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Mottled coloration of haploid-diploid and diploid-triploid mosaic amago salmon *Oncorhynchus masou ishikawae*

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ABSTRACT: Mottled amago salmon *Oncorhynchus masou ishikawe* with both yellow- and dark-pigmented skin occurred together with typical albino individuals in a commercial farm. Out of 12 mottled fish examined by DNA content flow-cytometry and erythrocytic nucleus size, three were diploid, eight were haploid-diploid mosaic and one was diploid-triploid mosaic. This fact indicates that the mottled coloration might link to polyploid mosaicism. Genotype of diploid and non-diploid cells at the albino locus was estimated in nine mature mottled fish by observing the frequency of wild-type and albino progeny when mating to homozygous albino (*aa*). One diploid and three haploid-diploid mosaic mottled fish were presumed to have mosaic genotype with both hemizygous '*a*' and heterozygous '*Aa*' cells (*a/Aa*), because the segregation ratio between two phenotypes was 1 : 1. Three other haploid-diploid mosaic fish were presumed to have mosaic genotype with both hemizygous '*a*' and homozygous '*AA*' cells (*a/AA*), because of exclusive occurrence of wild-type phenotype in the progeny. The diploid-triploid mosaic mottled fish was presumed to have mosaic genotypes '*aa/AAA*', '*aa/AAa*' or '*aa/Aaa*', because this fish yielded only albino progeny. One diploid mottled fish produced both two phenotypes but albino embryos appeared with much more frequency than the expectation, when assuming the genotype '*Aa*'. Thus, this fish was considered to have mosaic genotype '*Aa/aa*'.

KEY WORDS: **albino, amago salmon, haploid, mosaic, mottle, *Oncorhynchus*, triploid.**

INTRODUCTION

In rainbow trout *Oncorhynchus mykiss*, a genetic variation of body color showing yellow phenotype is referred to as an albino and/or a golden, which cannot generate melanin pigment normally.[1] A typical albino phenotype with red eyes was homozygous for recessive albinism gene '*a*',[2] but a golden phenotype with black eyes was determined by incomplete dominant gene '*G*'.[3] In other salmonids, the albinism has been reported in chinook salmon *O. tshawytscha*,[4] brook trout *Salvelinus fontinalis*,[5] lake trout *S. namaycush*[6] and Japanese huchen *Hucho perryi*.[7] In amago salmon *O. masou ishikawae*, two types of albinism were reported; one was a typical albino with red eyes,[8] while the other was an incomplete albino phenotype with lightly dark-pigmented eyes.[9]

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A number of unusual mottled amago salmon exhibiting both yellow and dark skin pigmentation and normal dark eyes were found in the salmon farm where the typical albino had been previously appeared.[8] Such an unique mottled phenotype has also been reported in pink salmon *O. gorbuscha*[10] and rainbow trout.[11,12] The mottled rainbow trout is suggested to be a mosaic that consists of two genetically different tissues. One example was considered a mosaic of golden (*G'G'*) and palomino (*GG'*) genotypes,[11] while the other case was a mosaic of albinism (*aa*) and wild-type (*Aa*) genotypes.[12] For albino-wild-type mosaicism, Galbreath and Plemmons[12] speculated that such a mosaicism might occur due to genetic mutation from wild-type '*A*' allele to albino '*a*' allele at the albinism locus within a cell(s) early in the embryonic development.

However, polyploid mosaicism also can be assumed as a cause of mottled coloration. If a haploid-diploid mosaic comprises both homozygous (*AA*) or heterozygous (*Aa*) diploid cells show-

ing wild-type phenotype and hemizygous (*a*) haploid cells showing albino pigmentation, such a mosaic individual may exhibit mottled coloration. Mosaicism comprising homozygous (*aa*) diploid cells and hemizygous (*A*) haploid cells may express mottled color phenotypes. In salmonids, haploid-diploid mosaic individuals have been reported to appear frequently in a normally fertilized[13–16] and chromosomally manipulated progeny.[17, 18]

In the present study, the relationship between polyploid mosaicism and mottled coloration was investigated in amago salmon with both yellow and dark parts in the body by measuring the cellular DNA content of various organs using flow-cytometry and the erythrocytic nucleus size. In addition, mosaicism of genotypes in the mottled salmon was then estimated based on segregation frequencies of two different phenotypes, wild-type and albino, occurred in the progeny.

MATERIALS AND METHODS

Fish specimens

In the Satoh Amago Salmon Farm (SASF), Seiyo City, Ehime Prefecture, Japan, two races of amago salmon are separately cultured. One is the native race, in which no albino has been found. The other yields typical albino. The latter race was introduced from the Aburabire-Kenkyujo Farm, Yusuhara Town, Kochi Prefecture. In 1997, approximately 100 wild-type and 30 albino individuals of this race were introduced and then reared in the SASF. All the albinos died, but about 60 wild-type individuals survived until maturation. When the surviving wild-type amago salmon were propagated by normal crosses, mottled individuals with both yellow and dark parts in the body appeared together with typical albino fish in the resultant progeny (Fig. 1). In 2000-year-class, three mottled fish were recorded. In 2001-year-class, approximately 20 mottled fish were found. In 2003-year-class, 14 mottled fish appeared in about 400 000 juveniles produced in this farm. These mottled and albino fish were reared to mature for 1 or 2 years. For this study, one wild-type fish (W1), three mottled fish (M1-M3) and two albinos (A1 and A4) in 2000-year-class, two wild-type fish (W2 and W3), nine mottled fish (M4-M12) and six albinos (A2, A3 and A5-A8) in 2001-year-class, and one wild-type fish (W4) in 2002-year-class were used. Wild-type fish used was from the native race that had never yielded albino. Hybrid amago salmon (H1-H4), which had been produced by a cross between wild-type and albino individuals in 2001,[8] exhibited normal coloration.

Body surface area measurement

The area of a lateral body and yellow patch of mottled amago salmon was measured in each side of the body using a video micrometer (VM-60; Olympus, Tokyo, Japan) on the photograph taken.

Ploidy determination and erythrocytic nuclei measurement

Relative DNA content of blood, skin, fin, liver, brain, sperm and eyed-embryos was measured by flow-cytometry using a Ploidy analyzer (PA; Partec GmbH, Münster, Germany). Blood and sperm were fixed with 70% ethylalcohol and 10% neutral formalin, respectively. Skin containing scales, epidermis and dermis was peeled and used for ploidy determination. Skin and fin samples were separately taken in both dark and yellow parts. Sample was homogenized in a microtube (1.5 mL) and then stained with 4′,6-Diamidino-2-phenylindole (DAPI) after unnucleation treatments by using a preparation kit provided by the manufacturer (Partec GmbH). Each sample of a wild-type amago salmon (native race) was used as the diploid standard with 2C value. When polyploid mosaicism was detected in a sample, the proportion of non-diploid cells was calculated based on number of cells counted by the flow-cytometer.

Blood smear preparation was made following a conventional procedure. Major diameter of nuclei was measured in a total of 50–200 erythrocytes from each fish, using a video micrometer (VM-60; Olympus). When unusually small or large erythrocytes were found in a preparation, total erythrocytes in a taken photograph were counted (2000–6432) and the proportion of unusual red blood cells was calculated.

Cross

When mottled fish mated to albino (M × A), crosses between wild-type individuals (W × W) and those between albino individuals (A × A), and those between wild-type and albino individuals (W × A) were carried out at the same time. Hybrid fish were used for preliminary sib mating (H × H) and backcrossing to albino (H × A). Each batch was placed in a separate box within a vertical tray incubator. At the eyed stage (cumulative temperature, 300°C days or more), dead and live eggs in each group were counted. Embryos with pigmented (wild-type) and unpigmented (albino) eyes were distinguished with naked eyes and the frequency of each phenotype was recorded. The

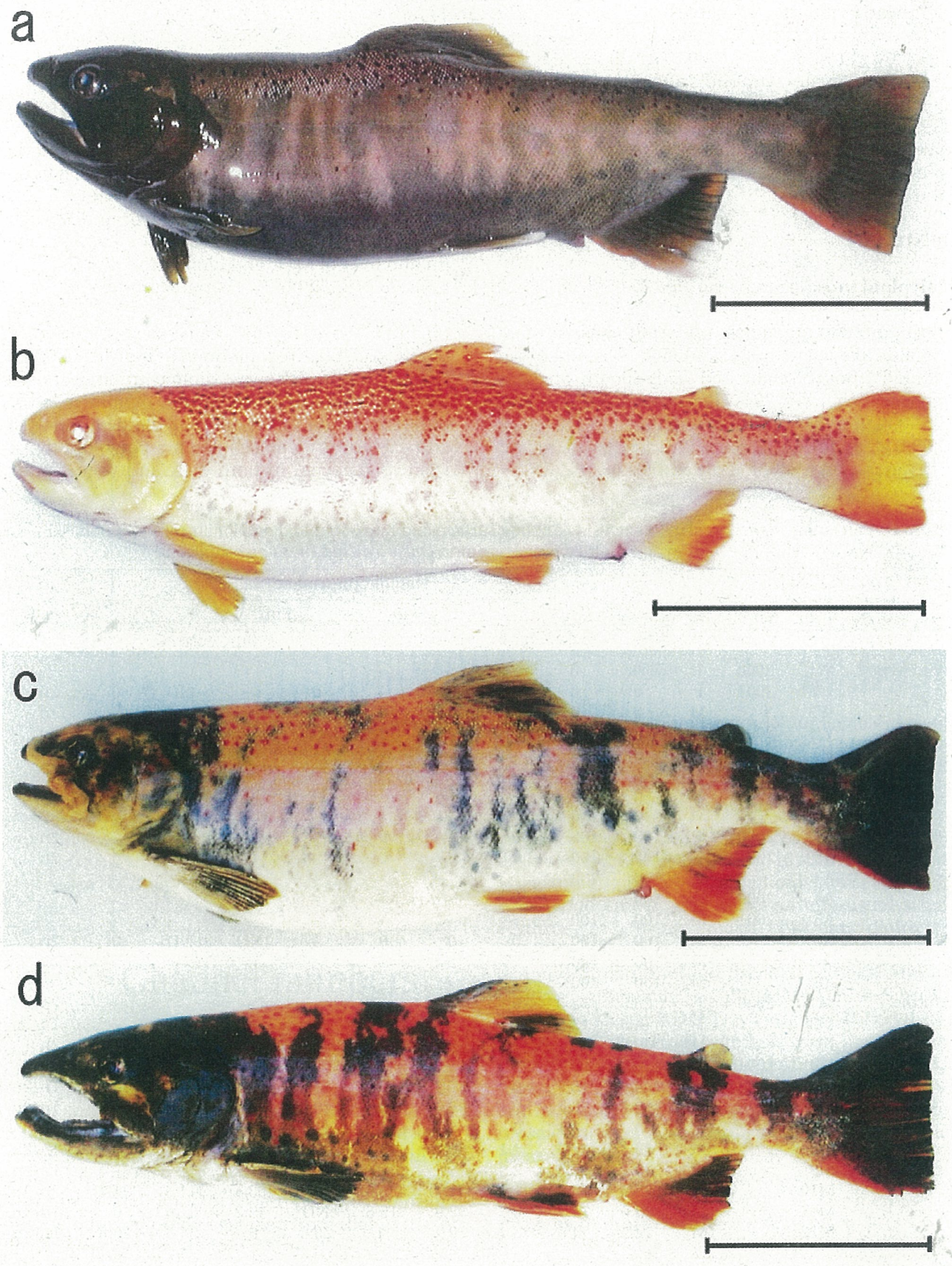

Fig. 1 (a) Wild type, (b) albino and (c,d) mottled amago salmon *Oncorhynchus masou ishikawae*. (c) Upper mottled fish is haploid-diploid mosaic (M6) and (d) lower one is diploid-triploid mosaic (M10). Scale bars indicate 100 mm.

significant difference between expected and observed number of each phenotype was assayed using by χ^2-test (d.f. = 1; $P < 0.05$).

At approximately 7 months after hatch, the sex of the progeny from mottled male was determined by the morphological observation of gonad with naked eyes, and the frequency of female and male in each batch was statistically investigated.

RESULTS

Polyploid mosaicism in mottled fish

In the mottled amago salmon examined, six were female, five were male and one was unknown (Table 1). Body weight and body length of these mottled fish were 284–717 g (mean 561.8 g) and 245–350 mm (314.2 mm) in females, and 113–600 g (353.6 g) and 190–325 mm (261.8 mm) in males. The lateral area of 11 mottled fish was 13 220.0–48 273.9 mm^2 (mean 31 839.7 mm^2). The proportion of yellow skin varied among individuals (3.1–70.7%) and was asymmetric between right and left side of the body in M5, M7, M8, M9, M10, M11 and M12 (Table 1).

Flow-cytometric investigation (Fig. 2) shows that albino (A1, A2 and A6) and three mottled fish (M1, M3 and M7) are diploid (Table 1). However, haploid cells were detected in: blood of M2; liver of M4; skin and fin of M5; blood, skin, fin, liver and brain of M6; blood, skin and brain of M8; blood of M9; skin, fin and liver of M11; and blood, skin, fin, liver and brain of M12 (Table 1). Therefore, these eight mottled fish were determined haploid-diploid mosaic (Fig. 2a). Since triploid cells were detected in blood, skin, fin, liver and brain of mottled fish M10 (Table 1), this fish was diploid-triploid mosaic (Fig. 2b). The proportion of non-diploid cells was different in the individual polyploid mosaic fish and each organ (Table 1). Such a polyploid mosaicism is detected in both dark and yellow parts,

Table 1 Percentage of yellow skin in each side of body surface and rate of haploid or triploid cells in blood, skin, fin, liver and brain of wild-type, albino and mottled amago salmon *Oncorhynchus masou ishikawae*

Pheno-type	Fish no.†	Year-class	Sex‡	Yellow skin (%)		Haploid (or triploid) cells (%)							Ploidy estimate
							Skin		Fin				
				Left	Right	Blood	Dark	Yellow	Dark	Yellow	Liver	Brain	
Wild–type	W1	2000	F	0	0	–	–	–	–	–	–	–	–
	W2	2001	F	0	0	0	0	–	0	–	ND	0	2n
	W3		M	0	0	0	0	–	–	–	–	–	2n
	W4	2002	M	0	0	0	0	–	0	–	ND	0	2n
	H1	2001	F	0	0	–	–	–	–	–	–	–	–
	H2		F	0	0	–	–	–	–	–	–	–	–
	H3		M	0	0	–	–	–	–	–	–	–	–
	H4		M	0	0	–	–	–	–	–	–	–	–
Albino	A1	2000	F	100	100	0	–	0	–	ND	–	–	2n
	A2	2001	F	100	100	0	–	0	–	0	ND	0	2n
	A3		F	100	100	–	–	–	–	–	–	–	–
	A4	2000	M	100	100	–	–	–	–	–	–	–	–
	A5	2001	M	100	100	–	–	–	–	–	–	–	–
	A6		M	100	100	0	0	0	–	ND	ND	0	2n
	A7		M	100	100	–	–	–	–	–	–	–	–
	A8		M	100	100	–	–	–	–	–	–	–	–
Mottle	M1	2000	F	29.0	32.0	0	–	–	–	–	–	–	2n
	M2		F	14.9	15.9	11.6	–	–	–	–	–	–	1n/2n
	M3		U	40.8	37.2	0	–	–	–	–	–	–	2n
	M4	2001	F	–	–	0	0	0	–	–	59.0	0	1n/2n
	M5		F	5.8	16.7	0	5.1	10.7	13.0	0	0	0	1n/2n
	M6		F	69.7	70.7	9.1	36.4	33.1	19.6	39.4	9.9	7.5	1n/2n
	M7		F	0	3.1	0	0	ND	ND	–	ND	ND	2n
	M8		M	13.3	2.6	4.1	50.0	0	–	–	–	21.2	1n/2n
	M9		M	30.7	17.8	2.3	–	–	–	–	–	–	1n/2n
	M10		M	29.3	52.8	(1.7)	(1.7)	(29.6)	(0)	(68.4)	(11.3)	(31.6)	2n/3n
	M11		M	23.0	14.7	0	32.9	15.6	ND	43.2	57.6	0	1n/2n
	M12		M	12.2	4.9	38.7	0	4.9	29.8	0	34.8	26.8	1n/2n

†A, Albino; H, hybrid fish between wild–type and albino; M, mottled; W, wild-type. ‡F, Female; M, male; U, unknown ; ND, no data due to failure of measurement. Parentheses means percentage of triploid cells.

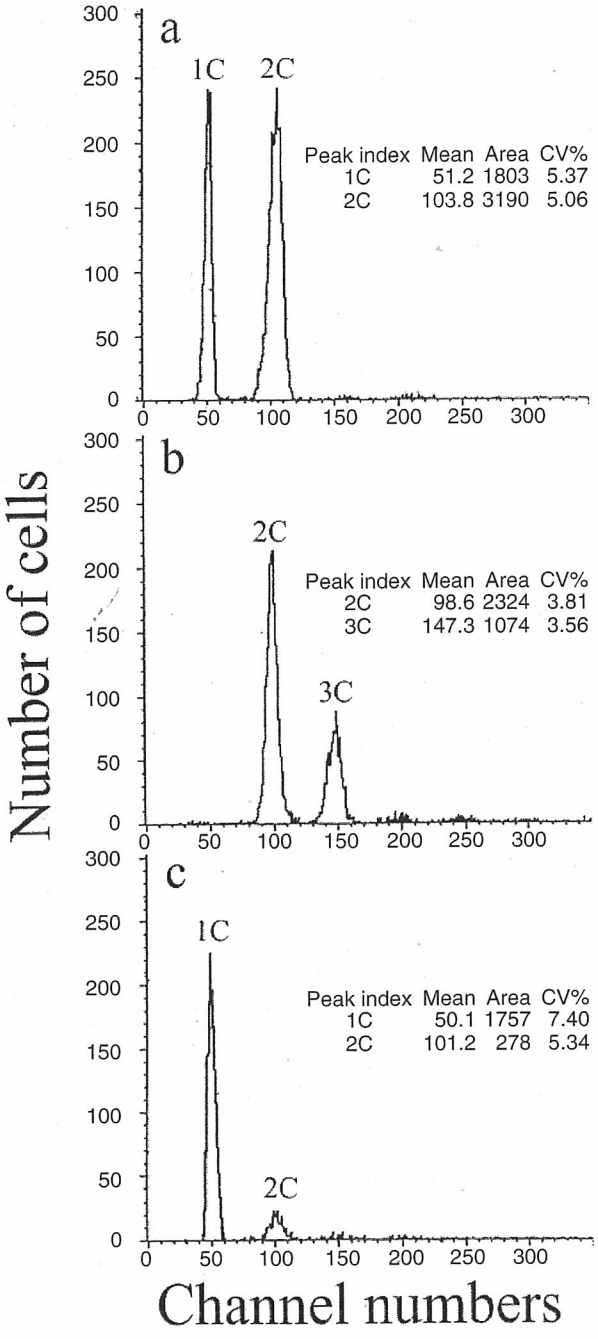

Fig. 2 DNA content of (a) erythrocytes in M12, and (b) liver and (c) sperm in M10 mottled amago salmon *Oncorhynchus masou ishikawae.*

separately taken from skin and fin of M6, and skin of M5, M10 and M11. Dark part taken from skin of M8 and fin of M5 and M12 and yellow part taken from fin of M10 and M11 were polyploid mosaic but the other colored part taken from that of these fish was exclusively diploid (Table 1).

Relative DNA content of sperm taken from haploid-diploid mosaic male M8, M11 and M12 and diploid-triploid mosaic male M10 showed a peak at 1C, exhibiting presence of haploid sperm (Fig. 2c).

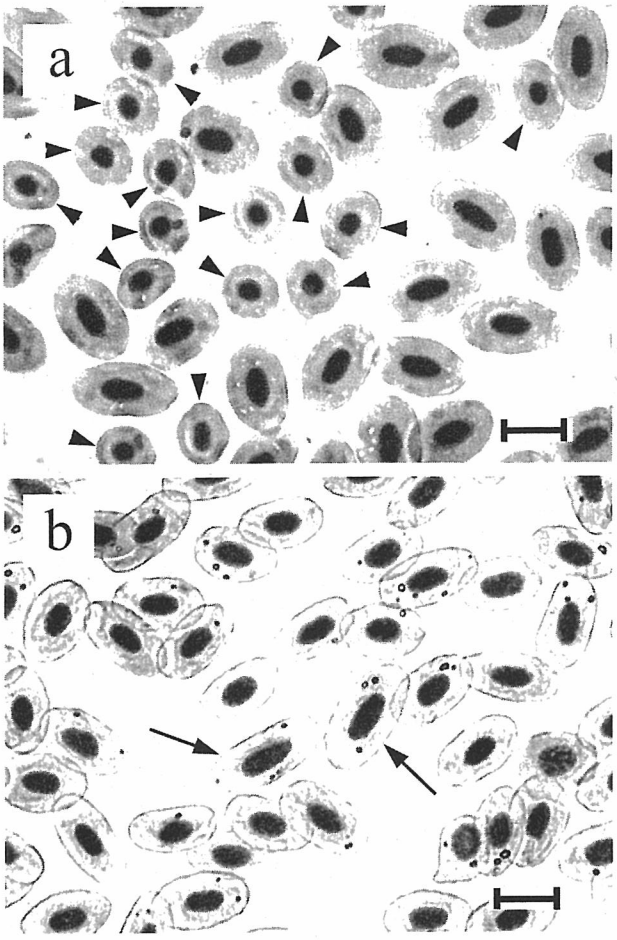

Fig. 3 Blood smear preparation of (a) haploid-diploid M12 and (b) diploid-triploid M10 mosaic amago salmon *Oncorhynchus masou ishikawae.* Note the smaller (arrow heads) and larger (arrows) erythrocytes within usual-sized cells. Scale bars indicate 10 μm.

In blood smear preparations, only usual-sized erythrocytes (mean major nuclear diameter [MMND] 6.1–6.7 μm) were observed in the albino fish and diploid mottled fish M1, M3 and M7. In contrast, unusually small (MMND 2.8–3.3 μm; Fig. 3a) and large (MMND 8.3 μm; Fig. 3b) erythrocytes were observed together with normal red blood cells (5.8–6.8 μm) in haploid-diploid mottled mosaic M2, M5, M6, M9 and M12 and diploid-triploid mosaic M10, respectively. Thus, polyploid mosaicism was also confirmed by erythrocytic measurements.

Progeny of mottled fish

The rate of normal eyed eggs, color phenotypes (wild-type and albino) and ploidy status of

Table 2 Number and ploidy status of wild-type and albino embryos, and χ^2 test for agreement with the predicted ratio among the progeny from mottled amago salmon *Oncorhynchus masou ishikawae*

Cross†	Hypothesized gynotype F‡	M‡	Normal eyed eggs (%)	Observed no. (expectation) Wild-type	Albino	$\chi^{2§}$	Diploid progeny/ eggs examined Wild	Albino
Preliminary mating								
H1 × A8	*Aa*	*aa*	86.8	127 (79.0)	31 (79.0)	58.33**	–	–
H2 × A8			81.5	179 (165.0)	151 (165.0)	2.38	–	–
A3 × H3	*aa*	*Aa*	85.2	105 (86.5)	68 (86.5)	7.91*	–	–
A3 × H4			56.2	97 (74.5)	52 (74.5)	13.59**	–	–
H1 × H3	*Aa*	*Aa*	92.8	178 (183.7)	67 (61.3)	0.71	–	–
H2 × H4			74.3	118 (114.7)	35 (38.3)	0.38	–	–
Experimental mating								
W1 × W3	*AA*	*AA*	75.4	129 (129.0)	0 (0)		–	–
W2 × W4	*AA*	*AA*	90.1	501 (501.0)	0 (0)		5/5	–
W2 × A6	*AA*	*aa*	88.5	346 (346.0)	0 (0)		5/5	–
A1 × W3	*aa*	*AA*	6.9	30 (30.0)	0 (0)		–	–
A2 × A6	*aa*	*aa*	96.6	0 (0)	309 (309.0)		–	5/5
W1 × M9	*AA*	*Aa*	89.8	185 (185.0)	0 (0)		–	–
M1 × A4	*Aa*	*aa*	90.6	81 (173.0)	265 (173.0)	97.85**	–	–
M2 × A5	*Aa*	*aa*	0.3	1 (1.0)	1 (1.0)	0	–	–
M5 × A6	*Aa*	*aa*	60.4	424 (413.5)	403 (413.5)	0.53	10/10	10/10
M6 × A6	*AA*	*aa*	75.6	502 (504.0)	2 (0)		10/10	2/2
M7 × A7	*Aa*	*aa*	75.5	441 (430.5)	420 (430.5)	0.51	–	–
A1 × M9	*aa*	*Aa*	9.4	23 (22.0)	21 (22.0)	0.09	–	–
A2 × M10	*aa*	*aa*	94.3	0 (0)	316 (316.0)		–	10/10
A2 × M11	*aa*	*AA*	92.0	333 (333.0)	0 (0)		10/10	–
A2 × M12	*aa*	*AA*	88.7	338 (338.0)	0 (0)		10/10	–

*$P < 0.01$; **$P < 0.001$.

†A, Albino; H, hybrid fish between wild-type and albino individuals; M, mottled; W, wild-type. ‡F, female; M, male; §$\chi^2 P_{0.05} = 3.84$ (d.f. = 1).

embryos are shown in Table 2. Phenotype distribution in the resultant progeny from W × W, W × A and A × A indicates that wild-type dark color gene '*A*' is dominant to albino gene '*a*', because of exclusive occurrence of wild-type. In the preliminary mating, hybrid (H) fish produced both wild-type and albino progeny. In the cross H2 × A8 (*Aa* × *aa*), the phenotype distribution was 1 : 1 as expected ($P = 0.12$). However, H1 × A8 gave significant excess of wild-type in phenotypic distribution ($P \leq 0.001$). The cross A3 × H3 and A3 × H4 (*aa* × *Aa*) produced statistically lower frequency of albino (68 and 52) than that of the expectation ($P < 0.01$). In the cross H × H, the segregation ratio between wild-type and albino phenotypes was 3 : 1 ($P = 0.39$ and 0.54), consisting with the presumption '*Aa* × *Aa*'.

In the crosses M × A and A × M, haploid-diploid mosaic male M11 and M12 produced only wild-type progeny, while diploid-triploid mosaic male M10 yielded only albino progeny. Diploid female M1 and M7, haploid-diploid mosaic female M2, M5 and M6 and haploid-diploid mosaic male M9 produced both wild-type and albino progeny. Wild-type and albino embryos from mottled fish M5, M6, M10, M11 and M12 were diploid (Table 2). These results indicate that diploid germ-line cells of M11 and M12 are homozygous '*AA*' genotype, while those of M10 are homozygous '*aa*' genotype. Since wild-type and albino appeared with 1:1 ratio in the progeny of M2, M5, M7 and M9 ($P = 0.47$–1.00), these fish are considered heterozygous '*Aa*' genotype in diploid germ-line cells. When genotype of diploid female M1 is assumed to be heterozygous '*Aa*', albinos appear much more frequency than the expectation (173) ($P \leq 0.001$). When M6 was assumed to have heterozygous '*Aa*' genotype, frequency of very few albino progeny was significantly different from the expectation (252) ($P \leq 0.001$). In contrast, when homozygous '*AA*' genotype was assumed in this female, genotype frequencies were fit to the expectation, but the occurrence of two albinos could not be explained in the cross '*AA* × *aa*'.

Mottled coloration was observed in none of the surviving 7-month-old progeny from mottled fish M10 and M11. In 10 juveniles from diploid-triploid mosaic M10, six female and four male were detected. In 56 juveniles from haploid-diploid

mosaic M11, 24 were female and 32 were male. The frequency of female and male in both batches agreed with the sex ratio 1:1 (χ^2 *cal.* = 0.40 and 1.14, P = 0.52 and 0.28).

DISCUSSION

In 12 yellow-dark mottled amago salmon examined, eight haploid-diploid mosaic and one diploid-triploid mosaic individuals were detected by flow-cytometry in several different tissues. No mosaicism was detected in wild-type and albino individuals so far examined. These results suggested that the mottled coloration might relate to a polyploid mosaicism. Although other three mottled fish were determined as diploid, polyploid mosaicism in other tissues could not be ruled out.

In fish, haploidy is lethal due to characteristic abnormalities collectively referred to as a haploid syndrome expressing edema, ascites, dwarfing, microphthalmia and microcephaly,[19] but haploid-diploid mosaic individual was proved to be viable. Since artificially produced haploid-diploid frog and goldfish chimeras were viable, additional diploid cells should compensate an inadequate function of haploid cells in organs.[20,21] In the normally cultured charr *Salvelinus leucomaenis* population, one live haploid-diploid mosaic individual, in which approximately 90% of blood, liver and spleen were haploid but almost all the brain cells were diploid, was found.[14] These observations indicated that an inviable haploid individual with diploid cells might recover viability. In kokanee salmon, however, haploid-diploid mosaic was frequently found in abnormal alevins.[15] This phenomenon suggests that survival capacity of haploid-diploid mosaic fish may relate to the critical proportion and distribution of diploid cells. One viable diploid-triploid mosaic amago salmon was detected in the present study. As eu-triploidy is not lethal,[19] triploidy has no deleterious influence on the development and survivability of diploid-triploid mosaic individual.

Kashiwagi[22] reported a very rare case that haploid frog laid eggs, but the resultant embryos developed abnormally. Because the haploid-diploid mosaic charr produced no normal oocytes, the germ-line cells were considered to be haploid.[14] In fish, triploid males generally produce a small quantity of aneuploid sperm, but the resultant progeny cannot survive due to aneuploidy.[23,24] These reports suggest that gametogenesis should not proceed normally in haploid and triploid germ-line cells in polyploid mosaics. Sperm taken from three haploid-diploid and one diploid-triploid mosaic males exhibited haploidy and the resultant progeny from haploid-diploid mosaic females fertilized with haploid sperm was diploid. These results suggest that normal gametogenesis should produce haploid sperm and eggs, because diploid germ-line cells were normally acted in the gonads of these mosaics.

When the hybrid (H) fish was presumed to have '*Aa*' genotype, the phenotype distribution in three batches (H1 × A8, A3 × H3 and A3 × H4) was statistically biased from the expectation, probably due to possible selective death of albino phenotype. Poor survival capacity of albino phenotype was reported in brook trout.[5]

When the genotype of mottled fish was presumed '*Aa*' in M2, M5, M7 and M9, '*aa*' in M10 and '*AA*' in M12, the observed frequency of wild-type and albino progeny was matched to the Mendelian inheritance. However, the phenotype distribution in the progeny of M1 and M6 was biased, when assuming '*AA*', '*Aa*' or '*aa*' genotype in these two individuals. The occurrence of unexpected two albino found in the progeny from mottled female M6 (*AA*) was difficult to explain. They might be contaminated or induced by very rare spontaneous diploid androgenesis.

On the other hand, when M6 was presumed to have very small numbers of '*Aa*' genotype cells, the appearance of albino is possible to explain. However, diploid mottled female M1 should have two kinds of genotypes (*Aa* and *aa*) in germ-line cells, because high frequencies of albino progeny can be explained by contribution of gametes from germ cells with '*aa*' genotype. This suggests that M1 might be a genotypic mosaic '*Aa/aa*', comprising two different cell types with '*Aa*' and '*aa*' genotype. Such a genotypic mosaicism exhibiting mottled coloration was reported in rainbow trout.[11,12]

The mottled amago salmon examined was presumed to have germ-line cells with '*AA*', '*Aa*' or '*aa*' genotype as a result of the cross examination. Because six out of nine mottled individuals were haploid-diploid mosaic, these mottled fish should have both hemizygous haploid '*a*' (yellow) cells and diploid '*AA*' or '*Aa*' (dark) cells. Therefore, genotypes at the albino locus of the haploid-diploid mosaic mottled amago salmon can be estimated as '*a/Aa*' in M2, M5 and M9 and '*a/AA*' in M6, M11 and M12. Although a haploid-diploid mosaic individual with homozygous diploid '*aa*' (yellow) cells and hemizygous haploid '*A*' (dark) cells might also exhibit mottled coloration, a mottled mosaic fish with '*A/aa*' genotype has not been suggested in this study.

Since germ-line cells in the diploid-triploid fish are estimated to have diploid '*aa*' genotype from the result of cross experiment, this mottled fish should comprise triploid cells with genotype '*AAA*',

'*AAa*' or '*Aaa*' showing dark coloration and diploid cells with genotype '*aa*' showing yellow coloration. In amago salmon, '*AAa*' and '*Aaa*' triploid genotypes, which had been induced by inhibiting the second meiotic division of the fertilized eggs in crosses between wild-type female (*AA*) and albino male (*aa*) and vice versa, gave dark-body pigmentation (Yamaki M, unpubl. data, 2001). Therefore, genotypes at the albino locus of diploid-triploid mottled amago salmon M10 is estimated '*aa/AAA*', '*aa/AAa*' or '*aa/Aaa*'. Because mottled fish M7 was diploid by flow-cytometry and the genotype was presumed '*Aa*' (dark) as a result of the mating to albino male, the yellow part of this female is possibly haploid cells '*a*', diploid cells '*aa*' or triploid cells '*aaa*' genotype.

As mentioned previously, the ploidy status of dark- and yellow-colored pigmentation parts in skin and fin of the individual mottled fish was estimated. However, as predicted, the haploidy of yellow parts has not been flow-cytometrically detected. This may be explained by mixing of non-pigment cells in the sample taken from whole skin and fin.

Sex ratio in the progeny of the diploid-triploid mosaic M10 and that in those of haploid-diploid mosaic M11 were 1 : 1 (female : male). Therefore, these two males should produce both X- and Y-bearing sperm, because the sex determination of amago salmon was reported to be male heterogamy (XY).[25] This result indicated that these polyploid mosaic males should develop from the normal karyogamy between female and male nucleus. Tanaka *et al.*[15] reported that haploid-diploid mosaic kokanee salmon *O. nerka* might be induced by the mitotic error, such as endomitosis (chromosome duplication without cytokinesis) which accidentally happened in one blastomere at the late stage of cleavage in spontaneous gynogenetic haploid embryo. However, such a mitotic division did not explain gonosomic heterozygosity (XY) and heterozygous genotype of the albino locus (*Aa*) in the polyploid mosaic fish. Therefore, the haploid-diploid mosaicism observed here is likely to have been induced by other cytological event.

There are a number of possible mechanisms for the origin of haploid-diploid and diploid-triploid mosaics. One possible mechanism is the inclusion of the second polar body (1n) into a blastmere. After the karyogamy occurred by the fusion of a female and a male pronucleus, haploid-diploid mosaic may be generated, when the retained second polar body independently develops. When the retained second polar body does not develop independently, but is incorporated into one of the daughter nuclei (2n) after the first cleavage, diploid-triploid mosaic may be produced. The other possible mechanism is genome loss. When genome loss happened during embryonic development in an usual diploid and a spontaneous triploid nucleus, haploid-diploid and diploid-triploid mosaics might arise, respectively. However, genome loss cannot be an origin of the genotype '*a/AA*' of haploid-diploid mosaic such as M6, M11 and M12. Binucleated oocytes and/or polyspermy can also be speculated. These mechanisms have been reported in chicken[26,27] and some mammals.[26,28–30] At present, cytological mechanism responsible for the occurrence of haploid-diploid and diploid-triploid mosaicisms still remain unclear and it is impossible to identify the mechanism operated for creation of mosaicisms. Further studies from the viewpoints of both cytogenetics and genetics are required to elucidate mechanisms of the occurrence of polyploid mosaics.

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