

Supporting Information

Miura et al. 10.1073/pnas.1012881107

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O.lati d(V)	:	TTTTLAFKFRHGVIVAVDSRASAGNYLASNDVNKVIIEINPYLLGTMGSGAADCQYWERLLAKECRLYLRLNNHRISVAAASKLLCNMMLGYRGMGLSVGSMICGW	:	104							
O.cele d(V)	:K.....S.....M.....	:	104							
O.curv d(V)1	:K.....S.....M.....	:	104							
O.curv d(V)2	:K.....S.....M.....	:	104							
O.curv d(V)3	:K.....S.....M.....	:	104							
O.danc d(V)	:K.....S.....M.....	:	104							
O.java d(V)1	:	R.....K.....S.....K.....M.....	:	104							
O.java d(V)2	:	R.....K.....S.....M.....	:	104							
O.java d(V)3	:K.....S.....M.....	:	104							
O.java d(V)4	:S.....M.....	:	104							
O.java d(V)5	:K.....N.....M.....	:	104							
O.marm d(V)	:K.....S.....M.....	:	104							
O.mata d(V)	:K.....S.....M.....	:	104							
O.minu d(V)1	:Q.....N.....Q.....	:	104							
O.minu d(V)2	:N.....SQ.....	:	104							
O.lati d(A)	:A.....K.....M.....	:	104							
O.cele d(A)	:A.....K.....S.....M.....	:	104							
O.curv d(A)	:A.....K.....M.....	:	104							
O.danc d(A)	:A.....K.....S.....M.....	:	104							
O.java d(A)1	:A.....K.....S.....M.....	:	104							
O.java d(A)2	:	DC.A.....K.....S.....K.....M.....	:	104							
O.marm d(A)	:	D.A.....K.....S.....M.....	:	104							
O.mata d(A)	:	A.....K.....S.....M.....	:	104							
O.minu d(A)	:	A.....H.....K.....Q.....M.....	:	104							
O.lati N	:	S.K.....T.S.I.TCEY.....K.....	:	104							
O.cele N1	:	M.K.....T.S.VDTCEY.....K.....S.....	:	104							
O.cele N2	:	K.....ISAGEY.....K.....	:	104							
O.curv N	:	S.K.....T.S.VDTCEY.....K.....	:	104							
O.danc N	:	K.....ISTGEY.....K.....	:	104							
O.java N	:	K.....ISTGEY.....K.....	:	104							
O.marm N	:	M.K.....T.S.VDTCEY.....K.....S.....	:	104							
O.mata N	:	M.K.....T.S.VDTCEY.....K.....S.....	:	104							
X.laevis-B	:	K.....S.I.LKA.....H.....Q.....S.....SS.....Q.....S.....	:	104							
shark-A	:	Q.....V.A.....Q.....K.....KQ.....S.....S.....CE.....M.....	:	104							
X.laevis-A	:	Q.....S.ISTIKF.....Q.....S.....M.....Q.....T.....	:	104							
shark-B	:	Q.....M.....S.ISTQMPK.....VF.....M.....Q.....I.K.....KK.....S.....A.VSE.K.....M.T.....	:	104							
Human	:	Q.....A.....S.ISALR.....C.....Y.....GE.....S.....S.....CQ.....M.....	:	104							

		#	#	#	#	#	##	#	##
O.lati d(V)	:	DKEGPGLYYVDDNGTRLNLSGRMFTSGCGNSYAYGVVDSYKEDMTVEEAYELGCRGIAHATHRDAYSGGVSVMYHMRDGMWIKVCKEDVSELIHRYREGMF	----	204					
O.cele d(V)	:G.....E.....V.....	----	204					
O.curv d(V)1	:G.....E.....V.....	----	204					
O.curv d(V)2	:G.....E.....V.....	----	204					
O.curv d(V)3	:	T.....	----	204					
O.danc d(V)	:	G.....E.....D.....	----	204					
O.java d(V)1	:	G.....D.....	----	204					
O.java d(V)2	:	G.....I.....D.....	----	204					
O.java d(V)3	:	G.....D.....	----	204					
O.java d(V)4	:	G.....D.....	----	204					
O.java d(V)5	:	G.....D.....	----	204					
O.marm d(V)	:	G.....E.....D.....	----	204					
O.mata d(V)	:	G.....E.....D.....	----	204					
O.minu d(V)1	:	G.....D.....	----	204					
O.minu d(V)2	:	G.....D.....	----	204					
O.lati d(A)	:	H.....	----	204					
O.cele d(A)	:K.....	----	204					
O.curv d(A)	:K.....	----	204					
O.danc d(A)	:	F.....K.....	----	204					
O.java d(A)1	:	F.....K.....	----	204					
O.java d(A)2	:	F.S.....K.....	----	204					
O.marm d(A)	:K.....	----	204					
O.mata d(A)	:K.....	----	204					
O.minu d(A)	:K.....	----	204					
O.lati N	:	I.....N.....S.SN.....L.....R.V.....S.V.....IQ.....D.....L.H.KK.....	----	204					
O.cele N1	:	I.....N.....S.SN.....L.....R.A.V.....S.V.....IQ.....D.....L.H.KK.....L.....	----	204					
O.cele N2	:	I.....N.....S.....L.....I.....R.....S.V.L.IQ.....D.....H.KQ.....	----	204					
O.curv N	:	I.....N.....S.SN.....L.....R.V.....S.V.....IQ.....D.....L.H.KK.....	----	204					
O.danc N	:	V.....S.....L.....I.....R.....S.V.L.IK.....D.....H.KK.....	----	204					
O.java N	:	V.....S.....L.....R.....S.V.L.IK.....D.....H.KK.....	----	204					
O.marm N	:	I.....N.....S.SN.....L.....R.A.V.....S.V.....IQ.....D.....L.H.KK.....L.....	----	204					
O.mata N	:	I.....N.....S.SN.....L.....R.A.V.....S.V.....IQ.....D.....L.H.KK.....L.....	----	204					
X.laevis-B	:	K.....C.DI.....S.....M.....R.F.L.P.....D.RHA.SY.....N.V.....K.....V.GEF.....LYK.A.EKCK---	205						
shark-A	:	K.....N.....V.....R.Y.L.P.....D.R.A.Y.....T.F.....S.D.G.HFK.AAEKQNLAQ	208						
X.laevis-A	:	K.....C.DI.....S.....M.....R.F.L.P.....D.R.A.SY.....C.L.K.....V.IGQF.....D.L.KFT.EKKN---	205						
shark-B	:	EK.....N.G.M.....S.....S.C.....M.....R.Y.L.K.....D.AQ.A.Y.....T.....N.....SQ.....GV.H.....TAETP---	204						
Human	:	K.....E.H.....N.....S.T.....M.....RPNLSP.....D.R.A.Y.....S.....V.....K.....L.V.EST.....D.L.Q.....ANQ---	204						

Fig. S1. Alignment of amino acid sequences of the mature peptides of proteasome subunit beta type 8 (*PSMB8*). *PSMB8* mature peptides of *Oryzias* species [two allelic lineages of *O. latipes* (NCBI accession nos. AB183488 and BA000027)], *Xenopus laevis* (NCBI accession nos. BAA07945 and BAA07954), two sequences of nurse shark (*Ginglymostoma cirratum*, NCBI accession nos. D64056 and D64057), and human (*Homo sapiens*, NCBI accession no. CAG46462) were aligned with ClustalX 2.0 (1). All *Oryzias PSMB8* sequences were determined in this study, except for two *O. latipes PSMB8N* and *PSMB8d(V)* sequences and the *O. dancena PSMB8N* sequence (NCBI accession no. FJ481084). Dots indicate identity with the residues in the uppermost sequence. *Residues involved in S1 pocket formation; #diagnostic residues for discrimination between the d and N allele types.

1. Larkin MA, et al. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948.

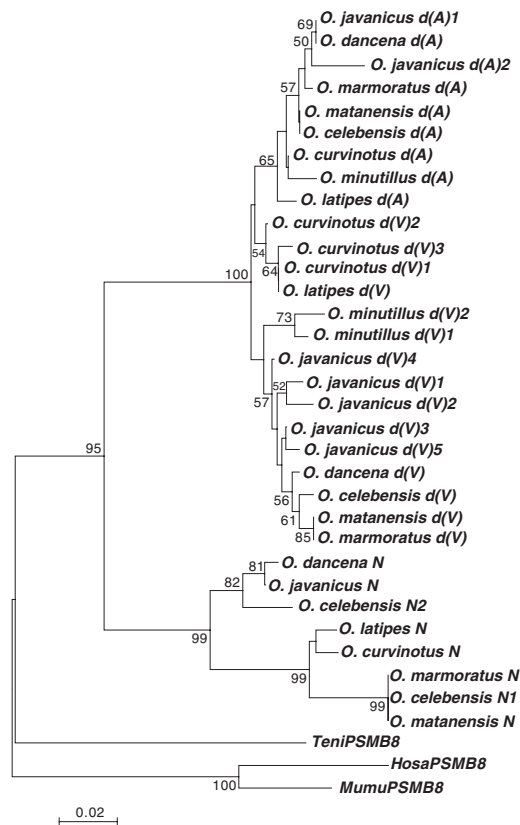


Fig. S2. Phylogenetic tree of *Oryzias PSMB8* alleles. The amino acid sequences of mature peptides of 204 residues were aligned by ClustalX 2.0 (1), and the phylogenetic tree was constructed by the NJ method (2). The evolutionary distances were computed using the p-distance model. The numbers on each branch represent bootstrap probabilities (>50%) based on 1,000 bootstrap trials. These evolutionary analyses were conducted in MEGA4 (3). The sequences of TeniPSMB8 (*Tetraodon nigroviridis*, NCBI accession no. CR697191), HosaPSMB8 (*Homo sapiens*, accession no. CR541661) and MumuPSMB8 (*Mus musculus*, NCBI accession no. BC013785) were used as an outgroup.

1. Larkin MA, et al. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948.
2. Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425.
3. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599.

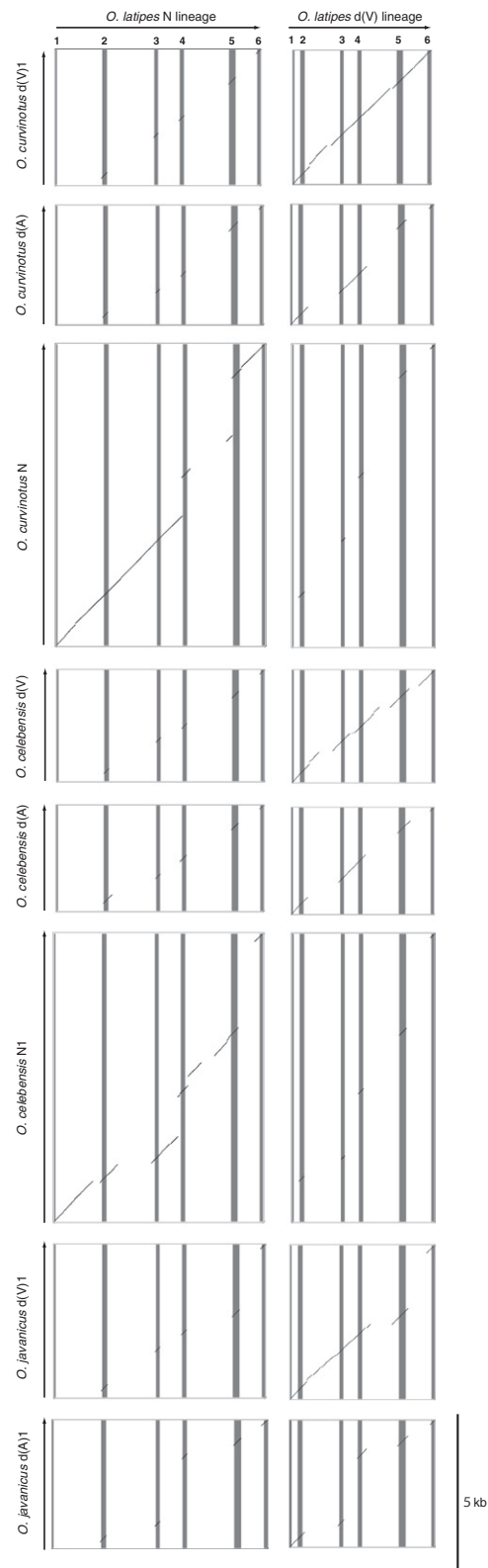


Fig. S3. Dot-plot comparison of the *Oryzias* *PSMB8* genes. The *PSMB8d(V)*, *PSMB8d(A)*, and *PSMB8N* lineages of *O. curvinotus* (the *latipes* group), *O. celebensis* (the *celebensis* group), and *O. javanicus* (the *javanicus* group) were compared with *PSMB8d(V)* and *PSMB8N* lineages of *O. latipes* (NCBI accession nos. AB183488 and BA000027). The horizontal and vertical axes represent the sequences of the *PSMB8d(V)* or *PSMB8N* of *O. latipes* and the other three *Oryzias* species, respectively. Each aligning gap-free segment with more than 50% identity is plotted. The exon positions of the *PSMB8d* or *PSMB8N* of *O. latipes* are shaded in the plot and are numbered above the plot. The arrows represent the transcriptional orientation of the alleles.

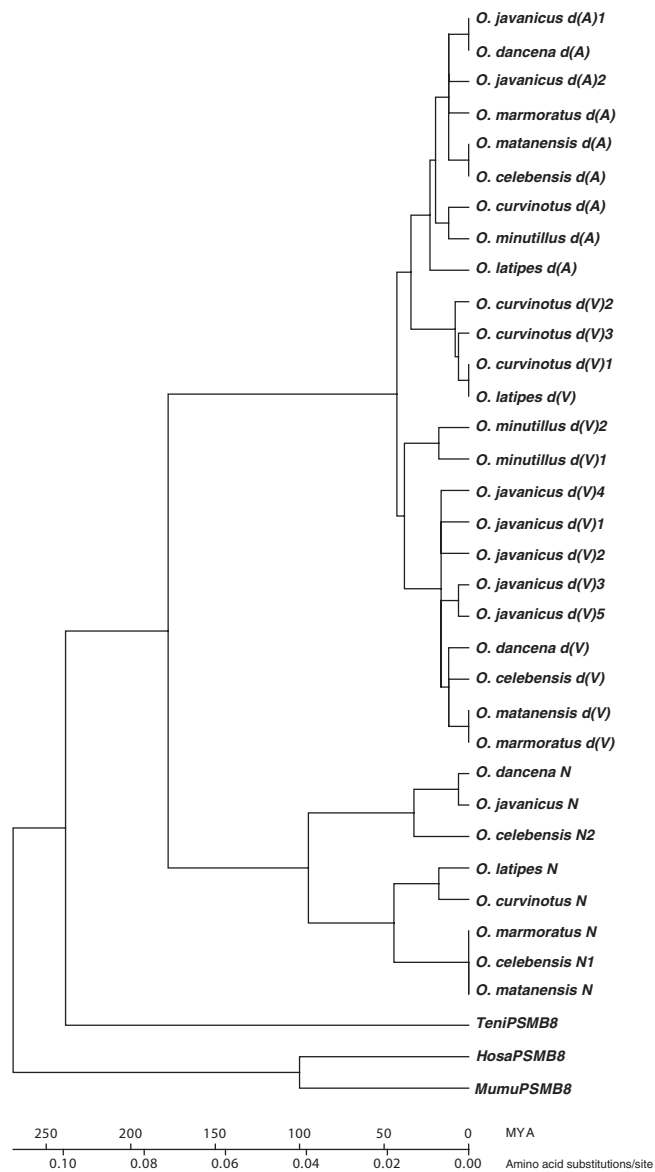


Fig. S4. Linearized tree of the *PSMB8* constructed under the assumption of molecular clock for the *PSMB8* alleles shown in Fig. S2. The linearized tree was constructed using Compute Linearized Tree in MEGA4 (1) based on the tree shown in Fig. S2. The scales under and above the horizontal line indicate amino acid substitutions per site and divergence time (Mya), respectively. One hundred million years was used as the divergence time between human and mouse.

1. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599.

Table S1. The number of specimens possessing each genotype in the *Oryzias* species

Species (no. specimens)	<i>PSMB8N/PSMB8N</i>	<i>PSMB8d/PSMB8d</i>	<i>PSMB8N/PSMB8d</i>
<i>O. curvinotus</i> (<i>n</i> = 69)	0	50	19
<i>O. celebensis</i> (<i>n</i> = 190)	36	74	80
<i>O. matanensis</i> (<i>n</i> = 106)	4	60	42
<i>O. marmoratus</i> (<i>n</i> = 106)	5	72	29
<i>O. javanicus</i> (<i>n</i> = 178)	20	119	39
<i>O. dancena</i> (<i>n</i> = 150)	0	150	0
<i>O. minutillus</i> (<i>n</i> = 244)	0	244	0