Genetic Studies on the Pacific Abalone—II

Excessive Homozygosity in Deficient Animals*¹Kazuo FUJINO*²

(Received January 28, 1978)

On the basis of observations of an excessive frequency of occurrence of homozygotes at the esterase F locus, an inbreeding structure was previously reported in the wild population of the Pacific abalone, *Haliotis discus hannai* Ino, in Iwate Prefecture. The occurrence of deficient or malnutritional animals, locally called "Rho-gai" or "Yase-gai" and of less commercial value, has long been known among fishermen. The relative weight of the soft parts of the body to the whole body weights made it possible to distinguish such deficient animals from normal ones. The deficient animals revealed a much higher frequency of homozygosity than that in normal animals. The results were interpreted as a reflection of an inbreeding structure called "inbreeding depression". Additional supporting evidence indicated that the overdominance of heterozygotes observed at the esterase M locus in normal animals was offset by the excessive homozygosity in deficient animals and turned to homozygote excess. On the basis of these observations, as well as the aging relations of fitness at the esterase M locus reported before, some general guidelines of selective breeding for increasing production and for genetic improvements of the Pacific abalone are suggested.

FUJINO¹⁾ described the evidence of inbreeding structure and overdominance in a wild population of the Pacific abalone, *Haliotis discus hannai* Ino, on the basis of observations of phenotypic frequencies of biochemical polymorphism. In the present paper, an evidence, that suggests an association between occurrence of deficient or malnutritional animals and the inbreeding structure, is described and a plan of selective breeding for increasing production is proposed.

Materials and Methods

Materials

Twenty-five specimens of the Pacific abalone, *H. discus hannai* Ino, were sampled from animals, which were taken commercially from waters around the Okkirai Peninsula, Iwate Prefecture in November and December 1977. Additional minty specimens were sampled from animals, which are called locally as "Rho-gai" or "Yase-gai" and were separated from the batch of commercial landings due to their deficient appearance and less commercial value. Each animal was measured for shell-length, weight of whole soft tissues and organs, and whole body weight for analysing production traits and distinguishing deficient animals from

normal ones. Length and weight data on the 39 specimens of *H. discus hannai* Ino, sampled from the west coast of the Rebun Island, Hokkaido in August 1977, were also used for the above purposes.

Analysis of Biochemical Polymorphism

Biochemical polymorphisms were analysed by following FUJINO¹⁾ and FUJINO *et al.*^{2,3)} Their phenotypic frequencies were analysed on the basis of the Laws of S. Wright's and Hardy-Weinberg's Genetic Equilibria. Proportional frequencies of homozygosity were compared between normal (healthy) and deficient (malnutritional) animals. For statistical analyses and synthesizing the strategy of selective breeding, used were also population data published before.¹⁾

Results and Discussions

Production Traits and Deficient Animals

Figure 1 summarizes the correlations between shell-length (L) and proportional weight of soft parts of body against the whole body weight (SP/BW) for the animals sampled from Iwate Prefecture and Hokkaido. The figure indicates that there is no apparent geographical difference in the correlation between the two locations sampled and that the proportion SP/BW increases

*¹ Supported in part by the grant from the Ministry of Education, the Government of Japan.

*² Kitasato University School of Fisheries Sciences, Sanriku, Iwate Prefecture 022-01 (藤野和男: 北里大学水産学部).

Fig. 1. Correlation between shell-length (L) and the proportional weight of soft parts (SP) against whole body weight (BW) of the Pacific abalone, where solid circles represent animals from the Rebut Island, open circles normal animals from Iwate Prefecture, and crosses deficient animals.

with the increment of shell-length and reaches a stationary value of 0.75 ± 0.03 (the lower rejection limit of 0.70 at 5% significant level) approximately at 8.5 cm of shell-length. In Fig. 1 are also plotted the individual values for deficient animals, which are equal or below the rejection limit shown above. Thus the deficient animals can be objectively distinguished from normal ones by the value of SP/BW in addition to their appearance. In contrast to such significant difference in the proportional weight of soft parts of body, little abnormality can be seen in the appearance of shells from deficient animals. Whether the physiological defects that resulted in malnutrition, were chronic or acute in nature, are of great interest to be studied from the view points of physiology and pathology in future.

Excessive Homozygosity in Deficient Animals

Table 1 compares the frequencies of phenotypes and those grouped into homozygotes and heterozygotes at the esterase two loci between normal and deficient animals. It can be stated from this table that the deficient animals reveal more homozygosity and less heterozygosity than the normal animals. A significant evidence for this view appears in the fact that the overdominance in heterozygotes in M locus¹¹ was offset by the excessive homozygosity and turned to homozygote excess (Table 1). The evidence strongly suggested that the occurrence of deficient animals was the reflection of inbreeding (called inbreeding depression⁴¹), which produced a high frequency of homozygosities with recessive and disadvantageous genes, that had been preserved in the "carriers" of hetero-

zygotes in the population. It is well known that such homozygous animals are generally less viable and more susceptible to unfavorable environmental conditions such as an occasional low or high water temperature and/or a critical competition for food due to overpopulation etc. Efforts have been made to obtain further evidence for the excessive homozygosity in deficient animals with the other loci of biochemical polymorphism.

A Proposed Strategy of Selective Breeding for Increasing Production

The inbreeding depression is now evident as one of potential causes for occurrence of malnutritional animals, called locally as "Rho-gai" or "Yase-gai", in the wild population from Iwate Prefecture by the two separate observations, one on inbreeding structure¹¹ and another on excessive homozygosity, described in the foregoing paragraph. Occurrence of malnutritional animals has long been known among fishermen in Iwate Prefecture as well as the other localities, where the Pacific abalone have been harvested. Neither systematic observations nor scientific research, however, has been conducted on the subject. A probable reason for the little attention to the subject seems to its apparent low frequency of occurrence among batch of commercial landings each time. Although it is unknown whether physiologically defective state causing malnutrition is chronic or acute, a cumulative proportion of deficient animals by the time of harvest (the legal smallest limit of shell-length is set as 9.0 cm

Table 1. Phenotype frequencies of esterase F and M loci in the normal and deficient animals from the wild population of the Pacific abalone

	Phenotypes												Total		Alleles			Number of animals of:	
	1				2				3						p	q	r		
	O	E	O	E	O	E	O	E	O	E	O	E	homo- zygotes	hetero- zygotes					
	O	E	O	E	O	E	O	E	O	E	O	E							
F locus #1	Normal animals	3	0.1	3	8.6	211	199.3	29	47.2	12	2.8	1	1.0	**259	0.019	0.877	0.104	226	33
	Deficient animals	3	0.3	3	7.9	76	70.2	4	10.6	4	0.4	0	0.6	90	0.050	0.883	0.067	**93	7
M locus	Normal animals	24	17.4	85	78.2	82	88.0	53	47.7	14	6.5	1	21.2	**259	0.259	0.583	0.158	120	139
	Deficient animals	17	7.6	18	29.4	39	28.9	6	14.7	10	1.9	0	7.5	90	0.289	0.567	0.144	**66	24

*1. Calculated under the condition of Hardy-Weinberg's genetic equilibrium; *2 cited from Fujino³¹; *3 deviation from normal animals, $\chi^2=2.17$, 1df, $P>0.1$; ** deviation from normal animals, $\chi^2=27.1$, 1df, $P<0.001$.

for the Okkirai district, Iwate Prefecture) is assumed to reach a quite high percentage. This assumption is based on the fact of the reversed proportion from heterozygote excess in normal animals to homozygote excess in deficient animals at the esterase M locus noted before.

Another observation on the aging relations of overdominance in the two heterozygotes 1.2 and 2.3 in esterase M locus is the base, by which the selective breeding is suggested for increasing production (Table 5, FUJINO³¹). Under the present legal limit of 9.0 cm of shell-length, for example, selective breeding between two homozygotes 2.2 and 2.3 for producing one hundred percent heterozygote 2.3 animals of the esterase M locus could result in a significant increase of harvests than the case of random mating in the number of animals developed from an equal number of seed animals. The results of the foregoing analyses now make it possible to propose the general guidelines for selective breeding for increasing production on the basis of a single foundation stock according to FISHER⁶¹ with some modifications as follows.

1. Selection of parental animals towards establishment of strains.

(1) Examination of homozygosity at as many biochemical polymorphisms as possible. Now at least seven loci have been known to be polymorphic in comparing with ten of the haploid chromosome number in the Pacific abalone (FUJINO, unpublished data). Homozygous animals in all the loci examined will be selected from a given foundation stock for producing progeny. With regard to the esterase M locus, only homozygotes 2.2 and 3.3 will be retained.

(2) Inbreeding and selection for superior performance. At first, mating among the selected animals to produce high homozygosity will be attempted and inbreeding will be practiced in the successive generations. Recessive and disadvantageous genes will become to be recognizable through phenotypic appearance and/or performance of the resulted homozygous animals. Animals with superior quality and performance traits such as high growth rate etc. will be retained. Such selection may have to be conducted repeatedly through several generations. For practice, biochemical polymorphism was to be examined through the progeny tests, until a new technique for testing biochemical polymorphisms will be developed without sacrificing parental animals.

(3) Establishment of strains or lines. A series

of repeated inbreeding for several generations or gynogenesis of the animals through the above procedures will be the way for establishment of a variety of strains or lines with high quality and performance. The established strains or lines must be maintained by inbreeding.

2. Production of heterozygous animals by crossing between different strains.

Crosses between two or more established strains will produce quality heterozygous animals for increasing production. For more specific genetic improvements, however, a number of works need to be conducted.

Acknowledgements

The cooperation of Messrs. Etsutaro TANAKA, Shojiro HIRATA, and Hiroshi KASHIWAZAKI, the officials of the Sanriku-machi, Mr. Keigo KUDO, the officials of the Hokkaido, and Mr. Kazuo

NAKAI, the Fishermen's Association of Okkirai in arranging and providing me with the research materials is greatly appreciated. The author is deeply indebted to Mrs. Takako KANAMORI and Miss Reiko AMANO for their careful technical assistances.

References

- 1) K. FUJINO: *Bull. Japan. Soc. Sci. Fish.*, **44**, 357-361 (1978).
- 2) K. FUJINO and N. NAGAYA: *Bull. Japan. Soc. Sci. Fish.*, **43**, 983-988 (1977).
- 3) K. FUJINO and N. NAGAYA: *Bull. Japan. Soc. Sci. Fish.*, **43**, 1455-1459 (1977).
- 4) L. E. METTLER and T. G. GREGG: *Population Genetics and Evolution*, Prentice-Hall, Inc. Englewood Cliffs, New Jersey, 1969 pp. 212.
- 5) R. A. FISHER: *The Theory of Inbreeding*, Second Edition, Academic Press, New York and London, 1965, pp. 150.