Differential Contribution of Oocytes to Triploid Offsprings due to Maternal Genotypes at Multiple Loci in the Pacific Abalone^{*1}

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(Received February 24, 1988)

Quantitative analyses of genotypic relationships between parents and their diploid and triploid offspring sib populations, induced by inhibiting release of first or second polar body, have made it possible to estimate rate of gene-centromere recombination at phosphogluconate dehydrogenase and phosphoglucumutase loci and to disclose differential contribution of oocytes to triploid offspring, in relation to the latter locus in the Pacific abalone *Haliotis discus hannai*.

In the present work, application of the similar analyses on parentage of genotypic compositions allowed to estimate rate of gene-centromere recombination and fitness or differential contribution of oocytes to triploid offspring at each of the loci coding electrophoretic and thermostability variations of phosphoglucose isomerase and esterase and to discuss probable integrated effect of genotypes at multiple loci of parental female animals on their relative contribution in production of triploid offsprings.

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The present paper analyses parentage in genotypic compositions to estimate rate of gene-centromere recombination, examine whether or not similar differential contribution of oocytes to triploid offsprings could be detected at the loci coding thermostability variations of phosphoglucose isomerase and esterase,³⁾ and discusses probable

integrated effect of genotypes at multiple loci of parental animals on their relative contribution in producing triploid offsprings.

Materials and Methods

Abalone Specimens

Five female and three male animals of the Pacific abalone *Haliotis discus hannai* collected from local waters of Iwate Prefecture, were used to produce normal diploid and triploid F₁ offspring sib populations in the mating no. 514 as reported before.¹⁾ Fertilization and induction of triploid animals were performed according to Arai *et al.*⁴⁾ The three groups of sib animals produced, that is, normal diploid (2n, untreated) and two kinds of triploid (3n·1 pb and 3n·2 pb, treated by cold

Table 1. Genotypic compositions at multiple loci of the parental animals used for mating no. 514 of the Pacific abalone

Locus	Parental animal identification (tag) number							
	Females					Males		
	B94	B111	B115	B145	B174	R89	R93	Y4543*
Pgdh	4·4	4·7	4·7	4·7	4·7	4·4	4·4	4·7
Pgi·S R/S	2 ^R ·2 ^R	2 ^R ·2 ^R	2 ^R ·2 ^R	1 ^S ·2 ^R	2 ^R ·2 ^R	2 ^R ·2 ^R	2 ^R ·2 ^R	2 ^R ·2 ^R
Es·F R/S	2 ^R ·3 ^S	2 ^R ·2 ^S	2 ^R ·2 ^S ₁	2 ^R ·2 ^S	2 ^S ·3 ^R	2 ^R ·2 ^S	2 ^R ·2 ^S	2 ^R ·2 ^S

* This animal was not available for electrophoretic analyses, so that the genotypes were estimated according to parent-F₁ normal diploid genotypic relations, as indicated in the following tables of the present paper as well as in the earlier work.¹⁾

^{*1} Genetic Studies on the Pacific Abalone XVI. Supported in part by the Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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shock at 3°C for 15 m for retaining first or second polar body respectively), were reared in separate tanks by group with running water at the Iwate Prefectural South Mariculture Center, Ofunato.

Samples were taken from each group at an age of approximately twenty-two month old. Ploidy status of individual animals and percentage induction of triploid animals were examined and estimated by observing karyotype from several individual specimens and zymograms at multiple loci on greater numbers of specimens as reported already,¹⁾ giving 86.0 both for 3n·1pb and 3n·2pb.

Analyses of Electrophoretic and Thermostability Variations

Electrophoretic and thermostability variations of enzymes were analysed on all the specimens sampled for phosphoglucose isomerase (EC 5.3.1.9. Pgi) according to Wilkins *et al.*⁵⁾ and esterase (EC 3.1.1.1. Es) according to Fujino and Nagaya⁶⁾ as well as for phosphoglucomutase (EC 2.7.5.1. Pgm) and phosphogluconate dehydrogenase (EC 1.1.1.44. Pgdh) according to Gosling⁷⁾ and Fujino and Sasaki⁸⁾ respectively. Parental animals of mating no. 514, which had not been available to electrophoretic analysis,¹⁾ became available for such purposes, except one male specimen (identification no. Y4543, see Table 1).

Estimating Proportions of Genotypes of Parental Animals in Production of F₁ Normal Diploid Population

Proportional contributions of female parents in producing their offsprings, by group of genotype at each locus, were estimated on the bases of analyses of genotypic relationships between parents and their diploid offspring population according to Fujino *et al.*¹⁾

Estimating Rate of Gene-centromere Recombination

On the bases of analyses on parent-F₁ diploid relationship, noted in the foregoing paragraph, a model on genotypic relations between parents and F₁ triploid sib populations was developed according to Fujino *et al.*¹⁾ After adopting the model with the observed frequencies of genotypes, rate of gene-centromere recombination and relative contributions or survivals of ootids were calculated according to Fujino *et al.*^{1,2)}

Results and Discussions

Parent-F₁ Diploid Offspring Relations of Genotypes

Genotypic compositions of parental animals of

Table 2. Pgi·S R/S and Es·F R/S genotypic frequencies in F₁ sib populations from mating no. 514 of the pacific abalone

Normal diploid ¹⁾ 2n				Triplets ²⁾											
				3n·1pb						3n·2pb					
Pgi·S R/S locus	R·R	R·S	S·S	To.	R·R·R	R·R·S	R·S·S	R·S·S	S·S·S	To.	R·R·R	R·R·S	R·S·S	S·S·S	To.
1·1	0	0	0	0	1·1·1	0	0	0	0	0	0	0	0	0	0
1·2	0	0	0	0	1·1·2	0	0	1*3	0	1	0	1*4	0	0	1
2·2	51	4	0	55	1·2·2	1	8·2	0	0	9	0	3*5	0	0	3
Total	52	8	0	60	2·2·2	36	3	0	0	39	43	3	0	0	46
Es·F R/S locus					Total	37	11	1	0	49	43	7	0	0	50
2·2	7	18	11	36	2·2·2	4	16	12	3	35	5	17	17	2	41
2·3	9	12*6	3	24	2·2·3	0	6	3	0	9	0	3	0	0	3
Total	16	30	14	60	2·3·3	1	2	0	0	3	1	3	0	0	4
					Total	5	24	15	3	47	6	23	17	2	48

Remarks, Comprising four of 1S·2R (*1), eight of 1S·2R·2R (*2), one of 1S·1S·2R (*3), one of 1R·1R·2R (*4), three of 1S·2R·2R (*5), and four of 2R·3S and eight of 2S·3R (*6).

Table 3. Parent- F_1 normal diploid offspring relations of Pgi·S R/S genotypes in mating no. 514

Parents	Females	Male
Genotype	$1^S \cdot 2^R$	$2^R \cdot 2^R$
Proportion	a	b
Allele frequency	$p_M^S = \frac{a}{4}$ $q_M^R = \frac{a}{4} + \frac{b}{2}$	$p_P = 0$ $q_P^R = 1$
, where $a+b=2$.		
$F_1 \cdot 2n$ offspring		
Genotype	$1^S \cdot 2^R: \frac{a}{4}$	
Proportion	$2^R \cdot 2^R: \frac{a}{4} + \frac{b}{2}$	

Table 4. Parent-triploid offspring relations of Pgi·S R/S genotypes in mating no. 514

Parents	Females	Male
Genotype	$1^S \cdot 2^R$	$2^R \cdot 2^R$
Proportion	a	b
Fitness	S	S_1
, where $a+b=aS+bS_1=2$.		
Gametes mated for producing $3n \cdot 1pb$ Eggs		
Genotype/allele	$1^S \cdot 1^S: \frac{aS}{4}x$	$p_P=0$
Proportion	$1^S \cdot 2^R: \frac{aS}{2}(1-x)$ $2^R \cdot 2^R: \frac{aS}{4}x + \frac{bS_1}{2}$	$q_P^R=1$
$F_1 \cdot 3n \cdot 1 pb$ offspring		
Genotype	$1^S \cdot 1^S \cdot 2^R: \frac{aS}{4}x$	
Proportion	$1^S \cdot 2^R \cdot 2^R: \frac{aS}{2}(1-x)$ $2^R \cdot 2^R \cdot 2^R: \frac{aS}{4}x + \frac{bS_1}{2}$	
Gametes mated for producing $3n \cdot 2 pb$ Eggs		
Genotype/allele	$1^S \cdot 1^S: \frac{aS}{4}(1-2x)$	$p_P=0$
Proportion	$1^S \cdot 2^R: aSx$ $2^R \cdot 2^R: \frac{aS}{4}(1-2x) + \frac{bS_1}{2}$	$q_P^R=1$
$F_1 \cdot 3n \cdot 2 pb$ offspring		
Genotype	$1^S \cdot 1^S \cdot 2^R: \frac{aS}{4}(1-2x)$	
Proportion	$1^S \cdot 2^R \cdot 2^R: aSx$ $2^R \cdot 2^R \cdot 2^R: \frac{aS}{4}(1-2x) + \frac{bS_1}{2}$	

Table 5. Parent- F_1 normal diploid offspring relations of Es·F R/S genotypes in mating no. 514

Parents	Females	Male
Genotype	$2^R \cdot 2^S$	$2^R \cdot 3^S$
Proportion	a	b
Allele frequency	$p_M^R = 0$ $q_M^R = \frac{a+b}{4}$ $q_M^S = \frac{a+c}{4}$ $r_M^R = \frac{c}{4}$ $r_M^S = \frac{b}{4}$	$p_P = 0$ $q_P^R = \frac{1}{2}$ $q_P^S = \frac{1}{2}$ $r_P = 0$
, where $a+b+c=2$.		
$F_1 \cdot 2n$ offspring		
Genotype	$2^R \cdot 2^R: \frac{a+b}{4} \cdot \frac{1}{2}$	
Proportion	$2^R \cdot 2^S: \frac{a+b}{4} \cdot \frac{1}{2} + \frac{a+c}{4} \cdot \frac{1}{2}$ $2^S \cdot 2^S: \frac{a+c}{4} \cdot \frac{1}{2}$ $2^R \cdot 3^R: \frac{c}{4} \cdot \frac{1}{2}$ $2^R \cdot 3^S: \frac{b}{4} \cdot \frac{1}{2}$ $2^S \cdot 3^R: \frac{c}{4} \cdot \frac{1}{2}$ $2^S \cdot 3^S: \frac{b}{4} \cdot \frac{1}{2}$	

mating no. 514 at Pgi·S and Es·F loci as well as at the other loci are shown in Table 1, where R and S represent thermo-resistant and thermo-sensitive alleles respectively, as defined before.^{3,5)} One specimen out of the three males was not available for electrophoretic analyses, then the genotypes of the specimens were estimated on the basis of parent- F_1 offspring relations according to Fujino *et al.*,¹⁾ as described in the following part of this text in detail. Table 2 summarizes the observed numbers of genotypes of electrophoretic and thermostability variations at Pgi·S and Es·F loci in the F_1 offsprings. Table 3 proposes a model for parent- F_1 normal diploid relationships of Pgi·S genotypes in mating no. 514. Parameters a and b denote proportions of two kinds of genotypes in female parents contributed to produce F_1 diploid population. After putting the observed frequencies of Pgi·S genotypes of F_1 normal diploid (Table 2) into the formulas of expected frequencies of the corresponding genotypes (Table 3), the values for the above parameters were

Table 6. Parent-triploid offspring relations of Es·F R/S genotypes in mating no. 514

Parents	Females			Male
Genotype	$2^R \cdot 2^S$	$2^R \cdot 3^S$	$2^S \cdot 3^R$	$2^R \cdot 2^S$
Proportion	a	b	c	
Fitness	S	S_1	S_2	
, where $a+b+c=aS+bS_1+cS_2=2$.				
Gametes mated for producing $3n \cdot 1$ pb Eggs Sperms				
Genotype/allele	$2^R \cdot 2^R$	$\frac{aS}{4}x + \frac{bS_1}{4}x$	$p_F=0$	
Proportion	$2^R \cdot 2^S$	$\frac{aS}{2}(1-x)$	$q_P^R = \frac{1}{2}$	
	$2^S \cdot 2^S$	$\frac{aS}{4}x + \frac{cS_2}{4}x$	$q_P^S = \frac{1}{2}$	
	$2^R \cdot 3^S$	$\frac{bS_1}{2}(1-x)$	$r_P=0$	
	$2^S \cdot 3^R$	$\frac{cS_2}{2}(1-x)$		
	$3^R \cdot 3^R$	$\frac{cS_2}{4}x$		
	$3^S \cdot 3^S$	$\frac{bS_1}{4}x$		
F_1 $3n \cdot 1$ pb offspring				
Genotype	$2^R \cdot 2^R \cdot 2^R$	$\frac{aS+bS_1}{8}x$		
Proportion	$2^R \cdot 2^R \cdot 2^S$	$\frac{aS+bS_1}{8}x + \frac{aS}{4}(1-x)$		
	$2^R \cdot 2^S \cdot 2^S$	$\frac{aS+cS_2}{8}x + \frac{aS}{4}(1-x)$		
	$2^S \cdot 2^S \cdot 2^S$	$\frac{aS+cS_2}{8}x$		
	$2^R \cdot 2^R \cdot 3^S$	$\frac{bS_1}{4}(1-x)$		
	$2^R \cdot 2^S \cdot 3^S$	$\frac{bS_1}{4}(1-x)$		
	$2^R \cdot 2^S \cdot 3^R$	$\frac{cS_2}{4}(1-x)$		
	$2^S \cdot 2^S \cdot 3^R$	$\frac{cS_2}{4}(1-x)$		
	$2^R \cdot 3^R \cdot 3^R$	$\frac{cS_2}{8}x$		
	$2^R \cdot 3^S \cdot 3^S$	$\frac{bS_1}{8}x$		
	$2^S \cdot 3^R \cdot 3^R$	$\frac{cS_2}{8}x$		
	$2^S \cdot 3^S \cdot 3^S$	$\frac{bS_1}{8}x$		

obtained as $a=0.332$ and $b=1.668$ (see Table 8). A good agreement between the observed and expected frequencies of genotypes of diploid (Table 7) verified that the proposed model of parentage and the calculated values of a and b were appropriate and correct.

Similar analyses on the parent- F_1 diploid relationships of genotypes at Es·F locus made it

Gametes mated for producing $3n \cdot 2$ pb Eggs Sperms			
Genotype/allele	$2^R \cdot 2^R$	$\frac{aS}{4}(1-2x)$	$p_F=0$
Proportion		$+\frac{bS_1}{4}(1-2x)$	
	$2^R \cdot 2^S$	aSx	$q_P^R = \frac{1}{2}$
	$2^S \cdot 2^S$	$\frac{aS}{4}(1-2x)$	$q_P^S = \frac{1}{2}$
		$+\frac{cS_2}{4}(1-2x)$	
	$2^R \cdot 3^S$	bS_1x	$r_P=0$
	$2^S \cdot 3^R$	cS_2x	
	$3^R \cdot 3^R$	$\frac{cS_2}{4}(1-2x)$	
	$3^S \cdot 3^S$	$\frac{bS_1}{4}(1-2x)$	
F_1 $3n \cdot 2$ pb offspring			
Genotype	$2^R \cdot 2^R \cdot 2^R$	$\frac{aS+bS_1}{8}(1-2x)$	
Proportion	$2^R \cdot 2^R \cdot 2^S$	$\frac{aS+bS_1}{8}(1-2x) + \frac{aS}{2}x$	
	$2^R \cdot 2^S \cdot 2^S$	$\frac{aS+cS_2}{8}(1-2x) + \frac{aS}{2}x$	
	$2^S \cdot 2^S \cdot 2^S$	$\frac{aS+cS_2}{8}(1-2x)$	
	$2^R \cdot 2^R \cdot 3^S$	$\frac{bS_1}{2}x$	
	$2^R \cdot 2^S \cdot 3^S$	$\frac{bS_1}{2}x$	
	$2^R \cdot 2^S \cdot 3^R$	$\frac{cS_2}{2}x$	
	$2^S \cdot 2^S \cdot 3^R$	$\frac{cS_2}{2}x$	
	$2^R \cdot 3^R \cdot 3^R$	$\frac{cS_2}{8}(1-2x)$	
	$2^R \cdot 3^S \cdot 3^S$	$\frac{bS_1}{8}(1-2x)$	
	$2^S \cdot 3^R \cdot 3^R$	$\frac{cS_2}{8}(1-2x)$	
	$2^S \cdot 3^S \cdot 3^S$	$\frac{bS_1}{8}(1-2x)$	

possible to propose a model for parentage as shown in Table 5. By putting the observed numbers of diploid genotypes (Table 2), proportions of the three kinds of the parental genotypes, contributed to produce offsprings, were calculated as $a=0.400$, $b=0.536$, and $c=1.064$. Application of these values to the formulas in Table 5 indicated a good agreement between the observed and expected

Table 7. Observed (O) and expected (E) frequencies of genotypes at Pgi·S and Es·F loci in F₁ offspring populations from mating no 514*¹

Normal diploid			Triploid* ²				
2n			3n·1pb			3n·2pb	
Pgi·S	O	E	Pgi·S	O	E	O	E
1·1	0	0.0	1·1·2	1	1.3	1	2.8
1·2	5	5.0	1·2·2	9	8.4	3	5.3
2·2	55	55.0	2·2·2	34	34.3	40	35.9
R·R	52	55.0	R·R·R	32	34.3	37	35.9
R·S	8	5.0	R·R·S	11	8.4	7	5.3
S·S	0	0.0	R·S·S	1	1.3	0	2.8
Total	60	60.0	S·S·S	0	0.0	0	0.0
			Total	44	44.0	44	44.0
Es·F	O	E	Es·F	O	E	O	E
2·2	36	36.0	2·2·2	30	31.1	35	32.6
2·3	24	24.0	2·2·3	9	10.1	3	7.8
			2·3·3	3	2.8	4	1.6
R·R	16	15.0	R·R·R	4	3.9	5	3.1
R·S	30	30.0	R·R·S	22	18.1	20	17.9
S·S	14	15.0	R·S·S	13	18.1	15	17.9
			S·S·S	3	3.9	2	3.1
Total	60	60.0	Total	42	42.0	42	42.0

*¹ No significant difference was seen between observed and expected frequencies.*² Observed numbers of homozygote animals were estimated by multiplying 0.86 (percentage triploidization) to those, shown in Table 2.**Table 8.** Parameters calculated from genotypic compositions at multiple loci in mating no. 514

Female parents		Survival rates of ootids		Rate of G-C
Genotype	Proportion	3n·1pb	3n·2pb	Recombination
Pgi·S locus				
1 ^S ·2 ^R	a=0.332	S =1.506	S =0.687	x=0.242
2 ^R ·2 ^R	b=1.668	S ₁ =0.899	S ₁ =1.062	
Es·F locus				
2 ^R ·2 ^S	a=0.400	S =3.215	S =3.695	x=0.355
2 ^R ·3 ^S	b=0.536	S ₁ =0.668* ¹	S ₁ =0.488* ¹	
2 ^S ·3 ^R	c=1.064	S ₂ =0.334* ¹	S ₂ =0.244* ¹	

*¹ Values calculated in the case of $S_1=2\cdot S_2$. Those in the other case of $S_1=S_2$ are 0.446 and 0.326 for 3n·1pb and 3n·2pb respectively.

frequencies of genotypes of diploid, verifying that the proposed model of parentage and the calculated values of parameters a , b , and c were appropriate and correct.

Rate of Gene-centromere Recombination and Differential Contribution of Oocytes to Triploid Offsprings

Table 4 presents the relationships of genotypes at Pgi·S locus between parents and the two kinds of triploid offspring sib populations of mating no. 514, developed on the basis of the model described in the foregoing paragraph, according to Fujino

*et al.*²³ In this table, additional parameters of fitness (adaptive value or relative contribution) of different thermostability genotype (R·S, S·R and R·R) are given. Genotype proportions in female gametes (ootids), having retained either first or second polar body, are also shown. By putting the observed frequencies of genotypes into the formulas of their expectation, rate of gene-centromere recombination (x) as well as fitness were obtained as $x=0.242$, $S=1.506$, $S_1=0.899$ and $S=0.687$, $S_1=1.062$, indicating a reverse relation between S and S_1 among the two cases of inhibiting release of first or second polar body, of which the

reason is not known yet. After applying these values to the formulas in Table 4, no significant difference was seen between the observed and expected frequencies of genotypes at Pgi·S R/S locus in the two kinds of triploid offsprings (Table 7).

Table 6 presents parent-triploid offspring relationships of genotypes at Es·F locus, developed on the basis of the model described in the foregoing paragraph. Symbols S , S_1 , and S_2 mean fitness, given for different genotypes of thermo-stability and/or electrophoretic variations. Genotype proportions in ootids, having retained either first or second polar body, are also shown. By putting the observed frequencies of genotypes into the formulas of their expectations, rate of gene-centromere recombination (x) as well as fitness were obtained as $x=0.355$, $S=3.215$, $S_1=0.668$, $S_2=0.334$ (or $S_1=S_2=0.446$) and $S=3.695$, $S_1=0.488$, $S_2=0.244$ (or $S_1=S_2=0.326$) for the two cases of inhibiting release of first or second polar body respectively. In the foregoing analyses, no specific data or information has been available with regard to quantitative relationship between S_1 and S_2 . Then, the values for S_1 and S_2 were calculated for the two cases under the assumption of $S_1=2 \cdot S_2$ or $S_1=S_2$ respectively (see Table 8). The results of the above analyses strongly suggested that the differences between the values of S and those of either S_1 or S_2 commonly observed in the two cases of inhibiting release of first or second polar body represent a marked differential contribution of ootids by genotype of female parents in production of triploid offsprings.

Some apparent deviations in the observed frequencies of occurrence of some genotypes, deficit of heterozygote, from their expectations both at Pgi·S and Es·F loci in the 3n·2pb samples (see Table 7) effected more or less on the estimates of rate of gene-centromere recombination (x) and/or

survival rate (S, S_1, S_2). Improved estimates of the parameters could be expected after analysing samples to be taken from other mating groups of more appropriate genotypic compositions in future. Apparent reverse relation between the values of S and $S_1(S_2)$ among the two sets of data calculated from 3n·1pb and 3n·2pb genotypic frequencies at Pgi·S locus suggested a necessity of analyses on 1) probable integrated effects or interference of multiple loci and 2) some differential cytoplasmic states between primary oocytes and secondary oocytes derived from identical oogonium.

Acknowledgements

Mr. Yorio Sugihara, the director of the Iwate Prefectural South Mariculture Center, Ofunato kindly provided us with the research materials and the facilities to support the present study. Careful technical assistance by Dr. Katsutoshi Arai in mating abalones for producing diploid and triploid sib populations are greatly appreciated.

References

- 1) K. Fujino, M. Kamakura, R. Mito, and K. Arai: *Nippon Suisan Gakkaishi*, **53**, 1759–1764 (1987).
- 2) K. Fujino, S. Nakajima, and H. Sawada: *Nippon Suisan Gakkaishi*, **54**, 953–958 (1988).
- 3) S. Okumura, K. Sasaki, and K. Fujino: *Nippon Suisan Gakkaishi*, **47**, 1627–1630 (1981).
- 4) K. Arai, F. Naito, and K. Fujino: *Nippon Suisan Gakkaishi*, **52**, 417–422 (1986).
- 5) N. P. Wilkins, K. Fujino, and K. Sasaki: *Nippon Suisan Gakkaishi*, **46**, 549–553 (1980).
- 6) K. Fujino and N. Nagaya: *Nippon Suisan Gakkaishi*, **43**, 983–988 (1977).
- 7) E. Gosling: *Nature*, **279**, 713–715 (1979).
- 8) K. Fujino and K. Sasaki: *Nippon Suisan Gakkaishi*, **50**, 11–15 (1984).