PRIMER NOTE

Microsatellite DNA markers for the intertidal copepod *Tigriopus californicus*

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Abstract

Eleven microsatellite loci were isolated and characterized for the intertidal copepod *Tigriopus californicus*. Primers reliably amplify alleles in inbred lines derived from two divergent populations previously shown to have genetic distances of 18% for the mitochondrial cytochrome oxidase I gene. The 11 loci provided markers for eight of the species' 12 chromosomes.

Keywords: copepoda, linkage, map, microsatellites, *Tigriopus californicus*

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The intertidal copepod *Tigriopus californicus* is commonly found in supralittoral pools ranging from Alaska to central Baja California. The remarkable genetic differentiation between populations of this species has been well-documented using allozymes as well as nuclear and mitochondrial DNA sequence data (Burton & Lee 1994; Burton 1998; Edmands 1999, 2001). Studies have also shown little to no variation exists within populations using these same genetic markers.

Despite the extensive genetic divergence observed between populations (mitochondrial sequence divergence ranges from 0 to 20%+) interpopulation crosses in the laboratory produce fertile hybrid offspring. F1 offspring from these crosses typically show slight heterosis, while F2 and backcross hybrids show reduced fitness measures in numerous characters relative to midparent values (Burton 1987; Edmands 1999). The genetic structure of natural populations in concert with the ease with which divergent populations can be bred and reared in the lab make *T. californicus* an important organism for investigating the genetic mechanisms involved in the development of post-zygotic reproductive incompatibilities. Herein we describe 11 microsatellite loci developed as part of a study investigating the genetics of hybrid breakdown between divergent populations of *T. californicus*.

Five of the 11 loci reported here (TC1202, TC1203, TC1555, TC1814, and TC56J2 in Table 1) were isolated using radioactively labelled probes [$(GA)_{15}$ $(CA)_{15}$ $(AAG)_{10}$ $(AAC)_{10}$] to screen an unenriched genomic library. Because detection of clones positive for repeats using this method was on the order of 1/1000 we turned to a modified version of the enrichment protocol described by Hamilton *et al*. (1999) to obtain additional microsatellite loci. Copepods were collected from Royal Palms, Palos Verdes, California (RP, 33°42′-N, 118°19′-W) and pooled for DNA extraction. Approximately 14 µg of genomic DNA was digested with *Hae*III, *Rsa*I, *Alu*I, and *Nhe*I restricion enzymes. The resulting 200–1000 bp fragments were treated with Klenow Polymerase and then dephosphorylated with shrimp alkaline phosphatase. The genomic DNA was then blunt-end ligated to the double stranded SNX linkers described by Hamilton *et al*. (1999), and cleaned with PCR columns (Qiagen). The ligated DNA was PCR amplified using the SNX-forward oligonucleotide as a primer. After PCR, the product was denatured and hybridized to one of two groups of biotin labelled oligonucleotides at 45 °C for 1 h. Group 1 consisted of $(GA)_{15}$, $(CA)_{15}$, $(AAG)_{10}$, and $(AAC)_{10}$ oligonucleotides, and group 2 consisted of $(TCAG)_8$, $(CATA)_8$, and $(GATA)_8$ oligonucleotides. Hybridization reactions were added to 300 µg of streptavidin-coated magnetic beads (Promega), mixed, and allowed to incubate at room temperature for 30 min. Fragments not containing microsatellites were eliminated by washing twice at room temperature and twice at 45 °C. Microsatellite enriched DNA was recovered from the magnetic beads by denaturation with 0.1 N NaOH at 60 °C, neutralized with an equal volume of 1 M tris buffer (pH 8.0), cleaned with a PCR column (Qiagen), and eluted in a final volume of 30 µL. A second

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Table 1 Characterization of 11 *Tigriopus californicus* microsatellite loci

Locus	Primer (5′–3′)	Repeat motif	Allele size				Linkage	GenBank Accession no.
			T_a	RP	Cloned	LB		
TC1202	F: GCGATGCGTGTAATAATGG R: TGATTTGTTACCTCGCCTGA	$(ACTC)_7$	55	162	198	188		AY643056
TC1203	F: GCGTTCAACTCTCGAAATCA R: TCCTTATCTCCTCATCCCATAGA	$(CAGA)_2(CA)_4(CT)_2(CACT)_4$	55	199	203	203		AY643057
TC56J2	F: CTCCCAACGCTGGTATTAGG R: AAGATGGGGCAAAGGAATTT	$(AC)_2GC(AC)_6$	55	216	216	208		AY643058
TC1555	F: GATTTGGTGTTGGAGACGCC R: GATCGACAAATCACACACAC	$(GT)_2A(TG)_5$	55	173	165	165		AY643059
TC1814	F: TTTTTCTGCTCGAGCGTTTT R: CCGTCGTCTCGAGCTCTTT	$(TG)_6$	55	160	153	150	TCS197	AY643060
TCS030	F: CATTCCCGAACGAAGACG R: TTAAAAGAACCAAACGCACG	$(AAC)_4AAT(AAC)_2$	55	62	65	68	TCS558	AY643061
TCS061	F: CCAACGACTGACGGGTCC R: ATCCGCGAGTCGAGAGTATG	$(GAA)_2(GT)_5 + (GTTT)_4(GT)_3$	55	377	377	392	TCS480	AY643062
TCS228	F: AATCGAGTTGGCATCCTTAGA R: GGTCATATCTTGCGATTGAGA	$(TTC)_5$	55	82	82	72		AY643063
TCS480	F: GCTGTCCACCCAACCAAC R: TGAAACTGCCAACAAGATCCATAC	$(GTCT)_{14}$	61	202	206	146	TCS061	AY643064
TCS558	F: CGAGAACATAACTTCAAACGAAAC R: GTACATCTGTGCATGGTCCAC	$(CT)_4 + (AC)_4 + (GT)_4$	55	116	116	110	TCS030	AY643065
TCS197	F: TGTTTGCCAACCAAAGTGAA R: CACAGTATGAAGAAGCCAGTCC	$(AACG)_8$	55	208	208	196	TC1814	AY643066

F, forward; R, reverse; T_a, optimized annealing temperature (°C); Linkage, same chromosome; Cloned, size of cloned allele; RP, inbred line derived from Royal Palms population; LB, inbred line derived from Laguna Beach population.

round of PCR was performed using 3 µL of the final elution as template and the SNX-forward oligonucleotide as a primer. These PCR products were digested with *Nhe*I and ligated into *Xba*I cut pBlueskrip II-SK-plasmid. Ligations were used to transform *Escherichia coli* XL1-Blue competent cells, which were subsequently grown on LB ampicilln plates. Recombinant plasmids were sequenced using universal M13 primers to confirm the presence of simple repeat motifs. Primers were designed from these sequences using the PRIMER3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

Successful PCR amplification for 11 loci in inbred lines derived from two genetically divergent populations are reported here. PCR was performed in a 10 µL reaction volume, and consisted of 1 × PCR buffer (Promega), 2.5 mM $MgCl_2$, 250 µM of each dNTP, 10 pmol each primer, and 0.25 units *Taq* polymerase (Promega). Forward primers were fluorescently labelled. Thermal cycling conditions were as follows: 5 min at 94 °C; 35 cycles of 30 s at 94 °C; 30 s at optimal annealing temperature (Table 1); 30 s at 72 °C followed by 5 min at 72 °C. Optimized annealing temperatures, primer sequences, repeat motif, allele sizes, and linkage information (i.e. placement on the same chromosome) are reported in Table 1. Allele sizes reported here are from inbred lines derived from two genetically divergent populations: Royal Palms, Palos Verdes, California (RP, 33°42′-N, 118°19′-W) and Laguna Beach, California (LB, 33°33′-N, 117°47′-W). We determined linkage relationships of the microsatlellite loci to the same chromosome by taking advantage of the lack of recombination in *T. californicus* females (Ar-Rushdi 1963). Non-recombinant backcross (F1 females × parental males) offspring were scored ($n = 22$) for each locus, and the observation of even a single non-parental genotype indicated that the loci are on different chromosomes (Table 1).

All 11 loci had distinct alleles and amplified consistently in inbred lines derived from two populations previously shown to have genetic distances of 18% for the mitochondrial cytochrome oxidase I gene (Edmands 2001; D. Peterson, unpublished data). Although primer sequences and repeat information are reported for the first time here, Edmands & Harrison (2003) previously surveyed variation at five of these loci (TCS197, TCS558, TC1202, TC1203, and TC56J2) for 50 individuals from each of six *T. californicus* populations (including Royal Palms). The number of alleles per locus ranged from four to nine, and observed heterozygosity values ranged from 0 for all loci in population SUN to 0.80 for TC1203 in population FHL. None of these loci deviated significantly from Hardy–Weinberg Equilibrium. In sum, these microsatellite loci may be useful for investigations of

genetic variation within and among natural populations of *T. californicus*, as well as in controlled breeding experiments aimed at understanding the genetic consequences of hybridization between divergent populations.

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