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TECHNICAL NOTE

Isolation and characterization of eight polymorphic microsatellite markers from the orange-fin anemonefish, *Amphiprion chrysopterus*

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Abstract Eight polymorphic microsatellite loci were isolated and characterized from the orange-fin anemonefish (*Amphiprion chrysopterus*). These loci provided markers with polymorphisms of 4–14 alleles per locus within 151 individuals from Moorea, French Polynesia. The expected heterozygosities ranged from 0.53 to 0.88. High variability suggests that these markers should be useful to study paternity, population structure, and connectivity in this species.

Keywords *Amphiprion chrysopterus* · Orange-fin anemonefish · Microsatellite

A common strategy to promote sustainability and conserve biodiversity on marine reefs involves the design and creation of spatially defined protected areas (Cowen et al. 2007). Crucial for the implementation of this and other management actions is the accurate knowledge of dispersal, self-recruitment, and connectivity among populations (Hauser and Carvalho 2008). Anemonefishes in the genus *Amphiprion* have been used as model species for such studies.

The largest species of *Amphiprion*, the orange-fin anemonefish *A. chrysopterus*, occurs in the central and western Pacific from French Polynesia to Micronesia, Papua New Guinea and the Philippines (Fautin and Allen 1997). As with most other anemonefish, reef-associated life stages of *A. chrysopterus* live on anemones from the moment larvae settle from the plankton (Fautin and Allen 1997). The ease with which various developmental stages can be sampled has enabled the use of anemonefishes in studies of dispersal and self-recruitment (e.g. Jones et al. 2005). Orange-fin anemonefish have been used in explorations of population and community dynamics (e.g., Schmitt and Holbrook 2003; Holbrook and Schmitt 2004), although issues related to connectivity and self-recruitment in this otherwise well studied species have not been resolved. The main goal of this study was to develop and characterize microsatellite markers from *Amphiprion chrysopterus*, to enable future behavioral and population genetic studies.

Total DNA was extracted using a DNeasy blood and tissue kit (QIAGEN) from approximately 20 mg of caudal fin. Genomic DNA (100 μg) was used by GIS (Genetic Identification Services, Chatsworth, California) to identify microsatellite loci. Genomic libraries were enriched for CA, CATC, TACA, TAGA repeat motifs. We tested 18 microsatellites for polymorphism. Amplification reactions were carried out using a fluorescently blue labeled forward primer and an unlabeled reverse primer in an Applied Biosystems GeneAmp PCR 9700 system in a total volume of 13 μl containing 11 μl of 1.1 PCR Mastermix (25 mM TAPS pH 9.5, 50 mM KCl, 2 mM $MgCl_2$, 200 μM each

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Table 1 Characterization of eight polymorphic microsatellite loci for the orange-fin anemonefish, *Amphiprion chrysopterus* for 151 specimens

Locus	Primer sequence (5′ to 3′)	Repeat	Ta	Na	As	RR (bp)	Ho (He)	GenBank
ACRY_A115	F: GACTCGTGTTCGGAGGAC	$(CA)_{27}$	57	12	228	212–242	0.69 (0.77)	GQ454916
	R: CGGGATAATAACGGAGAGC							
ACRY_A130	F: GCACTCAACACAAAGACCTTA	$(CA)_{24}$	57	12	167	160–194	0.79 (0.82)	GQ454917
	R: ACCCAAACAACATCCAGTC							
ACRY_A131	F: CCTCAGCAGTGTGAAATGA	$(CA)_{39}$	57	13	226	200–250	0.74 (0.81)	GQ454918
	R: CTCCACCTCTCTCTTCTTGAC							
ACRY_CF11	F: GCTGGTTACAACACCTTG	$(CT)_{15}(CA)_{16}$	60	8	198	180–206	0.71 (0.69)	GQ454919
	R: GTAATTGCTGCAAGACAG							
ACRY_D1	F: CCAAAAGTTTAGGAAGCTACC	$(GATA)_{25}$	57	13	325	273–341	0.78 (0.78)	GQ454920
	R: AACCAGACTGCCCTGATAC							
ACRY_D103	F: GTTGGCTAATGGTGCTGTG	$(GATA)_{13}$	57	4	247	239–251	0.84 (0.88)	GQ454921
	R: GATTCTGTGGTGGCATCAG							
ACRY_D108	F: GAAGGATGTGCTTTGTTGTTC	$(GATA)_{30}$	57	14	296	286–338	0.53 (0.53)	GQ454922
	R: GCTTTACGATTTTACAATGCAC							
ACRY_D114	F: TGTTCCAGCTCTGATATTTGAC	$(GATA)_{19}$	57	11	245	205–285	0.72 (0.76)	GQ454923
	R: TTGGCAGTGTTTTATACCTGTC							

Columns correspond to: microsatellite name (locus), primer sequence, (F) forward and (R) reverse primers, repeat motif, annealing temperature in degrees centigrade (Ta), number of alleles per locus (Na), amplification size of original clone (As), repeat size range in bp (RR), (Ho) observed and (He) expected heterozygosities and GenBank accession numbers. All microsatellites were tagged with blue fluorescent dye (from Sigma-Aldrich). All individuals were genotyped successfully except for D1 (two samples did not amplify)

dNTP, Taq 5 u/μl, Thermo Scientific), 0.2 μM primers and approximately 2 ng of DNA template. The following temperature profile was used: 3 min at 94°C, followed by 35 cycles of 30 s at 94°C for 40 s, 55–58°C for 40 s, 72°C for 40 s and a final extension of 72°C for 4 min. Each microsatellite amplification was diluted with $n H_2O$ (1:20), mixed with Applied Biosystems GeneScan 500 ROX and size standard, run on an ABI 3100 automated sequencer, and scored using the software GENEMAPPER version 3.7 (Applied Biosystems). We scanned our data for null alleles using MICROCHECKER (van Oosterhout et al. 2006), for each locus, calculated expected and observed heterozygosities, deviations from Hardy–Weinberg (HW) equilibrium and presence or absence of linkage disequilibrium using ARLEQUIN version 3.11 (Excoffier et al. 2005).

Four out of 15 microsatellites studied were found to be monomorphic and three others gave low amplification success. Table 1 summarizes the characteristics of the eight primer pairs of polymorphic loci developed from the orange-fin anemonefish. From a sample of 151 individuals collected in Moorea, all loci gave 100% successful amplifications except for locus D1 for which two samples were not amplified successfully. The number of alleles ranged from 4 to 17 per locus. No significant linkage disequilibrium was observed for any pair of loci ($P > 0.05$ for all comparisons). There was no evidence of null alleles for any of the markers. The large majority conformed to HW equilibrium except for A115. This result may be due to selection, reduction of gene flow, or other sources of pressure such as extensive population fluctuations.

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