequenced using Big Dye™ chemistry (PE Applied Biosystems) and electrophoresis on an ABI377. Full details of the enrichment protocol are provided by Bloor *et al*. (2001). Primers flanking the repeat regions were designed using PRIMER2 (S.J. Kemp, unpublished). We identified 85 putative positive clones from 288 colonies. Thirty-two positive clones were sequenced, yielding 28 separate microsatellites with suitable flanking sequence for primer design (three pairs of sequences were identical and primers could not be designed at one locus). Eleven of the remaining primer sets showed either inconsistent PCR amplification or multiple bands.

The remaining loci were tested for polymorphisms using DNA extracted from pelvic fin clips of 16 *N. nigroris* from the two forereef sites off Hoga island, southeast Sulawesi, Indonesia. Microsatellite alleles were amplified by PCR in a 10-μL reaction volume on a Dyad DNA engine (MJ Research Inc.). The PCR conditions were 1 min at 95 °C, six cycles of 30 s at 95 °C, 30 s at T_a and 45 s at 72 °C, 26 cycles of 30 s at 92 °C, 30 s at T_a and 55 s at 72 °C followed by 72 °C for 30 min (T_a is the annealing temperature at each locus; Table 1). Each reaction contained 75 mM Tris-HCl, 20 mM $(NH)_4SO_4$, 0.01% (v/v) Tween 20, 0.2 mM each dNTP, 1.5–3.5 mM $MgCl_2$ (Table 1), 10–50 ng template DNA, 10 pmol each primer and 0.25 U of *Taq* polymerase (ABgene). The forward primers were 5′ labelled with either 6-FAM, NED, PET or VIC fluorescent dyes (Applied Biosystems) for detection (Table 1). The PCR products were pooled with a GENESCAN 500 bp (LIZ) size standard (Applied Biosystems) and separated by capillary electrophoresis through a denaturing acrylamide gel on an ABI3100 automated sequencer (Applied Biosystems). We used ARLEQUIN version 2.001 (Schneider *et al*. 2000) to calculate observed (H_O) and expected (H_E) heterozygosities and linkage disequilibrium between all pairs of loci.

Two loci were monomorphic and 15 loci were variable and resolved distinct alleles within the expected size range. The observed number of alleles varied from two up to 23, H_O varied between 0.13 and 1.00 whilst H_E ranged between 0.24 and 0.98 (Table 1). Fourteen of the 105 possible pairwise

Table 2 Above diagonal, exact probability of linkage disequilibrium between 15 pairs of microsatellite loci isolated from the black-and-gold chromis, *Neoglyphidodon nigroris*; below diagonal, *indicates significant departure from linkage equilibrium after a sequential Bonferroni correction for multiple testing ($\alpha = 0.05$, $k = 14$)

	Microsatellite locus														
	12-002b	12-004	12-005	12-006	12-007	12-009	12-011	12-012	12-013	12-015	12-016	12-023	12-025	12-028	12-029
12-002b	—	0.000	0.003	0.936	0.375	0.000	0.001	0.018	0.003	0.433	1.000	0.000	0.317	0.291	0.001
12-004	*	—	0.488	0.691	0.124	0.006	0.061	0.153	0.470	0.083	0.584	0.001	0.996	0.032	0.019
12-005	*		—	0.795	0.250	0.107	0.031	0.523	0.466	0.150	0.896	0.019	0.295	0.045	0.245
12-006				—	0.207	0.734	0.927	0.150	0.483	0.129	0.518	0.630	0.112	0.230	0.013
12-007					—	0.580	0.523	0.592	0.027	0.396	0.565	0.058	0.228	0.687	0.918
12-009	*					—	0.006	0.818	0.139	0.543	0.835	0.001	0.753	0.001	0.002
12-011	*						—	0.822	0.078	0.097	0.898	0.002	0.745	0.011	0.256
12-012								—	0.163	0.150	1.000	0.001	0.192	0.088	0.812
12-013	*								—	0.148	0.425	0.033	0.245	0.144	0.315
12-015										—	0.008	0.060	0.642	0.001	0.516
12-016											—	1.000	0.901	0.201	0.842
12-023	*	*				*	*	*				—	0.298	0.016	0.006
12-025													—	0.697	0.138
12-028						*				*				—	0.486
12-029	*					*									—

comparisons between polymorphic loci showed significant linkage disequilibrium [after a sequential Bonferroni correction (Rice 1989) to maintain a type I error rate of $\alpha = 0.05$ for each locus]. Four of the loci (LIST12-006, LIST12-007, LIST12-016 and LIST12-025) did not demonstrate significant linkage disequilibrium with any other locus, whilst LIST12-002b and LIST12-023 were responsible for the majority of the significant departures from linkage equilibrium (see Table 2). These markers will allow us to discriminate between closely related individuals and also to assess the connectedness of different populations of the black-and-gold chromis.

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