HOKKAIDO UNIVERSITY

Title	Sexual dimorphism of gonadal structure and gene expression in germ cell-deficient loach, a teleost fish
Author(s)	Fujimoto, Takafumi; Nishimura, Toshiya; Goto-Kazeto, Rie et al.
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Table S1. Survival rates and the number of PGCs in larvae injected with different doses of *dnd* MO.

	No. of eggs used	No. of normal larvae	No. of abnormal larvae	Survival rates	No. of observed larvae	Mean No. of PGCs	s.d.	No. of PGCs observed in each larva
Intact control	48	46	1	0.958	12	48.5	7.6	60, 57, 54, 54, 53, 53, 48, 44, 41, 40, 40, 38
Control MO (1000-2000 pg / embryo)	48	44	3	0.917	11	50.5	13.0	68, 62, 59, 58, 58, 52, 49, 46, 42, 40, 21
dnd MO (250-500 pg / embryo)	48	46	0	0.958	12	10.2	8.7	28, 23, 17, 14, 8, 7, 7, 7, 7, 4, 0, 0
dnd MO (500-1000 pg / embryo)	48	45	2	0.938	12	7.8	14.5	44, 31, 9, 7, 1, 1, 0, 0, 0, 0, 0, 0
dnd MO (1000-2000 pg / embryo)	48	46	0	0.958	12	0.0	0.0	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0
dnd MO (2000-4000 pg / embryo)	48	42	2	0.875	12	0.0	0.0	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0

Table S2. Classification of gonads in 13-month-old loach following injection of *dnd* MO at the one-to two-cell stage.

	Sample size	Morphological feature of gonads			
		Ovary	Two testes	One testis	Atypical
Intact control	11	5	6	0	0
Control MO (1000-2000 pg / embryo)	11	1	10	0	0
dnd MO (250-500 pg / embryo)	8	5	2	0	1
dnd MO (500-1000 pg / embryo)	27	3	9	5	10
dnd MO (1000-2000 pg / embryo)	22	0	2	2	18
dnd MO (2000-4000 pg / embryo)	13	0	0	0	13

Table S3. Distribution patterns of *vasa*-positive cells of wild-type and *dnd* morphants at each stage.

	Stage	N	No. of *vasa*-positive individuals	Distribution of *vasa*-positive cells		
				Normal	Normal & ectopic	Ectopic
Wild-type	Embryonic shield	18	18	18	0	0
	2-4 somite	27	27	27	0	0
	20 somite	27	27	27	0	0
	30 somite	28	28	24	4[*]	0
	Larvae	14	14	14	0	0
dnd morphants	Embryonic shield	15	15	15	0	0
	2-4 somite	14	14	0	14[**]	0
	20 somite	15	15	1[***]	7[**]	7
	30 somite	18	17	1[***]	6[**]	10
	Larvae	18	0	0	0	0

[*]: A small number of *vasa*-positive cells were distributed in ectopic position, though most of *vasa*-positive cells were normal position.

[**]: Most of *vasa*-positive cells were distributed in ectopic position.

[***]: These embryos had a small number of *vasa*-positive cells distributed at normal position.

Table S4.　Sequences of primers used in the present study.

Primer	Purpose	Sequence (5'-3')
dead end-degenerate-F	Partial cloning	ACGGCGGCCCTCC(GATC)CC(GATC)GG(GATC)TGG
dead end-degenerate-R	Partial cloning	CCGGTCGATGTACTTGGC(AG)TA(GATC)GC(AG)AA
dead end-5' RACE-R1	RACE cloning	TCCGGTCGATGTACTTGGCGTAGGCGA
dead end-5' RACE-R2	RACE cloning	CTGGAAGAGCGGGATTAGGGTGTCCTCA
dead end-3' RACE-F1	RACE cloning	CTGCTACAGCTTTTCAACCCGCAGGCAC
dead end-3' RACE-F2	RACE cloning	TCCCGCTCTTCCAGAGAGTCGGCA
vasa-degenerate-F	Partial cloning	GGACGAGACCTGATGGCTTG(CT)GC(GATC)CA(AG)AC
vasa-degenerate-R	Partial cloning	TGTTCGTCCGATTCGGTG(GATC)AC(AG)TA(GATC)TC
vasa-5' RACE-R	RACE cloning	GTGCGTTCATTTCCTGTGGTTCTC
vasa-3' RACE-F	RACE cloning	GGTGGTTTATGGCGGGACAAACACTGGA
vasa-RT-F	RT-PCR	CTGAACCTGCCATGGATGAC
vasa-RT-R	RT-PCR	CTTCACCTCCTTTATAACCCTCAC
dmrt1-degenerate-F	Partial cloning	CGACGACAGCAGGCTCA(AG)GA(AG)GA(AG)GA
dmrt1-degenerate-R	Partial cloning	CGGTACTGAG(AG)(AG)GACA(CT)GTTGTGG
dmrt1-3' RACE-F1	RACE cloning	GAGGAGATGGGCATCTGCAATCCA
dmrt1-3' RACE-F2	RACE cloning	AGCTCCATCACCCACCACCACAAGT
dmrt1-3' RACE-F3	RACE cloning	TGAAAGCAGCTCAGAGTCCGGCACCTT
dmrt1-RT-F	RT-PCR	GAGATGGGCATCTGCAATCC
dmrt1-RT-R	RT-PCR	GCTGCTTTCACACTCCAGTC
foxl2-degenerate-F	Partial cloning	CCCTTTCTACGAGAAGAACAAGAA(AG)GG(GATC)TGGCA
foxl2-degenerate-R	Partial cloning	AGGCTGTCGGGAGCA(GATC)GC(AG)AA(CT)TG
foxl2-5' RACE-R1	RACE cloning	GCGCTGTTGGACGGTACCGGTGAG
foxl2-5' RACE-R2	RACE cloning	GGAGCCGAGAGGTAGCTGCCGTATCC
foxl2-3' RACE-F1	RACE cloning	GGATGAAGCGGCCGTTCAGACCGTCA
foxl2-3' RACE-F2	RACE cloning	AACGGCATGGGTCACCATCAGCACC
foxl2-3' RACE-F3	RACE cloning	TGTCTGAGGTTTCACCCGGTAGTGGA
foxl2-RT-F	RT-PCR	AAGCCGCCGTACTCTTATGTGG
foxl2-RT-R	RT-PCR	TCCCATTTGACAGGACGCGTAG
p450arom-degenerate-F	Partial cloning	ACATCGTGCGAGTGTGGAT(AT)AA(CT)GG
p450arom-degenerate-R	Partial cloning	GCTCGAGGTCCGCATCC(AG)AA(GATC)GG(CT)TG
p450arom-5' RACE-R1	RACE cloning	TCTGGACCAGCGATCACCATCTCC
p450arom-5' RACE-R2	RACE cloning	GGCGTCCTGCAACTCCTGAGCTTCT
p450arom-3' RACE-F1	RACE cloning	TCGGGATGCATGAACAGGGCATTA
p450arom-3' RACE-F2	RACE cloning	CAGTTCACCGATAGCCAGCGACAGG
p450arom-3' RACE-F3	RACE cloning	GGAATGTTAACGACGTGACATCGGAGTG
p450arom-RT-F	RT-PCR	CTGCAGGGAGTCACAGCTAC
p450arom-RT-R	RT-PCR	CAGTTCTGCAGCGAAGTCAAG
ß-actin-RT-F	RT-PCR	TTACCCACACCGTGCCCATCTAC
ß-actin-RT-R	RT-PCR	TACCGCAAGACTCCATACCCA