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Characterization of 24 Microsatellite Loci in Delta Smelt, *Hypomesus transpacificus*, and Their Cross-Species Amplification in Two Other Smelt Species of the Osmeridae Family

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Abstract

We characterized 24 polymorphic tetranucleotide microsatellite loci for delta smelt (*Hypomesus transpacificus*) endemic to the San Francisco Bay Estuary, California, USA. Screening of samples (n = 30) yielded two to 26 alleles per locus with observed levels of heterozygosity ranging from 0.17 to 1.0. Only one locus deviated from Hardy–Weinberg equilibrium, suggesting these individuals originate from a single panmictic population. Linkage disequilibrium was found in two pairs of loci after excluding the locus out of Hardy–Weinberg equilibrium. Twenty-two primer pairs cross-amplified in wakasagi smelt (*Hypomesus nipponensis*), and 15 primer pairs cross-amplified in longfin smelt (*Spirinchus thaleichthys*).

Keywords: Cross-species amplification, Delta smelt, *Hypomesus transpacificus*, Microsatellites, Osmeridae, Primers

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The delta smelt (Osmeridae: *Hypomesus transpacificus*) is an annual planktivorous fish endemic to the Sacramento–San Joaquin River delta and upper San Francisco Bay Estuary of central California (Moyle et al. 1992). Delta smelt have been in rapid decline since they were listed as threatened by the U.S. Fish and Wildlife Service (U.S. FWS) under the U.S. Endangered Species Act in 1993 (U.S. FWS 1993; Feyrer et al. 2007). A major threat to delta smelt is water diversion by the Federal and California State Water Projects, which export water from the delta to central and southern California for agricultural use and urban drinking water. Additional threats include reduced water quality from urban and agricultural runoff, and competition and predation by introduced species (Moyle et al. 1992; Feyrer et al. 2007). Microsatellite markers characterized for delta smelt will allow us to assess population structure and conduct genetic studies relevant to the conservation of this species.

Whole genomic DNA was extracted from fin tissue of delta smelt collected near Decker Island in the lower Sacramento River, California, using QIAGEN's DNeasy Tissue Kit protocol. Eight libraries enriched for tetranucleotide repeat motifs [$(AAAC_n$, $(CAGA)_n$, $(CATC)_n$, $(TAGA)_n$ (at two different annealing temperatures), $(AAAG)_n$, $(TACA)_n$, and $(TGAC)_n$] were constructed, screened, and sequenced by Genetic Identification Services according to Meredith & May (2002). The library with tetranucleotide repeat (CAGA) was particularly rich in microsatellites and 584 clones of that library were sequenced.

We analyzed sequences using SEQUENCHER version 4.7 (Gene Codes Corporation) to compare sequences for duplicates and employed MREPS version 2.5 (Kolpakov et al. 2003) to identify repeat regions. PRIMER 3 (Rozen & Skaletsky 2000) was used to create primer pairs flanking the repeat regions of interest for 163 loci. Primer pairs were initially tested on five delta smelt individuals to determine microsatellite amplification and polymorphism. Polymerase chain reaction (PCR) was performed with the following conditions: 5 ng DNA template, 1 × *Taq* DNA polymerase buffer B, 2.0 mm $MgCl_2$, 0.2 mm of each dNTP, 10 μm of each primer and 0.38 U *Taq* DNA polymerase (all reagents from Promega), for a total reaction volume of 10 μL. PCR was performed using a Bio-Rad DNA Engine Dyad thermal cycler under the following conditions: 95 °C

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Table 1 Characterization of 24 microsatellite loci in delta smelt (*Hypomesus transpacificus*) from the San Francisco Bay Estuary, California, USA. GenBank Accession numbers, primer sequences, fluorescent dye used to label primer, number of individuals genotyped, repeat motif, number of alleles, estimated allele size range (bp), observed and expected heterozygosities

Locus	GenBank Accession no.	Primer sequence (5'–3')	Dye	*n*	Repeat motif	No. of alleles	Estimated allele size range (bp)	H_O	H_E
HtrG103	EU621763	F: GCACGCATCATGTCAGAAATA R: *TCAGGCTAAGAGGACCTGGA	6-FAM	30	$(GACA)_{10}$	13	91–150	0.87	0.86
HtrG104	EU621764	F: GTGCTGACAGGTAGGCAGGT R: *CCGCATGGTAACAGGAAGTT	6-FAM	30	$(CAGA)_8(AG)_5$	6	113–160	0.53	0.60
HtrG105	EU621765	F: *CTGGGACAGACACCTCTGGT R: TCCCTAACCGCTAAACCATCT	6-FAM	5	$(CTGT)_8$	4	75–200	0.40	0.64
HtrG106	EU621766	F: *TCCCTCAAACCGTTTTTCAC R: GCTGGTAAGCTCGAGACTGG	6-FAM	24	$(GTCT)_6$	2	75–200	0.17	0.16
HtrG107	EU621767	F: *TGGACAGACACAGAGAAGCAG R: GGACATAGCTGGACCCTCAG	PET	25	$(CAGA)_7$	9	100–215	0.68	0.75
HtrG108	EU621768	F: *TTGGTACACGGCAACTGAAA R: AGCCCTGCCAGAGAGAGAAT	PET	22	$(GT)_9(TCTA)_8$	12	75–250	0.86	0.87
HtrG109	EU621769	F: *GGACAGCACAAAGTCCTGGT R: GACACTCACAGACAGTCTCATCG	PET	30	$(TCTG)_{11}(GTCT)_4$	15	145–218	0.90	0.89
HtrG110	EU621770	F: *AAACGTGTCTGGTGGTGTCA R: CCCACCCAGTCTGTCTGTTT	PET	28	$(CAGA)_{17}$	21	100–275	0.96	0.94
HtrG112	EU621771	F: *AGTCTTACGCGATCCACAGC R: ACTGTCTGTCTGCGGCTTTT	PET	29	$(CAGG)_4$	2	100–299	0.21	0.19
HtrG113	EU621772	F: *GCTGGCTGGCTAGCTGAC R: CGTCTTCCACCCTACATGCT	VIC	6	$(AGAC)_6$	3	100–300	0.50	0.68
HtrG114	EU621773	F: *ACCATGGGAGACAAGTCTGG R: TCACTGGCACAAACGAGAAG	VIC	28	$(TCTA)_5(TCTG)_{11}$	19	175–272	1.00	0.95
HtrG115	EU621774	F: *CTCTCCCTCCGTTTTGCTCT R: CTGGTCTTGCAACGTGTTTG	VIC	29	$(CTGT)_{18}$	12	175–240	0.79	0.90
HtrG116	EU621775	F: *CGCTTTTTAGCGTCTTCCAC R: GCTGGCTGGCTAGCTGAC	6-FAM	18	$(TGTC)_5$	3	175–250	0.33	0.37
HtrG117	EU621776	F: *CACACACTCCAAGAGCAGGA R: CTGTCTCTCTGCCCACCTTC	NED	24	$(GACA)_{17}$	12	150–300	0.96	0.91
HtrG118	EU621777	F: *GTTGCGGGATTCTTAAACCA R: CCCCAAAGAAGCCAGATGTA	VIC	30	$(ACAG)_5$	4	150–300	0.37	0.32
HtrG119	EU621778	F: *AAGCTTCTGCTGGACGAGAC R: ACTCCTACCGAACCGTGATG	NED	29	$(ACAG)_{21}$	26	179–272	0.97	0.96
HtrG120	EU621779	F: *ACAGCGAAACAACCACATCA R: GCGTGGTCTAGGCTTGAAAA	NED	30	$(AGAC)_6$	8	230–279	0.60	0.74
HtrG122	EU621780	F: *AACACATTGCAGCAAGGCTA R: TGACCTACGATTGGTGGAGA	NED	24	$(TGTC)_{30}$	8	250–300	0.42	0.86
HtrG123	EU621781	F: *TTAGCCAGTCAGTCATGTGGA R: GATCCCTTTTCATCCTGCAA	6-FAM	30	$(GACA)_{22}$	22	240–349	0.93	0.95
HtrG126	EU621782	F: GATCCCTTTTCATCCTGCAA R: *TTAGCCAGTCAGTCATGTGGA	6-FAM	30	$(TCTG)_{25}$	21	243–335	0.87	0.95
HtrG127	EU621783	F: GCATTCTTAGCCGTCTGGAG R: *CCCATTCCCTCCCCTATCT	6-FAM	30	$(AGAC)_3(ACAG)_{26}$	24	209–350	0.80	0.95
HtrG128	EU621784	F: *CTGCTCTGTTCCAATCAGCA R: GAAGCTGCCTGTCTGTCTAGC	6-FAM	19	$(ACAG)_{26}$	12	200–375	0.84	0.84
HtrG129	EU621785	F: *ACTGCCTGGAAGAGCACACT R: CAAAGTTCTGTGCAACTTGGAA	PET	28	$(TGTC)_5(CTGT)_7$	6	300–360	0.64	0.66
HtrG131	EU621786	F: *GAGAGAAGGGATGGGGAGTC R: GGCCAAGGGACAGTTCATAA	PET	27	$(CAGA)_{28}$	21	281–381	0.78	0.95

*labelled primer.

for 1 min, 30 cycles at 95 °C for 30 s, 50 °C for 1 min, 72 °C for 1 min, followed by 60 °C for 10 min, and held at 10 °C. Amplified products were diluted 1:1 with 98% formamide loading buffer, denatured at 95 °C for 2 min, and chilled immediately on ice before electrophoresis. PCR products were separated on a 5% denaturing polyacrylamide gel at 50 W for 70 min, visualized using the SYBR-Green-agarose overlay protocol (Rodzen et al. 1989), and scanned with a GE Healthcare FluorImager 595. Product sizes were estimated by comparison with a standard 400 bp ladder (The Gel Company).

Twenty-four of the 163 loci were polymorphic and well-resolved in the initial screening (Table 1). Those 24 loci were screened with an additional 25 delta smelt individuals (total n = 30) also collected near Decker Island. We also tested the 24 polymorphic loci for cross-species amplification in six individuals of longfin smelt (*Spirinchus thaleichthys*) and wakasagi smelt (*Hypomesus nipponensis*).

Multiplex PCR amplifications were performed using the same conditions described above for the initial screening, except that the cycle number was increased to 31 and 1 μm of fluorescently labeled primer [NED, VIC, and PET from Applied Biosystems (ABI), 6-FAM from Integrated DNA Technologies] was added into a total reaction volume of 15 μL. One microliter of multiplexed PCR product was run undiluted on an ABI 3130xl Genetic Analyzer with a LIZ600 size standard (ABI). GENEMAPPER version 4.0 (ABI) was used to analyze the electropherograms and allelic sizes were confirmed manually.

Data analysis was performed using genetic data analysis (GDA; Lewis & Zaykin 2001). MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004) was used to estimate the probability of the occurrence of null alleles. Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) significance was evaluated using Fisher's exact test with 10,000 permutations, and missing data discarded. Characteristics of the microsatellite loci amplifying in *H. transpacificus* are presented in Table 1. One locus, *HtrG122*, deviated from HWE expectations (P < 0.05) after applying sequential Bonferroni correction (Holm 1979). Heterozygote deficiency at this locus suggests the presence of null alleles (P < 0.001). However, 23 of 24 loci conform to HWE expectations, suggesting the 30 individuals included in the analysis may originate from a single panmictic population. Significant pairwise genotype LD (P < 0.05) was found in two pairs of loci after applying a sequential Bonferroni correction and excluding HtrG122: *HtrG115*/*HtrG131* and *HtrG127*/*HtrG131*.

Of the 24 primer pairs developed for delta smelt and tested for cross-amplification in *H. nipponensis* and *S. thaleichthys*, only one (4%) resulted in no amplification in either species. Fifteen (62.5%) of the 24 primer pairs amplified in *S. thaleichthys*, while 22 (91.6%) amplified in *H. nipponensis* (Table 2).

Table 2 Cross-species amplification results of 24 microsatellite loci for the smelt family Osmeridae, genus *Hypomesus* (*H. nipponensis*) and *Spirinchus* (*S. thaleichthys*). Species, sample size (n); 'U' indicates amplification but unclear; '—' indicates no amplification; number of alleles are given with numbers in parentheses indicating size range in bp

Locus ID	*H. nipponensis* (n = 6)	*S. thaleichthys* (n = 6)
HtrG103	U	2 (111–115)
HtrG104	2 (112–147)	—
HtrG105	1 (140)	1 (94)
HtrG106	1 (147)	—
HtrG107	3 (122–149)	U
HtrG108	4 (148–198)	U
HtrG109	4 (145–162)	1 (109)
HtrG110	2 (106–115)	1 (118)
HtrG112	1 (285)	U
HtrG113	2 (124–231)	2 (142–237)
HtrG114	1 (204)	1 (195)
HtrG115	U	—
HtrG116	—	—
HtrG117	U	U
HtrG118	7 (238–298)	8 (243–276)
HtrG119	—	U
HtrG120	2 (268–273)	—
HtrG122	2 (283–288)	—
HtrG123	12 (261–343)	—
HtrG126	4 (260–295)	U
HtrG127	3 (220–289)	—
HtrG128	10 (236–367)	U
HtrG129	U	U
HtrG131	5 (328–376)	—
Total no. of amplified loci	22	15

The microsatellite loci discussed here will be used to conduct genetic studies relevant to the conservation of delta smelt and related species.

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