Increased Variance of Quantitative Characters in the Two Types of Gynogenetic Diploids of Indonesian Common Carp

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Gynogenetic diploid induced by retaining second polar body and that by suppressing first cleavage of eggs of Indonesian common carp *Cyprinus carpio* were produced by inseminating eggs with ultra-violet ray irradiated sperm of Java carp *Puntius gonionotus* and giving heat shock treatment to the developing eggs. The morphometric and meristic characters of 3 month old fish were measured and counted. There was the prominent trend that the degree of phenotypic variation was larger in the mitotic- and meiotic-gynogenetic diploids than in the control-2N in the almost all morphological characters examined. These phenomena observed in variation of quantitative characters were explained by the function showing the relationship among phenotypic variance (V_p), genetic variance (V_g), environmental variance (V_e) and coefficient of inbreeding (F) in the first gynogenetic generation. The genetic implications for gynogenetic diploids were discussed in relation to genetic improvement of fish race in aquaculture.

Gynogenetic diploid induced by retaining the second polar body (abbreviated as meiotic-G2N) shows a different level of homozygosity in genotype composition by locus depending upon gene-centromere recombination rates.^{1,2)} In rainbow trout, for instance, it is infeasible to produce any line homozygous at all loci and genome by inducing gynogenesis followed by retention of the second polar body.¹⁾ However, gynogenesis by suppressing the first cleavage (abbreviated as mitotic-G2N) is able to produce offspring showing complete homozygosity both at all loci and genomes in the 1st generation because it is not given any effects of crossing over during meiosis.³⁻⁵⁾ Therefore, this method may have an advantage to produce pure lines in the 2nd generation.⁵⁾

It is expected that the coefficient of inbreeding (F) increases in gynogenetic diploid.⁶⁾ The genetic variance of quantitative characters will also increase in the first gynogenetic generation according to the formula of variance among inbreeding lines, $(1+F)V_g$,⁷⁾ if the variance among gynogenetic individuals could be considered as analogous with that among the inbreeding lines. Since mitotic-G2N takes a maximum value of F , it is expected the level of phenotypic variance will be always larger in the mitotic-G2N than in the meiotic-G2N as ascertained in ayu, *Plecoglossus altivelis*,⁸⁾

but the variance will decrease within a family in the second generation of G2Ns.⁵⁾ The variance analysis seemed to be useful for the estimation of heritability based on the degree of increase in genetic variance on the two types of gynogenetic diploid, and may give useful informations for the genetic improvement of fish race.⁵⁾

In this paper, we estimated the phenotypic variations of some quantitative characters and compared the values of the G2Ns with these of control-2N of Indonesian common carp stock to ascertain the phenomenon of increasing in variation being a remarkable genetic character of G2Ns,⁵⁾ to assess the degree of heritability in each quantitative character.

Materials and Methods

Five matured females of Majalaya stock, one of the most important stock in freshwater fish culture in Indonesia, were used to collect eggs for the experiments to induce gynogenetic diploids. A female being about 4 kg in body weight was given an injection of extract of 3 mg silver carp pituitary gland per kg body weight of the female to induce ovulation, and then put into the spawning pond together with a few males. Just after the first spawning action being considered as a signal for

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complete ovulation which is usually observed early in the morning, the female was transferred to the laboratory for egg collection by stripping the belly. The sperm from a male of java carp (tawes), *Puntius gonionotus*, was irradiated with ultraviolet light by the method of Taniguchi *et al.*,⁹⁾ and used for inseminating the common carp eggs. The initial time to heat shocking applied were 2 to 4 min after immersion of the eggs in the water temperature 25°C for induction of meiotic-G2N and 40 to 44 min for induction of mitotic-G2N. We applied heat shocking for 1.5 min at 40°C. The eggs were incubated in 80 l aquaria with recirculating system. The water temperature was 24–25°C. The recirculating system was stopped a few hours before hatching-out. As for the detailed procedures and verification of successfulness of gynogenesis in Indonesian common carp see Sumantadinata, *et al.*,¹¹⁾ The hatched fry were fed artemia nauplii for about ten days.

The two week old fry were transferred into 100 l concrete tanks with a double bottom and airlift system.¹²⁾ The density was 50 fry per tank for the first month, then the density was lowered to 25 fry per tank for the second and third months. During the first month rearing, the fry were fed moina, daphnia and the shrimp feed. In the second and third month, the fish were fed three times daily with larger sized shrimp feed and fresh tubifex worm.

The three month old fish were taken for meas-

urements and counts for morphological characters. The number of samples from the 5 experiments are presented in Table 1. The following morphometric and meristic characters were measured and counted: the relative values of body depth, body width, head length and intestine length to standard length, and the numbers of vertebrae, lateral line scales, gill rakers on the first gill arch, soft rays of dorsal, pectoral, ventral and anal fins.

The means and coefficients of variation of these morphometric and meristic characters were calculated for each experiment and for the pooled of all the five experiments. Meristic characters of the female parents were also counted.

Results

Variation in morphometric characters

The body sizes of gynogenetic offsprings at 3 month old were not different significantly from that of the control-2N as shown in the mean values of standard length in each experiment (Table 1). However, there was a trend that the coefficients of variation in body size were always greatest in the mitotic-G2N, followed by meiotic-G2N, and smallest in the control-2N.

The means and coefficients of variation for morphometric characters in the five experiments are summarized in Table 2 to compare these values of the two types of G2N with that of the control-2N. Mean proportional values of body

Table 1. Number (n) of samples, mean and coefficient of variation (CV) for standard length (cm) of the meiotic-G2N, mitotic-G2N and control-2N from 5 experiments

Experiment No.		Control-2N	Meiotic-G2N	Mitotic-G2N
1	n	50	53	11
	Mean	7.873	6.960	8.658
	CV (%)	8.9	9.9	17.7
2	n	46	39	17
	Mean	8.072	6.793	8.971
	CV (%)	8.6	11.4	23.5
3	n	53	47	31
	Mean	8.492	7.239	7.976
	CV (%)	6.6	13.4	23.0
4	n	50	46	17
	Mean	7.210	7.262	7.622
	CV (%)	12.5	17.0	19.4
5	n	50	50	51
	Mean	6.657	6.722	6.921
	CV (%)	9.7	11.7	14.7

Table 2. Mean and coefficient of variation (CV) of relative avlue of morphometric characters to standard length in the control-2N, meiotic-G2N and mitotic-G2N for 5 experiments

Types of offspring	Experiment no.										Overall population		
	1		2		3		4		5		Mean	CV (%)	V
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)			
Body depth													
C-2N	0.40	6.0	0.40	10.0	0.39	4.0	0.41	6.0	0.40	9.0	0.40	7.0	0.00078
Mei-G2N	0.40	8.0	0.41	6.0	0.41**	7.0	0.43**	9.0	0.44**	9.0	0.42**	8.0	0.00113
Mit-G2N	0.42*	6.0	0.44*	8.0	0.44**	6.0	0.47**	6.0	0.44**	8.0	0.44**	7.0	0.00095
Body width													
C-2N	0.21	8.0	0.21	11.0	0.20	7.0	0.22	6.0	0.21	8.0	0.21	8.0	0.00028
Mei-G2N	0.22**	9.0	0.22**	8.0	0.22**	16.0	0.24**	10.0	0.24**	8.0	0.23**	10.0	0.00053
Mit-G2N	0.23**	6.0	0.23**	9.0	0.23**	8.0	0.25**	8.0	0.25**	10.0	0.24**	9.0	0.00047
Head length													
C-2N	0.35	14.0	0.35	7.0	0.35	6.0	0.38	4.0	0.37	5.0	0.36	7.0	0.00064
Mei-G2N	0.37*	8.0	0.36	7.0	0.37**	7.0	0.38	5.0	0.38*	5.0	0.37**	6.0	0.00049
Mit-G2N	0.36	5.0	0.36	8.0	0.36	9.0	0.40	20.0	0.39**	5.0	0.38**	8.0	0.00092
Intestine length													
C-2N	1.71	13.0	1.63	16.0	1.74	13.0	1.66	9.0	1.70	11.0	1.69	12.0	0.04113
Mei-G2N	1.65	13.0	1.61	11.0	1.67	15.0	1.74*	14.0	1.92*	10.0	1.72	13.0	0.05000
Mit-G2N	1.82	19.0	1.70	28.0	1.71	16.0	1.93**	11.0	1.78*	14.0	1.78	16.0	0.08111

Student's t-test between C-2N and Mei-G2N or Mit-G2N, *: $P < 0.05$; **: $P < 0.01$.

Fig. 1. Comparison of the coefficients of variation among control-2N, meiotic-G2N, and mitotic-G2N in morphometric characters.

Fig. 2. Comparison of the frequency distribution pattern in vertebral counts among control-2N, meiotic-G2N and mitotic-G2N.

depth, body width and head length of two types of G2N were significantly larger than those of control-2N in overall samples. The trends were also

observed in respective experimental groups, although some exceptional cases were observed.

The coefficients of variation for the relative intestine length were largest in the mitotic-G2N, smallest in the control-2N and intermediate in the meiotic-G2N in the overall samples. This trend was not observed in the other morphometric characters (Table 2, Fig. 1).

Variation in Meristic Characters

Figs. 2 and 3 are examples of histograms of the pooled data of vertebral number and soft dorsal rays from five experiments to compare the distributional patterns of the two types of G2N with those of the control-2N. Histograms of the control-2N seemed to be a normal distribution. Distributional patterns of both types of G2N were deviated from these of control-2N and seemed to be more flattened in each character. The deviations in meristic count of the two types of G2N from the control-2N were directed to the left or to the smaller number of meristic counts. These

Fig. 3. Comparison of the frequency distribution pattern in soft ray counts of dorsal fin among control-2N, meiotic-G2N and mitotic G2N.

Table 3. Mean and coefficient of variation of meristic characters in the control-2N, meiotic-G2N, and mitotic-G2N of 5 experiments

Types of offspring	Experiment no.										Overall population		
	1		2		3		4		5		Mean	CV (%)	V
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)			
Vertebrae:													
C-2N	34.78	2.2	34.52	2.8	35.66	3.4	34.42	3.2	34.90	3.6	34.87	3.1	1.168
Mei-G2N	34.04*	3.7	34.74	2.7	34.89**	3.5	34.70	3.2	34.22*	3.7	34.49	3.4	1.423
Mit-G2N	33.91**	3.6	31.67**	5.8	34.61**	3.7	34.65	3.9	33.61**	5.5	33.76*	4.7	2.518
Dorsal fin:													
C-2N	17.74	2.9	17.63	5.0	17.72	3.0	17.88	3.1	17.08	4.9	17.61	3.9	0.472
Mei-G2N	17.62	3.1	17.21*	5.7	17.38*	5.1	17.30**	6.0	16.62*	5.6	17.23	5.0	0.742
Mit-G2N	16.82**	5.8	16.50*	10.2	17.06**	6.4	17.47	5.4	16.78	5.6	16.91*	6.4	1.171
Pectoral fin:													
C-2N	14.06	4.1	14.02	7.6	14.57	4.7	14.42	4.7	14.52	4.2	14.33	4.9	0.493
Mei-G2N	13.96	3.1	14.03	7.0	14.36	4.9	14.37	4.7	14.38	5.0	14.22	4.8	0.466
Mit-G2N	13.91	7.5	13.50	7.3	14.03*	5.3	14.64	4.1	14.35	5.0	14.16	5.5	0.607
Ventral fin:													
C-2N	7.94	3.9	7.85	4.6	7.96	3.4	7.94	3.0	7.94	1.7	7.94	3.3	0.069
Mei-G2N	7.91	3.7	7.62	8.8	7.89	3.9	7.76*	6.1	7.48**	7.2	7.74*	5.8	0.202
Mit-G2N	7.63	6.6	7.22*	11.2	7.77*	6.3	7.88	4.2	7.47**	8.3	7.58*	7.5	0.323
Anal fin:													
C-2N	5.94	4.0	5.74	10.1	5.96	3.2	6.00	0	5.86	0	5.92	3.4	0.041
Mei-G2N	5.98	2.3	5.59	12.8	5.89	6.3	5.89	5.3	5.86	6.9	5.85	6.4	0.140
Mit-G2N	5.81	6.9	5.50	11.3	5.71*	8.0	5.88	5.6	5.88	6.6	5.78	7.5	0.188
Lateral line scales:													
C-2N	32.98	2.5	32.09	4.2	32.36	3.5	33.04	2.9	32.38	4.1	32.58	3.3	1.156
Mei-G2N	31.98**	2.4	24.31**	50.4	32.09	5.0	32.63	3.6	32.76	3.7	31.02	11.4	12.505
Mit-G2N	31.91**	3.5	28.83	31.2	31.68	6.6	23.35	4.8	32.67	3.8	29.29	8.3	5.910
Gill rakers:													
C-2N													
C-2N	20.42	7.0	20.35	5.0	19.36	4.0	19.80	6.0	16.30	5.0	19.22	5.4	1.077
Mei-G2N	20.24	5.0	19.77	10.0	19.00	8.0	19.70	6.0	18.94**	5.0	19.53	6.6	1.661
Mit-G2N	20.00	10.0	18.83	27.0	19.16	10.0	20.94	9.0	19.39**	6.0	19.52	10.5	4.201

Student's t-test between C-2N and Mei-G2N or Mit-G2N. *: $P < 0.05$; **: $P < 0.01$.

Fig. 4. Comparison of the coefficient of variation values of control-2N, meiotic-G2N and mitotic-G2N in meristic characters.

distributional patterns in the two types of G2N being more flattened than the control-2N were observed in all of the meristic characters examined in this study.

Table 3 shows mean counts and the coefficients of variation of meristic characters in the control-2N and the two types of G2N. Mean values of some meristic characters of the two types of G2N were not significantly different from the control-2N. The significant decreases in the mean value were observed in a few characters of G2Ns; these are vertebral counts and numbers of fin rays. On the other hand, a significant increase in mean value was observed in gill raker counts of the G2Ns (Ex. 5).

Values of the coefficient of variation (CV) in meristic counts in overall samples were highest in the mitotic-G2N, lowest in the control-2N and intermediate in the meiotic-G2N with some exceptional cases (Fig. 4). These trends observed in the CV values of the meristic counts were more prominent than those of morphometric characters. These similar trends were also observed in each experiment. An exceptional case from the trend was observed in the counts of lateral line scales of the overall samples (Table 3).

The phenotypic variance (V_p) values of the mitotic-G2N were less than double of control-2N in the most morphometric characters and more than double of control-2N in the most meristic characters of overall samples with some exception (Tables 2 and 3).

Discussion

In the 1st generation of gynogenesis, genetic

variation in the quantitative characters of overall samples is generally expected to expand as an effects of segregation of genotypes, but it is reduced in the second generation of gynogenesis.⁵⁾ The phenomenon of expansion of variation has actually been observed in the 1st generation of G2N populations in ayu. This phenomenon is well explained by the formula of phenotypic variance in G2Ns as follows: $V_p = V_g(1+F) + V_e$ for meiotic-G2N, and $V_p = 2 \times V_g + V_e$ for mitotic-G2N.⁵⁾

As mentioned above, the fish examined here were reared under the same condition and same density using same sized concrete tanks with a double bottom and airlift system. Therefore, the environment condition given for each experimental group is considered to be the same, so that the effect of environmental conditions on morphological characters are equivalent principally for each experimental group. Differences between G2Ns and control-2N in the level of coefficients of variation were well explained by the formula cited above. The trend that the level of variation is highest in mitotic-G2N, and lowest in control-2N and intermediate in meiotic-G2N, was prominent in the meristic characters examined here. On the other hand, this trend was not so distinctly observed in the morphometric characters except by relative intestine length. According to the formula of phenotypic variance in G2Ns, it is suggested that the level of genetic variances for these morphometric characters are very low comparing with those of meristic characters and relative length of intestine examined.

Concerning to the mean values, the differences between G2Ns and control-2N can not be explained by the formula of phenotypic variance for the 1st generation of G2Ns cited above. The present data show a slight increase in the means of body proportions, but a decrease in the means of meristic counts from the control-2N to the meiotic-G2N and mitotic-G2N in the three month old fish. These changes in distributional pattern shown in Fig. 3 for examples also observed in the other meristic counts as "left direction effects". These changes in the mean values of morphometric and meristic characters suggest the presence of the effect of deleterious genes predicted in the in-breeding line.

Even though phenotypic variances in quantitative characters of meiotic- and mitotic-G2N increased, the level of expansion of variance must be less than double of the variance value of the control-2N as expected by the formula cited above.

However, the present study as well as study on ayu⁹⁾ showed that the variance values of the most quantitative characters in the first generation of two types of G2Ns were far larger than the control-2N (Table 3). These very large values of variance in carp and ayu may also suggest the effect of a deleterious gene on the variance of meristic characters of G2Ns as well as segregation of genotypes in polygenic characters.

Among the morphological characters examined in the present study, the lateral line scale and gill raker counts of experiment 2 and 3 showed the drastic increase of variance. These great increases of variance in lateral line scale counts were probably caused by the appearance of about 18% mirror carp of scattered type in the G2N offspring. The mirror carp of the scattered type may be segregated from the female parent having Ssnn genotype (scaled) for scale control. The large variance of gill rakers of experiment 2 and 3 may also be caused by the segregation of genes of mirror carp. The mirror carp commonly have lower number of gill rakers compared to the scaled type.¹³⁾

Differences between morphometric and meristic counts in the degree of expansion of variation in the first generation of two types of G2N may be due to the different level of heritability in these characters in the original population.

The genetic background of fish samples used for induction of G2Ns may also give an effect on the degree of increase of variation in the 1st generation of G2Ns. The spawners of ayu used for induction of the G2Ns were collected from the wild population which may include a wide range of variation with a low level of deleterious genes. On the other hand, the Indonesian common carp is domesticated and selected for very long years, so that the level of genetic variation may become low within the stocks and the deleterious genes may have also been already removed through domestication. The difference between fish species or fish races in the degree of increase of variation in the 1st generation of G2Ns should be well investigated.

The present study of phenotypic variation of quantitative characters in the two types of G2N gave evidence to the theory showing the relationship between F value and phenotypic variance in the two types of G2N mentioned above as well as the effects of inbreeding. Since the 1st genera-

tion of G2Ns are heterogenous and may include deleterious genes as suggested by the coefficients of variation in quantitative characters, the direct application of G2N offspring to practical pond culture is not reasonable and appropriate. It is important that the G2N offsprings are more profitable as the parents to get effective selection responses in the 2nd generation of meiotic-G2N or to produce clonal populations as the 2nd generation of the mitotic-G2N as already produced in ayu.* The authors will try to produce the second generation of gynogenetic offsprings to fix good traits and to establish useful clonal populations from the selected breeders of the 1st generation of G2Ns.

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