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Novel Polymorphic Microsatellites from Guppy *(Poecilia reticulata)* and their Utility in Swordtails (*Xiphophorus helleri*)

[1]Gen Hua Yue and [1,2]Laszlo Orban
[1]Temasek Life Sciences Laboratory, Singapore
[2]Department of Biological Sciences, National University of Singapore, Singapore

Abstract: Ten microsatellites were isolated from a genomic DNA library generated from guppy (*Poecilia reticulata; Poecilidae*) enriched for CA-repeats. All of the 10 microsatellites were polymorphic in guppy with an average allele number of 4.9/locus ranging from 2 to 14. All 10-primer pairs amplified specific products in green swordtail (*Xiphophorus helleri*) and 9 of the 10 microsatellites displayed polymorphism (average allele number: 4.1/locus with a scope between 2 and 8). Size range of alleles at most loci were similar between the two fish species. These microsatellites could be applied to breeding programs performed on these two species and possibly other poecilids and to genetic and ecological studies.

Key words: Cross-amplification, directional mutation, guppy, swordtail

INTRODUCTION

Guppy (*Poecilia reticulata; Poecilidae*) is a freshwater live-bearing species, with a main natural distribution in Guiana, Trinidad Island and Barbados. Selective breeding allowed guppy breeders to generate males exhibiting a wide range of patterns, shapes and colors, making these individuals popular for avid aquarists (Norman, 1982). Because of short generation interval, ease of breeding and establishment populations in a laboratory, as well as the availability of many different strains, the guppy is becoming a model organism for biological studies (Evans *et al.*, 2003; Houde, 1997; Morell, 2002). Genetic diversity and population structure of some domesticated and wild populations were studied by using RAPD (Khoo *et al.*, 2002), however microsatellites showed higher power than RAPD in population genetic studies for several species, including fish (Yue *et al.*, 2004). Some microsatellites have been isolated from the guppy genome earlier (Parker *et al.*, 1998) and used for studies on heterosis (Shikano and Taniguchi, 2002). Cross-species amplification of microsatellites is the simplest way to obtain polymorphic microsatellites (Gonz ez-P ez *et al.*, 2009; Nazareno *et al.*, 2009). In this paper we present 10 novel microsatellites from guppy and demonstrate their utility in green swordtail (*Xiphophorus helleri*), a good model fish species for studying sexual selection (Evans *et al.*, 2003; Hankison and Morris, 2003).

MATERIALS AND METHODS

A partial genomic DNA library enriched for CA-repeats was established in 2003 in Singapore using DNA from a single swordtail individual as described previously (Yue *et al.*, 2000). PCR-products from clones with inserts between 250 and 1000 bp size were cleaned using self-made glass milk, and sequenced using BigDye chemicals using an ABI 377 sequencer (ABI/PE) as described (Yue *et al.*, 2000). Forward and reverse sequences of each clone were aligned using the program Sequencher (Gene Codes). Primers were designed for each microsatellite locus by commercially available software Primer Premier (Primer Biosoft). One primer of each pair was labeled with a fluorescent dye: either 6FAM or Hex at the 5, end. PCR amplification of each locus was performed in a volume of 25 included 0.6 unit DNA polymerase (Finnzymes), 200 nM of each primer, 1 x PCR buffer (Finnzymes) containing 1.5 mM $MgCl_2$ and 50 of each dNTP. The PCR conditions were as follows: a preincubation at 94 ºC for 2 min, one cycle of 94 ºC for 30 sec, annealing temperature plus 2 ºC for 30 sec and 72 ºC for 30 sec, followed by 34 cycles of 94 ºC for 30 sec, annealing temperature (Table 1) for 30 sec and 72 ºC for 30 sec, then a final extension for 5 min. The PCR products were separated on an ABI 377 sequencer as described (Yue *et al.*, 2000). Gels were analyzed by using the software Genscan 3.0 and Genotyer 2.5 (ABI/PE). A total of 24 guppy individuals collected from 3 local fish farms, and 18 swordtail individuals from 2 farms were used to characterize the microsatellites. DNA was isolated from scales or fin clips using a very inexpensive and quick method developed by our lab (Yue *et al.*, 2004).

RESULTS AND DISCUSSION

Twenty-four clones were analyzed and 10 microsatellites were found (Table 1). Seven of the 10

Corresponding Author: Gen Hua Yue, Molecular Population Genetics Group, Temasek Life Sciences Laboratory, Singapore

Table 1: Characterization of 10 microsatellites in guppy (*Poecilia reticulata*) and polymorphism in swordtail (*Xiphophorus helleri*)

Locus GenBank No.	Repeat Motif	Primer (5 3	AT (ºC)	No. ofalleles	Size range(bp)	H_o	H_e
Pre01							
AY265993	$(GT)_4(CT)_2$-	F: CTATGGGGCCAGCGTAGTTTACC	60	3	172-176	0.85	0.56
	$(GT)_4$	R: GTGGCCACAGGGATCCAGTTAC		3	166-170	0.83	0.62
Pre02	$(GATA)_{38}$	F: AGGCAGGAGATAAAAGCAAAGAC	60	5	285-305	0.30	0.63
AY265994		R: GCCTTAGAAATTACTTGGGAAAAT		2	156-160	0.28	0.44
Pre03	$(GT)_{10}$	F: TGCAATGGCAGCAACTGAGA	60	2	157-159	0.41	0.48
AY265995		R: TTTGCCCTGATGTGTCCCATAA		2	157-159	0.50	0.44
Pre05	$(CA)_3ACCG$-/*-	F: AGCTGCTGCTGCCTCTCCAG	60	5	176-190	0.85	0.61
AY265996	$(CA)_5$	R: CCTCCCACGGTCCAAAAACA		4	184-190	0.39	0.56
Pre06	$(TG)_{10}CA$-	F: ACGCGACAGAATGAGACCTAAT	60	7	253-296	0.89	0.71
AY265997	$(TG)_4$	R: CGGCAACGCTGGAGAGTG		4	145-255	0.78	0.70
Pre08	$(GT)_9(GC)_2$-	F: TCTACCAAGGGGGAATTAGTGGAG	50	3	157-161	0.37	0.19
AY265998	$(GT)_6GC(GT)_2$	R: TGCAAACTGTATTGTTCTTTCACG		1	127	0.00	0.00
Pre09	$(GT)_8(G)_6(GT)_5$-	F: TTCCAAAAATGAATGTTTTATCAA	60	3	212-216	0.49	0.65
AY265999	$GC(GT)_2$	R: STAGGCTCATGGGTCATGG		3	182-214	0.33	0.37
Pre16	$(AC)_{24}$	F: GTAGCCCAACCCCAGGTTTT	60	14	253-325	0.63	0.90
AY266000		R: GCAAATATGTTTGGATGTTTGTGA		8	253-325	0.89	0.81
Pre17	$(TGCG)_{14}AGT$-	F: CTCCGTCACCCCGCCTGTTTG	60	4	131-177	0.52	0.44
AY2660013	$GT(TG)_7$	R: ACCCCGGGGAGCCAATTATCACA		5	113-125	0.67	0.77
Pre19	$(CA)_7TG(CA)_2$-	F: AGCAGATGCCAGGACAGTAAAGTG	50	3	265-269	0.48	0.44
AY266002	$(C)_4(CA)_5$	R: TGAGGGGGAAAGAAAGGAGGTAG		2	261-265	0.33	0.29

AT: Annealing temperature; H_O: observed heterozygosity; H_E : expected heterozygosity; F: forward; and R: reverse. For the boxes no. of alleles, size range, H_O and H_E, the information in first row is from guppy, whereas the one in the second row is from swordtail.

microsatellites were imperfect or compound microsatellites. All the microsatellites were polymorphic in guppy. The allele number ranged from 2 to 14 with an average of 4.9/locus. The most polymorphic marker was *Pre16* (14 alleles), whereas *Pre03* was the least polymorphic with 2 alleles. The average expected heterozygosity was 0.51 ranging from 0.19 for *Pre08* to 0.90 for *Pre16*. At several loci, a deficit of heterozygotes was seen, this could be mainly because the fact that the samples came from different farms. All the 10 microsatellites amplified specific products in green swordtail, nine of them showed polymorphism with an average allele number of 4.1/locus ranging from 2 to 8. *Pre16* was the most polymorphic marker among the cross-amplified ones in the green swordtail. The size of cross-amplified alleles at most loci except for *Pre02* were quite similar. These microsatellites could be applied to breeding programs performed on these two species and possibly other poecilids and to genetic, ecological studies and linkage mapping (Tripathi *et al.*, 2009).

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