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Simultaneous formation of haploid, diploid and triploid eggs in diploid-triploid mosaic loaches

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Running headline: **1N, 2N and 3N eggs of mosaic loaches**

ABSTRACT

In the loach *Misgurnus anguillicaudatus*, very few diploid-triploid mosaic individuals, which are generated by accidental incorporation of the sperm nucleus into diploid eggs produced by clonal diploid loach, occur in nature. Ploidy examination of gynogenetic progeny induced by activation with UV-irradiated goldfish sperm indicated that diploid-triploid mosaic females laid haploid, diploid and triploid eggs, simultaneously. In addition, triploid eggs exhibited larger egg sizes. Microsatellite genotyping of diploid-triploid mosaics revealed that triploid genotypes of mosaic mothers consisted of two alleles specific to the clonal diploid and one allele from normal diploid male. Diploid eggs from mosaic mother had genotypes absolutely identical to the diploid clone. Most genotypes of triploid eggs were identical to the mosaic mother, and one of three alleles of the mosaic mother was transmitted to haploid eggs. These results suggested that diploid germ cells, which had a clonal genome, were differentiated into clonal diploid eggs, and triploid and haploid eggs were produced from triploid germ cells in the same ovary of mosaic individuals.

Key words: *Misgurnus anguillicaudatus*; mosaic; clone; polyploid.

INTRODUCTION

In the loach *Misgurnus anguillicaudatus* (Teleostei: Cobitidae), bisexually reproducing diploids (2n=50) are most common in Japan, but a clonal lineage is found among diploid loaches collected from the wild population of Hokkaido Island, Japan (Morishima et al., 2002). Members of the clonal lineage produce unreduced diploid eggs by the mechanism of premeiotic endomitosis (Itono et al., 2006), which develop to genetically identical individuals without any genetic contribution of sperm donor by gynogenetic development (Itono et al., 2007). However, some unreduced eggs develop to triploid and/or diploid-triploid mosaic individuals through accidental incorporation of the sperm nucleus (Morishima et al., 2002, 2004; Itono et al., 2007).

The rare occurrence of natural diploid-triploid mosaic loaches comprising both diploid and triploid cell populations was previously reported in the same wild population (Morishima et al., 2004). In such diploid-triploid mosaic individuals, diploid cells are genetically identical to the somatic cells of the clonal loach and all triploid cells have diploid chromosome sets of the clone and a haploid chromosome set of the sperm donor. It was also reported that diploid-triploid mosaic males produced fertile diploid sperm, genetically identical to the clonal loach found in the wild population (Morishima et al., 2004). Sex determination of the loach

was inferred as a male heterogametic system with the XX female - XY male due to the exclusive occurrence of females in artificially induced gynogenetic populations (Suzuki et al., 1985). It is assumed that triploid germ cells possessing a Y-chromosome in the diploid-triploid mosaic should organize sterile testes caused by unusual meiosis as in natural triploid males (Oshima et al., 2005), whereas diploid clonal germ cells should differentiate to normal testes (Morishima et al., 2004). Thus, unreduced sperm might be produced from diploid clonal germ cells in the testes of diploid-triploid mosaic males (Morishima et al., 2004).

In contrast to mosaic males, no reproductive traits have been examined in mosaic females. In natural triploid loaches in Hokkaido, probably arisen from accidental sperm incorporation to clonal eggs, Oshima et al. (2005) found that at least four different types of eggs were formed. One triploid female laid both many aneuploids (1.1-1.7n) and very few triploid eggs simultaneously, whereas another laid many haploid and a few diploid eggs at the same time. In other cases, triploid females derived from crossing a normal diploid female and a natural tetraploid male, obtained from fish dealers (Arai et al., 1991), laid large-sized triploid and small-sized haploid eggs simultaneously (Matsubara et al., 1995). Similar formation of both triploid and haploid eggs was also observed in natural triploids in the wild population from Niigata prefecture, Honshu Island, Japan (Zhang & Arai, 1999).

Considering the presence of both clonal diploid and triploid cell populations in the ovary of diploid-triploid mosaic females, we hypothesize that they may generate diploid eggs originated from the diploid clone as well as various types of fertile eggs with different ploidies at the same time. The reproductive performance of diploid-triploid mosaic females is very interesting from the viewpoint of the evolutional history of clonal and polyploid lineages in the loach. In the present study, the reproductive capacity of diploid-triploid mosaic females was disclosed by examining the fertility and ploidy of eggs collected from these mosaic loaches, after inducing gynogenesis with UV-irradiated sperm and normal fertilization with functional sperm. Microsatellite genotypes of gynogenetically induced and normally fertilized progeny of these diploid-triploid mosaics were determined, so as to verify the genomic constitution of various types of eggs laid by mosaic females.

MATERIALS AND METHODS

FISH SPECIMENS

In June 2004, 133 loaches were collected from Memanbetsu town (designated by the conventional name before the consolidation of

municipalities in March 2006), Abashiri county, Hokkaido prefecture, Japan, and then they were sorted into diploid (*n*=120), triploid (*n*=12) and diploid-triploid mosaic individuals (*n*=1), based on relative DNA contents measured by flow cytometry as described below. The mosaic individual (2N/3Na) was a female and was selected as a parental fish for experimental crosses.

A clonal strain derived from unreduced eggs of the clonal loach (No.3, Morishima et al., 2002) previously identified among specimens taken from Memanbetsu town, was maintained at the Aquarium Center of the Graduate School of Fisheries Sciences, Hokkaido University, Hakodate city, Hokkaido, Japan. In January 2003, eggs of a mature clonal female taken from the clonal strain were fertilized with sperm of a normal diploid male collected from a paddy field in Ohno town (designated by the conventional name before the consolidation of municipalities in January 2006), Kameda county, Hokkaido prefecture. Surviving progeny (*n*=37) from this cross were sorted into diploid (*n*=20), triploid (*n*=12) and diploid-triploid mosaics (*n*=5) in May 2004. Two of five mosaic individuals (2N/3Nb and 2N/3Nc) were females, and these mosaic females were also used for experimental crosses.

In June 2005, eggs of the three mature diploid-triploid mosaics (2N/3Na, 2N/3Nb and 2N/3Nc) were used for experimental crosses. A normal diploid female (2Nf) and male (2Nm) collected from Kita village

(designated by the conventional name before the consolidation of municipalities in March 2006), Sorachi county, Hokkaido, were also used as parental fish.

EXPERIMENTAL CROSS AND INDUCED GYNOGENESIS

All the parental females were injected with human chorionic gonadotrophic hormone (hCG: Teikoku Hormone Mfg, Co. Ltd., Minato-ku, Tokyo) to induce ovulation according to the procedure described by Suzuki & Yamaguchi (1975). Sperm was obtained with a capillary tube by squeezing the abdomen (Morishima et al., 2002), and then diluted 100 times with physiological saline as described in Suzuki et al. (1985). For artificial gynogenesis, sperm of goldfish (*Carassius auratus*, Cyprinidae) from the Aquarium Center of the Graduate School of Fisheries Sciences, Hokkaido University was also obtained in the same manner, and then irradiated with ultraviolet rays (UV) following the procedure described by Suzuki et al. (1985).

A portion of eggs from each female was fertilized with sperm of the male loach (2Nm). The remaining eggs of each female were fertilized with UV-irradiated sperm of the goldfish to induce gynogenetic development. The cross involving normal diploid female (2Nf) and male (2Nm) was used

as control.

Egg diameters were measured about one hour after fertilization under a stereoscopic microscope using an ocular micrometer. Fertilized eggs were incubated in a plastic pan filled with freshwater at room temperature, and the hatching rate was calculated for each cross. One day after hatching, the relative DNA content of each progeny was determined by flow cytometry.

PLOIDY DETERMINATION

The relative DNA content of fry and erythrocytes was measured to determine the ploidy status by flow cytometry using a Ploidy Analyzer (Partec PA, Münster, Germany) after staining with DAPI (4’6-deamino-2-phenylindole) according to the procedure previously described by Morishima et al. (2002). As internal and external standards, erythrocytes of normal diploid loaches were used as a 2C value of normal diploidy.

MICROSATELLITE GENOTYPING

Mosaic individuals and their 7-day-old progeny from crosses, were genotyped at four microsatellite loci, *Mac3*, *Mac37*, *Mac49* and *Mac63,* as described in Morishima et al. (2001; 2007). DNA samples were extracted according to routine procedures as described by Asahida et al. (1996). Procedures for PCR amplification followed essentially Kim et al. (2007) and Morishima et al. (2007). Genotypes were determined as approximate allele sizes (base pairs) by an ABI 3130xl PRISM® Genetic Analyzer and GENEMAPPER software version 3.7 (Applied Biosystems, Foster, U.S.A) using com-LIZ™ 500 as the size standard.

RESULTS

PROPORTION OF DIPLOID AND TRIPLOID CELLS

Relative DNA contents of erythrocytes from the three mosaic individuals (2N/3Na, 2N/3Nb and 2N/3Nc) were flow-cytometrically examined (Fig. 1). In each individual, we detected both diploid (2C) and triploid (3C) cell populations. High frequencies of diploid cells were detected in 2N/3Na (85%) and 2N/3Nb (63%) [Fig. 1(a,b)], whereas 94% of the cells were triploid in 2N/3Nc [Fig. 1(c)]. These results indicated that the degree of diploid-triploid mosaicism was different among individuals.

FERTILITY OF EGGS

Mature ovulated eggs of diploid-triploid mosaic females (2N/3Na, 2N/3Nb and 2N/3Nc) were fertilized with sperm of normal diploid male (2Nm) and UV-irradiated goldfish sperm. Two discrete sizes were observed in the eggs of mosaics, while no multiple egg populations with apparently different sizes existed in normal diploid female 2Nf (mean 1.06 ± S.D. 0.02 mm, range 1.00-1.11, *n*=119). In the three mosaic females, large eggs (1.34 ± 0.02 mm, range 1.30-1.40 mm, *n*=17) were visually distinguishable from small eggs (0.83 ± 0.07 mm, range 0.68-1.02 mm, *n*=364). The rate of larger eggs was 3.8%, 0.8% and 0.9%, in 2N/3Na, 2N/3Nb and 2N/3Nc, respectively (Table I).

The hatching rate of small eggs produced by mosaic female 2N/3Nb when fertilized by sperm from the male 2Nm was 86.5%, approximately equivalent to that of the control cross (85.3%), whereas those of females 2N/3Na and 2N/3Nc showed lower hatching rates. In addition, large eggs of mosaic females fertilized by sperm of the male 2Nm showed 17-50% hatching rates (Table I).

When UV-irradiated goldfish sperm was used, eggs of normal diploid female showed a much lower hatching rate (18.8%) and the gynogenetic

progeny died. In contrast, the hatching rates of small eggs of the diploid-triploid mosaic females were higher (28.0-71.2%), and the gynogenetic progeny showed higher survival rates (Table I). Large eggs of mosaic females showed 16-40% hatching rates (Table I).

PLOIDY OF EGGS

All the gynogenetic progeny from eggs of normal diploid female (2Nf) were haploid, but the gynogenetic progeny derived from small eggs of mosaic females were both haploid and diploid (Table II). In small eggs, higher frequencies of diploid gynogenetic progeny were also shown in 2N/3Na (80%) and 2N/3Nb (53%), whereas 61% gynogenetic progeny were haploid in 2N/3Nc. In addition, all the gynogenetic progeny derived from larger eggs of mosaic females were triploid (Table II). Flow cytometical results of gynogenetic progeny concluded that diploid-triploid mosaic females formed haploid, diploid and triploid eggs, simultaneously.

In normally fertilized crosses, more than 90% of the progeny developed from small eggs of three diploid-triploid mosaic females were diploid, but six triploid and one diploid-triploid mosaic progeny were observed (Table II). Nine of ten progeny derived from large eggs of mosaic females were tetraploid, whereas one progeny of 2N/3Na was

triploid-tetraploid mosaic (Table II).

GENOTYPES OF GYNOGENETIC PROGENY

Microsatellite genotyping was carried out on gynogenetic progeny derived from crosses using UV-irradiated goldfish sperm (Table III). At each microsatellite locus, all diploid-triploid mosaic females (2N/3Na, 2N/3Nb and 2N/3Nc) comprised two alleles from the natural diploid clone and additional third allele; however, gynogenetic haploid progeny were not genetically examined, because they died due to abnormalities shortly after hatching. All the gynogenetic diploid progeny had two alleles identical to the clonal genotype at all the loci examined. Non-clonal third alleles of diploid-triploid mosaics were never transmitted to the gynogenetic diploid progeny. These results indicated that diploid-triploid mosaic females produced unreduced diploid eggs with the clonal genotype.

Six of seven gynogenetic triploid progeny exhibited genotypes comprising three alleles, identical to triploid somatic cells of diploid-triploid mosaic females; however, one gynogenetic triploid progeny from the mosaic female 2N/3Nc exhibited a different aclonal genotype at *Mac 49*. The results showed that mosaic females also laid

unreduced triploid eggs, together with haploid and unreduced diploid eggs. The occurrence of very few aclonal triploid eggs indicated the probable presence of genetic variation among unreduced triploid eggs.

GENOTYPES OF NORMALLY FERTILIZED PROGENY

In diploid progeny from experimental crosses between mosaic females (2N/3Na, 2N/3Nb and 2N/3Nc) and a normal diploid male (2Nm), two progeny of 2N/3Na, three progeny of 2N/3Nb and one progeny of 2N/3Nc gave genotypes at four loci, absolutely identical to the clonal diploid, without any genetic contribution of the sperm donor (Table IV). These results indicated that mosaic females produced unreduced diploid eggs, which should develop to a diploid clone, gynogenetically. The other diploid progeny of three mosaic females were not clonal, and had one allele out of all three alleles of mosaic females and one of two alleles of the paternal diploid (2Nm).

Triploid and diploid-triploid mosaic progeny had two alleles identical to the clonal genotype and one of the two alleles from their normal diploid male (Table IV). Thus, triploid and diploid-triploid mosaic progeny were generated by accidental incorporation of sperm into unreduced diploid eggs.

Tetraploid progeny had triploid genotypes comprising three alleles from the mosaic female and one additional allele from the normal male (Table IV). Seven of nine tetraploid progeny exhibited a triploid genotype genetically identical to the genotypes of the mosaic females and additional allele from the male, but other tetraploid progeny of the mosaic 2N/3Na showed a lack of one allele of the maternal genotype at one of four loci. For example, at the *Mac63* locus, one tetraploid progeny from the cross between 2N/3Na (*108/118/156*bp) and 2Nm (*120/180*bp) gave a genotype *108/108/156/180*bp or *108/156/156/180*bp, whereas the other tetraploid siblings had genotypes *108/118/120/156*bp and *108/118/156/180*bp. These results indicated that mosaic females laid unreduced triploid eggs, most of which were clonal but a few were aclonal.

One triploid-tetraploid mosaic progeny appeared in the cross between mosaic 2N/3Na female and 2Nm male. This had three alleles identical to the female and one allele from the male (Table IV).

DISCUSSION

Diploid-triploid mosaic females laid three types of mature eggs, reduced haploid, unreduced diploid and unreduced triploid eggs,

simultaneously. Most unreduced diploid eggs developed to clonal individuals gynogenetically without any genetic contribution of the sperm. However, the incorporation of sperm occurred accidentally as observed in unreduced eggs of wild clonal loaches (Morishima et al., 2002). In mosaic females, clonal diploid gametes should be derived from clonal diploid germ cells, as happens in diploid-triploid mosaic males, which produce fertile diploid spermatozoa derived exclusively from clonal diploid germ cells, whereas triploid germ cells produce no fertile sperm (Morishima et al., 2004). In the clonal loach, diploid eggs are produced by premeiotic endomitosis, which results in chromosome doubling before meiosis, followed by two quasinormal divisions (Itono et al., 2006). Diploid-triploid mosaic females are likely to produce clonal diploid eggs from its diploid germ cells by the same unreduced oogenetic system.

In the present study, all the aclonal diploid progeny included one allele derived from the triploid genotype of mosaic females, whereas triploid progeny from gynogenetic induction and tetraploid progeny from normal fertilization exhibited three alleles derived from triploid genotypes of diploid-triploid mosaic females in their triploid and tetraploid genotypes. Therefore, both reduced haploid and unreduced triploid eggs were also produced in mosaic females, and these eggs might be formed from the triploid germ cells. Zhang et al. (1998) suggested that unreduced triploid eggs are formed by quasinormal meiosis of hexaploid germ cells,

generated by premeiotic endomitosis, while haploid eggs are produced from bivalents, formed by two chromosome elements with high affinities, after eliminating univalents in triploid germ cells. The production of haploid eggs by triploid females was also reported in the hybrid spined loach between *Cobitis hankugensis* (*C. sinensis*) and *C. longicorpus* (Kim & Lee, 2000; Saitoh et al., 2004), while simultaneous egg formation of both haploid and unreduced triploid eggs has been reported in the natural triploid minnow *Squalius alburnoides* (Alves et al., 2004), triploid hybrid (diploid female × tetraploid male) loach (Matsubara et al., 1995; Zhang & Arai, 1996; Zhang et al., 1998) and natural triploid loach (Zhang & Arai, 1999).

In premeiotic endomitosis, identical chromosomes are formed by duplication due to mitosis without cytokinesis before the beginning of meiosis. These sister chromosomes should pair like homologous chromosomes in normal meiosis; therefore, the recombination between two identical sister chromosomes should not yield any genetic variation because the exchanges occur between equal elements (Dawley, 1989). In the present study, some triploid eggs, which are generated by unreduced oogenesis of triploid germ cells in diploid-triploid mosaic females, gave a variation in one of the four microsatellite loci, whereas the others were absolutely identical to the mosaic females. Thus, premeiotic endomitosis could yield a small amount of genetic variation in the formation of triploid

eggs. Preferential pairing between sister replicates doubled from each chromosome should be regular in this system because of their strong affinity, but accidental synapses and recombination between non-sister homologous chromosomes could happen in a few members of the chromosome set to produce aclonal eggs; however, no aclonal indiviuals have been identified in progeny gynogenetically developed from unreduced eggs of the natural clonal loach in the Memanbetsu population (Morishima et al., 2002). A small number of very similar but aclonal individuals have been identified by DNA fingerprinting among triploid (diploid female × natural tetraploid male) progeny gynogenetically induced from triploid eggs of triploid loaches, which were expected to be genetically identical among sib progeny due to unreduced oogenesis through premeiotic endomitosis (Arai & Mukaino, 1997; Momotani et al., 2002). Aclonal individuals were also found by DNA markers among gynogenetic diploid progeny induced from unreduced eggs of the diploid loach (Arai et al., 2000), which was a source of natural triploid loaches in Hirokami villege, Niigata prefecture (Zhang & Arai, 1999).

Dawley & Goddard (1988) discussed that mosaicism probably occurred by “genome loss” or “delayed fertilization”. The former can be seen in meiosis of hybridogenetic unisexuals, in which the paternal genome is discarded during oogenesis, e.g. *Poeciliopsis* (Schultz, 1969;

Cimino, 1972) and *Rana esculenta* (Graf & Müller, 1979). In the latter case, the sperm nucleus, which fails to fuse with the female pronucleus before the first mitotic division, will re-activate to become a female pronucleus-like structure, and then fuse with the maternal nucleus in the later embryonic stage, as discussed in *Carassius langsdorfii* (Mada et al., 2001) and *Poecilia formosa* (Lamatsch et al., 2002). Since the transformation of a spermatozoon to a male pronucleus-like structure in a blastomere of two or more advanced stages of clonal embryos was cytologically observed by Itono et al. (2007), mosaic individuals from clonal eggs should be generated by such "delayed fertilization" in embryonic stages. Therefore, it is expected that "delayed fertilization" in the earlier embryonic stage may give rise to the higher frequency of diploid cell population in mosaic individuals. Different ratios of diploid and triploid cell populations and different frequencies of various types of eggs among mosaic individuals might be explained by the time of "delayed fertilization" in the course of embryonic stages.

In the present study, gynogenetic progeny from large eggs of mosaic females were triploid. In addition, fertilization of such eggs with normal haploid sperm produced tetraploid progeny. Therefore, large eggs correspond to unreduced triploid eggs. In contrast, the difference in size between haploid and diploid eggs was not clear.

From a view of evolutionary biology, sexual reproduction is more

advantageous than asexuality, because the absence of recombination gives rise to the accumulation of deleterious genetic load and should lead to rapid extinction of asexual lineages (Gabriel et al., 1993). However, there is a paradox that large numbers of asexual lineages exist and survive in various species of lower vertebrates (Vrijenhoek, 1989; Alves et al., 2001; Arai, 2003). Therefore, the studies on reproduction in the asexual lineages may get an insight into the mechanism how these asexual animals may compensate for the disadvantages. In the present study, however, the simultaneous formation of reduced haploid, unreduced diploid and unreduced triploid eggs by diploid-triploid mosaic females, that are probably generated by accidental incorporation of sperm nucleus to a blastomere of clonal diploid embryo (Itono et al., 2007), suggests the flexibility of asexual reproduction in the clonal lineage of *Misgurnus* loach, as follows. Similar flexibility is also suggested from various gametes of triploid loach females, appeared after syngamy of unreduced diploid eggs and normal haploid sperm (Oshima et al., 2005).

The diploid progeny could appear after the normal fertilization of reduced haploid eggs from triploid and/or diploid-triploid mosaic females with normal haploid sperm from sexual lineages in nature and such diploid progeny could restore sexual reproduction due to normal meiotic process. Therefore, the production of reduced haploid eggs by diploid-triploid (present study) and triploids (Oshima et al., 2005) are

considered a stepping stone to reach the sexual lineage from the asexuality via triploid situation. Such a route from asexual to sexual has been elucidated in *Phoxinus eos-neogaeus* complex (Dawley & Goddard, 1988; Goddard & Schultz, 1993) and Iberian minnow *Leuciscus alburnoides* complex (Alves et al., 2001). Production of reduced haploid eggs in triploid females was also reported in *Cobitis hankugensis-longicorpus* complex (Saitoh et al., 2004).

The formation of unreduced triploid eggs was found in diploid-triploid mosaic females, as previously reported in natural triploid loach (Oshima et al., 2005; Zhang & Arai, 1999). When these unreduced triploid eggs were fertilized by normal haploid sperm, tetraploid progeny appeared as shown in the present study and Oshima et al. (2005). This is a typical route to tetraploidy in asexually reproducing vertebrates via syngamy between triploid eggs and haploid sperm from sympatric bisexual lineages (Dawley, 1989). At present, however, reproductive mode of such tetraploid individuals remains unknown. Thus, further studies are required to disclose whether such tetraploid loaches are able to generate diploid gametes or not.

The formation of unreduced diploid eggs with clonal genotypes in diploid-triploid mosaic females provides a new route to tetraploidy via syngamy with clonal diploid sperm, which are formed in rare diploid-triploid mosaic males (Morishima et al., 2004) and sex-reversed

clonal males (Yoshikawa et al., 2007). This type of tetraploids is theoretically able to appear by the syngamy of unreduced diploid eggs from the clone and unreduced diploid sperm (Morishima et al., 2004; Yoshikawa et al., 2007).

As mentioned above, new genetic materials can be introduced to various progenies of the clonal lineage from sympatric sexually reproducing lineages via triploidy and/or diploid-triploid mosaicism and this flexibility may be one of the mechanisms to compensate deleterious mutations in clonal lineages in the loach. An involvement of similar mechanisms to maintain asexual lineages has been proposed in Iberian minnow *L.alburnoides* complex (Alves et al., 1999; 2001).

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Figure caption

FIG.1. Flow cytometry for erythrocytes from diploid-triploid mosaics (a: 2N/3Na, b: 2N/3Nb and c: 2N/3Nc).

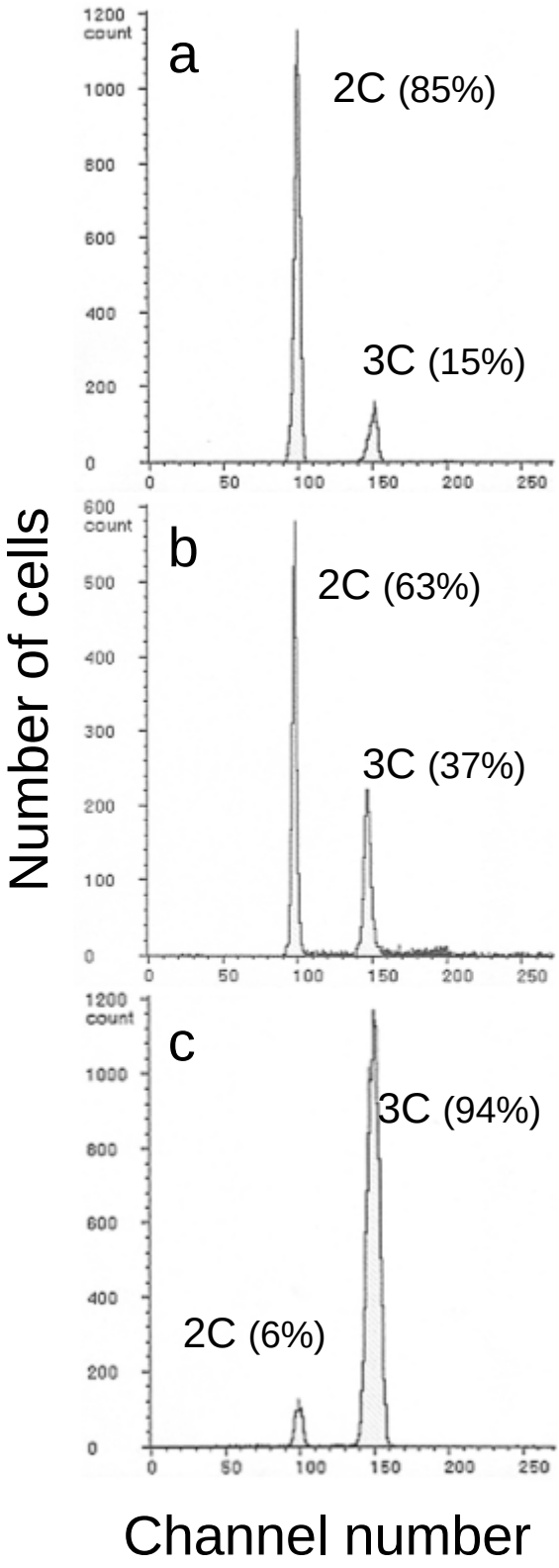
a
2C (85%)
3C (15%)
b
2C (63%)
3C (37%)
c
3C (94%)
2C (6%)
Number of cells
Channel number

Fig.1

TABLE I. Developmental capacity of progeny from three diploid–triploid females, and a normal diploid female

Exp.	Cross		Total egg no.	Small eggs			Large eggs		
	Female	Male		No.	Hatch no. (%)	Survival no. (%) *	No.	Hatch no. (%)	Survival no. (%) *
Induced gynogenesis	2Nf	UV	96	96	18 (18.8)	0 (0)	–	–	–
	2N/3Na	UV	690	665	186 (28.0)	140 (75.3)	25	4 (16.0)	N.D.
	2N/3Nb	UV	497	493	351 (71.2)	169 (48.1)	4	1 (25.0)	N.D.
	2N/3Nc	UV	665	660	288 (43.6)	108 (37.5)	5	2 (40.0)	N.D.
Normal fertilization	2Nf	2Nm	401	401	342 (85.3)	333 (97.4)	–	–	–
	2N/3Na	2Nm	960	923	609 (66.0)	554 (91.0)	37	7 (18.9)	N.D.
	2N/3Nb	2Nm	727	721	624 (86.5)	571 (91.5)	6	1 (16.6)	N.D.
	2N/3Nc	2Nm	381	377	157 (41.6)	135 (86.0)	4	2 (50.0)	N.D.

* 7 days after hatching.

TABLE II. DNA content of progeny from three diploid–triploid females, and a normal diploid female

Exp.	Cross		Relative DNA content of progeny							Total
			Small eggs				Large eggs			
	Female	Male	1C	2C	3C	2C/3C	3C	4C	3C/4C	
Induced gynogenesis	2Nf	UV	18	0	0	0	0	0	0	18
	2N/3Na	UV	4	16	0	0	4	0	0	24
	2N/3Nb	UV	19	21	0	0	1	0	0	41
	2N/3Nc	UV	17	11	0	0	2	0	0	30
Normal fertilization	2Nf	2Nm	0	20	0	0	0	0	0	20
	2N/3Na	2Nm	0	28	2	0	0	6	1	37
	2N/3Nb	2Nm	0	32	2	1	0	1	0	36
	2N/3Nc	2Nm	0	24	2	0	0	2	0	28

TABLE III. Microsatellite genotypes in induced gynogenetic progeny developed from eggs of three mosaic females after fertilization with UV-irradiated sperm

			Genotype			
Specimens	Ploidy	*n*	*Mac3*	*Mac37*	*Mac49*	*Mac63*
Female (2N/3Na)	2n/3n	–	*102*/*116*/*144*	*88*/*102*/*108*	*102*/*108*/*112*	*108*/*118*/*156*
Gynogens	2n	5	*102*/*144*	*102*/*108*	*108*/*112*	*118*/*156*
	3n	4	*102*/*116*/*144*	*88*/*102*/*108*	*102*/*108*/*112*	*108*/*118*/*156*
Female (2N/3Nb)	2n/3n	–	*102*/*106*/*144*	*102*/*108*/*114*	*108*/*112*/*120*	*118*/*120*/*156*
Gynogens	2n	5	*102*/*144*	*102*/*108*	*108*/*112*	*118*/*156*
	3n	1	*102*/*106*/*144*	*102*/*108*/*114*	*108*/*112*/*120*	*118*/*120*/*156*
Female (2N/3Nc)	2n/3n	–	*102*/*106*/*144*	*102*/*108*/*114*	*108*/*112*/*120*	*118*/*156*/*170*
Gynogens	2n	5	*102*/*144*	*102*/*108*	*108*/*112*	*118*/*156*
	3n	1	*102*/*106*/*144*	*102*/*108*/*114*	*108*/*112*/*120*	*118*/*156*/*170*
	3n	1	*102*/*106*/*144*	*102*/*108*/*114*	*108*/*120* *	*118*/*156*/*170*

Genotypes of mosaic females were represented by triploid genotypes. Underlined alleles originated from the diploid clone loach. * triploid genotype, *108/108/120* or *108/120/120*.

TABLE IV. Microsatellite genotype in progeny of normal fertilization using eggs of diploid–triploid mosaic loaches

Specimens	Ploidy	*n*	Genotype			
			Mac3	*Mac37*	*Mac49*	*Mac63*
Female (2N/3Na)	2n/3n	–	*102*/*116*/*144*	*88*/*102*/*108*	*102*/*108*/*112*	*108*/*118*/*156*
Male (2Nm)	2n	–	*108*/*110*	*100*/*106*	*118*/*120*	*120*/*180*
Progeny	2n	2	*102*/*144*	*102*/*108*	*108*/*112*	*118*/*156*
	2n	1	*110*/*116*	*88*/*106*	*112*/*120*	*108*/*120*
	2n	1	*102*/*110*	*102*/*106*	*108*/*118*	*108*/*120*
	2n	1	*110*/*116*	*88*/*106*	*102*/*118*	*118*/*120*
	2n	1	*110*/*116*	*88*/*106*	*102*/*120*	*108*/*180*
	3n	1	*102*/*110*/*144*	*102*/*106*/*108*	*108*/*112*/*120*	*118*/*120*/*156*
	3n	1	*102*/*110*/*144*	*102*/*106*/*108*	*108*/*112*/*118*	*118*/*156*/*180*
	4n	1	*102*/*110*/*116*/*144*	*88*/*102*/*106*/*108*	*102*/*108*/*112*/*118*	*108*/*118*/*120*/*156*
	4n	1	*102*/*110*/*116*/*144*	*88*/*102*/*106*/*108*	*102*/*108*/*112*/*120*	*108*/*118*/*120*/*156*
	4n	1	*102*/*110*/*116*/*144*	*88*/*102*/*106*/*108*	*102*/*108*/*112*/*120*	*108*/*118*/*156*/*180*
	4n	1	*102*/*110*/*116*/*144*	*88*/*106*/*108* [*1]	*102*/*108*/*112*/*118*	*108*/*118*/*120*/*156*
	4n	1	*102*/*110*/*116*/*144*	*88*/*102*/*106*/*108*	*102*/*108*/*112*/*120*	*108*/*156*/*180* [*2]
	4n	1	*102*/*110*/*116*/*144*	*88*/*102*/*106*/*108*	*102*/*108*/*112*/*120*	*108*/*118*/*156*/*180*
	3n/4n	1	*102*/*110*/*116*/*144*	*88*/*102*/*106*/*108*	*102*/*108*/*112*/*118*	*108*/*118*/*120*/*156*
Female (2N/3Nb)	2n/3n	–	*102*/*106*/*144*	*102*/*108*/*114*	*108*/*112*/*120*	*118*/*120*/*156*
Male (2Nm)	2n	–	*108*/*110*	*100*/*106*	*118*/*120*	*120*/*180*
Progeny	2n	3	*102*/*144*	*102*/*108*	*108*/*112*	*118*/*156*
	2n	1	*102*/*108*	*106*/*114*	*118*/*120*	*156*/*180*
	2n	1	*102*/*108*	*100*/*108*	*120* [*3]	*120*/*180*
	2n	1	*106*/*110*	*106*/*114*	*112*/*120*	*156*/*180*
	2n	1	*106*/*110*	*106*/*114*	*118*/*120*	*120*/*156*
	2n	1	*106*/*110*	*106*/*108*	*118*/*120*	*120*/*156*
	2n	1	*108*/*144*	*100*/*108*	*120* [*3]	*120*/*156*
	3n	1	*102*/*110*/*144*	*102*/*106*/*108*	*108*/*112*/*120*	*118*/*156*/*180*
	3n	1	*102*/*110*/*144*	*100*/*102*/*108*	*108*/*112*/*118*	*118*/*156*/*180*
	4n	1	*102*/*106*/*110*/*144*	*100*/*102*/*108*/*114*	*108*/*112*/*118*/*120*	*118*/*120*/*156*/*180*
	2n/3n	1	*102*/*108*/*144*	*100*/*102*/*108*	*108*/*112*/*120*	*118*/*156*/*180*
Female (2N/3Nc)	2n/3n	–	*102*/*106*/*144*	*102*/*108*/*114*	*108*/*112*/*120*	*118*/*156*/*170*
Male (2Nm)	2n	–	*108*/*110*	*100*/*106*	*118*/*120*	*120*/*180*
Progeny	2n	1	*102*/*144*	*102*/*108*	*108*/*112*	*118*/*156*
	2n	1	*110*/*144*	*106*/*108*	*112*/*118*	*156*/*180*
	2n	1	*106*/*110*	*106*/*108*	*118*/*120*	*170*/*180*
	2n	1	*108*/*144*	*100*/*108*	*120* [*3]	*120*/*156*
	2n	1	*102*/*108*	*100*/*102*	*118*/*120*	*170*/*180*
	2n	1	*102*/*108*	*100*/*102*	*108*/*120*	*170*/*180*
	2n	1	*110*/*144*	*106*/*114*	*120* [*3]	*156*/*180*
	2n	1	*110*/*144*	*106*/*114*	*118*/*120*	*170*/*180*
	2n	1	*108*/*144*	*100*/*108*	*118*/*120*	*120*/*156*
	3n	1	*102*/*108*/*144*	*102*/*106*/*108*	*108*/*112*/*120*	*118*/*156*/*180*
	3n	1	*102*/*108*/*144*	*100*/*102*/*108*	*108*/*112*/*118*	*118*/*120*/*156*
	4n	1	*102*/*106*/*108*/*144*	*100*/*102*/*108*/*114*	*108*/*112*/*118*/*120*	*118*/*120*/*156*/*170*
	4n	1	*102*/*106*/*108*/*144*	*102*/*106*/*108*/*114*	*108*/*112*/*118*/*120*	*118*/*120*/*156*/*170*

Genotypes of mosaic females were represented by triploid genotypes. Underlined alleles originated from the diploid clone loach. [*1] tetraploid genotype, *88/88/106/108* or *88/106/108/108*; [*2] tetraploid genotype, *108/108/156/180* or *108/156/156/180*; [*3] diploid genotype, *120/120*.