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PRIMER NOTE

Characterization of tetranucleotide microsatellite markers in guppy (*Poecilia reticulata*)

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Abstract

Ten tetranucleotide microsatellite loci are characterized for guppy, *Poecilia reticulata*, an important model species in the study of adaptation and mating systems. Loci were isolated following a microsatellite enrichment procedure using probe-labelled magnetic beads. These microsatellites were designed for use in examining gene flow, reproductive isolation, and parentage within natural guppy populations.

Keywords: guppy, microsatellite, *Poecilia reticulata*, Tetranucleotide

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Trinidadian guppies (*Poecilia reticulata*) have become a model system in the study of evolutionary ecology and of mating systems (reviews: Endler 1995; Houde 1997). The principal attraction of this system is the remarkable variation within and among populations in colour, life history, morphology, and behaviour (Haskins & Haskins 1951; Reznick & Endler 1982; Endler & Houde 1995; Reznick *et al*. 1997). Much of the variation among populations reflects sexual selection and adaptation to alternative selective environments, particularly the contrast between 'high predation' and 'low predation'. The 10 tetranucleotide microsatellite loci described here were developed as part of an investigation into mating systems, local adaptation, and population structure of wild guppies. They should also prove useful for determining parentage in natural populations, thereby allowing detailed studies of natural and sexual selection in the wild. While there are a number of dinucleotide microsatellites designed for *P. reticulata* (e.g. Becher *et al*. 2002; Watanabe *et al*. 2003) and cross-species primers reported to work in *P. reticulata* (e.g. Parker *et al*. 1998; Yue & Orban 2004), we prefer tetranucleotide microsatellites because of their reliability of scoring and usefulness for population genetic analyses.

Genomic DNA was purified from muscle tissue of wild guppies using DNeasy Kit (QIAGEN). DNA from one individual was used to create microsatellite-enriched libraries for CATC, AAAG and GACA repeats, following previously published protocols (Hamilton *et al*. 1999; Diniz *et al*. 2004). The microsatellite libraries were cloned using pDrive cloning vector (QIAGEN), transformed into MAX Efficiency DH5 alpha Competent Cells (Invitrogen), and plated on imMedia Amp Blue agar (Invitrogen). Positive colonies were screened for suitably sized inserts (400–1000 bp) by direct polymerase chain reaction (PCR) amplification of colony picks using M13 primers under standard PCR conditions, and imaged with agarose electrophoresis. Plasmid DNAs were isolated using QIAprep Miniprep Kit (QIAGEN) and sequenced on CEQ 8000 (Beckman Coulter). PRIMER3 software (Rozen & Skaletsky 2000) was used for primer design.

We tested the 10 loci on guppies collected at upstream sites in the Marianne River, on the north slope of the Northern Range of Trinidad and Tobago. Guppies from the collection sites experience low-predation conditions, and are expected to show low variability compared with downstream, high-predation populations. This expectation was based on likely founder effects associated with rare colonization events above waterfalls. Individual *P. reticulata* were nondestructively sampled in the field by collecting a portion of the caudal fin in 90% alcohol. Individuals were allowed to regenerate caudal fins for a week before they were returned to the wild. In the lab, fin clips were removed from ethanol, dried on paper towels (tissue dry weight 0.2–1.4 mg) and DNA was isolated following Elphinstone *et al*. (2003), modified to work with a 96-well filter plate. Individuals

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Table 1 Characteristics of nine microsatellite loci in *Poecilia reticulata*. T_a, annealing temperature; n, number of individuals; H_O, observed heterozygosity, H_E, expected heterozygosity

Locus GenBank no.	Repeat motif	Primer sequence (5′–3′)	T_a (°C)*	No. alleles	Size range (bp)	H_O	H_E
Pre1	$(CATC)_{32}$	TGCTTAATTGCCATTGAAAAC	60–65TD	13	197–249	0.7941	0.8964
AY830939		CAGAACGTTAGCTGGTGATGG		$n = 34$			
Pre7**	$(CCTT)_4CCTA(CCTT)_7$	AAGACGGTTCACGAGGGTTG	60–55TD	4	138–154	0.2508	0.2391
AY 830940		GACAGGGCAGAGGTGACAAG		$n = 46$			
Pre8**	$(CTGT)_9$	CTTGTCACCTCTGCCCTGTC	65–60TD	14	177–289	0.8925	0.8750
AY830940		AGCAACCGAGGCAGAAAG		$n = 48$			
Pre9	$(CAGA)_{13}$	TTGCAAGTCAGTTGATGGTTG	65–60TD	7	176–200	0.7701	0.7660
AY830941		TGCCCTAGGGATGAGAAAAG		$n = 47$			
Pre13	$(CTGT)_{18}$	ACAGTACTGTCTGTCTGTCT	62	17	107–191	0.9200	0.9048
AY830942		TGTTTGAGACACTCATGGTGAAG		$n = 42$			
Pre15	$(GATG)_{16}$	CTGAGGGACCAGGATGTTAAG	60–55TD	12	227–275	0.8235	0.9008
AY830943		CCATAAACACGCAAACCAAC		$n = 34$			
Pre17	$(CATC)_8$	TGAAAGGATAGTCCAGATTTTTAAGAG	55–50TD	7	160–188	0.7222	0.7496
AY830944		CGACCCCACTAAGGGACAAG		$n = 36$			
Pre18	$(CAAA)_6$	CTGTCACGCACTACAACCAAG	65–60TD	3	111–133	0.0414	0.0208
AY830945		TTTCAAACACGTCCCTGATG		$n = 48$			
Pre26	$(GATG)_{19}$	GCTGACCCCAGAAAAGTGG	65–60TD	7	129–209	0.6852	0.7833
AY830946		TGGGACTTTCATGAGACTTGG		$n = 54$			
Pre29	$(GTAG)_7$	ACCCGGAACATTAGCTGGAG	60–55TD	4	198–210	0.4415	0.4688
AY830947		GCAGAAAGAAAACCTGCAAAC		$n = 32$			

*TD indicates a touchdown PCR, **locus Pre7 and Pre8 may be linked, see text.

were genotyped using PCR amplifications of 5- or 10-μL volumes containing 20–100 ng DNA, 2.0 mM $MgCl_2$, 50 μM each dNTP, 0.5 U *Taq* DNA polymerase (Applied Biosystems), 0.3–0.5 μM each primer (forward primers were 5′ end-labelled with IR700 or IR800 dye), and 1× PCR buffer (10 mm Tris-HCl, pH 8.3; 50 mm KCl). We used touchdown PCR in order to increase the opportunity to multiplex PCRs. Touchdown PCR conditions included: 95 °C for 3min, followed by 5 cycles: 95 °C for 20 s, primer specific T_a (Table 1) minus 1 °C per cycle for 20 s, 72 °C for 20 s, followed by 25 cycles where the T_a was held constant at 5 °C below the starting T_a. Reactions were run in either MJ Research or Eppendorf thermocyclers and imaged on IR2 DNA Analysers (Licor).

Observed and expected heterozygosities, Hardy–Weinberg equilibrium (HWE), and linkage disequilibrium (LD) were calculated using GENEPOP (version 3.4, Raymond & Rousset 1995). No departures from HWE were detected (data not shown). We found some evidence of LD between loci Pre9 and Pre15 (χ^2, d.f. = 2, $P = 0.00045$), although these loci are not in LD in a larger data set of 900 samples (data not shown). Surprisingly, Pre7 and Pre8 were not in LD, even though the two microsatellite arrays are only 105 bases apart on the same clone. The microsatellite loci presented here are being used to examine reproductive isolation among natural guppy populations.

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