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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, CA, 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 US Fish and Wildlife Service (USFWS) under the US Endangered Species Act in 1993 (USFWS 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, CA, 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) $_n$ 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 MgCl₂, 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, CA, 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	<i>n</i>	Repeat motif	No. of alleles	Estimated allele size range (bp)	<i>H</i> _O	<i>H</i> _E
<i>HtrG103</i>	EU621763	F: GCACGCATCATGTTCAGAAATA R: *TCAGGCTAAGAGGACCTGGGA	6-FAM	30	(GACA) ₁₀	13	91-150	0.87	0.86
<i>HtrG104</i>	EU621764	F: GTGCTGACAGGTAGGCAGGT R: *CCGCATGGTAACAGGAAGTT	6-FAM	30	(CAGA) ₈ (AG) ₅	6	113-160	0.53	0.60
<i>HtrG105</i>	EU621765	F: *CTGGGACACAGACCTCTGGT R: TCCCTAACCGCTAAACCATCT	6-FAM	5	(CTGT) ₈	4	75-200	0.40	0.64
<i>HtrG106</i>	EU621766	F: *TCCCTCAAACCGTTTTCAC R: GCTGGTAAGCTCGAGACTGG	6-FAM	24	(GTCT) ₆	2	75-200	0.17	0.16
<i>HtrG107</i>	EU621767	F: *TGGACAGACACAGAGAAGCAG R: GGACATAGCTGGACCCTCAG	PET	25	(CAGA) ₇	9	100-215	0.68	0.75
<i>HtrG108</i>	EU621768	F: *TTGGTACACGGCAACTGAAA R: AGCCCTGCCAGAGAGAGAAT	PET	22	(GT) ₉ (TCTA) ₈	12	75-250	0.86	0.87
<i>HtrG109</i>	EU621769	F: *GGACAGCACAAAGTCTGGT R: GACACTCACAGACAGTCTCATCG	PET	30	(TCTG) ₁₁ (GTCT) ₄	15	145-218	0.90	0.89
<i>HtrG110</i>	EU621770	F: *AAACGTGTCTGGTGGTGTCA R: CCCACCAGTCTGTCTGTTT	PET	28	(CAGA) ₁₇	21	100-275	0.96	0.94
<i>HtrG112</i>	EU621771	F: *AGTCTTACCGATCCACAGC R: ACTGTCTGTCTGCGGCTTTT	PET	29	(CAGG) ₄	2	100-299	0.21	0.19
<i>HtrG113</i>	EU621772	F: *GCTGGCTGGCTAGCTGAC R: CGTCTTCCACCCTACATGCT	VIC	6	(AGAC) ₆	3	100-300	0.50	0.68
<i>HtrG114</i>	EU621773	F: *ACCATGGGAGACAAGTCTGG R: TCACTGGCACAACAGAGAAG	VIC	28	(TCTA) ₅ (TCTG) ₁₁	19	175-272	1.00	0.95
<i>HtrG115</i>	EU621774	F: *CTCTCCCTCCGTTTGTCTCT R: CTGGTCTTGCAACGTGTTTG	VIC	29	(CTGT) ₁₈	12	175-240	0.79	0.90
<i>HtrG116</i>	EU621775	F: *CGCTTTTGTAGCGTCTTCCAC R: GCTGGCTGGCTAGCTGAC	6-FAM	18	(TGTC) ₅	3	175-250	0.33	0.37
<i>HtrG117</i>	EU621776	F: *CACACACTCCAAGAGCAGGA R: CTGTCTCTCTGCCACCTTC	NED	24	(GACA) ₁₇	12	150-300	0.96	0.91
<i>HtrG118</i>	EU621777	F: *GTTGCGGGATTCTTAAACCA R: CCCCAAAGAAGCCAGATGTA	VIC	30	(ACAG) ₅	4	150-300	0.37	0.32
<i>HtrG119</i>	EU621778	F: *AAGCTTCTGCTGGACGAGAC R: ACTCCTACCGAACCGTGATG	NED	29	(ACAG) ₂₁	26	179-272	0.97	0.96
<i>HtrG120</i>	EU621779	F: *ACAGCGAAACAACCACATCA R: GCGTGGTCTAGGCTTGAAAA	NED	30	(AGAC) ₆	8	230-279	0.60	0.74
<i>HtrG122</i>	EU621780	F: *AACACATTGCAGCAAGGCTA R: TGACCTACGATTGGTGGAGA	NED	24	(TGTC) ₃₀	8	250-300	0.42	0.86
<i>HtrG123</i>	EU621781	F: *TTAGCCAGTCAGTCATGTGGA R: GATCCCTTTTCATCCTGCAA	6-FAM	30	(GACA) ₂₂	22	240-349	0.93	0.95
<i>HtrG126</i>	EU621782	F: GATCCCTTTTCATCCTGCAA R: *TTAGCCAGTCAGTCATGTGGA	6-FAM	30	(TCTG) ₂₅	21	243-335	0.87	0.95
<i>HtrG127</i>	EU621783	F: GCATTCCTTAGCCGCTCTGGAG R: *CCCATTCCCTCCCTATCT	6-FAM	30	(AGAC) ₃ (ACAG) ₂₆	24	209-350	0.80	0.95
<i>HtrG128</i>	EU621784	F: *CTGCTCTGTTCCAATCAGCA R: GAAGCTGCCTGTCTGTCTAGC	6-FAM	19	(ACAG) ₂₆	12	200-375	0.84	0.84
<i>HtrG129</i>	EU621785	F: *ACTGCCTGGAAGAGCACACT R: CAAAGTTCTGTGCAACTTGAA	PET	28	(TGTC) ₅ (CTGT) ₇	6	300-360	0.64	0.66
<i>HtrG131</i>	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 μ M 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 μ L. 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	<i>H. nipponensis</i> ($n = 6$)	<i>S. thaleichthys</i> ($n = 6$)
<i>HtrG103</i>	U	2 (111–115)
<i>HtrG104</i>	2 (112–147)	—
<i>HtrG105</i>	1 (140)	1 (94)
<i>HtrG106</i>	1 (147)	—
<i>HtrG107</i>	3 (122–149)	U
<i>HtrG108</i>	4 (148–198)	U
<i>HtrG109</i>	4 (145–162)	1 (109)
<i>HtrG110</i>	2 (106–115)	1 (118)
<i>HtrG112</i>	1 (285)	U
<i>HtrG113</i>	2 (124–231)	2 (142–237)
<i>HtrG114</i>	1 (204)	1 (195)
<i>HtrG115</i>	U	—
<i>HtrG116</i>	—	—
<i>HtrG117</i>	U	U
<i>HtrG118</i>	7 (238–298)	8 (243–276)
<i>HtrG119</i>	—	U
<i>HtrG120</i>	2 (268–273)	—
<i>HtrG122</i>	2 (283–288)	—
<i>HtrG123</i>	12 (261–343)	—
<i>HtrG126</i>	4 (260–295)	U
<i>HtrG127</i>	3 (220–289)	—
<i>HtrG128</i>	10 (236–367)	U
<i>HtrG129</i>	U	U
<i>HtrG131</i>	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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