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# Bilaterally Asymmetrical Testes in Fishes of the Genus *Oryzias*

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**ABSTRACT**—From a comparative morphological viewpoint, we examined the development of gonads of 9 species of teleost fish, genus *Oryzias*, in which three types of bilateral asymmetry were discriminated. The first type occurs in *O. celebensis*, *O. javanicus*, *O. marmoratus*, *O. mekongensis*, *O. melastigma*, and *O. minutillus*, in which testes are completely asymmetrical. Their gonadal rudiments are constructed only on the right side and develop into testes of a unilobate form. The second type is exhibited in *O. curvinotus* and *O. luzonensis*, where gonadal rudiments appear unilateral only on the right side. But testicular tissue occurs on the left side of the dorsal mesentery during the development, and adult testes become bilobate. The testes of *O. latipes* belong to the third type. They initiated from bilateral gonadal rudiments to develop into bilobate testes. These results almost correspond with the phylogenetical classification of *Oryzias* fishes based on the chromosome morphology, indicating that alleles of the gene(s) controlling the symmetry of gonads in these fish originated just after the time when the phylogenetic differentiation occurred. The possible process of the development of the various types of the asymmetrical gonads was discussed.

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## INTRODUCTION

Vertebrate has bilaterally symmetrical body form in principal, however, all organs do not always develop symmetrically. For example, heart is situated on the left and liver on the right. Recently, evidence for bilaterally asymmetrical gene expression preceding any gross morphological manifestation of the left-right axis has been reported in chick (Levin *et al.*, 1995) and mouse (Meno *et al.*, 1996; Collingnon *et al.*, 1996; Lowe *et al.*, 1996). However, little information is available regarding the initial left-right determinant.

Avian gonad has been known to be one of such bilaterally asymmetrical organs; only the left gonad becomes functional and the right becomes atrophied to be rudimentary. From the fact that removal of the left gonads in the developing chicks induced the compensatory development of the right, the endocrinological interactions between gonads on the both sides are involved in the gonadal asymmetry in birds (Haffen, 1977). But it is not known how only the left is determined to develop into a functional ovary.

In the previous report (Hamaguchi, 1983), we described the asymmetrical development of gonads in the fish, *Oryzias celebensis*, in which gonadal rudiments are formed only on the right side of the dorsal mesentery. We also found that the developmental processes of the asymmetrical gonads in *O. celebensis* are quite different from those of avian gonads.

The development of fish gonads has been investigated morphologically in various species (Johnston, 1951),

extensively in *Oryzias latipes* (Gamo, 1961; Satoh and Egami, 1972; Hamaguchi, 1982; Kanamori *et al.*, 1985). Gonads are composed of germ cells and somatic cells which originate from coelomic epithelial cells. Primordial germ cells originate in the laterally extraembryonic region, and migrate to the area of the presumptive dorsal mesentery through lateral plate mesoderm. Then, a morphogenetic activity operates to open the body cavity, into which germ cells surrounded with somatic cells protrude from dorsal mesentery to develop into gonadal rudiments. In *Oryzias latipes*, gonadal rudiments are formed on the both side of the mesentery, whereas only on the right side in *O. celebensis* (Hamaguchi, 1983). There is no information about the mechanism by which the gonads in *O. celebensis* develop only on the right side and do not on the left.

About a dozen of species of fishes in genus *Oryzias* are known to inhabit the south-east Asia, and the phylogenetic relation among these species has been investigated (Sakaizumi, 1985a, b; Uwa *et al.*, 1983; Uwa, 1986; Uwa and Parenti, 1988; Uwa *et al.*, 1988). It has also been clarified that *O. latipes* includes four genetically distinct groups, the Northern population, the Southern population, the East Korean population and the China-West Korean population (Sakaizumi *et al.*, 1983; Sakaizumi, 1986; Sakaizumi and Jeon, 1987). From a comparative morphological viewpoint, we examined the development of gonads of 9 species of *Oryzias* fishes to know that all fishes except *O. latipes* have unilateral gonadal rudiments. The intraspecific difference among groups of *O. latipes* was also noted in the bilateral distribution of the germ

cells. In the present article, we will describe the observations on the asymmetry of gonads in *Oryzias* fishes, and discuss on the possible mechanism controlling the asymmetry of the gonads of these fishes.

## MATERIALS AND METHODS

Fishes used were those from the stocks of wild populations of *Oryzias* species. The locations at which founder fish of these stocks were collected is shown in Fig. 1. The stocks used are enumerated below. In parentheses, the names of institutes at which each stock has been maintained are indicated.

*O. latipes*: The Northern population: 1, Niigata (Univ. Tokyo); 2, Himi (Niigata Univ.); and 3, Maiduru (Niigata Univ.). The Southern population: 4, Nagashima (Tokyo Univ.); 5, Tottori (Tokyo Univ.); 6, Sanyo (Tokyo Univ.); 7, Hisayama (Tokyo Univ.); 8, Hiwaki (Tokyo Univ.); 9, Gushikami (Tokyo Univ.); and *d-rR* strain (Niigata Univ.). The East Korea population: 10, Yangbuk (Niigata Univ.); 11, Maam (Niigata Univ.); 12, Sunchon (Niigata Univ.); and 13, Chinso (Niigata Univ.). The China-West Korea population: 14, Puyong (Niigata Univ.); 15, Beijing (Tokyo Univ.); 16, Shanghai (Tokyo Univ.); and 17, Kunming (Niigata Univ.). *O. curvinotus*: 18, Hong Kong (Niigata Univ.). *O. luzonensis*: 19, Solsona (Niigata Univ.). *O. mekongensis*: 20, Yang Talat (Shinshu Univ.). *O. celebensis*: 21, Ujung Pandang (Aichi Univ. Educ.). *O. marmoratus*: 22, Timampu (Niigata Univ.). *O. minutillus*: 23, Bangkok (Shinshu Univ.). *O. javanicus*: 24, Jakarta (Aichi Univ. Educ.). *O. melastigma*: 25, Chidambaran (Aichi Univ. Educ.).

Fish were reared in an air-conditioned room at  $27\pm 2^\circ\text{C}$  with a long photoperiod (14-hr light, 10-hr dark). Naturally spawned eggs were collected and kept at  $27\pm 2^\circ\text{C}$ . Newly hatched fry were fixed in Bouin's solution *in toto*, and used for microscopic observations. Fixed materials were embedded in paraffin and serial transverse sections were made. From careful observations on all the serial sections, the numbers of germ cells in gonads on the right and the left sides of the dorsal mesentery were determined. Testes from adult male fish of these species were also fixed to be served for the histological observations.

## RESULTS

### *Oryzias latipes*

In *Oryzias* fishes, females initiate oogenesis just before hatching; oogonia begin to proliferate actively and oocytes at meiotic prophase begin to appear. While, male germ cells are at a mitotically dormant stage for 10 days after hatching. Consequently, the germ cell number in the female is about twice as large as that in the male at hatching (Sato and Egami, 1972). The sex of the fry can be determined by the absence of oocytes as well as the total number of germ cells. In the present study the total number of germ cells and their bilateral distributions in the male were examined.

The gonads in *O. latipes* developed on the both sides of the dorsal mesentery, though slightly larger on the right (Fig. 2a). In Fig. 3, the numbers of germ cells per fish and the percentage of the germ cell on the right side are shown. About 50–75% of germ cells were on the right side in the Northern, the Southern and the East Korean populations. In the China-West Korean population the asymmetrical distribution was more conspicuous, and 75–100% of germ cells were situated on the right side. The difference between the China-West Korean population and other populations of *O. latipes* was significant (t-test,  $p < 0.001$ ). In the China-West Korean population, fish of Kunming had the most asymmetrical gonads.

The total number of germ cells in most fish was within the range from 50 to 125, though rather variable among individuals. Fish from Sunchon of the East Korean population contained a markedly large number of germ cells. In females of Sunchon, presumptive ovaries contained much more germ cells, some of which were at the early stage of meiotic prophase, that is, the distinction between male and females was conspicuous. A detailed analysis of the kinetics of the germ cell proliferation in the male of the Sunchon medaka is now in progress.

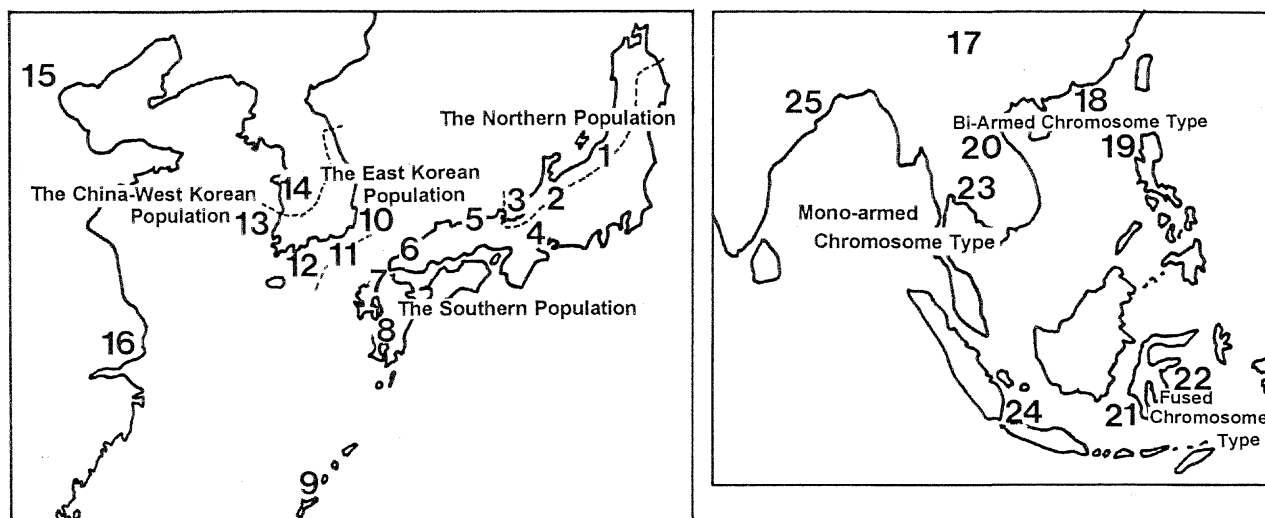


Fig. 1. Geographic distribution of *Oryzias* species examined. Numbers are explained in Materials and Methods.

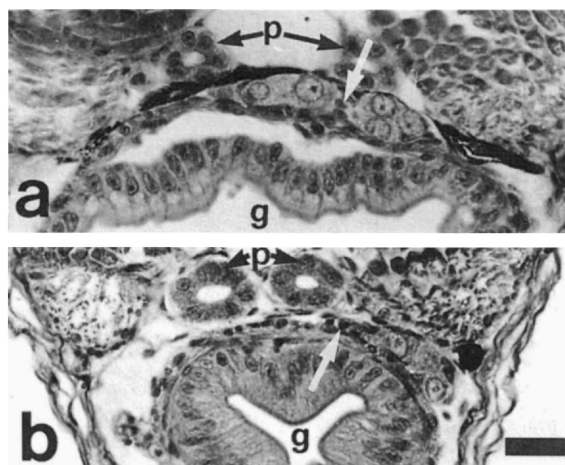


Fig. 2. Sections of bilobed and unilobed gonadal rudiments in the fry just after hatching. a, bilobed type (*Oryzias latipes*, Mauduru). b, unilobed type (*O. marmoratus*). g, gut; p, pronephric duct. White arrows, dorsal mesentery. Bar represents 20  $\mu$ m.

The testes of adult fish of these 4 populations were also examined on their transverse sections. The efferent duct was situated at the dorsal mesentery, on the both sides of which spermatogenic tissues could be observed (Fig. 4a).

#### Other *Oryzias* species

The fry of *O. curvinotus*, *O. luzonensis*, *O. mekongensis*, *O. celebensis*, *O. marmoratus*, *O. javanicus*, *O. minutillus*, and *O. melastigma* were fixed just after hatching and examined histologically. Observations revealed that the gonadal rudiments developed only on the right side of the dorsal mesentery in all these fishes (Fig. 2b). The results of germ cell counting on serial sections are shown in Table 1. In all *Oryzias* species except *O. latipes*, the gonads started to develop from a completely unilateral form.

The total number of germ cells in most fish was within the range from 50 to 125. In all fry of *O. mekongensis* at hatching oocytes could be observed in their gonads, therefore it was impossible to distinguish males from female by the absence of oocytes. The data of *O. mekongensis* in Table 1 designate the germ-cell numbers of all fish counted including males and females. Oocytes disappeared within 10 days in the gonads of about a half of fish which are considered to be male.

The complete serial sections of testes from the adult fish were made and examined by microscopy. The testes of *O. latipes* were almost bilaterally symmetrical (Fig. 4a). *O. curvinotus* and *O. luzonensis* had bilobed and asymmetrical testes though their gonadal rudiments were unilateral. The left testes in adult fish of these species were distinctly smaller than the right, but they do fulfil their function of producing sperm (Fig. 4b). The testes in adult fish of other species were unilobed; spermatogenic tissue was situated only on the right side of the dorsal mesentery where the efferent duct was there (Fig. 4c).

#### Inter- and intra-specific hybrids

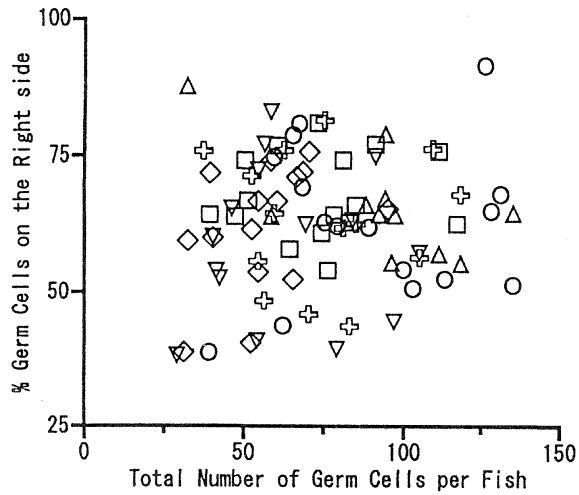
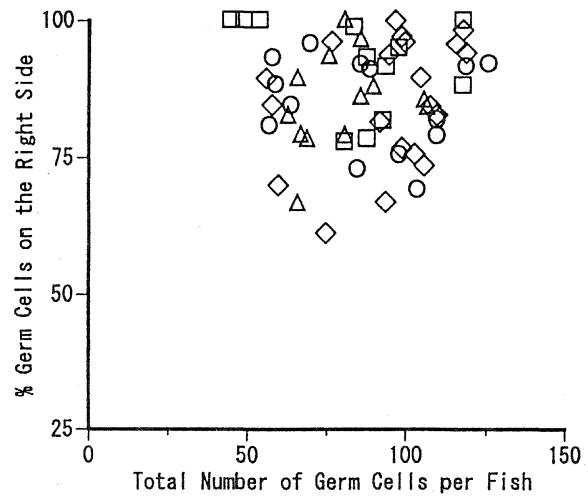
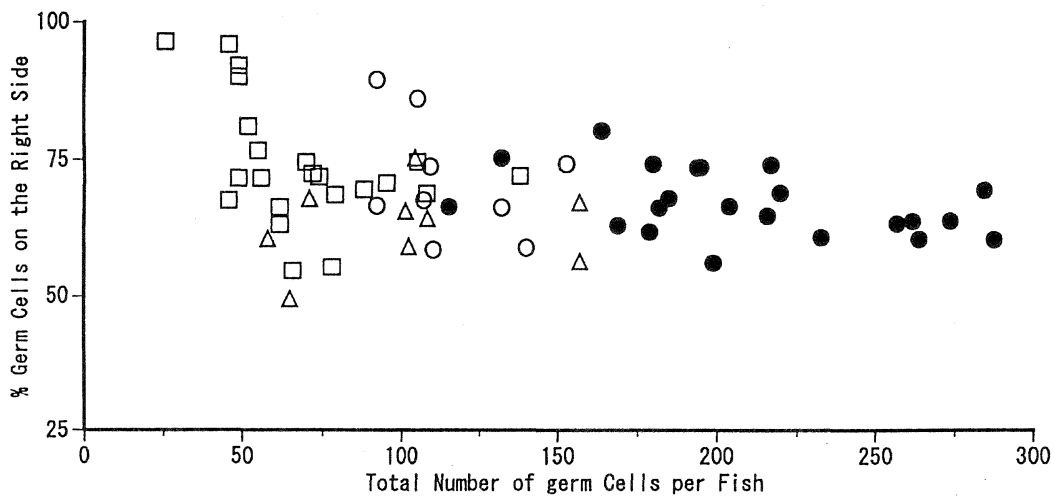
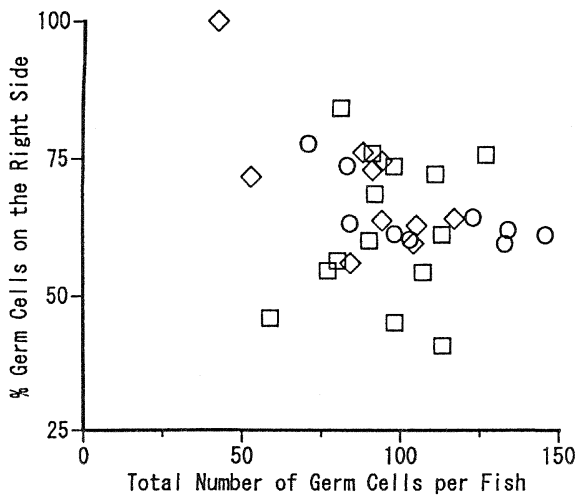
Inter- and intra-specific hybrid fish were made and the bilateral symmetry of the distribution of germ cells in the fry just after hatching was examined (Table 2). In all hybrid fish gonadal rudiments developed on the both sides of the dorsal mesentery, but the degree of bilateral asymmetry was varied according to the species used for crossing. In *O. latipes-celebensis*, the percentage of the germ cell number on the right side was almost the same as that in *O. latipes*. On the other hand, in *O. latipes-curvinotus* and *O. curvinotus-latipes*, the percentage of the germ cell number on the right side showed almost the intermediate values between those of their parents. The intra-specific cross between the China-West Korea population and the Southern population of *O. latipes* also produced fry with intermediately asymmetrical gonads.

## DISCUSSION

From the present study, three types of bilateral asymmetry can be discriminated in the testes of *Oryzias* fishes. The first type occurs in *O. celebensis*, *O. javanicus*, *O. marmoratus*, *O. mekongensis*, *O. melastigma*, and *O. minutillus*, in which testes are completely asymmetrical. Their gonadal rudiments are constructed only on the right side and develop into testes of a unilobed form. The second type is exhibited in *O. curvinotus* and *O. luzonensis*, where gonadal rudiments appear unilateral only on the right side. But during the development of fry, testicular tissue occurs on the left side of the dorsal mesentery, and adult testes of these fishes become bilobed. The testes of *O. latipes* belong to the third type. They initiated from bilateral gonadal rudiments to develop into bilobed testes.

Based on the analysis of the morphology of chromosomes, Uwa (1986) classified *Oryzias* fishes into three groups, mono-armed chromosome type (*O. javanicus*, *O. melastigma*, *O. minutillus*), bi-armed chromosome type (*O. mekongensis*, *O. curvinotus*, *O. luzonensis*, *O. latipes*) and fused chromosome type (*O. celebensis*, *O. marmoratus*). The grouping of *Oryzias* species examined in the present study almost correspond to Uwa's phylogenetical classification of *Oryzias* fishes. Except for *O. mekongensis* with bi-armed chromosomes, fishes of mono-armed and fused chromosome types had unilateral testes, whereas, the testes of fishes with bi-armed chromosomes were bilateral with various degrees of asymmetry.

In fishes of bi-armed chromosome type, *O. curvinotus* and *O. luzonensis* can be discerned from *O. latipes* from their unilateral gonadal rudiments. By the analysis of the phylogeny of fishes of bi-armed chromosome type using molecular evolutionary methods, the close relationship between *O. curvinotus* and *O. luzonensis* is demonstrated (Matsuda and Sakaizumi, in preparation). The present study also showed that fishes of the China-West Korean population in *O. latipes* had extremely asymmetrical gonadal rudiments, and could be discriminated from those of other populations. It is known that fishes of the China-West Korean population have a

**(A) The Southern Population****(B) The China-West Korean Population****(C) The East Korean Population****(D) The Northern Population**

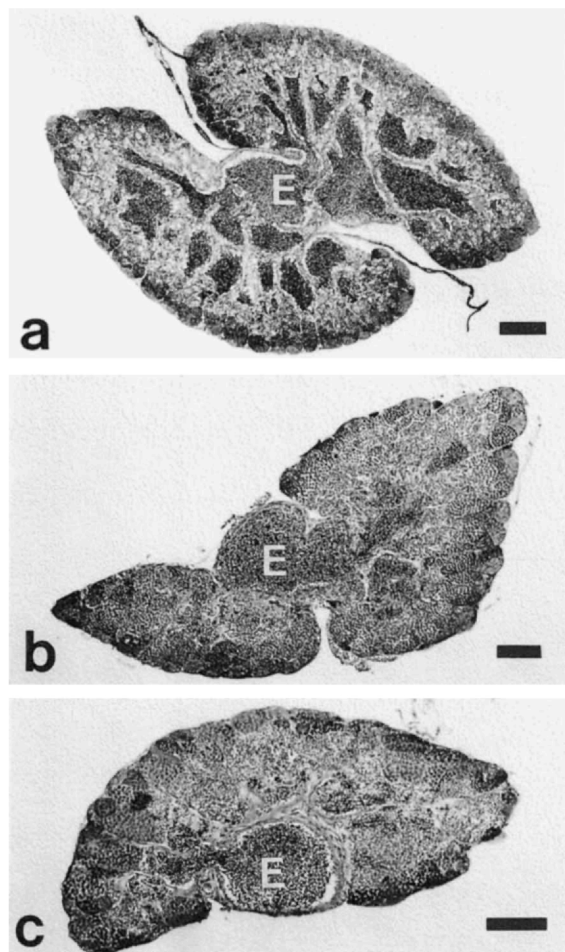


Fig. 4. Sections of testes from the adult fish of three *Oryzias* species. a) An almost symmetrical bilateral testis of *O. latipes* (*d-rR*). Bar: 200  $\mu\text{m}$ . b) An asymmetrical bilateral testis of *O. luzonensis*. Bar: 100  $\mu\text{m}$ . c) A unilateral testis of *O. marmoratus*. Bar: 100  $\mu\text{m}$ . E, efferent duct.

chromosome number and morphological characteristics different from those of other populations of *O. latipes* (Uwa and Jeon, 1987).

The correspondence between phylogenetic classification of *Oryzias* fishes and the grouping by the bilateral asymmetry of testes suggests that genetic mechanisms controlling the symmetry of the gonads began to differ among these fishes about the time when the phylogenetic differentiation occurred among fishes of mono-armed, bi-armed and fused chromosome types. The only exception in fishes of bi-armed chromosome type was *O. mekongensis*, that has a unilateral

testis. It is obscure how to evaluate this discrepancy.

The present results lead us to the hypothesis that two steps of developmental process are involved in the manifestation of the asymmetry in *Oryzias* gonads. The first step determines whether gonads are bilateral or unilateral. In this sense, fishes of mono-armed and fused chromosome types are considered to be unilateral, and fishes of bi-armed chromosome type are bilateral. The fact that gonads in *O. latipes-celebensis* were bilateral and that the percentage of the germ cells on the right side was almost the same with *O. latipes*, suggests that the bilateral is dominant over the unilateral. The second step is involved in the bilateral distribution of germ cells at the formation of the gonad. In the present observations, the gonads in *O. curvinotus* and *O. luzonensis* were unilateral at their rudimentary stage, but develop into bilateral testes. This fact may be interpreted by the following considerations: the gonadal architecture of these fish is potentially bilateral, but the bilateral distribution of germ cells is extremely asymmetrical and the left gonads remain empty at the rudimentary stage. By the consequence of the overgrowth of germ cells on the right side, some germ cells enter into the empty left gonads, and the left lobe of the testes begins to develop. In the fish of the unilateral gonad group the left gonad is originally absent, so germ cells cannot overflow over the dorsal mesentery.

In fishes of bi-armed chromosome group, the various extents of asymmetry of germ cell distribution were observed. In hybrids between fishes of the bi-armed chromosome group, the degree of the asymmetry of germ cell distribution was shown to be intermediate between their parental fishes. These facts imply that the bilateral distribution of germ cells is controlled by polygenes.

In order to verify the hypothesis, a detailed morphological investigation as to the difference between the unilateral gonadal rudiments in fishes of mono-armed chromosome group and those in fishes of bi-armed chromosome group, and the study on the bilateral symmetry of the gonadal rudiments of the hybrid fish among fishes with unilateral testes are now in progress.

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Fig. 3. Total numbers of germ cells per fish just after hatching and percentages of germ cells on the right side. Each dot designated individual fish. (A) The Southern population.  $\nabla$ :Nagashima,  $\oplus$ :Tottori,  $\diamond$ :Sanyo,  $\triangle$ :Hisayama,  $\circ$ :Hiwaki,  $\square$ :Gushikami. (B) The China-West Korean population.  $\circ$ :Puyong,  $\diamond$ :Beijing,  $\triangle$ :Shanghai,  $\square$ :Kunming. (C) The East Korean population.  $\square$ :Yangbuk,  $\circ$ :Maam,  $\bullet$ :Sunchon,  $\triangle$ :Chinso. (D) The Northern population.  $\square$ :Niigata,  $\diamond$ :Himi,  $\circ$ :Maiduru.

Table 1. Bilaterally asymmetrical distribution of germ cells in *Oryzias* fishes just after hatching

	No. of fish examined	Total no. of germ cells per fish	% of germ cells on the right
<i>Oryzias latipes</i>			
Southern Population	87	74.3±26.8	63.3±11.9
Northern Population	33	96.5±22.9	65.2±11.8
East Korean Population	62	130.7±70.6	69.2± 9.8
without Sunchon	40	87.2±33.6	70.5±11.1
Sunchon	22	209.7±47.4	66.7± 6.0
China/West Korean Population	59	87.7±20.6	86.6± 9.8
<i>O. curvinotus</i>	12	62.3±20.5	100
<i>O. luzonensis</i>	6	37.0± 6.3	100
<i>O. mekongensis</i>	28	162.7±80.0	100
<i>O. celebensis</i>	8	69.1±29.1	100
<i>O. marmoratus</i>	15	40.9±13.4	99.0
<i>O. javanicus</i>	6	112.2±22.4	100
<i>O. minutillus</i>	10	85.3±30.2	99.9
<i>O. melastigma</i>	4	115.8±47.6	100

Table 2. Bilaterally asymmetrical distribution of germ cells in *Oryzias* fishes and their inter- and intra-specific hybrids

	No. of fish examined	Total no. of germ cells per fish	% of germ cells on the right
<i>Oryzias latipes</i>			
Southern Population			
<i>d-rR</i> strain	10	77.4±14.7	60.3±11.4
Nagashima	15	63.0±23.1	58.7±14.2
Northern Population			
Niigata	14	95.5±17.9	61.9±13.2
China/West Korean Population			
Beijing	20	94.4±19.4	85.6±11.4
<i>O. celebensis</i>			
Ujung Pandang	8	69.1±29.1	100
<i>O. curvinotus</i>			
Hong Kong	12	62.3±20.5	100
Interspecific Hybrids			
<i>O. latipes-celebensis</i>			
<i>d-rR</i> X Ujung Pandang	13	56.1±13.8	62.0±12.2
<i>O. latipes-curvinotus</i>			
Nagashima X Hong Kong	14	44.5±15.6	84.8±11.8
Niigata X Hong Kong	8	89.5±20.7	94.4± 3.7
<i>O. curvinotus-latipes</i>			
Hong Kong X Nagashima	10	88.7±22.8	76.2±17.9
Hong Kong X Niigata	13	89.0±16.8	80.1±11.9
Intraspecific Hybrids			
Beijing X Nagashima	7	106.1±19.7	68.9± 7.2
Nagashima X Beijing	11	84.0±21.0	72.0±11.6

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