第2減数分裂阻止によるエゾアワビの雌性発生2倍体の誘導

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Induction of Gynogenetic Diploid by Inhibiting Second Meiosis in the Pacific Abalone*1

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Successful inductions of gynogenesis and of the two kinds of triploid animals of the Pacific abalone *Haliotis discus hannai* have been reported. After careful re-examination of various conditions developed before, selected were irradiation of spermatozoa by ultraviolet ray of a total dose of 1,200 erg/mm^2 for genetic inactivation of paternal genomes and cold shock of zygote at 3°C for 15 min, starting 32 min after fertilization for retention of second polar body. A set of such treatments resulted in low survival rates of both planktonic and settled larvae but appreciable numbers of viable gynogenetic diploid, indicating 50 to 60 percentage induction of gynogenetic diploid among the animals survived. Simultaneous induction of some aneuploid animals was observed through the analyses of isozyme genotypes. Comparison of the shell-length distributions between the sib animals demonstrated that gynogenetic diploid animals revealed enlarged variances and marked deviations in mean values towards either bigger or smaller direction in comparison with those of normal diploid animals.

Induction of gynogenetic diploid could be one of the most useful technologies in breeding to expedite establishment of certain strains of superior performance and removal of recessive-deleterious genes from seedling population in aquacultural species.[1] Successful induction of gynogenesis in the pacific abalone has been reported.[2] The present paper examined various conditions of treatments of gametes and zygotes to induce gynogenetic diploid of the pacific abalone, *Haliotis discus hannai*, and evaluated some of the performance of the animals induced.

Materials and Methods

Abalone Egg and Sperm Specimens

Egg and sperm specimens were obtained by inducing spawning with sterilized and heated sea water, according to a series of procedures reported before,[2] from the animals of the pacific abalone, sampled locally from the coast of Iwate Prefecture and reared at the Iwate South Mariculture Center, Ofunato. After taking from single male, sperm suspension was prepared with a concentration of 4×10^6 spermatozoa per m*l* of sea water and successively kept in dark and cool (10° to 15°C) conditions until being treated with ultraviolet ray irradiation or used for fertilization directly without such treatment. The Thoma's cell-counter was used to determine concentration of spermatozoa. Egg specimens taken from two female animals were pooled with sea water in a plastic tank and kept at 20°C in dark until being used for fertilization.

Ultraviolet-Ray Irradiation of Spermatozoa and Fertilization

For the ultraviolet-ray irradiation of sperm, the apparatus and conditions of the treatments, described before,[2] were modified as follows. The number of ultraviolet ray (UV) lump was increased from one in the earlier case to three to give more homogeneous illumination to the layer of sperm suspension placed in petri dish. The three lumps commercially available (Toshiba GL 15, 253.7 nm of UV wave length) were fixed in parallel with each other on the surface of the ceiling of the

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wooden box described before, giving illumination of approximately 60 erg/mm^2·s in average at the surface of sperm suspension placed in petri dish. The distance between the centre of the lumps and the dish was approximately 25 cm in comparison with the 32 cm of that of the earlier apparatus. Sperm suspension in petri dish was exposed to the irradiation for 20 s, giving a dose of approximately 1,200 erg/mm^2, which is almost equivalent to that described before[2] as being optimum one for genetic inactivation of spermatozoa of the pacific abalone (refer Arai *et al.*,[2] Fig. 2, case of 30 s irradiation). Thus the dose of irradiation was fixed in spite of the increased intensity of source of UV light and of shortened time of irradiation. Immediately after the treatment, the irradiated sperm suspension was mixed with suspension of eggs for fertilization according to Arai *et al.*[2] Another batch of eggs was fertilized by untreated sperm to produce normal diploid sib animals.

Treatment of Zygotes for Gynogenetic Diploidization

Low temperature treatment of zygotes was applied to inhibit release of second polar body for diploidization according to Arai *et al.*[3] as follows. Inseminated eggs were placed carefully in a plastic cylinder, 5 cm in diameter and 6 cm deep, with a sheet of screen of 40 μm mesh at the bottom. Then the zygotes were cold-shocked at 3°C for 15 min, starting 32 min after fertilization, according to the conditions of treatments for inducing triploid animals retaining the second polar body in the pacific abalone. One part of zygotes inseminated by UV-irradiated sperm was not treated by cold shock for diploidization to examine the effect of UV-irradiation on sperm by observing survival rate of the zygotes induced. The successive handling procedures of zygotes and hatched larvae were practiced in the normal way. These treatments were carried out at room temperature.

Observations on Rates of Fertilization and Survival of Larvae

When large numbers of eggs were obtained from pairs of parental animals in a mating experiment, they were subdivided into two or three lots for a series of successive treatments. The rate of fertilization of eggs was observed soon after completion of practice of fertilization on each experimental group among the three: normal diploid (norm·2n), gynogenetic haploid (gyno·n), and gynogenetic diploid (G2n·2pb). The rate of survival of planktonic larvae was also observed four days after fertilization on each group noted above.

Determination of Ploidy Status of Offspring Animals

Two groups of sib animals of normal diploid and those hatched after treatment for inducing gynogenetic diploid from each mating were reared in separate tanks with running sea water after settlement of larvae. At approximately six to six and a half months old, specimens of animals were sampled from each group. Analyses on isozyme variations in parental animals were conducted by starch-gel electrophoresis on several loci to select suitable ones to examine genotypic relationships between parents and their offsprings. Among the loci examined, selected were three loci: phosphoglucomutase (EC 2.7.5.1, Pgm), phosphogluconate dehydrogenase (EC 1.1.1, 44, Pgdh), and leucine aminopeptidase (EC 3.4.1.1, Lap). Tissue sample of either muscle or digestive diverticula from fresh or frozen specimen was used for analyses of isozyme variations at the loci noted above by starch-gel electrophoresis according to Wilkins *et al.*,[4] Fujino and Sasaki,[5] Fujino,[6] and Shaw and Prasad.[7] Genotypes were read from zymograms obtained and were used to examine the ploidy status of individual animals.

Results

Improved Treatment of Sperm Suspension for Genetic Inactivation

A series of careful examination of UV-illumination at the surface of sperm suspension placed in a petri dish indicated a reduced variations of the dose of UV ray, ranging from 1,020 to 1,340 erg/mm^2, under the conditions of the treatment of the present work in comparison with those, varied from approximately 300 to 2,300 erg/mm^2, under the conditions of the treatment by our earlier apparatus. Table 1 summarizes the rates of fertilization of eggs and of survival of planktonic larvae, observed at one and four days after fertilization respectively. It was commonly demonstrated in all lots of the experimental groups in the mating nos. 906 and 909, that the group of eggs/animals treated for inducing gynogenetic haploid (Gyno·n) revealed zero survival after successful fertilization in comparison with the appreciable survivals of the group of normal diploid animals (Norm·2n)

Table 1. Rates of fertilization of eggs and survival of planktonic larvae in some selected experimental mating groups of the pacific abalone

Mating and lot no.*4	Experimental group	Rate of fertilization (A, $\times 10^3$)*5	Rate of survival (B, $\times 10^3$)*6
906–1	Norm·2n*1	99.1% (114)	35.3% (113)
	Gyno·n*2	87.6 (250)	0.0 (219)
	G2n·2pb*3	89.6 (914)	3.3 (819)
906–2	Norm·2n	99.1 (110)	26.2 (109)
	Gyno·n	86.8 (235)	0.0 (204)
	G2n·2pb	87.6 (1,052)	4.6 (922)
909–1	Norm·2n	88.3 (196)	42.8 (173)
	Gyno·n	18.4 (147)	0.0 (27)
	G2n·2pb	14.1 (341)	4.2 (48)
909–2	Norm·2n	50.8 (61)	64.5 (31)
	Gyno·n	20.8 (120)	0.0 (25)
	G2n·2pb	19.6 (567)	9.0 (111)
909–3	Norm·2n	48.4 (128)	32.3 (62)
	Gyno·n	18.4 (141)	0.0 (26)
	G2n·2pb	16.7 (522)	4.6 (87)

Remarks, *1, *2, *3, These three groups denote animals of normal diploid (untreated), those treated for inducing gynogenetic haploid, and those for gynogenetic diploid retaining second polar body respectively.

*4, Eggs were subdivided into two or three lots before fertilization in the mating nos. 906 and 909 respectively.

*5, *6, Figures A and B in parenthesis indicate numbers of eggs used for fertilization (A) and those fertilized successfully (B), based on the observations made soon after fertilization.

Table 2. Rate of survival of animals after settlement in some selected experimental mating groups of the pacific abalone

Mating no.*1	Experimental group	No. of animals settled ($\times 10^3$)	Rate of survival (%) at age of approximately:		
			one month,	three month,	six month
906	Norm·2n	13	47.7	16.2	12.6 (2,100)*2
	G2n·2pb	70	1.0	0.3	0.1 (225)
909	Norm·2n	18	83.3	19.6	17.5 (3,500)
	G2n·2pb	16	2.4	0.6	0.4 (80)

*1 Animals of each experimental group from two lots in mating no. 906 or three lots in mating no. 909 were pooled into single lot each before beginning of settlement of larvae (refer Table 1).

*2 Figures in parenthesis indicate numbers of animals survived at age of approximately seven month old.

Table 3. Genotypic compositions at multiple loci of the parental animals used for mating nos. 906 and 909 of the pacific abalone

	Mating no. 906			*Mating no. 909*		
	Identification number of parental animals					
Locus*	Females		Male	Females		Male
	Y300	Y314	B208	R34	R1	B222
Lap·S	2·2	2·2	4·4	2·4	2·2	2·4
Pgm·F	3·3	1·3	1·2	2·3	3·3	3·3
Pgm·S	3·4	4·4	3·4	2·2	3·3	2·2
Pgi·S	2·2	2·2	2·2	2·2	2·2	2·2
Pgdh	4·4	4·4	4·4	4·4	4·4	4·7
Me	1·2	1·1	1·1	1·2	1·2	1·1
α-Gpdh	1·2	1·2	1·2	1·2	2·2	2·2
To	1·1	1·2	1·2	1·1	1·1	1·1

* Examined were isozymes of phosphogluconate dehydrogenase (Pgdh), phosphoglucomutase (Pgm), phosphoglucose isomerase (Pgi), esterase (Es), leucineaminopeptidase (Lap), malic enzyme (Me), alpha-glycerophosphate dehydrogenase (α-Gpdh), and superoxide dismutase (To). Thermostability variations were not examined.

Table 4. Isozyme genotype compositions observed in parents and their offspring populations of mating no. 906 of the pacific abalone

Parents Identification no.		Females Y314	Females Y300	Male B208
Locus	Lap·S	2·2	2·2	4·4
	Pgm·F	1·3	3·3	1·2

F_1 offsprings

Normal diploid

Genotype	No. of animals observed
Lap·S	
2·2	0
2·4	20
Total	20
Pgm·F	
1·1	0
1·2	1
1·3	12
2·3	7
Total	20

Diploid resulted from treatment for inducing gynogenetic diploid

Genotype	No. of animals observed
Lap·S	
2·2	12
(2·4)*1	3
Total	15
Pgm·F	
1·1	0
1·3	5
(2·3)*1	1
3·3	9
Total	15

Contingency tables of genotypic occurrences at the two loci in offsprings

Normal diploid

Lap·S	Pgm·F 1·2	1·3	2·3	Total
2·4	1	12	7	20
Total	1	12	7	20

Gynogenetic diploid/aneuploid

Lap·S	Pgm·F 1·1	1·3	(2·3)*1	(1·3·3)*2	(2·3·3)*2	3·3	Total
2·2	0	3	0	0	2	9	14
(2·4)*1	0	2	1	2	1	0	6
Total	0	5	1	2	3	9	20

*1 Genotypes in parenthesis were theoretically denied as gynogenetic diploid from the mating.
*2 These five animals were not included in the numbers of diploid animals described above.

Induction of Gynogenetic Diploid

It was also demonstrated commonly in all the lots of the experimental group in the mating nos. 906 and 909 (Table 1) that the group treated for inducing gynogenetic diploid (G2n·2pb) retaining second polar body resulted in to induce some viable animals, although the rates of survival were low. Animals of each experimental group (norm·2n and G2n·2pb) from the two lots of the mating no. 906 and those from the three lots of mating no. 909, shown in Table 1, were each pooled into a single lot before the beginning of settlement of planktonic larvae for a series of successive observations. Table 2 shows the rates of survival of animals of each experimental group in the mating nos. 906 and 909 at the ages of one, three, and six month old, demonstrating the decreasing but appreciable numbers of animals survived.

Table 3 summarizes the genotypic compositions at multiple loci in the parental animals used in mating nos. 906 and 909. On the bases of the genotypic relations between female and male animals, the two loci selected were of Pgm·F and Lap·S in mating no. 906 and one locus of Pgdh in mating no. 909 to examine qualitatively and/or quantitatively whether or not gynogenetic diploids were successfully induced after a series of the treatments. Table 4 presents the relationships of genotype compositions at the selected loci between the parents and their offspring animals as well as those between the selected two loci in the form of contingency table in mating no. 906. At Lap·S locus, only genotypes 2·4 and 2·2 are theoretically possible in normal diploid and gynogenetic diploid offspring animals respectively, when a complete genetic inactivation of spermatozoa is attained. The occurrence or genotype 2·4 in the animals produced after the treatments for inducing gynogenetic diploid suggests that genetic inactivation of sperm suspension was

Table 5. Parent-F_1 offspring relations of genotypes at Pgm·F locus in mating no. 906 of the pacific abalone

Parents	Females		Male
Genotype	1·3	3·3	1·2
proportion	a	b	
Allele	1 . . . p_M: $\frac{a}{4}$		p_P: $\frac{1}{2}$
frequency	2 . . . q_M: 0		q_P: $\frac{1}{2}$
	3 . . . r_M: $\frac{a}{4}+\frac{b}{2}$		r_P: 0
, where a+b=2.			
F_1 normal 2n offspring			
Genotype	1·1: $\frac{a}{8}$		
proportion	1·2: $\frac{a}{8}$		
	1·3: $\frac{a+2b}{8}$		
	2·3: $\frac{a+2b}{8}$		
Gametes mated for producing G2n·2pb*	eggs	UV-irradiated sperms	
Genotype	1·1: $\frac{a}{4}(1-2x)$	p_P	genetically
proportion	1·3: ax	q_P	inactivated
	3·3: $\frac{a}{4}(1-2x)+\frac{b}{2}$	r_P : 0	
F_1 G2n·2pb* offspring			
Genotype	1·1: $\frac{a}{4}(1-2x)$		
proportion	1·3: ax		
	3·3: $\frac{a}{4}(1-2x)+\frac{b}{2}$		

* Gynogenetic diploid retaining second polar body.

not completely attained. Similarly, genotypes of 1·1, 1·2, 1·3, and 2·3 and those of 1·3 and 3·3 at Pgm·F locus are theoretically possible in normal and gynogenetic diploid animals respectively. Occurrence of genotype 2·3 in the animals treated for inducing gynogenetic diploid suggests the incompleteness in genetic inactivation of sperm suspension, supporting the observations on the Lap·S genotypes noted above.

The relation of genotypic occurrence between the two loci of Pgm·F and Lap·S in the animals produced after the treatments for inducing gynogenetic diploid, shown in the form of contingency table in Table 4, indicated that some animals revealed triploid-like zymogram (genotypes 1·3·3) at Pgm·F locus but diploid-like zymogram (genotype 2·4) at Lap·S locus (refer Fig. 1 in Fujino *et al.*[8]), suggesting the occurrence of aneuploid animals. This observation supports the statement noted in the foregoing paragraph, which suggested an incomplete genetic inactivation of sperm suspension. Thus, the number of gynogenetic diploid animals induced can be accounted approximately 12 animals or less among 20 those produced from the mating no. 906.

Table 5 presents the parent-F_1 diploid offspring relations of genotypes at Pgm·F locus, developed according to Fujino *et al.*[9,10] After putting the observed frequencies of occurrence of genotypes of normal diploid animals (Table 4) into the formulas of the table, proportional contributions of the two female animals were calculated as a=0.2 and b=1.8. Application of these values as well as that of the rate of gene-centromere recombination,

Table 6. Isozyme genotype compositions observed in parents and their offspring populations of mating no. 909 of the pacific abalone

Parents Identification no.		Females R34 and R1		Male B222
Isozyme locus	Pgdh	4·4		4·7
F_1 offspring				
Normal diploid			Diploid resulted from treatment for inducing gynogenetic diploid	
Genotype at Pgdh locus	No. of animals observed		Genotype	No. of animals observed
4·4	23		4·4	24
4·7	7		(4·7)*	6
Total	30		Total	30

* Genotype in parenthesis was theoretically denied as gynogenetic diploid from the mating.

Table 7. Parent-F_1 offspring relations of genotypes at Pgdh locus in mating no. 909 of the pacific abalone

Parents	Females	Male
Identification no.	R34 and R1	B222
genotype	4·4	4·7
allele	4 . . . $p_M=1.000$	$p_P=\frac{1}{2}$
frequency	7 . . . $q_M=0.000$	$q_P=\frac{1}{2}$
F_1 normal diploid		
genotype proportion		4·4: $\frac{1}{2}$
		4·7: $\frac{1}{2}$
gametes mated for inducing G2n·2pb	egg	UV irradiated sperm*
genotype	4·4	$p_P=\frac{1}{2}(1-c)$
		$q_P=\frac{1}{2}(1-c)$
F_1 gynogenetic diploid/other diploid		
genotype		4·4: $\frac{1+c}{2}$
proportion		4·7: $\frac{1-c}{2}$

* c, proportion of sperm genetically inactivated.

x=0.396, reported earlier[9] to the formulas of genotype proportions of gynogenetic diploid (G2n·2pb) resulted in suggesting the possible inclusion of normal diploid in the three animals of genotype 1·3, shown in the contingency table of Table 4. Together with the statements in the foregoing paragraph, percentage induction of gynogenetic diploid in mating no. 906 was estimated as $50.0\left(=\frac{1+9}{20}\right)$. On the bases of a series of foregoing analyses, the expected frequencies of occurrence of genotypes at Pgm·F locus were calculated as summarized in Table 8.

Table 6 presents the relationships of genotype compositions at Pgdh locus observed between the parents and their offspring animals in mating no. 909. Occurrence of genotype 4·7 in the animals produced after the treatments for inducing gynogenetic diploid suggests that genetic inactivation of sperm suspension was not completely attained similarly to the case of the mating no. 906. Table 7 presents the parent-F_1 diploid offspring relations of genotypes at Pgdh locus, developed according to Fujino *et al.*[9,10] After putting the observed frequencies of occurrence of genotypes observed (Table 6) after the treatment for inducing gyno-

Table 8. Observed and expected frequencies of genotypes at some selected loci in the offsprings of mating nos. 906 and 909 of the pacific abalone

Pgm·F locus in mating no. **906**

Normal diploid			Gynogenetic diploid		
Genotype	obs.	exp.*1	Genotype	obs.	exp.*1
1·1	0	0.5	1·1	0	0.1
1·2	1	0.5	1·3	1	0.8
1·3	12	9.5	3·3	9	9.1
2·3	7	9.5			
Total	20	20.0	Total	10	10.0

Pgdh locus in mating no. **909**

Normal diploid			Gynogenetic diploid		
Genotype	obs.	exp.*2	Genotype	obs.	exp.
4·4	23	15.0	4·4	18	18.0
4·7	7	15.0			
Total	30	30.0	Total	18	18.0

*1 Expected frequencies were calculated on the basis of the values of parameters: a=0.20, b=1.80, and x=0.396 (see text for more detail).

*2 $x^2=8.533$, 1 df, 0.01→P.

genetic diploid, percentage induction of gynogenetic diploid was estimated as 60.0 in the mating no. 909. The expected frequencies of occurrence of genotypes at Pgdh locus in the mating were summarized in Table 8 together with the observed figures.

Performance of Gynogenetic Diploid

Fig. 1 compares the shell-length distributions between normal diploid and animals produced after the treatment for inducing gynogenetic diploid retaining second polar body in the mating nos. 906 and 909 of the pacific abalone. In mating no. 906, the animals produced after the treatment reveal much greater variance in the distribution of shell-length ($s^2=22.9$) in comparison with that of normal diploid sib animals ($s^2=2.3$), indicating a smaller mean value (10.3 mm) in gynogenetic diploid (G2n·2pb) and the bigger value in aneuploid animals (16.5 mm). In mating no. 909, the animals produced after the treatment reveal a much bigger mean value (21.2 mm) than that of normal diploid (11.7 mm) with the greater variance ($s^2=10.2$ to 12.3) to that of normal diploid ($s^2=2.9$). No significant difference was shown in either the mean value/or variance between G2n·2pb and aneuploid animals in mating no. 909.

Discussions

Percentage Induction of Gynogenetic Diploid

In the present study, a series of the treatments of genetic inactivation of spermatozoa by UV ray irradiation with an approximate dose of 1,200 erg/mm^2 and of retaining polar body by cold shock of fertilized eggs at 3°C for 15 min resulted in successful induction of gynogenetic diploid, retained second polar body, indicating a range of 50 to 60 percentage induction of its occurrence among the animals examined at an age of approximately six month old. Analyses of isozyme genotypes proved the occurrence of aneuploid animals other than gynogenetic diploid, despite a reduced range of variations of dosage of UV ray irradiation. The result of the observations suggested the neccessity of further effort for technical improvement towards more homogeneous UV illumination throughout sperm suspension preparations.

The reason for the significant deveiations in genotypic frequency distribution at Pdgh locus in normal diploid of mating no. 909, shown in Table 8, is not known yet. A more careful examination of additional mating data together with the evidence obtained from the earlier observations on the wild population[5] as well as that from the earlier mating experiment[11] could provide an overall view of any dynamic features of genotypic compositions at Pgdh locus in the species in the

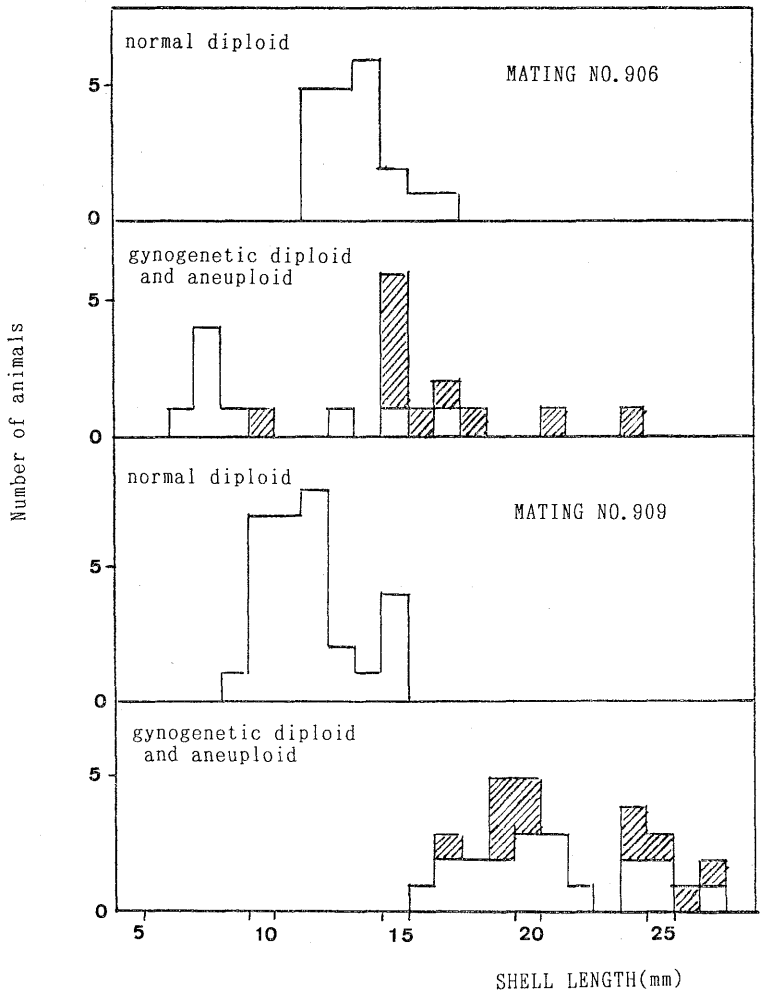

Fig. 1. Comparison of shell-length distributions between normal diploid, gynogenetic diploid, and aneuploid (hatched) sib animals in mating nos. 906 and 909 of the pacific abalone. Mean value (x) and standard deviations (s) were calculated for the shell-length distributions in mm: 13.6±1.5, 10.3±3.9, and 16.5±4.0 in normal diploid, gynogenetic diploid, and aneuploid animals respectively in mating no. 906 as well as 11.7±1.7, 20.7±3.2, and 21.8±3.5 in the same three sib animals respectively as the above in mating no. 909.

future.

Segregation of Shell-length Distribution due to Manipulations

Comparisons of shell-length distributions between normal diploid and sib animals induced after the treatment indicated greater variances and shifts of mean values towards either bigger or smaller directions in the animals of gynogenetic diploid as well as aneuploid induced. While, Fujino[12] demonstrated shifts in degrees of heterozygosity/homozygosity in genome/isozyme genotype compositions of ootids due to manipulations, indicating increased homozygoisty in ootids retaining second polar body. The above observations in the present study together with the shifts of homozygosity/heterozygosity, theoretically demonstrated in the above, proved the phenomenon of segregation of maternal genomes in the offspring populations due to manipulations.[13]

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References

1) K. Fujino: in "Recent Prog. in Breed, Sci. **28**" (ed. by Jap. Soc. Breed.), Keigaku Publ. Co., Tokyo, 1987. pp. 75–86.
2) K. Arai, F. Naito, H. Sasaki, and K. Fujino: *Nippon Suisan Gakkaishi*, **50**, 2019–2023 (1984).
3) K. Arai, F. Naito, and K. Fujino: *Nippon Suisan Gakkaishi*, **52**, 417–422 (1986).
4) N. P. Wilkins, K. Fujino, and K. Sasaki: *Nippon Suisan Gakkaishi*, **46**, 549–553 (1980).
5) K. Fujino and K. Sasaki: *Nippon Suisan Gakkaishi*, **50**, 11–15 (1984).
6) K. Fujino: *Proc. XVI Intern. Conf. Anim. Bl. Gr. Biochem. Polymor.*, 245–256 (1979).
7) C. R. Shaw and R. Prasad: *Biochem. Genet.*, **4**, 297–320 (1970).
8) K. Fujino, R. Kaneko, T. Ikeda, K. Arai, and S. Okumura: *Nippon Suisan Gakkaishi*, **55**, 1875–1883 (1989).
9) K. Fujino, S. Nakajima, and H. Sawada: *Nippon Suisan Gakkaishi*, **54**, 953–958 (1988).
10) K. Fujino, S. Nakajima, and T. Takahashi: *Nippon Suisan Gakkaishi*, **54**, 2049–2054 (1988).
11) K. Fujino, M. Kamakura, R. Mito, and K. Arai: *Nippon Suisan Gakkaishi*, **53**, 1759–1764 (1987).
12) K. Fujino: *Fish Genetics and Breed. Sci.* **13**, 1–7 (1988).
13) S. Wright: *Ann. Eugen.*, **15**, 323–354 (1951).