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Field Survey of Sex-Reversals in the Medaka, *Oryzias latipes*: Genotypic Sexing of Wild Populations

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ABSTRACT—The medaka, *Oryzias latipes*, has an XX/XY sex determination mechanism. A Y-linked DM domain gene, *DMY*, has been isolated by positional cloning as a prime candidate for the sex-determining gene. Furthermore, the crucial role of *DMY* during male development was established by studying two wild-derived XY female mutants. In this study, to find new *DMY* and sex-determination related gene mutations, we conducted a broad survey of the genotypic sex (*DMY*-negative or *DMY*-positive) of wild fish. We examined 2274 wild-caught fish from 40 localities throughout Japan, and 730 fish from 69 wild stocks from Japan, Korea, China, and Taiwan. The phenotypic sex type agreed with the genotypic sex of most fish, while 26 *DMY*-positive (XY) females and 15 *DMY*-negative (XX) males were found from 13 and 8 localities, respectively. Sixteen XY sex-reversals from 11 localities were mated with XY males of inbred strains, and the genotypic and phenotypic sexes of the F_1 progeny were analyzed. All these XY sex-reversals produced XY females in the F_1 generation, and all F_1 XY females had the maternal Y chromosome. These results show that *DMY* is a common sex-determining gene in wild populations of *O. latipes* and that all XY sex-reversals investigated had a *DMY* or *DMY*-linked gene mutation.

Key words: sex determination, *DMY*, wild population, sex-reversal, medaka

INTRODUCTION

In vertebrates, the Y chromosome gene, *SRY*, has been identified as the testis-determining gene in mammals (Sinclair *et al*., 1990; Gubbay *et al*., 1990), however until recently, no equivalent gene or sex-determining genes had been identified in non-mammalian vertebrates. In the medaka, *Oryzias latipes*, which has an XX/XY sex-determining system (Aida, 1921), a Y-linked gene, *DMY*, was found to be a prime candidate for the sex-determining gene using positional cloning. *DMY* encoded a protein that contains a DM-domain, which was originally described as a DNA-binding motif found in two proteins, DSX and MAB-3, involved in sexual development in *Drosophila melanogaster* and *Caenorhabditis elegans*, respectively (Raymond *et al*., 1998). Vertebrates have also several DM-domain genes, and one of these, *DMRT1* (*DM-related transcription factor 1*), has been implicated in male sexual development in mammals, birds, reptiles and fish (Raymond *et al*., 1999; Smith *et al*., 1999; De Grandi *et al*., 2000; Guan *et al*., 2000; Kettlewell *et al*., 2000; Marchand *et al*., 2000; Moniot *et al*., 2000). The cDNA sequences of medaka *DMY* and *DMRT1* indicated the high similarity (83%) and *DMY* appears to have originated through a duplication event of an autosomal segment containing the *DMRT1* region (Nanda *et al*., 2002).

DMY expression was observed only in genotypic (XY) males during gonadal sex differentiation, and a loss-of-function *DMY* mutation and depressed *DMY* expression mutation resulted in XY females (Matsuda *et al*., 2002). When a DNA fragment containing *DMY* was introduced as a transgene, testis developed in chromosomally (XX) female medaka (Matsuda *et al*., manuscript submitted for publication). These results demonstrate that *DMY* is both necessary and sufficient in determining testis formation in medaka. *DMY* is the first sex-determining gene to have been identified in non-mammalian vertebrates.

Sex-reversal mutants are important in revealing the molecular function of *DMY* and identifying other genes that are involved in sex determination. Analyses of these mutants allow us to extend our understanding of the function of genes and cell differentiation during sex determination. Two XY sex-reversed females with naturally occurring *DMY* mutations have already been found by screening wild medaka populations (Matsuda *et al*., 2002). This suggests

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that other spontaneous gene mutations involved in the sex-determining pathway can be identified from surveys in the wild.

A variety of sex-determining systems can coexist in closely related species or even within a single lower vertebrate species. Both the male-heterogametic XX/XY and female-heterogametic ZZ/ZW systems have been investigated within the *Xiphophorus* genus (reviewed in Volff and Schartl, 2001) and at the species level in *Rana rugosa* (Nishioka *et al*., 1993, 1994). Wild medaka populations comprise four genetically divergent groups (Sakaizumi, 1986; Sakaizumi and Joen, 1987; Sakaizumi *et al*., 1983), while the presence of *DMY* has been confirmed only in several medaka strains and wild populations classified in the Northern or Southern Populations (Matsuda *et al*., 2002). Therefore, it is possible that some populations have a sex-determining system that is not determined by *DMY*. On the other hand, artificial sex-reversals as a result of exogenous sex steroids (Yamamoto, 1953, 1958) or heat stresses (Gresik and Hamilton, 1977) have been reported in medaka. This suggests that in addition to the role of sex-determining genes, the natural environment might affect the phenotypic sex of wild medaka.

In this study, we conducted a broad survey of the *DMY* gene in wild medaka populations to locate any new gene mutations involved in the sex-determining pathway. Consequently, we report that approximately 1% of the wild medaka surveyed were sex-reversals and that all XY sex-reversals examined had a *DMY* or *DMY*-linked mutation.

MATERIALS 0D METHODS

Fish: We surveyed 2274 wild-caught fish at 40 localities throughout Japan (Table 1), collected from 2001 to 2003, and 730 fish from 69 wild stocks at the Faculty of Science, Niigata University. The wild stocks were collected from 55 localities in Japan (#41 to #95 in Table 2), 12 in Korea (#96 to #107), one in China (#108), and one in Taiwan (#109) between 1986 and 2001, and have been maintained thereafter.

Sexing of the wild fish: Phenotypic sex was judged from secondary sex characters, namely, the shape of the dorsal and anal fins, and papillary processes on the male anal fin rays. Genotypic sex, XY or XX, was determined by the presence or absence of the *DMY* gene using PCR from caudal fin clip DNA extracted according to Shinomiya *et al*. (1999). PCR was performed with the following primers for *DMY* and *DMRT1*: PG17.5, CCGGGTGCCCAAGTGCTCCCGCTG, and PG17.6, GATCGTCCCTCCACAGAGAAGAGA at an annealing temperature of 55°C. PCR products were analyzed by electrophoresis in a 1% agarose gel. Other primers for *DMY*, ex3.1, GCAACAGAGAGTTGGATTTACGTCTCA, ex3.2, CTTTTGACTTCAGTTTGACACATCAATG, ex6.1, GTCATTAACACAACGCACAACAACTT, and ex6.2 AAAAACCAGAAGACCCGAGAGGAAG were also used (see Results). The positions of these primers in the *DMY*

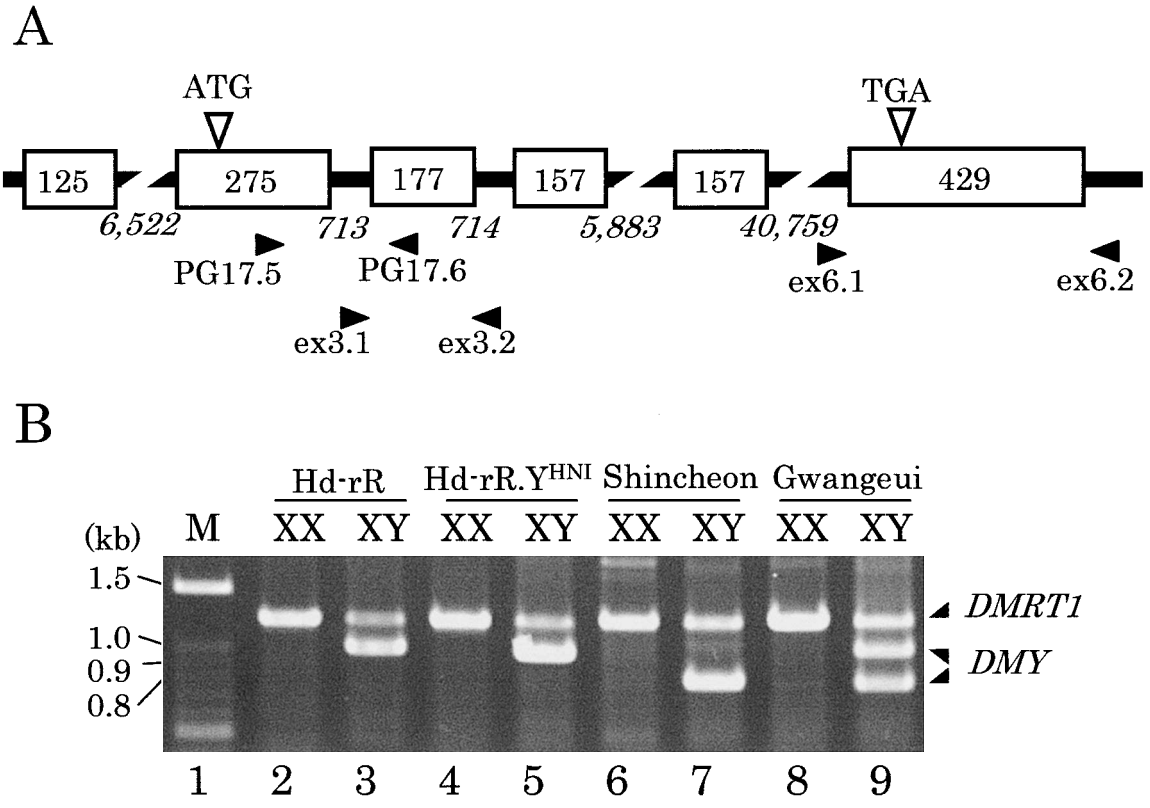

Fig. 1. Genotypic sexing with PCR. (A) *DMY* structure of the Hd-rR.Y^{HNI} strain and positions of the primers used. Open boxes and horizontal lines indicate exons and introns respectively. Translation start and stop sites are shown by ATG and TGA. Numbers represent nucleotide sequence length (bp). The primer positions are illustrated by solid arrowheads. **(B)** One percent agarose gel electrophoresis of the *DMY* and *DMRT1* PCR products. Individuals with only the *DMRT1* band were judged XX (lanes 2, 4, 6, 8), while those with both *DMRT1* and *DMY* bands were judged XY (lanes 3, 5, 7, 9).

Table 1. Phenotypic and genotypic sexes of the wild-caught medaka

	Collection site	Total	Female		Male		Population[1)]
			XX	XY	XX	XY	
1 .	Nakazato, Aomori Pref.	16	8	0	0	8	N
2 .	Aomori1, Aomori Pref.	80	43	0	0	37	N
3 .	Aomori2, Aomori Pref.	75	35	2	0	38	N
4 .	Ikawa, Akita Pref.	38	21	0	0	17	N
5 .	Nikaho, Akita Pref.	100	54	0	0	46	N
6 .	Kisakata, Akita Pref.	68	39	0	0	29	N
7 .	Tsuruoka, Yamagata Pref.	12	6	0	0	6	N
8 .	Aizu-wakamatsu, Fukushima Pref.	102	48	2	0	52	N
9 .	Aizu-bange, Fukushima Pref.	6	4	1	0	1	N
10 .	Shibata, Niigata Pref.	120	56	0	0	64	N
11 .	Kajikawa, Niigata Pref.	100	41	2	0	57	N
12 .	Toyosaka, Niigata Pref.	15	9	0	0	6	N
13 .	Sasakami, Niigata Pref.	61	41	0	0	20	N
14 .	Niigata1, Niigata Pref.	138	77	0	0	61	N
15 .	Niigata2, Niigata Pref.	67	33	0	1	33	N
16 .	Maki, Niigata Pref.	15	7	0	0	8	N
17 .	Shirone, Niigata Pref.	295	151	2	7	135	N
18 .	Ojiya2, Niigata Pref.	3	2	0	0	1	N
19 .	Kashiwazaki, Niigata Pref.	10	5	0	0	5	N
20 .	Arai, Niigata Pref.	100	43	0	0	57	N
21 .	Itoigawa, Niigata Pref.	10	5	0	0	5	N
22 .	Inawashiro, Fukushima Pref.	65	38	0	0	27	N
23 .	Suzu, Ishikawa Pref.	5	1	1	0	3	N
24 .	Wajima, Ishikawa Pref.	10	5	0	0	5	N
25 .	Noto, Ishikawa Pref.	10	5	0	0	5	N
26 .	Awara, Fukui Pref.	128	85	1	0	42	N
27 .	Kanazu, Fukui Pref.	17	9	0	0	8	N
28 .	Tsuruga, Fukui Pref.	95	50	0	0	45	N
29 .	Mito1, Ibaraki Pref.	98	45	0	0	53	S
30 .	Mito2, Ibaraki Pref.	39	21	0	1	17	S
31 .	Oarai, Ibaraki Pref.	81	34	0	0	47	S
32 .	Nagareyama, Chiba Pref.	24	12	0	0	12	S
33 .	Akishima, Tokyo Pref.	10	7	0	0	3	S
34 .	Toda, Saitama Pref.	10	5	0	0	5	S
35 .	Odawara Kanagawa Pref.	112	54	0	0	58	S
36 .	Inuyama, Aichi Pref.	28	18	0	0	10	S
37 .	Maibara, Shiga Pref.	12	7	0	0	5	S
38 .	Kitakyushu, Fukuoka Pref.	9	4	1	1	3	S
39 .	Onga, Fukuoka Pref.	63	25	0	0	38	S
40 .	Mageshima, Kagoshima Pref.	27	21	0	1	5	S
		2274	1174	12	11	1077	

[1)] N, Northern Population; S, Southern Population (Sakaizumi *et al.*, 1983; Sakaizumi, 1986).

Table 2. Phenotypic and genotypic sexes of the wild stocks

	Collection site	Total	Female		Male		Population[1)]
			XX	XY	XX	XY	
41 .	Higashidori, Aomori Pref.	10	5	0	0	5	N
42 .	Honjo, Akita Pref.	10	5	0	0	5	N
43 .	Teradomari, Niigata Pref.	10	5	0	1	4	N
44 .	Ojiya1, Niigata Pref.	9	6	0	0	3	N
45 .	Hamochi, Niigata Pref.	10	5	0	0	5	N
46 .	Aikawa, Niigata Pref.	5	3	0	0	2	N
47 .	Inawashiro, Fukushima Pref.	10	5	0	0	5	N
48 .	Kurobe, Toyama Pref.	34	8	6	0	20	N
49 .	Nanao, Ishikawa Pref.	10	5	0	0	5	N
50 .	Hanamaki, Iwate Pref.	10	5	0	0	5	S
51 .	Kesennuma, Miyagi Pref.	10	3	2	0	5	S
52 .	Sendai, Miyagi Pref.	10	5	0	0	5	S
53 .	Mooka, Tochigi Pref.	10	5	0	0	5	S
54 .	Kawachi, Tochigi Pref.	10	5	0	0	5	S
55 .	Setagaya, Tokyo Pref.	10	5	0	0	5	S
56 .	Hatoyama, Saitama Pref.	10	5	0	0	5	S
57 .	Atsugi1, Kanagawa Pref.	7	1	0	2	4	S
58 .	Atsugi2, Kanagawa Pref.	7	5	0	0	2	S
59 .	Odawara, Kanagawa Pref.	10	5	0	0	5	S
60 .	Hakone, Kanagawa Pref.	11	5	0	0	6	S
61 .	Fuji-yoshida, Yamanashi Pref.	10	5	0	0	5	S
62 .	Fuji, Shizuoka Pref.	10	4	0	0	6	S
63 .	Saori, Aichi Pref.	10	5	0	0	5	S
64 .	Hikone, Shiga Pref.	10	2	0	0	8	S
65 .	Toba, Mie Pref.	15	9	0	0	6	S
66 .	Kumano, Mie Pref.	10	5	0	0	5	S
67 .	Maizuru, Kyoto Pref.	10	5	0	0	5	N
68 .	Ine, Kyoto Pref.	10	5	0	0	5	N
69 .	Tango, Kyoto Pref.	10	5	0	0	5	S
70 .	Amino, Kyoto Pref.	10	6	0	0	4	S
71 .	Kumihama1, Kyoto Pref.	10	5	0	0	5	H
72 .	Kumihama2, Kyoto Pref.	10	5	0	0	5	H
73 .	Hikami, Hyogo Pref.	10	6	0	0	4	S
74 .	Kinosaki, Hyogo Pref.	10	5	0	0	5	H
75 .	Toyooka1, Hyogo Pref.	10	5	0	0	5	H
76 .	Toyooka2, Hyogo Pref.	20	10	0	0	10	H
77 .	Hamasaka, Hyogo Pref.	10	5	0	0	5	H
78 .	Saigo, Shimane Pref.	15	7	3	0	5	S
79 .	Sakurae, Shimane Pref.	10	5	0	0	5	S
80 .	Miyoshi, Hiroshima Pref.	10	5	0	0	5	S
81 .	Iwakuni, Yamaguchi Pref.	10	5	0	0	5	S
82 .	Hohoku, Yamaguchi Pref.	10	5	0	0	5	S
83 .	Aki, Kochi Pref.	10	4	1	0	5	S
84 .	Kamiura, Ehime Pref.	10	5	0	0	5	S
85 .	Kashima, Saga Pref.	10	5	0	0	5	S
86 .	Ashibe, Nagasaki Pref.	10	5	0	0	5	S
87 .	Saiki, Oita Pref.	10	5	0	0	5	S
88 .	Saito, Miyazaki Pref.	10	5	0	0	5	S
89 .	Kushima, Miyazaki Pref.	10	4	0	0	6	S
90 .	Oura, Kagoshima Pref.	10	3	2	0	5	S
91 .	Sato, Kagoshima Pref.	9	4	0	0	5	S
92 .	Kikai, Kagoshima Pref.	10	5	0	0	5	S
93 .	Ogimi, Okinawa Pref.	3	2	0	0	1	S
94 .	Ishikawa, Okinawa Pref.	10	5	0	0	5	S
95 .	Ginoza, Okinawa Pref.	10	5	0	0	5	S
96 .	Samsan, Korea	10	5	0	0	5	W
97 .	Daebu, Korea	10	5	0	0	5	W
98 .	Paltan, Korea	9	3	0	0	6	W
99 .	Guhang, Korea	10	5	0	0	5	W
100 .	Shincheon, Korea	10	5	0	0	5	W
101 .	Gwangeui, Korea	37	21	0	1	15	W
102 .	Suncheon, Korea	10	5	0	0	5	W
103 .	Jinseo, Korea	9	4	0	0	5	E
104 .	Jisan, Korea	10	5	0	0	5	E
105 .	Heunghae, Korea	10	3	0	0	7	E
106 .	Yeoncho, Korea	7	5	0	0	2	E
107 .	Sokcho, Korea	10	5	0	0	5	E
108 .	Kunming, China	9	5	0	0	4	W
109 .	Ilan, Taiwan	4	3	0	0	1	W
		730	351	14	4	361	

[1)] N, Northern Population; S, Southern Population; H, Hybrid Population between N and S; W, China-West Korean Population; E, East Korean Population (Sakaizumi, 1986; Sakaizumi, 1984; Sakaizumi and Joen, 1987; Sakaizumi *et al.*, 1983).

gene are shown in Fig. 1A.

Progeny tests: All XY females from the wild populations were mated with XY males of an inbred strain, from either Hd-rR (Hyodo-Taguchi, 1996) or Hd-rR.Y^{HNI} (Matsuda *et al*., 1998). The F_1 progeny from each pair were grown, and their phenotypic and genotypic sexes were determined. The *DMY* gene of the Northern Population had 21 nucleotide deletions in intron 2 compared to the Southern Population. Therefore, XY females from the Northern Population were mated with XY Hd-rR males (Southern Population), and those from the Southern Population were mated with XY Hd-rR.Y^{HNI} males, which had the HNI(Northern Population)-derived *DMY* gene. Four genotypes were distinguished in the F_1 progeny (XX, XY^m, XY^p, Y^mY^p; Y^m, maternal and Y^p, paternal) by separating the *DMY* PCR products with 10% vertical polyacrylamide slab gels in the buffer system of Davis (1964).

RESULTS

Genotypic sexing of wild populations

Since the nucleotide sequence of *DMY* is similar to that of *DMRT1*, many PCR primers can be used on both genes. For genotypic sexing, we used PG17.5 and PG17.6 primers and judged individuals with only the *DMRT1* fragments to be XX (Fig. 1B, lane2, 4, 6, 8) and those with both *DMY* and *DMRT1* fragments to be XY (Fig. 1B, lane3, 5, 7, 9). We also used two other *DMY* primer sets, ex3.1 and ex3.2 or ex6.1 and ex6.2, when males had only the *DMRT1* fragments (XX males). In all such cases, no *DMY* fragments were detected (data not shown).

The PCR *DMY* products in most individuals were identified in the 1.0 kb vicinity on the 1% agarose gel, though the fragments were approximately 0.8 kb long in fish from Shincheon (#100 in Table 2) in Korea, and Kunming (#108) in China. In Gwangeui (#101), Korea, two *DMY* bands of 1.0 kb and 0.8 kb were identified (Fig. 1B).

The genotypic sex of the natural populations was investigated using 2274 wild-caught fish (Table 1). Of the females, 1174 (99.0%) were XX, while 12 (1.0%) from Aomori2 (#3), Aizu-wakamatsu (#8), Aizu-bange (#9), Kajikawa (#11), Shirone (#17), Suzu (#23), Awara (#26), and Kitakyushu (#38) were XY. Of the males, 1077 (99.0%) were XY, while 11 (1.0%) from Niigata2 (#15), Shirone (#17), Mito2 (#30), Kitakyushu (#38), and Mageshima (#40) were XX.

The 69 wild stocks investigated contained 11 stocks from the Northern Population, 38 from the Southern Population, six from the Hybrid Population (Sakaizumi, 1984), nine from the East Korean Population, and five from the China-West Korean Population (Table 2). XY females were identified in five stocks, Kurobe (#48), Kesennuma (#51), Saigo (#78), Aki (#83) and Oura (#90). While, XX males were found in 3 stocks, Teradomari (#43), Atsugi1 (#57) and Gwangeui (#101). A total of 14 XY females and 4 XX males were found in the wild stocks.

Progeny of the XY females

To clarify the cause of the XY sex-reversals, a total of 16 XY sex-reversals from 11 localities were mated with XY males of an inbred strain, either Hd-rR (females from #3, #8, #9, #17, #23, #26, and #48), or Hd-rR.Y^{HNI} (#51, #78, #83, and #90). The genotypic and phenotypic sexes of the F_1 progeny were then analyzed (Table 3). All XX individuals in the F_1 progeny from each pair were female and all XY^p and Y^mY^p were male, while all or some of the XY^m were female. The XY^m F_1 from Aomori2 (#3), Aizu-bange (#9), Suzu

Table 3. Genotypic and phenotypic sexes of the F1progeny from the XY females

Collection site[1)], and ID no. of XY female		Total	XX		XY^m		XY^p		Y^mY^p	
			Female	Male	Female	Male	Female	Male	Female	Male
3 .	Aomori2, 001	106	11	0	37	0	0	32	0	26
3 .	Aomori2, 002	92	21	0	27	0	0	25	0	19
8 .	Aizu-wakamatsu, 034	101	34	0	16	5	0	19	0	27
8 .	Aizu-wakamatsu, 066	114	33	0	22	4	0	28	0	27
9 .	Aizu-bange, 001	53	10	0	14	0	0	18	0	11
17 .	Shirone, 001	92	26	0	18	10	0	27	0	11
23 .	Suzu, 001	58	13	0	10	0	0	22	0	13
26 .	Awara, 001	86	26	0	23	0	0	16	0	21
48 .	Kurobe, 002	29	8	0	7	0	0	6	0	8
51 .	Kesennuma, 002	70	19	0	4	13	0	12	0	22
78 .	Saigo, 001	55	12	0	13	0	0	18	0	12
78 .	Saigo, 002	75	13	0	22	0	0	23	0	17
78 .	Saigo, 003	29	8	0	11	0	0	3	0	7
83 .	Aki, 001	78	21	0	19	0	0	13	0	25
90 .	Oura, 001	62	21	0	13	0	0	28	0	0
90 .	Oura, 002	26	5	0	6	0	0	15	0	0

[1)] The numbers of the collection sites correspond to those in Tables 1 and 2.

(#23), Awara (#26), Kurobe (#48), Saigo (#78), Aki (#83) and Oura (#90) were all female, while the XY^m F_1 of Aizuwakamatsu (#8), Shirone (#17) and Kesennuma (#51) contained both males and females. In all crosses, the occurrence of XY sex-reversals in the F_1 progeny was linked to the maternal Y chromosome, while the paternal Y chromosome derived from the inbred strains resulted in all F_1 male individuals (XY^p, Y^mY^p).

DISCUSSION

In the present study, we conducted a broad survey of the genotypic sex of a total of 3004 wild medaka from 109 localities covering the four genetically divergent groups of this species (Sakaizumi, 1986; Sakaizumi and Joen, 1987; Sakaizumi *et al*., 1983). Of the 1540 males, 1525 (99%) had the *DMY* gene (XY), while of the 1464 females, 1438 (96%) did not (XX). This intimate relationship between male and female gender and the presence or absence of the *DMY* gene strongly suggests that *DMY* is a common sex-determining gene in wild medaka populations.

Multiple fragment patterns were identified in the PCR products of *DMY*. In most populations a long fragment was detected, while individuals from Shincheon (#100) and Kunming (#108) had a short fragment. Fifteen out of 16 males from Gwangeui (#101) had both fragment types (Fig. 1B) (one male had no *DMY* fragments), indicating that the Gwangeui population had two *DMY* gene on the Y chromosome. The short fragment was only detected in populations classified in the China-West Korean Population. Furthermore, molecular phylogenetic analysis of the *DMY* gene among the four major medaka groups demonstrated that the two types of *DMY* from the China-West Korean Population were most closely related. In XY males from Gwangeui with two types of *DMY*, only the short type was expressed at the sex-differentiating stage (Sato *et al*., in preparation).

The frequency of sex-reversed fish in wild-caught medaka was 1.0% (23 out of 2274, Table1), while 18 sex-reversals were found in eight of the 69 wild stocks (Table 2). All XY sex-reversals (n=16) produced XY females in their F_1 progeny, demonstrating that all these sex-reversals resulted from a gene mutation involved in sex-determination. This suggests that XY sex-reversal resulted from environmental factors hardly occurs in natural habitats. All XY F_1 females had maternally-derived *DMY* genes, indicating that the mutation was linked to the *DMY* gene. The mutant gene is inferred in *DMY* itself because *DMY* is the only functional gene in the Y-specific segment (Nanda *et al*., 2002).

Two types of XY sex-reversals existed with regards to the sex of the XY F_1 progeny. Twelve out of 16 XY females produced an all-female XY^m progeny, while four yielded both male and female XY^m progenies. One XY female from Awara that produced an all-female XY^m progeny (#26 in Table3) was found to have a *DMY* mutation causing a frameshift and premature termination of the *DMY* protein. Another XY female from Shirone that yielded a high proportion of XY^m female offspring (#17) had reduced *DMY* expression (Matsuda *et al*., 2002). These results suggest that the XY females found in this study are a promising tool for gaining an understanding of the biochemical function and regulatory regions of the *DMY* gene, just as *SRY* mutants have in human studies (reviewed in Koopman, 2001; McElreavey *et al*., 1995; Vilain and McCabe, 1998). The sequences and expression patterns of the *DMY* gene of other XY sex-reversals are now under investigation.

In humans, approximately 15% of XY females have mutations in the coding region of *SRY* (Hawkins, 1995), and XY sex-reversals with mutations in other genes related to testis development have also been reported (reviewed in Ahmed and Hughes, 2002; Vilain and McCabe, 1998). On the other hand, all XY sex-reversals in medaka were linked to the *DMY* gene. A loss-of-function *DMY* mutation is thought to induce dominant sex-reversal in XY individuals, and furthermore, to be transmitted from XY females to their offspring because XY medaka females are fertile (Yamamoto, 1953) unlike mammals. *DMY* mutations might not be harmful and thus survive in natural habitats and wild stocks.

Fifteen XX males were observed in this survey. One of these XX males produced XX males in its backcross progeny, suggesting that XX sex-reversal results from a recessive mutation(s). We are now mapping the gene(s) that causes XX sex-reversal, which is expected to be involved in sex-determination.

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