Fisheries Science 65(5), 736–741 (1999)

Live Haploid-diploid Mosaic Charr *Salvelinus leucomaenis*

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(Received April 2, 1999)

A live haploid-diploid mosaic charr *Salvelinus leucomaenis*, in which 89% of erythrocytes were unusually small, was found by chance. This individual was externally normal in shape and grew to the adult size (335 mm body length, 324 g body weight). Flow cytometry for DNA content revealed that large population of cells in blood, liver, and spleen were haploid, whereas those in brain were diploid. Thus, this individual was concluded to be a mosaic consisted of two types of cells with different ploidies. These results suggested that haploid cells may have normal function in the mosaic environment including diploid cells. Histological observation showed that this mosaic charr was a female with ovaries filled with degenerating and degenerated oocytes which showed various abnormalities and was likely to lack normal reproductive ability, even if it had survived longer.

Key words: haploid, mosaic, charr, *Salvelinus*

Haploid individuals have been produced in many fish species by the technique of induced gynogenesis[1–14] and androgenesis.[1,3,15–18] These studies have shown that haploids die during embryonic development before or soon after hatching or early larval stages before the beginning of feeding, due to characteristic abnormalities collectively referred to as "haploid syndrome" including edema, ascites, dwarfing, microphthalmia, and microcephaly. However, a very few viable haploids were observed in the induced gynogenesis of tilapine fish *Oreochromis mossambicus*, but they exhibited severe deformities such as caudal fracture and were unable to swim, feed, and escape from predators.[19] Similar results have been seen in amphibian species. Most haploids reveal abnormal development and die sooner or later,[20–22] but a very few haploids can survive for long time beyond metamorphosis[20–22] and reach maturation.[22] Thus, viable haploidy is considered to be possible, but exceptional in lower vertebrates.

On the other hand, normal and viable mosaics including haploid cells have been reported in fish,[23] frogs,[24] and birds.[25] These suggest that haploid organs may become viable and functional in a diploid environment. This has been further evidenced in frogs by the artificial production of viable haploid-diploid longitudinal chimeras which consisted of haploid and diploid halves.[26,27] Haploid newts were reared beyond the time of metamorphosis and some attained their sexual maturity by means of parabiosis with normal diploids.[28]

In June, 1997 at a fish farm managed by one of the authors (the late K. Kawakami) in Kochi Prefecture, we happened to find an externally normal but cytologically mosaic charr *Salvelinus leucomaenis* which included large population of unusually small erythrocytes together with small number of normal ones. This putative haploid-diploid mosaic individual is quite interesting material to elucidate whether the viability of haploid fish was related with the existence of diploid cells. However, this individual became inert in October, 1998. As the mosaic charr was in critical condition, it was killed to examine ploidy status and biological characteristics together with a normal diploid charr as control. This paper presents details of the mosaic ploidy levels flow-cytometrically estimated by DNA content in erythrocytes and various tissues including liver, spleen, and brain of this specimen. Histological observation of the gonad was also conducted to conclude reproductive capacity of this mosaic charr.

Materials and Methods

Specimens

Two specimens of Japanese charr *Salvelinus leucomaenis* (a putative mosaic and a normal diploid control #1, both about 25 cm in total length) were obtained from the fish farm (Aburabire Kennkyujo) managed by one of the authors (the late K. Kawakami), in Yusuhara Town, Kochi Prefecture. They had been reared in the pond until October 26, 1998, when the mosaic was in the critical stage and thus killed for the present study. The other normal diploid charr (control #2) was also obtained from the same place in October 29, 1998 as the diploid standard in flow cytometry. The exact origin and rearing history of these specimens were unknown because of the sudden death of

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the farm manager (the late K. Kawakami).

Erythrocyte Measurements

Blood samples were obtained from the caudal vein of the mosaic and control #1 charr and blood smears were fixed on slides with methanol and stained with Giemsa. Blood smear slides were photographed and numbers of normal-sized and small-sized erythrocytes were counted on the printed photograph. Major diameter of erythrocytic cells was measured using the video-recorder equipped micrometer (VM-60, Olympus Co. Ltd.).

Flow Cytometry for DNA Content

Brain, gills, heart, liver, spleen, digestive tract, muscle and blood were removed from the mosaic and the control #2 and separately taken into the micro tubes with Eagle's MEM medium (Nissui). Then, they were transported to Laboratory of Aquaculture, Faculty of Applied Biological Science, Hiroshima University, Higashi-hiroshima, Hiroshima Prefecture and measured by FACS Calibur flow cytometer (Beckton-Dickinson) after preparation according to the method described by Zhang and Arai.[29]

Egg Measurements and Histology

The ovary of the putative mosaic was fixed with 10% neutralized formalin and embedded in paraffin. Sections (10 μm) were stained with Delafield's hematoxylin-eosin. A part of fixed eggs were measured for diameter using Nikon Profile Projector Model DP-302 (Nihon Kogaku, Co. Ltd.).

Results

The putative haploid-diploid individual was not different from normal charr in body shape (Fig. 1, Table 1). No abnormalities were observed in external appearence and inner structure.

In blood smear preparation, two types of erythrocytes, small and normal, were seen (Fig. 2). The ratio between small- and normal-sized erythrocytes was 4974 (89.3%): 593 (10.7%) in a randomly chosen area on the smear slide.

Table 1. Measurements of mosaic and normal specimens of charr, *Salvelinus leucomaenis*

Measurements	Mosaic	In % HL	Normal (#2)	In % HL
Total length (TL)	38.5 cm	513.3	38.0 cm	513.5
Body length (BL)	33.5 cm	446.7	33.5 cm	452.7
Head length (HL)	7.5 cm	100.0	7.4 cm	100.0
Snout length (SL)	2.0 cm	26.9	1.9 cm	25.7
Body depth (BD)	4.6 cm	59.3	5.4 cm	73.0
Body width (BWI)	3.0 cm	40.0	3.3 cm	44.6
Body weight (BW)	324.00 g	—	326.00 g	—
Liver weight (LW)	2.50 g	—	2.50 g	—
Gonad weight (GW)	2.10 g*1	—	0.20 g*2	—
GSI*3	0.60%	—	0.06%	—

*1 ovaries;
*2 testes;
*3 gonad somatic index, GW/BW × 100.

Major diameter of normal erythrocytes (mean 16.09 ± standard deviation 1.30 μm, $n=35$) of the putative mosaic was not significantly different from that (15.52 ± 0.93 μm, $n=250$) of the normal diploid, whereas that of small erythrocytes (10.95 ± 0.93 μm, $n=215$) of the mosaic was significantly smaller than the normal erythrocytes observed in the diploid charr.

Of tissues flow-cytometrically assayed, blood, liver, spleen, and brain gave clear histograms of DNA content, but gills, heart, muscle, and digestive tract did not, probably due to the presence of debris in samples. In blood, liver, spleen, and brain sample, the normal diploid charr (#2) exhibited prominent peaks at the same channel numbers (Fig. 3a, c, e, g). In contrast, the mosaic charr showed a prominent peak at 1C and a very low peak at 2C (Fig. 3b), when the DNA content of erythrocytes from the normal charr was 2C (Fig. 3a). This indicates that the mosaic individual includes a very large population of haploid erythrocytes and a small population of diploid erythro-

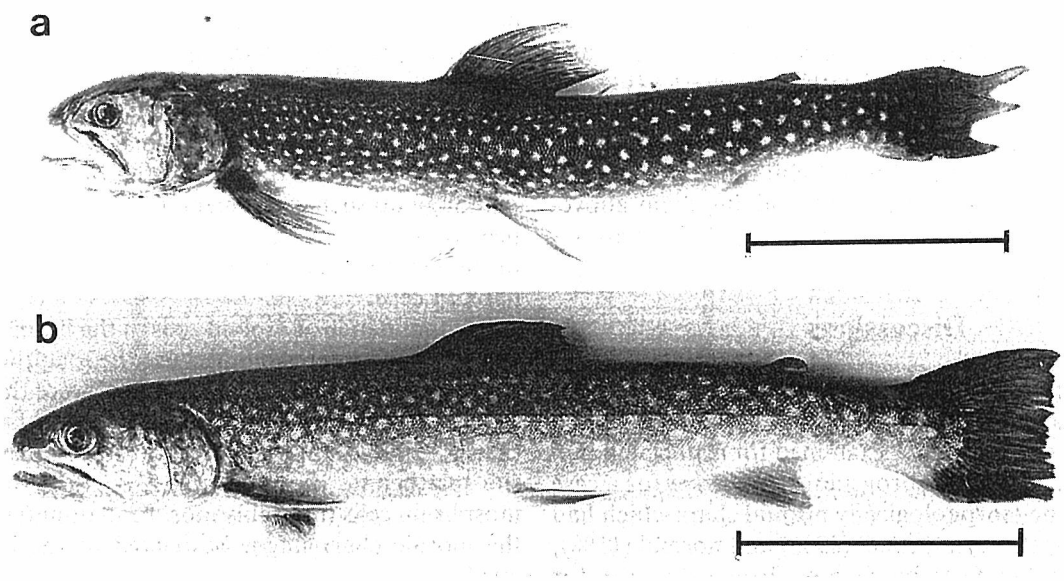

Fig. 1. External appearance of haploid-diploid mosaic (a) and normal control individual (b) of the Japanese charr *Salvelinus leucomaenis*. Scale indicates 10 cm.

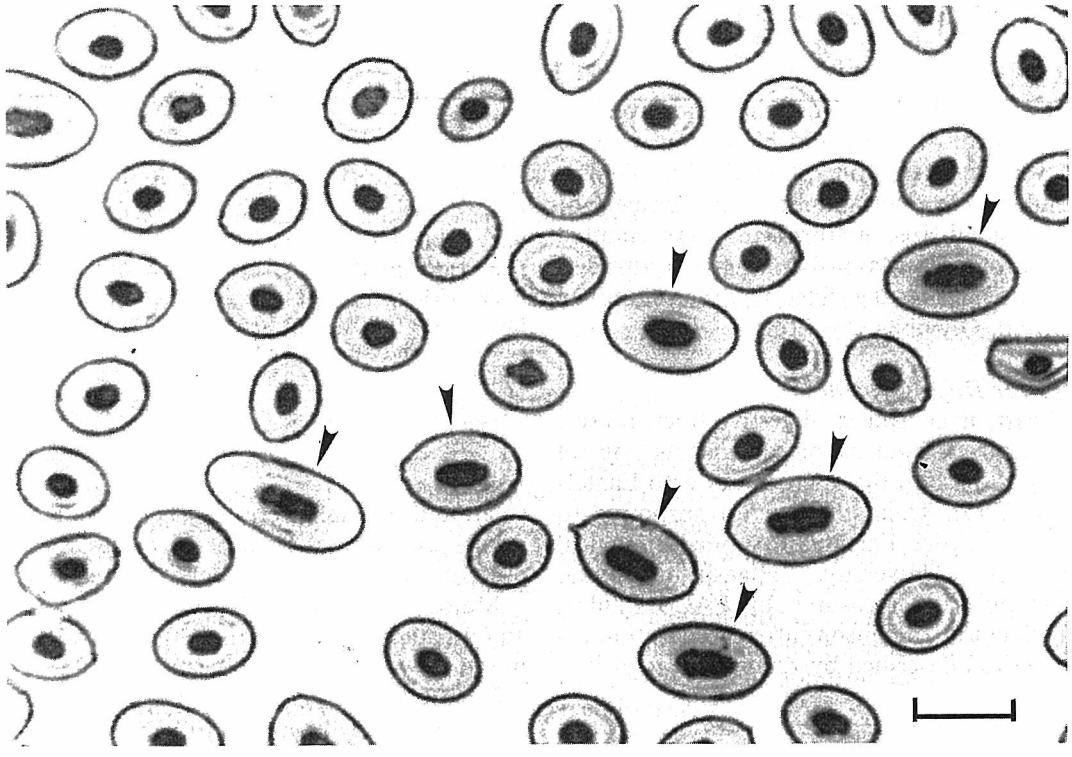

Fig. 2. Two types of erythrocytes, small and normal (arrow heads), observed in the putative haplo-diploid mosaic charr. Scale indicates 10 μm.

cytes, as predicted from the observation of blood smear slides. Similar results were also observed in spleen and liver samples, both of which exhibited predominance of haploid cells (Fig. 3d, f). On the other hand, most cells from the brain of the mosaic individual were diploidy, as observed in normal control diploid charr (Fig. 3g, h). A very small population of haploid cells was flow-cytometrically observed in the brain of this mosaic individual.

The mosaic charr was a female with low GSI value (Table 1). This fish had ovaries which contained not only growing oocytes at the perinucleolus and the yolk vesicle stages, but also degenerating oocytes with hypertrophied granulosa cells, vacuolation of yolk, and zona radiata broken into fragments (Fig. 4). Degenerated oocytes with empty and atretic follicles were also found within the ovaries (Fig. 4). Average diameter of eggs was 0.63 mm ($n=100$, range 0.07 to 1.42 mm) and two modes of egg diameter were seen at 0 to 0.2 mm range and 1.2 to 1.4 mm range (Fig. 5). No fully matured oocytes were present, judging from abovementioned histological observation and egg size measurements.

Discussions

There is a consensus that haploids cannot develop beyond the larval stages after the beginning of feeding and are inviable in teleosts, so far examined for artificial induction of gynogenesis and androgenesis,[1-18] except for a case in Tilapia.[19] The morphologically normal charr which had two types of erythrocytes, small (89%) and normal (11%), was found in a fish farm by chance. Frow cytometry for DNA content in this individual revealed that a large population of cells in blood, liver, and spleen were haploid, but those in the brain were diploid. These results showed that this fish was a live haploid-diploid mosaic with a great predominance of haploidy at least in the blood, liver, and spleen.

Presence of such a live haploid-diploid mosaic shows that diploid cells, tissues, or parts act as prophylatic and haploid blood, liver, spleen, and probably other cells have normal function in the mosaic environment including small populations of diploid cells. Drastic improvement of the poor viability of haploid individuals by addition of diploid tissues has been evidenced in amphibian species by successful production of viable longitudinal diploid-haploid chimeras[26,27] and viable haploids experimentally united in parabiosis with diploid individual.[28] Kashiwagi and Kashiwagi[27] concluded that the better development of diploid-haploid chimeras might be due to the removal of haploid syndromes such as edema owing to the existence of diploid tissues in the anterior half of the frog embryos. Although no such experimental-embryological works have been done in teleosts, it is very likely that the diploid cells or tissues may be removing harmful effects caused by haploid cells.

Predominance of diploid cells in the brain of the mosaic charr is quite interesting because the longitudinal chimera frogs which showed the best survival were those produced by exchanging the anterior part of haploid embryos with that of diploid embryos after cutting transversely at the site just behind the pronephros.[27] Thus, the diploidy of most brain cells (probably most head or anterior region) of the mosaic charr might be related to the improved survival.

The present haploid-diploid charr was a female and its ovaries were filled with a considerable number of degener-

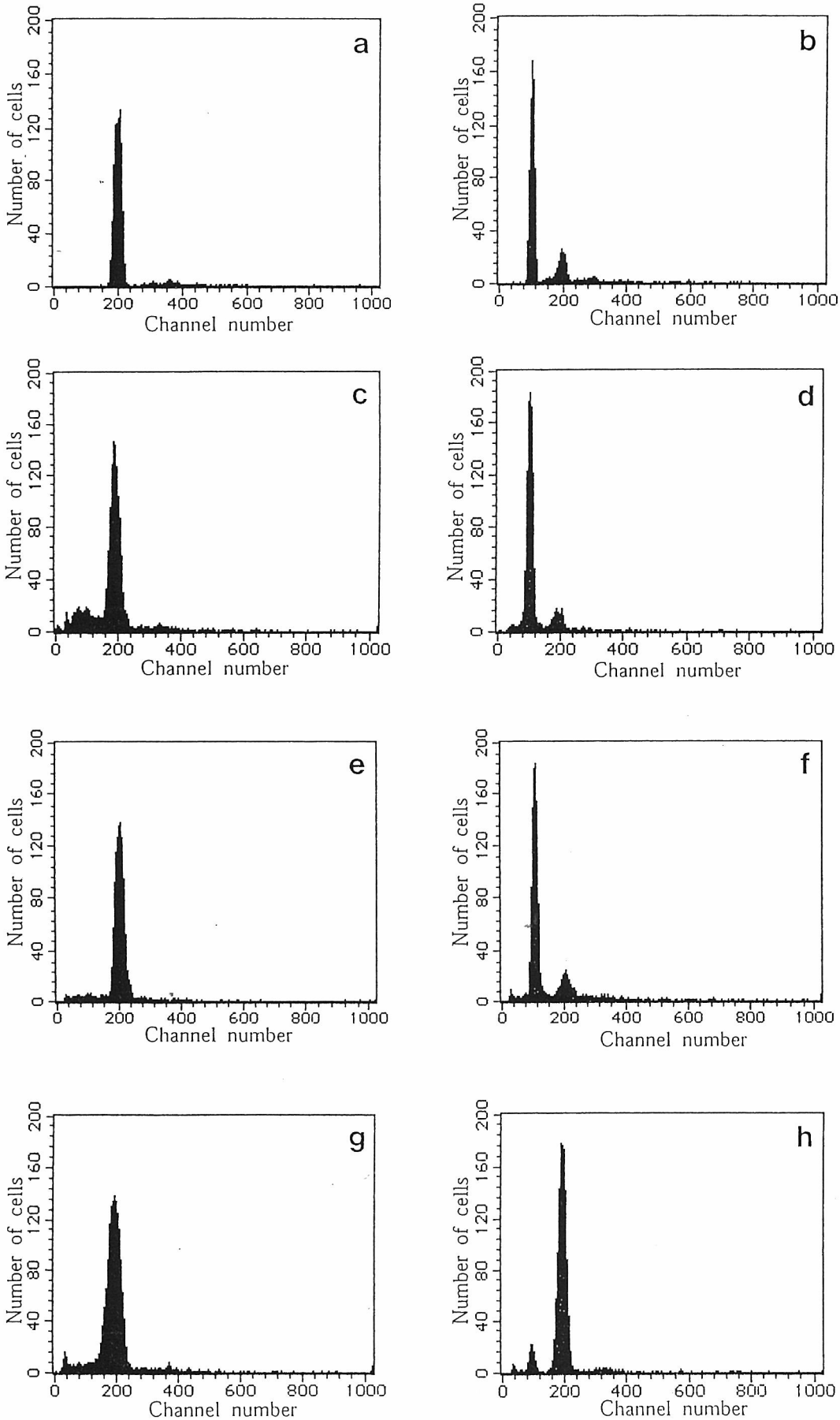

Fig. 3. Flow cytometric histograms for DNA content of blood, liver, spleen, and brain samples (from top to bottom) of the normal control (a, c, e, g) and the mosaic charr (b, d, f, h).

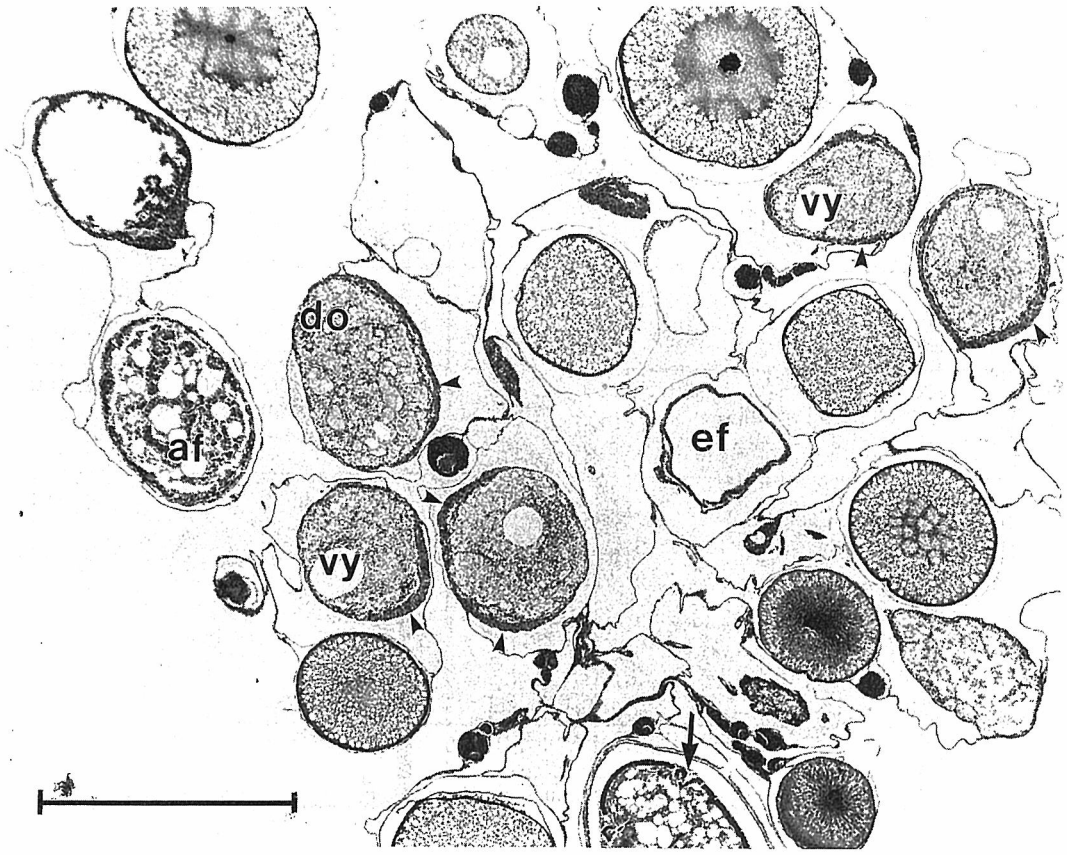

Fig. 4. Cross section of ovaries of the haploid-diploid charr.
do, degenerating oocytes; ef, empty follicle; af, atretic follicle; vy, vacuolation of yolk. Arrow heads indicate hypertrophied granulosa cells. Arrow denotes zona radiata broken into fragments. Scale indicates 1000 μm.

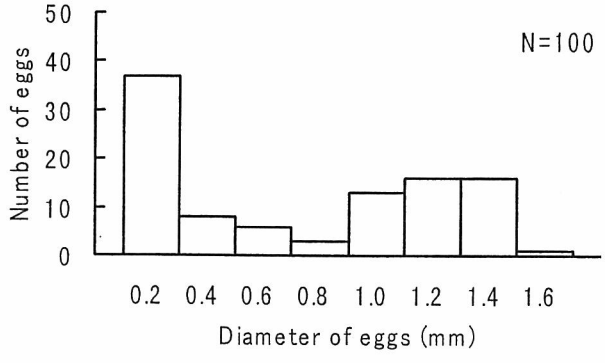

Fig. 5. Distribution of egg diameters of the haploid-diploid mosaic charr.

ating and degenerated oocytes together with large number of growing oocytes which contained no yolk globules. The diameters of oocytes measured in this mosaic were distributed between 0.07 and 1.42 mm (average 0.63 mm) and are smaller than the normal size of mature eggs (average diameter 4.3 mm) reported in the Japanese charr.[30] The GSI (0.6%) of the present mosaic female is lower than the average GSI (18.1%, $n=21$) measured in the Japanese charr females with similar average body weight (476.9 g).[30] The fact described above seemed to show that all the oocytes degenerate in the ovaries of mosaic charr, before vitellogenesis begins in them. Morphological abnormalities of oocytes were often observed in the young stage of oogenesis before or soon after vitellogenesis. Thus, this mosaic charr is likely to have been lacking reproductive ability, even if it had survived longer. Since similar observation in the gonadal development was reported in the three-year-old haploid frog[21] and the anterior diploid-posterior haploid chmera frogs,[27] it is supposed that most oocytes of the mosaic charr might develop from haploid germ cell lines. However, there was a possibility of the occurrence of mature eggs in haploid ovaries of the mosaic charr, because Kashiwagi[22] reported a very rare case wherein the haploid frog laid eggs and the resultant embryos normally cleaved after fertilization with normal sperm but arrested at the late blastula stage. The possibility of normal gametogenesis cannot be excluded in the gonadal development of haploid-diploid mosaic individual, because diploid germ cells of the mosaic are likely to form functional gametes.

Haploid-diploid mosaic may arise by several different mechanisms which have been explained in detail by Miller *et al.*[23] They stated probable involvement of a) meiotic error such as karyogamy followed by independent development of the second polar body leading to generation of both diploid and haploid cells, b) mitotic segregation error involving haploid genome complements which might lead to haploid or diploid cell subpopulations, and c) polyspermy. In chicken, the polyspermy is a normal occurrence and is the most likely origin of haploid cells in the mosaic. Considerably frequent occurrence of mosaicism was reported in certain strains of chicken and the involve-

ment of genetic factors was suggested.[25] However, it was impossible to identify the cause which induced mosaicism including haploidy in the present study, due to the lack of information about the origin, genetic background, and ploidy status of the fish farm strain. Further descriptive and experimental studies on mosaic fish are needed to elucidate the occurrence of mosaicism.

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research (C) to K. A. (No. 10660181) from the Ministry of Education, Science, Sports and Culture, Japan and by that to M. Y. from Towa Food Research Promoting Fund (Towashokuhin Kenkyushinkoukai). We are very grateful to Center for Gene Science, Hiroshima University for providing technical expertise for use of the FACS Calibur flow cytometer. We also thank Messrs. M. Satoh and H. Satoh, Satoh Amago Salmon Farm for their careful rearing of the mosaic charr in the Satoh Amago Farm.

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