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GENETICS AND BREEDING OF AQUATIC CREATURES

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QUANTITATIVE GENETICS

Introduction

Mendel's Law of heredity, a first step in modern genetics, was applied initially only to qualitative characters showing discontinuous variations. It had been discussed whether or not it was good or bad to apply this law to a quantitative character showing continuous variations, covering many biological variations. Thereafter, the multiple genes (polygene) theory was introduced by combining the biological statistics with Mendel's Law of heredity; and later, it was considered that a quantitative character could also be governed by the chromosome genes. Furthermore, the genetics of quantitative character were established on the basis of the introduction of population genetics, which have advanced tremendously in recent years; also on the basis of study of the theory of breeding crops and livestock. There are many unfavorable aspects to using creatures living in water as research materials for genetics and breeding studies, because extensive facilities and much labor are needed to culture and breed aquatic creatures and also because of purely technical difficulties. However, in recent years, the theory of quantitative genetics began

In closing, we express our deep appreciation to the Committee of the Meeting of the Japan Fisheries Society, held in the Autumn of 1978, the Chairmen and Scientists who attended the discussions.

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December, 1978

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2. Invertebrates..... K. Numachi (Tokyo University Institute of Oceanography)

3. Fish ..... K. Suzuki (Freshwater Fisheries Research Laboratory, at Ueda)

III. Discussion ..... K. Fujino, Chairman (Kitazato University, Department of Fisheries)

Closing speech ..... S. Sudo (Tohoku University, Department of Agriculture)

In Part I, the results of basic studies of aqua-genetics from many aspects were presented. In Part II, the actual state of aquaculture was presented. In the former, recent results concerning the following studies were presented and various possibilities of applying these studies were suggested: quantitative genetics, the purpose of which is to study quantitative character which is of great importance in the breeding studies; population genetics—the biochemical polymorphisms , useful as indicators, are mainly studied; cytogenetics as a basic study of chromosomal engineering; and, finally, genetic effects on wild populations by water pollution. In the latter, the recent states of agar, invertebrates and fish were introduced. Throughout these speeches, the necessity of accumulation and organization of basic knowledge relative to the resources and the techniques for breeding, as well as the importance of developing strategic measures for breeding studies were stressed.

I N T R O D U C T I O N

In 1965, a Symposium concerning aquaculture was held by the Japan Fisheries Society and the studies and techniques related to this field were discussed. Since then, the basic studies related to aquaculture and, in particular, to isozyme genetics, population genetics and cytogenetics have been developed remarkably. On the other hand, techniques of aquaculture have also advanced greatly and it is now considered that genetic improvements of species are very important. It is also considered important to have genetic control of wild populations and, in fact, there is a possibility to control them. Twice in 1966 and again twice in 1975, Meetings were held by the Japan Aqua-Resources Preservation Society concerning aquaculture, in cooperation with scientists interested in genetics and breeding of aqua-creatures. Methods, techniques, plans for possible aquaculture and other problems were discussed.

Relative to the progress just described, a Symposium was held by the Japan Fisheries Society in order to learn the present-day state of aquaculture and breeding and in order to develop aquaculture and to use the latest studies. The contents of speeches and discussions are in this book. This Symposium was held on October 9, 1978, at the Tokai University (Shimizu City, Shizuoka Prefecture) according to the following program.

"GENETICS AND BREEDING OF AQUA-CREATURES NOW AND IN THE FUTURE"

Opening speech ..... M. Nomura (Tokyo University for Fisheries)

I. Progress in aqua-genetics..... S. Sudo, Chairman (Tohoku University, Department of Agriculture)

1. Genetics of quantitative characters ..... K. Wada (Pearl Research Laboratory)

2. Biochemical polymorphisms and population genetics..... K. Fujino (Kitazato University Department of Fisheries)

In contrast to the quantitative character, there is a qualitative character showing a discontinuous variation. Historically, as mentioned previously, the inheritance of this qualitative character was explained in the genetic theory by Mendel. Thereafter, on the basis of the introduction of the biological statistics and also considering the aspect of multiple genes, it was established that a quantitative character is also governed by the chromosome genes, similarly to the case of the qualitative character. The genes governing quantitative characters are called polygenes. It is considered that many genes, each with a limited influence, complement each other and participate in the manifestation of a character. As will be described in the following, concerning the statistical studies of a quantitative character, it is necessary to bear in mind the fact that the probability patterns include many hypotheses, like a normal pattern of distribution, as mentioned previously.

2. Phenotype and genotype values and the partition of their variances*

(*square of standard deviation)

Differing from the qualitative character, the genotype of a quantitative character cannot be assumed from the measurements. Therefore, a genotype value (effect of genes: G) and a phenotype value (practical measurement: P) are used as quantitative aspects. The phenotype value is expressed as the sum of the genotype value and the non-genetic effect, the effect of the environment (E):

$$P = G + E \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

G is a portion determined by the genotype governing the quantitative character of a certain individual. E is a variance determined by the environmental effect on the character. E is also called the

to be applied to aqua-creatures in order to study the breeding and homogeneity of experimental aquatic creatures.

In this paper, this theory, its application and the problems involved are discussed.

1. Quantitative character and polygenes

A measurable character showing continuous variations, from morphological character (like: length, weight, or color tone) to a physiological reaction, is called a quantitative character. Generally, the economically important aspects, like growth and the resultant volume of production, often reflect the above variations. In order to further the studies, it is desirable that many statistical individuals be measured, as statistical analyses are prerequisites. The method of genetic analysis of a quantitative character, now described, is based on the assumption that the character variation shows normal distribution. However, the method of quantitative genetics can also be applied to measurements showing asymmetric distribution by approximating the normal distribution and transforming the scales, like the transformation of a logarithm. Even when the measurements can be expressed only by whole numbers, like the numbers of intermuscular bones, it is considered to be a quantitative character showing continuous variations. Furthermore, concerning sickness, susceptibility to illness is an "all or none" character: whether to become ill or not. Based on the idea of becoming ill, when the potential of this character exceeds a certain limit, it is intended to treat this as a quantitative character.

V_G, V_E, V_A, V_D and V_I are respectively: a genotype variance, an environmental variance, an additive genetic variance, a dominant variance, and an epi-hypostatic variance (epistasis variance).

Partitioning of the phenotype variances is usually achieved by analysis of the variances. This is a basic study to analyze the quantitative character genetically. Especially, the ratio of the additive genotype variance is important for breeding, as will be described later. For the purpose of this analysis, the method of comparing a pure line or an inbred line with hybrid groups and also the methods of crossings, utilising the data on twins and experimental plans for twins, are used. Contingency tables of the variance components of various kinds of populations were obtained. However, when G and E are not independent and there are both a correlation and an interaction between G and E, the partition of variances cannot be used. Concerning the correlation between the genotype and the environment, it was theorized how to estimate the component of variance.

In the following, an example is given of the application of this theory to the aquatic creatures. Concerning the following three characters: the weight after spawning, the number of eggs spawned and the size of the eggs, Gall (3) estimated the additive phenotype, dominant and epistasis variances, using domestic Rainbow trout, bred in California, USA (two lines of sub-population).

Ten of each of the H and V lines were combined and crossed (♀ 1 to ♂ 1). As the offspring of each line, a reciprocal cross F_1; two reciprocal crosses F_2 (♀ HV x ♂ VH and ♀ VH x ♂ HV); and two

random error. Using statistical language, the sum of all E of the individuals, belonging to one population, equals zero and, when the average of the population is calculated, the phenotype value equals the genotype value.

On the other hand, the portion determined genetically (G) is partitioned as follows: (1) a portion which is proportionate to the number of genes, that is, a portion determined by the additive effect of the genes - additive genotype value (A); (2) a portion determined by a dominant phenomenon - dominant variance (D); and (3) a portion determined by the correlation between the genes or between the genotypes on the different gene loci - epi-hypostatic or epistasis variances (I). Thus, equation (1) can be shown as:

$$P = A + D + I + E \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

The main purpose of genetic studies of a quantitative character is to establish the contributing degree of each component of the phenotype to the variation of the character. Therefore, it is necessary to partition the variances found in the phenotype values and to know the ratio of each component. Thereby, the genetic characteristics of the character are clarified.

If each component of the equation (2) is independent, the phenotype variance (V_P) is the sum of the variances of each component comprising the phenotype:

$$\begin{aligned} V_P &= V_G + V_E \\ &= V_A + V_D + V_I + V_E \quad \ldots\ldots\ldots\ldots\ldots \quad (3) \end{aligned}$$

and the outbreeding) (9,10); the shell-shape of Tamakibi (Littorina saxatilis), naturally bred snails from different habitats (estimation of the genetic variance by analysis of the co-variances of the full-sib, the parents and the offspring) (11); the growth of the larvae of Murasakigai (Mytilus edulis) in water of low salinity (estimation of the genetic variance by analysis of the variance of the half-sib and full-sib) (12); etc.

Furthermore, concerning the reciprocal action of the genotype and the environment, there are reports of experimental studies, using carp bred in Europe and in China, by Moav (13,14). Many studies concerning the genetic analysis of quantitative characters have just been started. The development of these studies is expected in the near future.

3. Heritability

The indices which show to what extent variations in the quantitative characters are determined by genetic factors, are useful for analyses of the quantitative characters genetically. These indices are called heritability (h^2), and, using the components of the variances in equation (3), it is defined as:

$$h^2_B = \frac{V_G}{V_P} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

This is called heritability in a broad sense. When using only the additive genetic variance V_A in the three components of the genotype variance V_G, it is called heritability in the narrow sense:

$$h^2_N = \frac{V_A}{V_P} .$$

Using the additive genetic variance, heritability is practical when used in selective breeding.

back crosses (♀ HV x ♂ H and ♀ VH x ♂ V) were obtained. These crossings were done twice. In total, 1,604 trout, fully 2 years old, were checked with regard to the characters just described. The variance was partitioned by analysis of the generation means. As the result, it was observed that the dominant variance and the epistasis variance were remarkable in the weight of the fish and the size of the eggs; and the additive genetic variance was clearly expressed in the number of eggs.

Furthermore, with regard to many species of aquatic creatures, partitioning of the environmental variance and the genotype variance was made using population data. These studies, excluding studies relating to estimation of the heritability described under the following heading of Paragraph 3, are as follows:

> The recapture rate of Salmo salar bred in the Atlantic Ocean for stocking (estimation of the presence of the additive genetic variance by analysis of the variance of half-sib*) (4), (FIGURE 1.3);and the weight of the fish when they were recaptured (estimation of the presence of the genetic variance by analysis of the variance of full-sib**) (5), (FIGURE 1.2); the weight and the body length of a guppy (Lebistes reticulatus) (estimation of the ratio of the additive genetic variance by analysis of the variance of the full-sib and the half-sib) (6); the number of the intermuscular bones of carp (estimation of the presence of the genetic variance by analysis of the variance of the full-sib) (7); and (estimation of the genetic variance by the comparison of several different lines and their hybrids) (8); the growth rate of carp (estimation of the genetic variance by analysis of the full-sib crosses, the inbreeding

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* half-sib = same mother, different father or vice-versa

**same mother and same father

These methods are classified into: (1) a method to estimate the correlation or the regression between the parents and the offspring; (2) a method to analyze the variances in the full-sib or the half-sib; (3) a method to estimate the correlation between the full-sib or the half-sib; (4) a method to estimate the result of the selection experiment; (5) a method to estimate the variance of twins. From these five methods, the method to apply depends on the breeding mode of the experimental subject and the degree of difficulty in the breeding and in the controlling of the culture. Concerning the aquatic creatures, method (2) above has been used frequently so far. An explanation of the relationship of the full-sib, analysis of the variance, the composition of the mean squares (MS) and an estimation of heritability are shown in FIGURE 1.2 and TABLE 1.1. Above these, are shown the half-sib (FIGURE 1.3 and Table 1.2). As shown in TABLES 1.1 and 1.2, the heritability obtained by analysis of the variance of the full-sib is heritability in the broad sense. In order to estimate heritability in the narrow sense, it is necessary to effect analysis of variance of the half-sib.

However, as shown in FIGURES 1.2 and 1.3, in order to carry out this analytical experiment of the half-sib, many facilities for culturing and much labor are involved. For the statistical treatment, it is necessary to increase the number of crossings as per the accuracy required. Thus, the method of estimation to be applied is decided according to the experimental scale available and the accuracy of the estimation required.

3.1 Heritability and selective breeding

Heritability shows the heritable character of a certain type and, at the same time, shows the population character. Therefore, it is important in the field of breeding, as are the genetic analysis of a quantitative character and artificial selection. As an example, with regard to a character with high heritability, the effect of artificial selection appears quickly and individual selection is appropriate as the method of selection. On the other hand, it is considered that family selection is suitable for a character with low heritability. Generally, it is considered that there is the effect of selection in a character with heritability higher than 0.2. When heritability is applied to selective breeding in practice, there is another important aspect. That is, that there are many cases when the heritability obtained with regard to a certain character differs according to the method of estimation used, the genetic background of the population and the environment in which the population was settled. When a certain value is obtained for the heritability, it should be noted that this value was estimated, using a specific population in a specific environment. Therefore, in order to estimate heritability as the index for selective breeding, it is recommended to use a specific biological population (line) and a specific facility or controlling method (environment) in each culture-ground or hatching-ground.

3.2 Estimation of heritability

Many methods have been established to estimate heritability. Basically, as described in Paragraph 2, the method applied is to study how much more similar are each of the closely related individuals than those of remote relationship and to obtain a genetic variance.

The genetic correlation can be often either an advantage or an obstacle to breeding. A favorable correlation can be utilized, while a ralationship of an unfavorable combination has to be broken. It is possible to break the correlation due to the linkage. However, the correlation caused by pleiotropism of the genes remains without solution.

Genetic correlation, environmental correlation and phenotype correlation are defined as follows:

$$\text{Genetic correlation: } \gamma G = \frac{\text{Cov}\ (G_1, G_2)}{\sqrt{V_{G_1} \cdot V_{G_2}}} \quad \ldots\ldots\ (5)$$

$$\text{Environmental correlation: } \gamma E = \frac{\text{Cov}\ (E_1, E_2)}{\sqrt{V_{E_1} \cdot V_{E_2}}} \quad \ldots\ldots\ (6)$$

$$\text{Phenotype correlation: } \gamma P = \frac{\text{Cov}\ (P_1, P_2)}{\sqrt{V_{P_1} \cdot V_{P_2}}} \quad \ldots\ldots\ (7)$$

Covariances G_1, G_2, E_1, E_2, P_1 and P_2 are the genetic, environmental and phenotype covariances of characters 1 and 2, respectively. By the same token, V_{G_1}, V_{G_2}, V_{E_1}, V_{E_2}, V_{P_1} and V_{P_2} are the genetic, environmental and phenotype variances of characters 1 and 2. Apparently, from these definitions, in order to estimate these correlations, the correlation of the two characters has to be estimated. For this purpose, the following methods are used.

(1) a method based on the correlation or the regression between the parents and the offspring; and

(2) a method based on analyses of variances and covariances of the population related to the species.

3.3 Heritability estimated in the aquatic creatures

The aquatic reatures, the heritability of which has been estimated so far, are within a wide range, from fish and shells to weeds. Their heritability, taken from recent reports and arranged, are shown in TABLE 1.3. Studying This TABLE, one can see that there are many cases when heritability was iestimated mainly for the purpose of breeding in the industry of fisheries; also there are some cases when estimations were made from the point of view of experiments with animals and the classification of their lines. With regard to diversity of species, fish are the most numerous and, in particular, freshwater fish, like Rainbow trout. The heritability of their many characters has already been established.

4. Correlation of quantitative characters

The two econimically important characters are often found in either positive or negative correlations. The correlation between the phenotypes of two quantitative characters (phenotype correlation) is partitioned into an environmental correlation and a genetic correlation, according to the cause.

The genetic correlation includes all correlations expiring from genetic causes. Furthermore, it is considered that there are cases with a close linkage among the genes governing this character and that there are other cases when the same one gene (a group) relates to the expression of two different characters (pleiotropism of genes). Concerning a population crossed at random, it is considered that the genetic correlation, due to linkage, will reach the linkage equilibrium, and that many cases are caused by the pleiotropism of the genes.

according to whether it is carried out artificially or spontaneously. In many cases of artificial selection, it is difficult to exclude the influences of natural selection. Basically, selection is a change in the frequency of the genes. Since this cannot be measured directly in the quantitative character, the means, the variances and the covariances of the population are used to judge the effects of such selection

Artificial selection is a most important breeding method and it has been used traditionally since olden times. The situation has not changed even today. Concerning aquatic creatures, many actual cases were recorded before quantitative genetics were developed. There are quite a few cases affecting industry, mainly of freshwater fi viz.: carp, crucian carp and Rainbow trout (27). However, from the point of view of quantitative genetics, there are many cases when imperfections are found in the genetic records, that is, the uncertainty of the selection methods, the lack of a control group (genealogical), and no estimation of genetic gain (selective response). In order to carry out a selective experiment of quantitative characters, it is more convenient to study the results theoretically, if the selection method is clarified and the control group is used as described in the following; also, the results thus obtained can be used for prediction of the selection effects.

5.1 Prediction of selection difference, strength and response

Generally, when an individual with a desirable phenotype is selected at the rate of P% from a population with a mean value of quantitative character as Po, and the next generation is cultured, the difference between the mean value of the individual selected, Ps,

In the experiment with Rainbow trout, described previously, Gall (3) estimated the genetic correlation, the environmental correlation and the phenotype correlation (using the method of analyses of the variances) of the body weights after spawning, the number of eggs spawned, the volume of the eggs and the size of the eggs (egg diameter), also the number of eggs per 100 g. of body weight (TABLE 1.4).

As the result, a comparatively strong genetic correlation was found between the volume of eggs and the diameter of the eggs, the number of eggs or the body weight. Generally, it is considered that there is a strong correlation between the diameter of the eggs and the number of eggs or the number of eggs per unit of body weight. However, in the experiment above, the correlation between the diameter of the eggs and the number of eggs was found to be low. Furthermore, the volume of eggs should be considered in order to carry out selective breeding. Concerning the population to be used for experiments, it is considered that an improvement of the reproduction character and also an increase of body weight, will be obtained by selection of the volume of eggs. Concerning carp, Moav and Wohlfarth (10) reported examples of the correlation between the growth rates obtained by different culture methods; also, Wohlfarth et al. (26) reported examples of a correlation between the ability to escape from seines and the growth rate.

5. Artificial selection

Selection is designed to give a breeding rate to a specific individual, which is different from that of other individuals. The method is called an artificial selection, or a natural selection,

responding to the sizes and the selective strengths of various populations, were calculated (28).

5.2 Selection experiment with aquatic creatures

Donaldson and Olson (29) reported their results of the selection breeding of about six generations for 23 years with regard to increases in body weight, body length and the number of eggs of Rainbow trout. Remarkable progress was observed in all these characters. Also, Donadson and Menasveta (30) carried out selection experiments with Masunosuke, used for stocking, and reported that the progress of the characters (as, for instance, the growth rate) was observed.

Ryman (31) conducted selection experiments throughout nine generations with regard to the body weight of guppies of 63 days old. As the basic population for the selection, 597 individuals were used, the additive genetic variances of which had been scarcely discovered by the analysis of variances of their sib in the different experiments. The selection was made in two lines of the body weight: heavy and light. The number of individuals selected was not constant, 1-6 fish from one water tank (3-12 fish of only full-sib were put in one water tank and the number of water tanks was 8-131 for one generation).

The number of individuals measured was 28-566 females and 24-469 males for one generation. At the time when the distinction between the males and the females was clear, the males and the females were cultured separately. Also, their genetic gains were obtained separately. A summary of the results is given in FIGURE 1.5. The genetic gain shows little difference between the males and females.

and Po is called a selective difference (ΔP) (FIGURE 1.4). P is the selective strength and the difference, ΔG, between the mean value of the younger generation, P_1, and Po is the genetic gain or the selection response. If all variances in the character of the parental population are additive genetic variances, P_1 should agree with Ps. However, generally, such a case cannot exist, and P_1 leans to Po. FIGURE 1.4 shows a breakage selection, in which all indivuduals with more than a certain value of phenotype are slected. k is called the breakage point in a selection.

The relationship between ΔG and ΔP can be written, using heritability (h^2) as follows:

$$\Delta G = h^2 \Delta P \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Since using this equation, the selection response of one generation can be predicted from the selection difference and the heritability, it is a useful formula for the development of selective breeding. The selective difference is expressed by the unit of the character (gr., cm., etc.). In case of different variances and when different characters are compared, the selection difference is expressed by the unit of a standard deviation, in order to compare the selective strength. When the standard deviation of the character in the parental generation is σP and $i = \Delta P/\sigma p$, in the case of a normal distribution, $i = z/p$. Where z is the height of the vertical axis at the breakage point in FIGURE 1.4 and P is the selective strength. Thus, equation (8) can be written as follows:

$$\Delta G = i\sigma p h^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (9)$$

i is a standardized selective difference, the values of which, cor-

Furthermore, every year from 1966 to 1970, egg collections were repeated from a parent, taken from the original population at random, and were used as control groups for the whole (the crossbred control). The selective strength was 15-45%, which was as moderate as possible, in order to avoid a random genetic drift. 43-375 fish were used as parents. After the second generation, the parents of each line were divided into 2-6 sub-groups, when the selection, collection of eggs, crossings and cultivation of the offspring were made separately. In order to avoid inbreeding depression as much as possible, when crossing, the males and the females obtained from different sub-groups of each line were combined. The offspring were cultured in 3-5 culture grounds. The sub-group mean values and those in the culture grounds were corrected by a multiple-nursing method developed in the different experiments (33) and shown in FIGURE 1.6.

As shown in FIGURE 1.6, similarly to the experiments described previously (9,32), the selection response has a negative tendency with regard to the body weight gains in the "large" direction.

According to Moav (15), it was considered that the above results were caused by the fact that the population used for this experiment reached a selection plateau, retaining large genetic variations of the character and, therefore, the genetic and evolutionary mechanisms of this character were studied. On the other hand, it was reported that the selection response in the "small" direction of the increase in body weight was observed and the genetic rate realized was about 0.3, computed from the regressions obtained until around the third generation, at the time when the response ceased.

However, this difference is very small, when compared with the variation between the generations. It was reported that the heritability realized, estimated from the genetic gain, was below 10%. Applying the analysis of variances and considering the fact that a highly significant difference was observed in one litter of the line, it is thought that the additive genetic variances were very few and some other genetic variances must be important for the character under investigation.

Moav and Wohlfarth (9,32) conducted selection experiments in three directions, concerning increases in the weights of one year old carp bred in Israel: large, small and random. As the result, the selective effects were scarcely found in the "large" direction, but it was in evidence in the "small" direction. In this experiment, it was arranged that the original parents of the population should be kept as a control, from which offspring were bred again and also, in order to exclude the influence of inbreeding depression, the individuals selected from the two groups were crossed. Since this experiment was on a small scale and the selection was for only one generation, thereafter, more accurate experiments were conducted in a similar manner throughout 5 generations, concerning the increase in the body weight of one year old fish (15). FIGURE 1.6 shows a summary of the results. At first, in 1965, the selection of 30,000 fingerlings from 110 females and 21 males, was made. From the following year, a group with a large increase in body weight (High), a group with a small increase in body weight (Low), and a control group (High R and Low R), extracted at random from each line, were separated.

but the ratio of the homozygotes increases, when compared with the case of a random-crossing. The difference in the gene frequency due to inbreeding, when compared with a case of continuous random crossing, is related to the inbreeding coefficient (F).

When the frequency of the allelomorphs "A" and "a" are "p" and "q" respectively, in the case of random crossing, the gene frequency is as follows, according to the Law of Hardy Weinberg:

$$AA : Aa : aa = p^2 : 2pq : q^2.$$

Concerning the inbreeding population with inbreeding coefficient F, the gene frequency is as follows:

$$AA : Aa : aa = p^2 + Fpq : 2pq - 2Fpq : q^2 + Fpq.$$

Fujino (41,42) estimated the inbreeding coefficient of a natural population of Ezoawabi, using the above facts and the gene frequency governing variations in the esterase isoenzyme. Furthermore, he considered that the appearance of abnormal shells must have been caused by the inbreeding depression, because the ratio of homozygocytes is large in the abnormal shells, called "lean" (rogai, in Japanese).

Ryman (4) compared the recapture rates between families of Salmo salar , of the lines which were not inbred (the coefficient: F=0) and of the inbred lines, the F of which is known. The recapture rate of the inbred line was significantly low, even with regard to the variations caused by the state of the rivers and years. It was

This selection experiment was highly precise and detailed in the records; also, it was well planned with regard to the breeding mode and the genetic background. Selection experiments with carp are being conducted in the USSR, concerning their resistance to sickness and also concerning their growth rate (34).

Wada (35) continued the selection experiments of the coefficient of the shell width, using one group from the population of the full-sib Pinctada, which had been used previously for evaluation of the heritability (22). Wada reported that the result obtained from the first generation (selection from only the "large" direction) almost agreed with the selection response of one generation estimated from equation (9), using the heritability computed previously.

6. Inbreeding and crossing

The phenomena of inbreeding depression and heterosis have been known since olden times and, in particular, the latter has been applied in breeding. Concerning aquatic creatures, many reports are available of experiments, using Copepoda (36), oysters (37,38), carp (9,13,14,15,39) and Rainbow trout (40).

The inbreeding coefficient shows the rate of inbreeding which takes place in a certain population and, theoretically, it is defined as the probability that the allelomorphs, being combined, are homologous with their ancestors. Actually, the coefficient of the ancestor, as a starting point, is considered to be zero and the coefficient is shown in a relative degree to it. In a population, in which inbreeding has taken place, the ratio of the heterozygotes decreases,

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considered that this might have been caused by the low survival rate of the inbred lines.

So far, the basic theory and recent research concerning the genetics of quantitative characters have been described. Until recently, the practical application of quantitative genetics has been limited to only one part of the freshwater fish. However, as artificial breeding and the culture method of larvae have been developed, applications of genetics to marine creatures are being found. This also applies to the analyses of wild populations, like natural selection and the problems of evolution. In the future, the importance of quantitative genetics will increase in the field of stocking in the industry of fisheries and in their application to the breeding theory in the aquaculture. In order to meet this situation, it is desirable to accumulate more genetic information concerning many kinds of characters among the various species.

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FIGURE 1: FREQUENCY DISTRIBUTION OF SHELL CHARACTER VARIATION OF PINCTADA FUCATA OBTAINED FROM ONE PAIR OF PARENTS (ONE YEAR OLD)

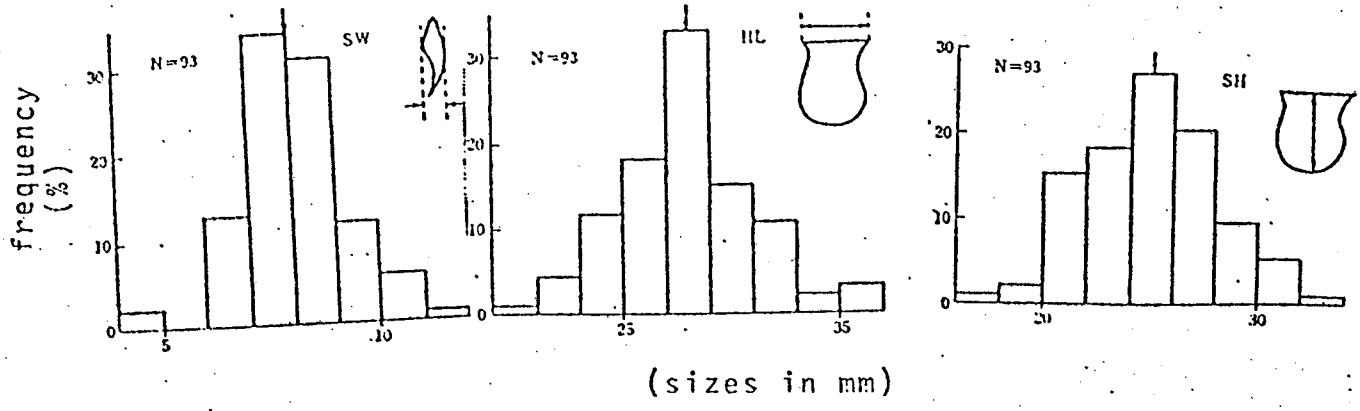
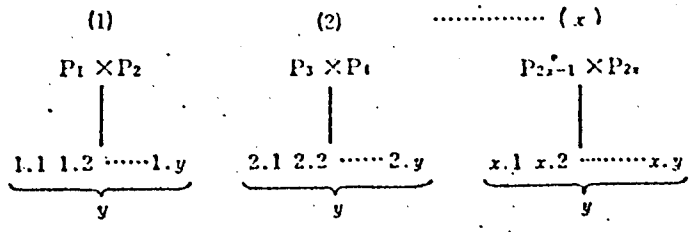
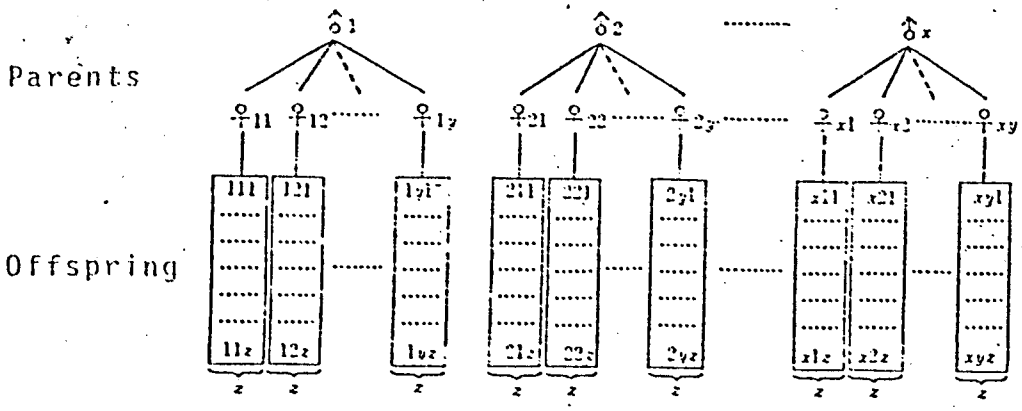


FIGURE 2: RELATIONSHIP BETWEEN FULL-SIBS



X = number of crossings
Y = number of offspring per one parent combination

FIGURE 3: DIAGRAM SHOWING RELATIONSHIP BETWEEN HALF-SIBS



X = number of fathers
Y = number of mothers per one father
Z = number of offspring from one pair of parents

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FIGURE 6: PROGRESS OF SELECTION EXPERIMENT FOR WEIGHT GAIN IN CARP (Moav et al., 15)

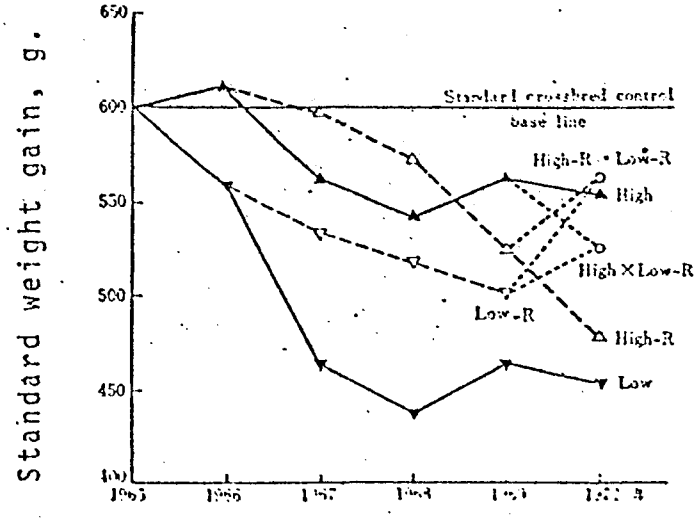
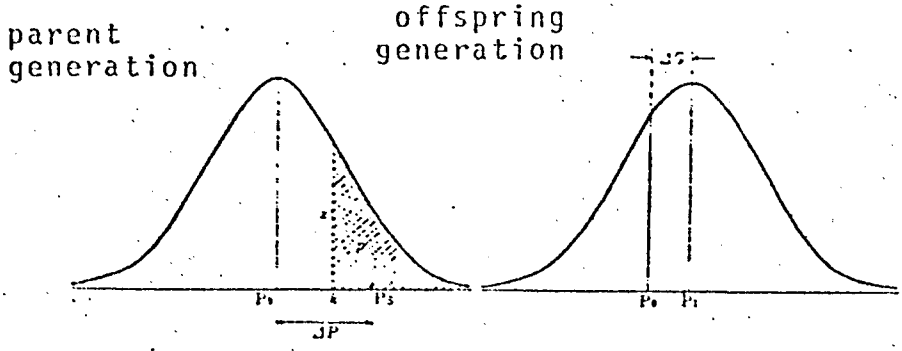


FIGURE 4: VARIATION IN POPULATION-MEANS DUE TO SELECTION



Horizontal axis = measurement of quantitative character
Vertical axis = correlation frequency

PO = population-means of parent generation
PS = means of individuals selected
P_1 = population-means of offspring generation
R = breakage point in selection
k = selection strength
$\Delta P = PS - P_o$: selective difference
$\Delta G = P_1 - P_o$: genetic gain (selective response)

FIGURE 5: PROGRESS OF SELECTION EXPERIMENT FOR BODY WEIGHT OF GUPPIES OF 63 DAYS OLD

solid line: large direction
broken line: small direction (Ryman, 1973, 31)

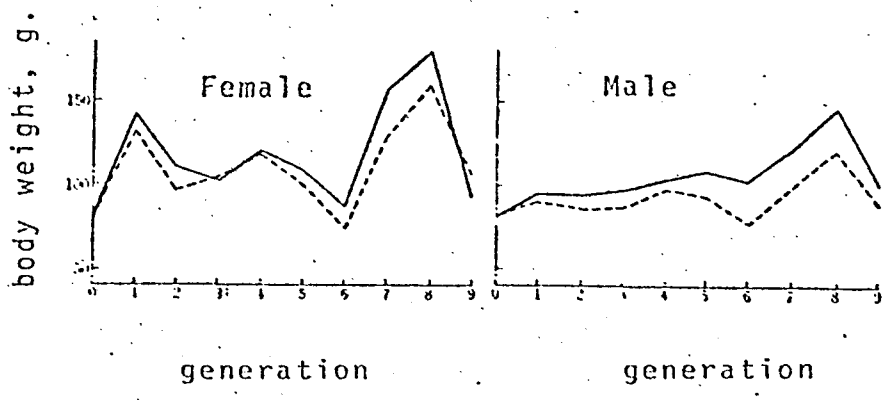


TABLE 2: ANALYSIS OF VARIANCE OF HALF-SIB, COMPOSITION OF SQUARE MEANS AND ESTIMATION OF HERITABILITY

Factor	Freedom degree	Square means	Square means composition
Between fathers	x - 1	MS_S	$\sigma_W2+Z\sigma_d2+YZ\sigma_S2$
Between mothers	x(y-1)	MS_D	$\sigma_W2 + Z\sigma_d2$
Between s i b s	xy(z - 1)	MS_W	σ_W2

Heritability from father: $h^2_N = \frac{4\sigma_S2}{\sigma_W2 + \sigma_S2 + \sigma_d2}$

Heritability from mother: $h^2N = \frac{4\sigma_d2}{\sigma_W2 + \sigma_S2 + \sigma_d2}$

Heritability from both father and mother: $h^2_N = \frac{2(\sigma_S2 + \sigma_d2)}{\sigma_W2 + \sigma_S2 + \sigma_d2}$

σ_W2 is variance in sib

σ_S2 is variance between fathers

σ_d2 is variance between mothers

for X, Y and Z - refer to FIGURE 3

TABLE 1: ANALYSIS OF VARIANCE OF FULL-SIB, COMPOSITION OF SQUARE MEANS AND ESTIMATION OF HERITABILITY

Factor	Freedom degree	Square means	Square means composition
Between sibs	x - 1	MS_S	$\sigma_W^2 + Y\sigma_b^2$
In one sib	X(y - 1)	MS_W	σ_W^2

Heritability: $h_B^2 = \frac{2\sigma b^2}{\sigma_S^2 + \sigma_W^2}$

σ_{W2} is variance in sibs

σb^2 is variance between sibs

for X and Y - refer to FIGURE 2

TABLE 3 (continued)

	Matured individuals, ratio in their 2nd year	h_S^2 = 0 - 0.09 h_D^2 = 0.01		
	Ditto, in their 3rd year	h_S^2 = 0.47 - 0.74 h_D^2 = 0.00	Half-sib, variance analysis	Moller et al.[18]
	(Estimation of body weight and length)			
Salmon bred in the Atlantic Ocean Salmo salar	Fish recaptured after 2 years in stock, body weight	0.22	Full-sib, variance analysis	Ryman[5]
	Resistance to Vibrio disease	h_S^2 = 0.1151±.0570 h_D^2 = 0.0747±.0497	Half-sib, variance analysis	Gjedrem[19]
Hybrids:				
River trout (Salvelinus fontonalis) and Laketrout (Salvelinus namaycush)	Non-eyed eggs, survival rate	0.06 ± .07		
	Eyed-eggs, survival rate	0.09 ± .11		
	Fingerlings, survival rate	0.41 ± .18		
	Resistance to bluesuck disease	0.76 ± .28	Half-sib, variance analysis (from mother and father)	Ayles[20]
	Resistance to malformation	0.03 ± .07		
Shells				
Crassostrea gigas	12 month growth Shell height (H) Shell length (L) Shell width (W) (Estimations of: H+L+W, L/H; survival and settling rates of spat; of above characters, and whole and shell weights of 18 months)	0.81 ± .27 0.81 ± .07 1.17 ± .05	Full-sib, variance analysis	Lannan[21]

TABLE 3

HERITABILITY REALIZED IN AQUATIC CREATURES

Species	Character	Heritability	Estimation method	Bibliography
Fish Group				
Carp	Weight gain	0.0	Selection (large)	Moav & Wohlfarth[9]
Cyprinus carpio	(for 4-5 months)	0.2-0.3	Selection (small)	
	body-weight, 150th day	$h^2_S = 0.17 \pm .110$		
Rainbow trout		$h^2_D = 0.00 \pm .068$		
Salmo gairdnerii	body-length, 150th day	$h^2_S = 0.17 \pm .157$	Half-sib, variance analysis	Aulstad et al.[16]
		$h^2_D = 0.07 \pm .101$		
	(Estimation of body weight and length on 280th day)			
	Body-weight of fish 2 years old	0.20		
	Egg size of above fish	0.21		
	Number of their eggs	0.19	Full-sib, variance analysis	Gall[3]
	Quantity of eggs	0.20		
	Number of eggs per body-weight	0.20		
	Non-eyed eggs, mortality	$h^2_S = 0.18 \pm .081$		
		$h^2_D = 0.86 \pm .141$		
	Mortality of eyed-eggs	$h^2_S = 0.15 \pm .056$	Half-sib, variance analysis	Kanis et al.[17]
		$h^2_D = 0.27 \pm .045$		
	Fingerlings, mortality	$h^2_S = 0.14 \pm .031$		
		$h^2_D = 0.06 \pm .012$		

TABLE 3 (continued)

	♀	$h_S^2 = 0.24$		
		$h_D^2 = 0.93$		
	♂		0.18 ± .104	Regression relationship between parent and offspring
	♀		0.17 ± .061	
	(Estimation of characters above at 15°C)			
Algae				
Kelp	Stalk length in an area with rough waves	0.10		
Laminaria saccharina	Diameter of sulcate of stalk-portion	0.078		Full-sib, variance analysis (estimation of regression relationship beteen parent and offspring) Chapman[25]
(L.longicruris)	Stock-portion length in an area with calm water	0.64		
	Diameter of sulcate of stalk-portion	0.239		

Concerning heritability obtained by analysis of variance of half-sib, h_S^2 was estimated from the componer variance of the father; whereas, h_D^2 was estimated from the component variance of the mother; (reference to TABLE 2).

TABLE 3 (continued)

Pinctada fucata	Characters after 1 year			
	Shell height (H)	0.32 ± .29		
	Length of hinge line (L)	0.05 ± .08		
	Shell width (W)	0.36 ± .32		
	Coefficient of shell height $\left(\frac{H}{H+L+W}\right)$	0.58 ± .43	Full-sib, variance analysis	Wada[22]
	Coefficient of hinge line length: $\left(\frac{L}{H+L+W}\right)$	0.57 ± .43		
	Coefficient of shell width: $\left(\frac{W}{H+L+W}\right)$	0.49 ± .39		
	(Estimations of: above character, shell weight wet and dry; also color of pearl-layer of 2nd yr.)			
American oyster: Crassostrea virginica	Growth of spat (size) 6th day after fertilization	0.09 - 0.51	Full-sib, variance analysis	Newkirk et al.[2]
	Ditto on 16th day	0.60		
	Ditto on 6th day	0.26 - 0.39	Half-sib, variance analysis	
	Ditto on 16th day	0.50		
Eurytemora herdmani	Maturation day (10°C) ♂	$h_S^2 = 0.10$		
		$h_D^2 = 0.42$		
	♀	$h_S^2 = 0.20$		
		$(h_D^2 = 0.00)$		
	Mortality before maturation (10°C)	$h_S^2 = 0.01 \pm .107$		
		$h_D^2 = 0.08 \pm .111$	Half-sib, variance analysis	McLaren[24]
	Sex ratio (10°C)	$h_S^2 = 0.11 \pm .218$		
		$h_D^2 = 0.13 \pm .221$		
	Mature body-size (10°C) ♂	$(h_S^2 = 0.00)$		
		$h_D^2 = 0.75$		

ARTICLE 2

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BIOCHEMICAL POLYMORPHISMS AND POPULATION GENETICS

INTRODUCTION

At the beginning of the twentieth century, studies of the biochemical polymorphism of marine animals were stimulated by the discovery (1,2) of the A B O blood group system and Forssman's antigen; the blood group of domestic animals, discovered in 1920, and also the development of studies of the genetic polymorphisms of proteins by gel-electrophoresis. At first, the studies concerning the blood group were commenced in Japan and the USA, around 1950. These were followed by expansion of the studies concerning protein polymorphism, about 1965; finally, these studies reached an outstanding development in many countries of the world(1). The number of species of marine animals studied was about 200, which greatly exceeds the number of domestic animals and man combined. Although polymorphism had been applied to the identification, classification and evolution studies of the breeding populations of fish and other marine animals as an index-character (2,3,4), these studies generally remained only within the range of development of analytical techniques and descriptions of the variations of polymorphism. Studies of the biochemical significance of polymorphism remained few.

TABLE 1 shows an application of protein polymorphism of the blood group to marine animals, as compared with the cases of man and

TABLE 4: PHENOTYPE CORRELATION (UPPER LINE), ENVIRONMENTAL CORRELATION (MIDDLE LINE) AND GENETIC CORRELATION (LOWER LINE) BETWEEN THE REPRODUCTION CHARACTERS OF RAINBOW TROUT.

(Gall, 1975, 3)

Character	Number of eggs	Volume of eggs	Body-weight	Number of eggs per 100 g. of body-weight
Size of eggs	.15 .20 .09±.17	-.45 -.38 -.55±.12	-.19 .01 -.46±.13	.26 .12 .47±.13
Number of eggs		.78 .78 .77±.07	.35 .33 .39±.14	.51 .58 .41±.14
Volume of eggs			.43 .34 .56±.11	- - -
Body-weight				-.50 -.42 -.61±.10

ing combinations of antiserums: Anti. $S_3(0_3)$ - Anti. Y_2, Anti. S_2 - Anti Y_2, Anti. Y_2 - Anti.Y_1, and Anti-S_2 - Ant. S_1 - Anti. Y_2. The numbers of their allelomorphs are also shown. Models 5-8 show the basic electrophoretic pattern of the protein or the enzyme poly-morphism, and they correspond to the case when the number of allelomorphs is two (monomer) and three (monomer, dimer, tetramer).

When the allelomorphs , determining the polymorphism, are equal without any dominant or recessive relationship, the number of allelomorphs is n; the number of phenotypes is $n(n+1)/2$. This case is shown in models 1,5,6,7 and 8. Since either dominant or recessive relationship is found between the allelomorphs in models 2 and 4, the number of phenotypes is lower than in the former cases. However, in either case , whether the relationship between the allelomorphs is equal, dominant or recessive, the gene frequency and also the theoretical frequency of the phenotypes (i.e. the expected values) can be obtained from the observed values of the phenotype frequency found in the population, as will be described later (ref.: Hardy-Weinberg's Law). As model 3 is a sub-type, the Hardy-Weinberg's Law cannot be applied to this case. Also, as this model is not related directly to the following study, the explanation for this model is omitted here.

When the entire population is genetically homogeneous and also crosses at random, a certain genetic equilibrium is established among the phenotypes appearing. For example, in the case of models 1 or 5 in FIGURE 21, when the three phenotypes are A, a and Aa and when the frequencies of the two allelomorphs, A and a, determining these

domestic animals. Since the analytic technique of protein polymorphism of the blood group has now been developed sufficiently well and already has been reported in books, in this paper, the basic aspects required for the analysis of population genetics mainly are discussed, as well as the biological significance of polymorphism. In addition, the possibility of the application of genetic polymorphism to the breeding of aquatic creatures in the future will be studied.

1. Basic aspects - Genetics and the Analytical Technique of Polymorphism

The blood grouping of animals is polymorphism classified according to the genetic variations of the immunological specificity of antigenic substances on the surface of cell membranes of the red blood cells. The classification of the blood type depends upon the reactions of the agglutination or the hemolysis of the red blood cells to the type-specific antibodies in various antiserums.

The polymorphism of water-soluble proteins and enzymes is classified by the genetic differences in the charge and the size of the molecules. These differences are shown by gel-electrophoresis and by electrophoretic differences in the pattern of histochemical staining. Concerning marine life, the analysis of polymorphism has been studied for many proteins and enzymes.

FIGURE 1 shows the basic pattern of the blood types actually observed in marine animals, also its electrophoretic differences. Models 1-4 are found, for example, in the Y blood type system of Katsuwonus pelamis (4). These models correspond to each of the follow-

In this TABLE, F is an index showing the degree of inbreeding and is called the inbreeding coefficient. It becomes closer to F = 1 from F = 0, with crossings at random, as the inbreeding progresses.

TABLE 4 shows the genetic frequency in Hardy-Weinberg's equilibrium, inbreeding and fully homozygote populations. When using the inbreeding coefficient F, the frequency of heterozygotes (A/a) in an inbreeding population decreases by 2Fpq, when compared with the value of a Hardy-Weinberg's equilibrium-population, and the frequencies of the homozygotes (A/A and a/a) increase each by Fpq. This relationship is called S. Wright's equilibrium condition and, based on the data obtained from a population, it can be used to estimate the inbreeding coefficient.

Differing from the above, the inbreeding coefficient also can be calculated as a possibility when the two genes of the homologous gene locus in a specific individual are precisely the same genes derived from the same ancestral gene. However, the relative description is omitted here (Mettler et al.,5).

2. Biological Significance of Polymorphism

It has not yet been clarified whether or not there are any biochemical or physiological differences in the genetic polymorphism of man's or the animal blood groups or in the proteins and enzymes of animals and plants; also whether there are any differences or not in the breeding and survival rates in the polymorphism, that is, whether or not there is an adaptive value. However, recently, concerning the aquatic creatures, the existences of the above aspects were reported one after the other. The main aspects from these reports are described

phenotypes, are each p and q(p+q=1), the freqency of these three phenotypes is formed as $p^2A + 2pqAa + q^2a = 1$ (FIGURE 2.2). This relationship is known as Hardy-Weinberg's Law concerning the genetic equilibrium.

TABLE 2·2 shows the method to obtain the genetic frequency from the observed values of the phenotype frequency and permits calculation of the expected frequency of genotypes (or phenotypes) in the cases of the three allelomorphs. In many reports concerning the populations of aquatic creatures, generally the frequency observed agrees very well with the expected frequency. Therefore, the universal validity of Hardy-Weinberg's Law is confirmed.

In contrast to the random crossing of the individuals in a population, as described, large populations are divided, more or less, into smaller sub-populations, according to the conditions which exist in the areas occupied by them. As the result, it has actually been observed in the natural groups that the crossings are made between individuals of closer relationship than the average in a population. This is called inbreeding and is accompanied by several important and interesting genetic phenomena. The most remarkable is an increase in the number of homozygotes.

The closer the blood relationship, the higher the rate of increase in the number of homozygotes. Similarly to the examples in FIGURE 2.2, TABLE 2.3 shows the increase in the homozygotes (A/A and a/a) and also a decrease in the heterozygotes (A/a), when a Hardy-Weinberg's equilibrium-population with two allelomorphs, A and a (p=q=0.5) participated to the highest degree in the inbreeding, i.e. a regular self-fertilization.

polymorphism of Katsuwonus varies according to the age. A similar phenomenon was observed in the esterase polymorphism in the digestive diverticulum of Haliotis discus hannai INO (8).

(3) Growth rate or the rate of weight gain

As described previously in (2), according to McIntyre et al. (7), the growth rate of coho salmon with type AA of the serum transferrin polymorphism is higher than that with type AC. Similar phenomena were also reported concerning the serum transferrin polymorphism in Rainbow trout, Salmo gairdnerii (9). , and also Lake trout, Salvelinus namaycush (10).

(4) Adaptability to more expansive areas

According to Koehn (11,12), the enzyme activity of three types of the serum esterase, Es-$I^{a/a}$, Es-$I^{a/b}$ and Es-$I^{b/b}$, in freshwater fish bred in North America, Catostomus clakii, shows a correlation to the temperature. Es-$I^{b/b}$ showed high activity at low temperature; Es-$I^{a/a}$ at high temperature; and Es-$I^{a/b}$ at medium temperature. On the other hand, concerning the frequency of the presence of the three types in a wild population, Es-$I^{a/a}$, the activity of which increases at high temperature, appeared with great frequency in the low latitudes (i.e. the areas with high temperatures); but Es-$I^{b/b}$ appeared infrequently. The relative frequency of the polymorphism varied with the different latitudes and, in the high latitudes (i.e. areas with low temperatures), the frequency was contrary to that in the low latitudes. Also, it is reported that the results show the existence of an adaptive value due to the differences in the physiological functions of enzymes (FIGURE24).

in the following.

(1) Fertilization rate

Fujino et al. (6) compared the observed frequency (O) of the serum transferrin of Katsuwonus bred in the Pacific Ocean and its expected frequency (E) obtained on the basis of Hardy-Weinberg's Law. Then it was established that, concerning the three phenotypes (2, 2.3, 3), the O/E value is smaller than 1 in homozygotes (types 2 and 3), but that it is larger than 1 in heterozygotes (type 2.3), and that in both cases, it comes close to 1 with the increase in body length (age) (TABLE25). This shows the difference in the relative survival rates in the transferrin polymorphism of Katsuwonus, in particular, the presence of over-dominance of the heterozygotes (heterosis). That is, the fertilization rate or the hatching rate is highest in type 2.3, which are heterozygotes, followed by types 2 and 3, or homozygotes; while the relative survival rate during the growing process after hatching shows a drastic decrease in the heterozygotes rather than in the homozygotes. FIGURE23 shows the adapted values in the different polymorphisms, calculated using TABLE25. This FIGURE also shows the differences in the fertilization rates (or the hatching rates) among the polymorphisms during the developmental stage.

(2) Survival rate

McIntyre et al. (7) observed an artificially crossed population of coho salmon, Oncorhynchus kisutch, before stocking, and reported that, in the serum transferrin polymorphism, AA, AC and CC, both the survival and growth rates of type AA were higher than of type AC. As described previously in (1), the relative survival rate of the serum transferrin

and the ability to carry oxygen by the hemoglobin polymorphism of a hybrid of Lepomis cyanellus x Chaenobryttus gulosus (16), the phenomenon of heterosis has been reported.

On the other hand, it is expected that the correlation between the polymorphism and the immunity to disease, found in domestic animals (for example, the B-type blood group in chickens) will be found in aquatic creatures in the near future. In general, it is considered that the correlation between biological polymorphism and the biological characteristics is caused by (1) the pleiotropism of the genetic function; (2) linkage and (3)super dominance of heterozygotes. Also, some scientists of molecular genetics, population genetics and evolution consider that a specific physiological difference cannot exist between individuals with the enzyme polymorphism showing the same catalysis to certain specific biochemical reactions. However, as shown in the electrophoric mobility, there are obvious differences in the physiological character among the polymorphism molecules. That is, according to the different physiochemical conditions of the body fluid (one of the factors of the body-interior) the enzyme polymorphism functions from its production area and moves at a different rate of velocity to another area to be consumed. It is considered that this aspect to some extent may cause different concentrations of the enzyme polymorphism; and, therefore, may also cause different physiological activities.

When considering the above, without regard to the different physiochemical qualities in the polymorphism molecules, the opinion that there is no difference in the physiological activities among

FIGURE25 shows the existence of adaptive values represented by the correlation between the latitudinal temperatures and the genetic frequency of the three enzymes of the American eel, Anguilla rostrata, sorbitol dehydronase enzyme (SDH), phosphohexose isomerase (PHI) and alcohol dehydrogenase (ADH) (13). As described, it is considered that the phenomenon of the correlation between the adaptive values of the polymorphism and the latitudes (i.e. temperatures), is one of the important basic factors in the study of adaptability throughout the more expansive areas for the purpose of breeding.

(5) Other physiological activities

Concerning the allelomorphs Idh Ha^B and Idh Ha^A of the LDH polymorphism in the liver of Rainbow trout, Tsuyuki et al. (14) bred a homozygous line by artificial crossing and compared the swimming durability experimentally. As the result, the mean time, required until 50% of the fish tested, of all lines, became exhausted in a certain velocity of flow, is 2.3 times longer in the group of the Ha^A Ha^A than in the group of the type Ha^B Ha^B. Furthermore, concerning Steelhead (catadromous Rainbow trout) from a wild population, the three types Ha^A Ha^A, Ha^A Ha^B and Ha^B Ha^B, were compared, similarly to the above. No significant differences were found among these three types, or between Ha^A Ha^B and Ha^B Ha^B.

However, differences were found between the two groups bred in different areas. That is, the mean time required until 50% of fish bred in the Thompson River became exhausted was 3.8 times longer than that of the fish bred in the Vedder River. Furthermore, concerning the oxygen affinity of the hemoglobin polymorphism of rabbints (15),

establishing species and lines. The practical theory and the methods involved have already been discussed in the paper, "Biochemical identification of fish species" (4).

(2) Analysis of breeding structure, specifically inbreedin As described in a paragraph of Hardy-Weinberg's Law, according to reports concerning polymorphism in aquatic creatures, many migrating fish with strong swimming stamina, breed at random in large wild populations. However, recently, the overdominance of homozygotes in Mussels mytilus californianus (20), lobsters (Homarus americanus) (21) and Ezoawabi (Haliotis discus hannai, INO), which are invertebrates, has been reported frequently. The overdominance of homozygotes means that the observed frequency in homozygotes is higher than expected, based on Hardy-Weinberg's Law of genetic equilibrium. However, the observed frequency in heterozygotes is lower than expected. With regard to the cause of this, the following two cases can be considered.

One is the case of breeding by individuals belonging to several sub-populations with different genetic frequencies; and the other is the case of inbreeding by the individuals described above. In the former case, generally it is difficult to check the deviations from the equilibrium, using samples obtainable, unless the difference in the genetic frequencies between the populations is significant.

Concerning these relationships, Fujino (22) made quantitative analyses. In both cases, this phenomenon occurs when the entire population is not homogeneous but consists of several small sub-populations. If this is in evidence, then it is possible to distinguish the above two cases.

individuals with different phenotypes, seems too weak to be persuasive. The future accumulation of relative data on the above aspects will provide a solution.

3. Utilization of Polymorphism for Genetics and Breeding

There are many reports about the relationship between polymorphism and biological character, but, as has been described earlier, some of these reports cannot withstand criticism of their sampling or their interpretation of data cited. There are many examples of marine life, in which polymorphism is correlated with biological characters. These observations encouraged the use of polymorphism for selective breeding. This section will deal with the problem of whether polymorphism, without adaptive values, is useful in genetics and in breeding.

(1) Identification of wild populations, species and lines. With advances in breeding, although some species with certain characteristics may have been cultivated, the wild populations of these species are still sources of genes for making other new types with different characteristics. This has been demonstrated in the breeding of agricultural crops and domestic animals. In particular, concerning aquatic creatures, the breeding studies of which are still in the state of infancy, the volume of knowledge accumulated systematically about them is bound to affect the future development of the breeding studies.

The genetic frequency of polymorphism is useful as an index for identification and classification of wild populations and for

abnormal shells are distinguished from normal shells when using the ratio between the weight of the soft tissue and the weight of the entire body (FIGURE2.6). As the result, the following aspects were clarified (23).

(3) The overdominance of homozygotes in the F zone was more noticeable in the abnormal shells than in the normal shells.

(4) Although the normal shells showed a superdominance of heterozygotes in the M zone, the absormal shells showed an overdominance of homozygotes in the same zone. The difference between both groups reached a statistically significant level. Based on the observation results above, it was reported that the presence of the abnormal shells is a phenomenon connected with the inbreeding depression, relative to the inbreeding structure. [Life activity decreases due to inbreeding. As a cause, it is considered that inferior and harmful genes, as heterozygotes (carrier), in a population with superior and normal genes will appear due to the increase in homozygotes accompanied by inbreeding.] That is, accompanying the progress of the homozygosis in all genetic loci by inbreeding, the recessive and harmful genes also become homozygotes. Thus, individuals with low physiological activity stand out.

For example, it is considered that, when physiochemical or biological environmental factors become worse (such as a drastic change in water temperature or food shortage due to overcrowding), the individuals with such low activity develop abnormal shells due to physiological disorders and malnutrition.

In order to distinguish these two cases, the investigation area has to be selected properly, as samples must be collected repeatedly from the parent population. Then, studies are made as to whether or not the distribution of the genetic frequencies of the samples converge to a certain value; also, whether or not they are divided into the same number of sub-populations as expected. These aspects have not been studied sufficiently in the report concerning Mussels mytilus californianus, and lobsters (Homarus americanus) mentioned above. In the following, the results concerning Ezoawabi (Haliotis discus hannai INO) observed by the author, as well as their application to breeding, are described.

As part of the genetic study of Ezoawabi, using the samples obtained repeatedly from their wild population in the water area around a peninsula, located at Sanriku-cho, Iwate Prefecture, Fujino (8) analyzed the esterase-polymorphism in the digestive diverticula and clarified the following: (1) six phenotypes controlled by three equal allelomorphs were found in the F zone. The appearance rate showed a remarkable overdominance of homozygotes and also showed inbreeding in the wild population. (2) Six phenotypes controlled by three equal allelomorphs in the M zone, which genetically is independent from the F zone, and also a superdominance of heterozygotes was observed.

Also, abnormal shells of little commercial value, which are called "Rogai" (lean shells) by fishermen , were infrequently found in catches (usually, 1-2%); these were compared with normal shells. Fishermen can extract these abnormal shells from their catches when shipping by observing the appearance of the shells. Furthermore, these

genotypes of nine genetic loci in each normal shell (Fujino, 24). In this TABLE, there are four individual shells with homozygotes at all their nine genetic loci* (Nos. 2, 16, 17 and 20). It is expected that individual shells with homozygotes at all nine loci of polymorphism, including a genetic locus determining unknown performance traits, have a higher probability to be homozygotes than other shells, because the homozygosis, due to inbreeding, affects all genetic loci, as was mentioned previously. [* At present, no linkage has been found in these gene loci.]

As an example of performance traits, the ratio of the weight of the soft body-portion to the whole body-weight, SP/BW (FIGURE 26), was selected and the distribution frequency of this value is shown in FIGURE 27, according to: abnormal shells, normal shells and shells with homozygotes at all nine genetic loci.

As is obvious from this FIGURE, the mean value X_H of the shells with homozygotes at all nine genetic loci is higher than the mean value X_N of normal shells. This fact shows that the individual shells with homozygotes at all genetic loci have greater probabilities of having dominant/superior genes in the homozygotes at genetic loci, considered to be polygenes, determining the performance traits, than the other shells.

Therefore, genetic improvement is expected by selection and breeding this type of individual shells. (At present, polymorphism is checked after breeding, sacrificing parent shells. The shells needed are retained, but the shells bred but not needed are abandoned. However, it may be possible to breed selectively without destroying the

(5) Fujino (8) also confirmed that the superdominance was of heterozygotes found in the M zone of the esterase of normal shells, therefore, the adaptive value of polymorphism shows a correlation to age (TABLE 26). This is the same phenomenon as the adaptive values of polymorphism of the serum transferrin in Katsuwonus, shown in FIGURE 23, which varied according to age.

Recently, the constituents of the digestive diverticula of Ezoawabi (Haliotis discus hannai INO) and also of their shell muscles were analyzed further. Biochemical polymorphisms were found in 7 genetic loci: leucin aminopeptidase (Lap), tetrazolium-oxidase (To), malate dehydrogenase (Mdh), phosphoglucose-isomerase (Pgi), malic enzyme (Me), etc. In most of these, the overdominance of homozygotes was found, caused by inbreeding. Consequently, polymorphism was found at nine genetic loci of Ezoawabi, including the esterase mentioned previously (24) (this number is close to the haploid number of Ezoawabi, n = 10).

Fujino (23) adapted the observation results of the wild populati- mentioned (1-5), to the essential part of breeding studies in agricultural crops, particularly corn (Fisher, 25). He also presented a "Selective breeding guide how to increase the production of Ezoawabi" (TABLE 27). The case when more than one, or many, individuals, comprising one wild population, are used as experimental material, was studied. When experimental material is used from other wild populations, an accumulation of systematic data concerning their characteristics is needed. The application of polymorphism was studied in order to establish species with superior performance traits, suitable for the selection of parent shells. TABLE 28 shows the examination results of the

lyzed quantitatively (28,29,30). According to these analyses, it has been reported that the increase in the appearance rate of malformation, the decrease in the rate of feeding-efficiency, or the rate of weight gain, and the decrease in the survival rate were observed more noticeably in the inbreeding group, as compared with the outbreeding group.

It has also been reported that the damage due to inbreeding reached 24-31% in fish body-weight and 16-22% in the number of surviving fish. As inbreeding depression appears in many other aspects as well, this effect is often more extensive.

Ihssen (30) urged the necessity in aquaculture to avoid the bad effects, caused by the inbreeding depression in seed production. In the following, a case when parent fish (shells), obtained from a wild population, are used for seed production is described. As already mentioned, it has been clarified that there are some aquatic creatures which inbreed in their wild state. This fact shows that, although there are some differences, there is a possibility for progress in inbreeding frequency by seed production.

It is considered that the facts described above show the necessity to study genetically the problems, including malformation accompanying the seed production of aquatic creatures. Biochemical polymorphism will be useful as an index, showing the inbreeding progress-degree.

(4) Others

Together with the method of cell genetics, biochemical polymorphism can be used for identification of the genetic loci and for drawing

parent shells, if polymorphism can be tested by taking a small portion of tissue, using anesthesia.) In this case, it is considered that $X_H - X_H$ corresponds to the response to selection (26), the genetic gain, or the selective advance, when selection is made in general.

Also, with the increase in the number of genetic loci of biochemical polymorphisms as an index, more genetic gains can be expected.

Using the offspring generation obtained as above, after elimination of the inferior or harmful genes, appearing infrequently, due to repetitive inbreeding (shown in 2(2) in the Practice of Selective Breeding in TABLE2.7) or parthenogenesis, new species are established. So far, the case of SP/BW has been described as a selective index. A similar method can be adopted to the growth rate and other quantitative characters (FIGURE 2.8).

As mentioned previously, repetitive breeding is necessary in order to establish species. It is considered that inbreeding in the wild population of Ezoawabi is one way of breeding artificially. It is also considered that utilization of biochemical polymorphism can be an effective supplementary method, when the inbreeding structure is followed by artificial breeding.

(3) Index of inbreeding in aquaculture

At present, in order to procure parent fish (shells) for seed production, the following two ways are followed: they are obtained directly from a wild population and also by means of artificial breeding. Recently, concerning the inbreeding depression of Rainbow trout, a typical case of the latter, the results of comparison of the characters between lines with different frequencies on inbreeding were ana-

Then, the application methods must be different for the cases when there are differences in the biochemical and physiological activities of polymorphisms and when adaptive values exist, i.e. different from the method used in a neutral case. A neutral case will play an important role as an index in the monitoring of the breeding structure and its effect on wild populations, particularly concerning the inbreeding structure or artificial inbreeding in aquaculture.

Therefore, no developments in the genetic and breeding studies of aquatic creatures can be expected, if only analysis of the phenomena is realized from a narrow point of view. It is considered that the overall attitude must be one of dedication and a multi-faceted pattern of studies on a wide scale is indispensable.

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chromosome diagrams, using hereroploid, chromosome polymorphism, or polymorphism in interspecific hybrids, as experimental materials.

Furthermore, in recent years, a correlation between the presence of function and the activity of enzymes, accompanied by the differentiation of organs and tissues in their different stages of development, has been perceived, and also its application to developmental genetics (31).

4. Summary

When looking back over the last quarter of a centuary, studies of biochemical polymorphism, which started as studies of blood groups and developed to studies of protein and enzyme polymorphisms, have advanced greatly. However, it is regrettable that studies of polymorphism of antibodies, including the blood groups, have lagged somewhat behind due to technical difficulties and the lack of well-defined purpose.

It is considered that the genetic and breeding studies concerning immunity to diseases in aquatic creatures, also studies of the correlation between the serological character and the sensibility as a receptor of bacteria and viruses, are very interesting and important problems.

In the future, biochemical polymorphism must increase its usefulness in the different fields of genetics of aquatic creatures also in breeding studies as an index-character.

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TABLE 2.1: BLOOD GROUPS AND PROTEIN POLYMORPHISM IN MEN, DOMESTIC ANIMALS AND AQUATIC CREATURES (Fujino,1)

I t e m s	M e n	Domestic animals	Aquatic
Evolution and population biology	o Anthropology	o Evolution o Identification of species and wild populations	o Evolution, phylogeny and classification o Identification of wild populations (families) and species o Analysis of the breeding structure of populations and identification of inbreeding lines
Correlation between physiological characteristics	o Clinical medicine . Blood transfusion . Abortion, stillbirth and erythroblastosis fetalis . Twins, chimera . Stomach cancer and duodenal ulcer incidences o Anthrobiology . Analysis of chromosome mutation structure	o Clinical veterinary medicine . Hemolytic diseases prevention in newly-borns . Identification of freemartins o Correlation between commercially valuable characters . Correlation between blood type B of chickens and body-weight, and rates of: survival, egg-laying and disease . Effect of heterosis	o Adaptive values . Heterosis . Correlation between adaptive values of serum esterase polymorphism of fish, Numerigoi family, and temperatures . Heterosis found in polymorphism of Conomurex and Haliotis discus hannai In . Correlation between heterosis found in polymorphism of Katsuwonus and Haliotis discus hannai Ino and age
Identification of individuals and parental relations	o Legal medicine . Identification of parental relations . Identification of individuals	o Animal husbandry . Identification of parental relations . Registration of pedigrees	None at present

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TABLE 2.3: THE DECREASE IN HETEROZYGOTES WHEN A BALANCED POPULATION ($p = q = 1/2$) CHANGES TO REGULAR SELF-FERTILIZATION. WHEN ALL THE OFFSPRING OF SELF-FERTILIZATION ARE CONSIDERED, THE GENE FREQUENCIES ARE NOT CHANGED BY INBREEDING. THE INBREEDING COEFFICIENT INCREASES WITH EVERY GENERATION UNTIL ALL THE INDIVIDUALS BECOME HOMO.
CONCERNING THE CASES SHOWN IN THIS TABLE, AS THE INITIAL GENE FREQUENCY IS 1/2 EACH, IT IS EXPECTED THAT THE RATIO BETWEEN BOTH HOMOZYGOTES IS EQUAL, WHEN $F = 1$.
ONE SHOULD NOTE THAT, WITH REPEATED INBREEDING, THE WAY IN WHICH F COMES CLOSE TO 1.0 BECOMES SLOWER IN EACH GENERATION.
(Mettler, 5*)

Generation	GENOTYPE FREQUENCY				
	A/A	A/a	a/a	F	q
0	1/4	1/2	1/4	0	1/2
1	3/8	1/4	3/8	1/2	1/2
2	7/16	1/8	7/16	3/4	1/2
3	15/32	1/16	15/32	7/8	1/2
4	31/64	1/32	31/64	15/16	1/2
n	$\frac{1-(1/2)^n}{2}$	$(1/2)^n$	$\frac{1-(1/2)^n}{2}$	$1-(1/2)^n$	1/2
∞	1/2	0	1/2	1	1/2

TABLE 2.2: CALCULATION METHOD OF THE GENE FREQUENCY AND THE EXPECTED FREQUENCY OF PHENOTYPES FROM THE OBSERVED FREQUENCY OF PHENOTYPES IN POLYMORPHISM, CONTROLLING 3 EQUAL ALLELOMORPHS.

THE OBSERVED FREQUENCY IS OBTAINED BY DIVIDING THE NUMBER OF INDIVIDUALS WITH PHENOTYPES BY THE TOTAL NUMBER OF INDIVIDUALS INVESTIGATED.

THE SUM OF THE OBSERVED FREQUENCIES IS 1.0; also, $p + q + r = 1.0$.

PHENOTYPES

	1	1·2	2	2·3	3	1·3
Observed frequency	$\overline{1}$	$\overline{1\cdot 2}$	$\overline{2}$	$\overline{2\cdot 3}$	$\overline{3}$	$\overline{1\cdot 3}$
Expected frequency	p^2	$2pq$	q^2	$2qr$	r^2	$2pr$

Genes	Frequency
A^1	$p = 1 + 1/2(\overline{1\cdot 2} + \overline{1\cdot 3})$.
A^2	$q = 2 + 1/2(\overline{1\cdot 2} + \overline{2\cdot 3})$.
A^3	$r = 3 + 1/2(\overline{2\cdot 3} + \overline{1\cdot 3})$.

TABLE 2.5: RATIO BETWEEN OBSERVED VALUES (O) AND EXPECTED VALUES (E) OF TRANSFERRIN IN KATSUWONUS

(Fujino and Kang,6)

Fluke-distance (cm.)	Phenotypes										Number of fish	Gene frequency			Phenotypes O/E		
	1・2		2		2・3		3		1・3								
	O	E	O	E	O	E	O	E	O	E		$T^1_{s/}$	$T^2_{s/}$	$T^3_{s/}$	2	2・3	3
31~40	0	0.0	21	23.0	23	19.0	2	4.0	0	0.0	46	0.000	0.707	0.392	0.91	1.21	0.56
41~50	4	3.5	201	209.3	169	152.9	20	23.0	1	1.3	395	0.005	0.723	0.266	0.96	1.11	0.71
51~60	1	0.6	60	64.3	74	65.9	13	16.9	0	0.3	148	0.003	0.659	0.338	0.93	1.12	0.77
61~70	0	0.0	53	59.5	50	47.2	8	9.3	0	0.0	116	0.000	0.716	0.284	0.97	1.06	0.86
71~80	1	1.4	33	38.8	38	35.9	7	8.3	1	0.6	85	0.012	0.676	0.312	0.93	1.06	0.84

TABLE 2.4: GENOTYPE FREQUENCY IN THE CASE WHEN THERE IS NO INBREEDING IN A POPULATION BRED AT RANDOM; IN THE CASE WHEN INBREEDING TAKES PLACE PARTIALLY; AND IN THE CASE OF COMPLETE INBREEDING.

(Mettler, 5)

Number of inbred generations	F	Genotype frequency			
		A/A	A/a	a/a	
0 (Hardy-Weinberg equilibrium)	$F=0$	p^2	$2pq$	q^2	population)
1 and over 2 (Wright's equil.)	$1>F>0$	p^2+Fpq	$2pq-2Fpq$	q^2+Fpq	population)
Infinity (completely homozygotic)	$F=1$	p^2+pq	0	q^2+pq	

TABLE 2.7: SELECTIVE BREEDING GUIDE AS TO HOW TO INCREASE THE PRODUCTION OF HALIOTIS DISCUS HANNAI,INO

(Fujino, 23)

A. GENETIC ASPECTS

1. Inbreeding in wild populations of Haliotis discus hannai,Ino.
2. Presence of abnormal shells called Rogai (i.e. lean shells), is a phenomenon of inbreeding depression.
3. Survival rate of Rogai is low.
4. Phenomenon of superdominance of heterozygotes.
5. Correlation between adaptive values of heterozygotes and age.

B. PRACTICE OF SELECTIVE BREEDING

1. Materials ... more than one individual, comprising one wild population.
2. Selection of parent shells to establish species.
   (1) Selection of homozygotes in all biochemical polymorphisms investigated.
   Type 2.2 and type 3.3, concerning esterase at M-gene locus
   (2) High ratio of homozygotes by repetitive inbreeding.
   Elimination of inferior and harmful genes.
   Preservation of individuals with superior characters.
   (3) Establishment of species and lines by repetitive inbreeding and parthenogenetic development.
3. Hybrids from the breeding of different species.
   By breeding two or more established species with superior characters, or lines, it is possible to produce and supply consistently homogeneous seeds of hybrids with superior characters.

TABLE 2.6: ADAPTIVE VALUES OF PHENOTYPES OF ESTERASE POLYMORPHISM IN M ZONE OF HALIOTIS DISCUS HANNAI, INO

(Fujino, 8)

SHELL LENGTH (cm.)	PHENOTYPES			
	1•2	2	2•3	Others
7.0~ 9.4	1	0.36	0.50	0.29
9.5~10.4	0.87	0.36	1	0.27
10.5~15.4	0.64	0.33	1	0.27
TOTAL	0.96	0.41	1	0.32

FIGURE 2.1: BLOOD GROUP PATTERNS (models 1-4) AND ELECTROPHORIC PATTERNS (models 5-8) OF AQUATIC CREATURES

(Fujino,1)

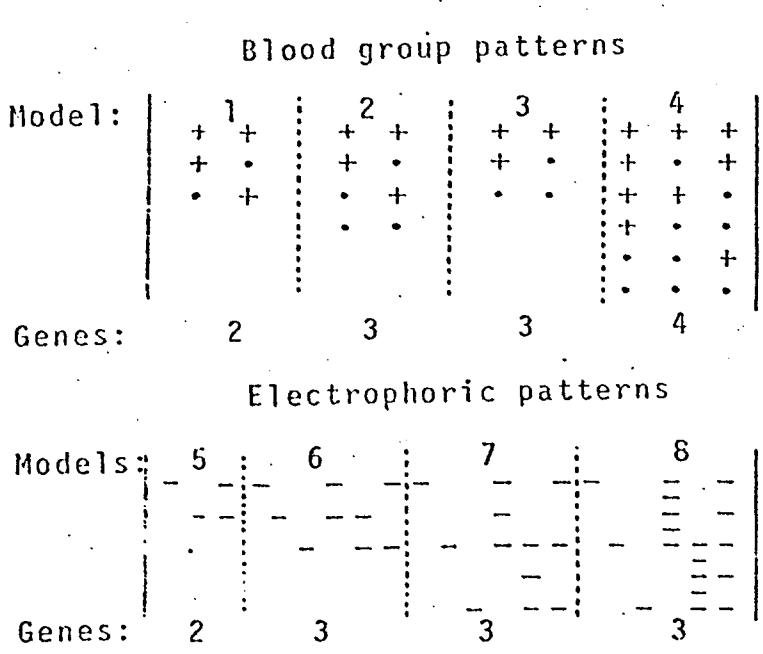


TABLE 2.8: GENOTYPES OF BIOCHEMICAL POLYMORPHISMS IN 9 GENE LOCI OF HALIOTIS DISCUS HANNAI, INO.

(Fujino, 24)

* all the homozygotes at 9 gene loci

\# heterozygotes at gene loci

SAMPLE NUMBER	Es-M	Lap-S	To-M	To-S	Mdh-F	Mdh-S	Pgi	Me
1	2/2	1/2#	4/4	1/1	1/1	2/2	2/2	2/2	1/2#
2*	2/2	1/1	3/3	1/1	1/1	2/2	2/2	2/2	1/1
3	2/2	1/1	4/4	1/2#	1/1	2/2	2/2	2/2	1/1
4	2/2	1/2#	2/4#	1/2#	1/1	2/2	2/2	2/2	1/2#
5	2/2	1/2#	4/4	1/1	1/1	2/2	2/2	2/2	1/1
6	2/2	1/1	2/4#	1/1	1/1	2/2	2/2	1/2#	1/1
7	2/2	1/2#	4/4	2/2	1/1	2/2	2/2	2/2	1/1
8	2/2	2/2	3/3	2/2	1/1	2/2	2/2	1/2#	1/1
9	2/2	1/2#	6/6	1/2#	1/1	2/2	2/2	2/2	1/1
10	3/3	1/2#	4/4	1/2#	1/1	2/2	2/2	2/2	1/1
11	2/2	1/1	3/5#	1/2#	1/1	2/2	2/2	1/1	1/2#
12	2/2	2/3#	4/4	1/2#	1/1	2/2	2/2	2/2	1/1
13	2/2	1/1	4/4	1/2#	1/1	2/2	2/2	2/2	1/1
14	2/2	1/2#	4/4	1/1	1/1	2/2	2/2	2/2	1/1
15	2/2	1/2#	4/4	1/1	1/1	2/2	2/2	2/2	1/1
16*	2/2	2/2	4/4	2/2	1/1	2/2	2/2	2/2	1/1
17*	2/2	1/1	4/4	2/2	1/1	2/2	2/2	2/2	1/1
18	2/2	1/2#	3/3	1/2#	1/1	2/2	2/2	2/2	1/1
19	2/2	1/1	2/4#	1/1	1/1	2/2	2/2	2/2	1/2#
20*	2/2	1/1	4/4	1/1	1/1	2/2	2/2	2/2	1/1
21	2/2	1/1	2/4#	2/2	1/1	2/2	2/2	2/2	1/1
22	2/2	1/1	4/4	1/2#	1/1	2/2	2/2	2/2	1/2#
23	2/2	1/2#	2/4#	1/1	1/1	2/2	2/2	2/2	1/1
24	2/2	1/2#	2/4#	1/1	1/1	2/2	2/2	2/2	1/1
25	2/2	2/2	2/4#	1/2#	1/1	2/2	2/2	2/2	1/1

FIGURE 2.3: RELATIONSHIP BETWEEN THE SURVIVAL RATE AND THE BODY LENGTH (AGE) IN SERUM TRANSFERRIN POLYMORPHISM OF KATSUWONUS BRED IN THE SEA NEAR HAWAII.

AGES WERE ESTIMATED FROM THE GROWTH-CURVES OF VON BERTALANFFY.

(Fujino,6)

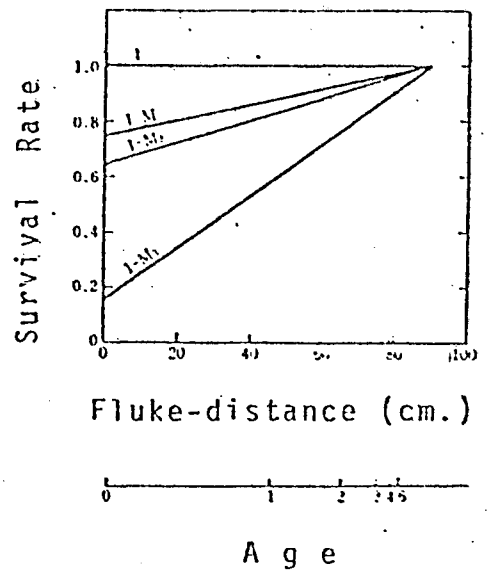


FIGURE 2.2: EQUILIBRIUM POPULATION.
THIS FIGURE SHOWS EQUILIBRIUM POPULATION RESULTING FROM A RANDOM COMBINATION OF THE GROUPS WHICH SUPPLY GAMETES. EACH SIDE OF THE SQUARE IS TAKEN TO BE 1.
BOTH THE UPPER SIDE AND THE LEFT SIDE OF THE SQUARE ARE DIVIDED INTO 2 PORTIONS AT A PLACE WHICH SHOWS GENE FREQUENCIES OF A GROUP WITH MALE GAMETES AND A GROUP WITH FEMALE GAMETES ($p = 0.6$, in A; and $q = 0.4$, in a). THUS, THE SQUARE IS DIVIDED INTO 4 PORTIONS BY A VERTICAL LINE AND A HORIZONTAL LINE WHICH START FROM THE DIVISION POINTS AT THE UPPER AND LEFT SIDES.
THESE 4 AREAS SHOW THE RELATIVE RATIOS OF GENOTYPES IN THE OFFSPRING OF RANDOM COMBINATIONS OF A GROUP WITH SPERM GAMETES AND A GROUP WITH EGG GAMETES.
THE AREA OF THE SQUARE IS 1.0. THUS, THE SUM OF THE GENOTYPE AREAS IS ALSO 1.0. (L.E. Mettler et al., 5)

GROUP WITH SPERM GAMETES

GROUP WITH EGG GAMETES

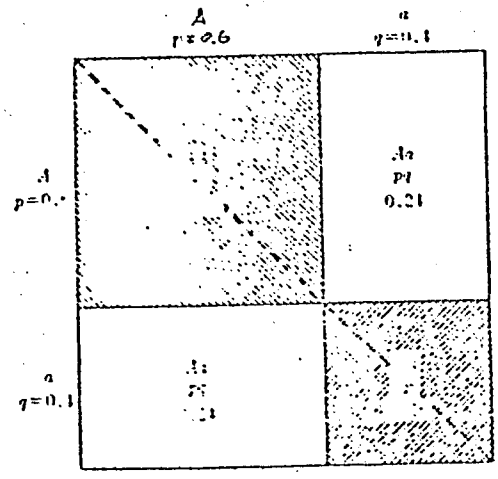


FIGURE 2.5: CORRELATION BETWEEN THE GENE FREQUENCY IN THE ENZYME POLYMORPHISM OF THE AMERICAN EEL ANGUILLA ROSTRATA AND THE GEOGRAPHICAL LATITUDES. (Williams, 13)

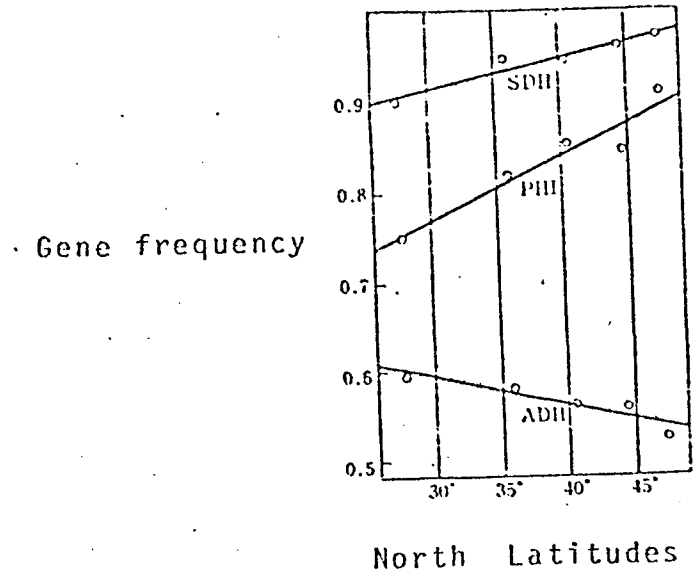


FIGURE 2.4: CORRELATION BETWEEN ADAPTIVE VALUES AND TEMPERATURES IN THE SERUM ESTERASE OF CATOSTOMUS CLARKII, FRESH WATER FISH BRED IN U.S.A.

(Koehn, 11)

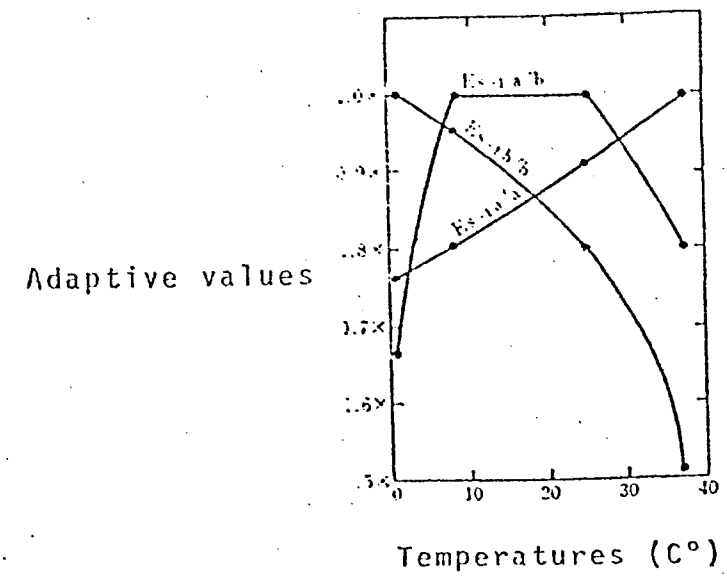


FIGURE 2.7: COMPARISON BETWEEN THE DISTRIBUTION OF SP/BW IN SHELLS WITH ONLY HOMOZYGOTES, NORMAL SHELLS AND ABNORMAL SHELLS.

X_H AND X_N SHOW THE MEAN VALUES OF SP/BW DISTRIBUTION IN THE FORMER TWO GROUPS.

(Fujino, 24)

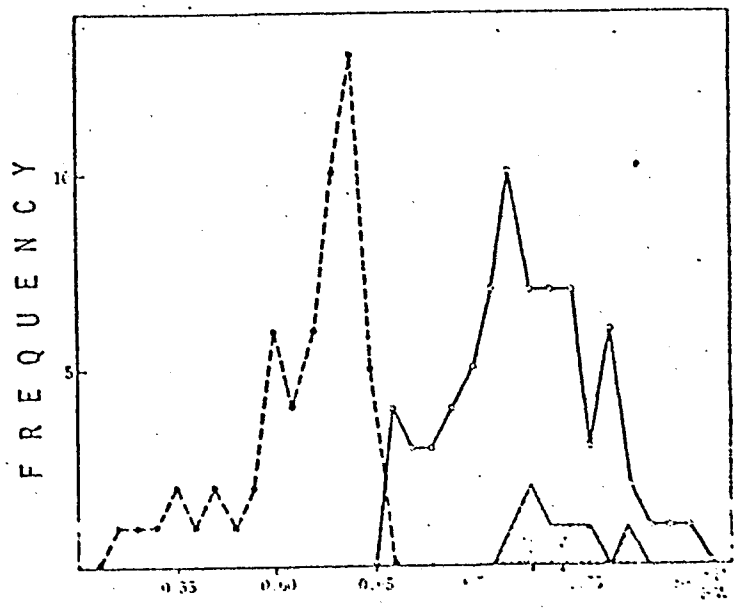
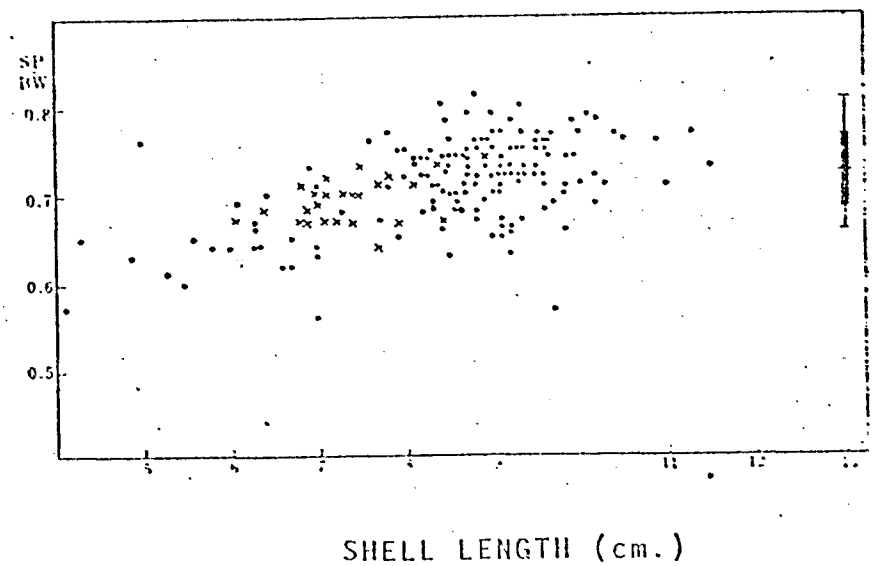


FIGURE 2.6: CORRELATION BETWEEN THE SHELL LENGTH (L) AND THE RATIO OF THE SOFT TISSUE WEIGHT TO THE BODY WEIGHT (SP/BW) IN NORMAL SHELLS OF HALIOTIS DISCUS HANNAI, INO.
BLACK DOTS SHOW THE SHELLS BRED AT REINBU ISLAND AND IN IWATE PREFECTURE.
X SHOW THE SHELLS BRED AT OKUJIRI ISLAND.
CONCERNING SHELLS WITH LENGTHS GREATER THAN 8.5 cm., THE MEAN VALUES OF SP/BW, THE STANDARD DEVIATION, AND THE LIMIT VALUE OF DISCARDS [SHELLS WITH 2.5% (LOWER LIMIT) AND WITH 5% (UPPER LIMIT) SIGNIFICANT LEVELS] ARE SHOWN.
IN ABNORMAL SHELLS, THEIR LOWER LIMIT VALUES ARE BELOW THE ABOVE LIMIT VALUE.

(Fujino, 24)



ARTICLE 3

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FISH CYTO-GENETICS

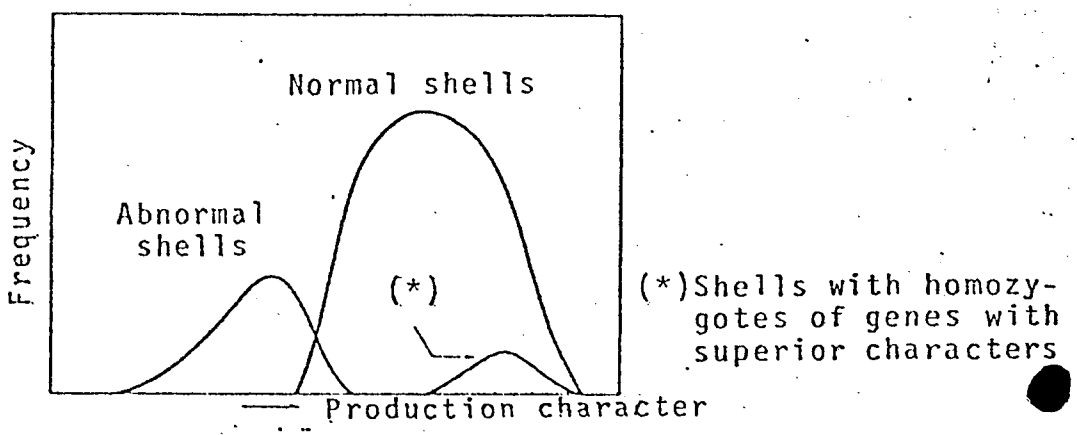
1. Past and present aspects of chromosome studies of fish

It was very difficult to find chromosomes in fish and a paraffin section method was used for all chromosome studies. In order to make preparations for fixation and staining, specific training and experience were prerequisites. In particular, it was considered that chromosomes could not be found in any tissues other than the gonads of men and other animals.

For studies of fish chromosomes, their gonads were used. However, the period during which these chromosomes can be observed is extremely brief. Thus, it was considered to be best to fix a male gonad about one month prior to spawning. Cell division scarcely occurs in the gonads before this period; and sperm fullness, but no cell divisions are found in the gonads after this period.

Also, as the spawning period differs according to the fish species, fish collection was hard work. Furthermore, as chromosome patterns on the prepared samples, made by the paraffin section method, were extremely small, it was very difficult to observe them. Most of

FIGURE 2.8: COMPARISON BETWEEN THE DISTRIBUTION OF PRODUCTION CHARACTERS IN THREE GROUPS: SHELLS WITH HOMOZYGOTES OF GENES WITH SUPERIOR CHARACTERS, NORMAL SHELLS AND ABNORMAL SHELLS.



However, with such short term cultures, chromosomes can be prepared only once, and it was difficult to obtain a sufficient number of cells from mitosis.

Concerning the chromosomal studies of mammalia, recently, various banding techniques were developed, such as G - banding and C - banding. Thus, not only identification of every chromosome, but also partial identification became possible. In order to perform banding techniques, as above, it is necessary to obtain good chromosomal preparations. Therefore, the author and others studied how to establish cultured cell lines from fish tissues and they succeeded in establishing cell lines from a mixed culture of scales and fins (Hayashi et al., 9) and from cultures of eyed embryos (Ojima et al., 10). At present, using the above techniques, the chromosomal studies of fish are advancing rapidly from various aspects.

More than 300 research papers concerning fish chromosomes have been presented throughout the world since 1960. The number of species investigated was 727. Since the number of all species of fish is about 20,000, it is considered that only about 3% have been studied. Usually, the fish family is divided into Chondrichthyes and Osteichthyes. Cyclostomata are also included in the fish family. Gosline (11) divided Teleostei of Osteichthyes into 3 large groups: the lower, intermediate and higher groups. In order to further chromosomal studies of fish, the author classified the fish family into 8 groups, by adding Cyclostomata, Salachii and also Palaeonisci, Chondrostei and Holostei, which are Osteichthyes, to the above three groups. Fish species, the chromosomes of which had already been studied, together with their classifications are shown in TABLE 3.1.

the chromosome patterns were observed as dots, or sometimes as short rods. As these patterns were not presented sufficiently well by microphotography, in most of the cases they were sketched.

In 1952, for the first time, Hsu (1) and Pomerat (2) reported their studies concerning men's chromosomes, using cells cultured in vitro. Thereafter, studies of chromosomes of mammalia developed rapidly. Studies concerning the chromosomes of fish, stimulated by the above studies, were developed from about 1963. During the early stages of these studies, the chromosomes were investigated in the following manner: colchicine was injected into the abdominal cavity and, 4-5 hours later, the kidneys and testes were mashed.

Thereafter, the dry-air method was used for studies of the chromosomes of men; this method was adopted also to study the chromosomes of the fish family. Due to the difficulty of preparations for analysis of karyotypes, using the mashing method, no papers were written concerning this method. Reports concerning Carassius and goldfish by Ojima et al. (3) in 1966, formed the first studies in the world, using the dry-air method, and also making a precise analysis of karyotypes.

Cells of fish were cultured for the first time by Wolf et al. (4) in 1960. In 1961, cultured cell lines were established by Clem et al. (5). However, these cells were not cultured for the purpose of chromosome studies. In order to study chromosomes, short-term culture methods were developed, such as blood culture (Ojima et al.,6), scale culture (Ojima et al., 7), and kidney culture (Yamamoto et al.,8).

inactive X-chromosome in the two X-chromosomes in men and mice. Chromosomes stained darkly by the C-band staining procedure are constitutive heterochromosomes. They are found universally, tend to delay replication of DNA and contain many tandem duplications of DNA. Also, it was reported that one portion of the long arm of the Y-chromosomes of men and gorillas can be stained darkly by C-band. The chromosomal number of Carassius (Funa) is basically 2n, 100. By performing C-band staining on Kinbuna (Carassius auratus subsp.), Ginbuna (C. a. langsdorfii), Nigorobuna (C. a. grandoculis) Nagabuna (C. a. buergeri), and Genguro-buna (C. a. cuvieri), Ueda et al. (27, 1978) clarified that two short arms of the second chromosome with sub-median centromeres were stained darkly in the female Kinbuna (Carassius auratus subsp.) (this will be referred to as the marker in the following); and only one short arm of the chromosome was stained darkly in the case of a male Kinbuna (FIGURE 3.1 a,b).

According to Kobayashi et al. (12,13) and Ojima et al. (14), it is known that some Ginbuna (C. a. langsdorfii) are comprised only of females and they breed by gynogenesis, a type of parthenogenesis. Also, it is known that many chromosomes of Ginbuna (C. a. langsdorfii) are triploidy and 150 ± , and the DNA content per one cell is 1.5 times that of other species of Carassius. (There are some other groups with diploidy, having both males and females; and also some other groups with tetraploidy, having only females.) Usually, there are three pairs of homologous chromosomes in a triploidy; however, in Ginbuna (C. a. langsdorfii), only two marker chromosomes are shown by C-band staining (FIGURE 3.1 c). Therefore, it is considered that the chromosomal numbers of Ginbuna (C. a. langsdorfii) are not 3n and 150 ±, but that it

Cyclostomata and Salachii have scarcely been studied so far. As the numbers of species of Chondrostei and Holostei of Osteichthyes are few, it is considered that they have been investigated sufficiently. It was obvious that, in the case of the lower group of Teleostei, most studies were concentrated on Cypriniformes and Salmo-oncorhynchus. Concerning the intermediate group, the studies were concentrated on Oryzias; and, finally, with regard to the higher group, the studies of Percopsiformes were overwhelming. That is, the fish groups which have been studied a great deal, are easily collected and conveniently cultured; also, most of them are freshwater fish; to which facts should be added that their orders include many species.

During these several years, the banding techniques of chromosomes, such as G - banding and C - banding, have been developed remarkably, mainly using mammalia. However, these techniques were scarcely applied in studies of fish chromosomes because: (1) the chromosomal size of fish is small; and (2) it was difficult to obtain a stable metaphase which could withstand banding due to inadequate techniques in the chromosomal preparations. However, as described previously, the cell lines of fish have recently been established and, therefore, the latter problem (2) has been solved.

At present, the G-band staining of fish chromosomes has been successful only to a certain extent, but, concerning the C-band staining, one after another, very interesting facts have been found. Chromosomes contain both euchromatin and heterochromatin. Heterochromatin can be divided into functional heterochromatin and constitutive heterochromatin. In a typical example of the former, there is one genetically

the origins of Wakin and Ryukin. However, since the presence of their marker chromosomes, revealed by C-band staining, agrees with the case of Kinbuna (C. a. subsp.), the origin of Goldfish might be fish of the Kinbuna (C. a. subsp.) family, the original habitat of which is in China. The author wants to visit China in order to study Funa (Carassius), bred in China.

2. Fish chromosomes and evolution

Of the 727 species of fish already reported, the chromosomal numbers have been described only for about 200 species. Concerning the remaining species (approximately 500), their karyotypes were analyzed. Previously, the author classified fish families into 8 group systems, basing these on Gosline's systems. In the following, the relationship between chromosomes and evolution is discussed, using these systems. TABLE 3.2 shows this relationship. Concerning Cyclostomata, the lowest chromosomal number is 2n and 36 in Paramyxine atami, and the highest is 2n and 168 in Petromyzon marinus. Interestingly, Petromyzoniformes, in both the Northern and Southern hemispheres, have a polyploidal relationship. Salachii have scarcely been studied. Only 11 species of about 1500 species of Salachii have been reported. The lowest chromosomal number is 2n and 28 in Narcine brasiliensis; and the highest is 2n and 98 in Raja clavata. However, results may be different, subject to more studies in the future. Concerning Chondrostei of Osteichthyes, the smallest chromosomal number is 2n and 36 in Polypterus palmas; and the greatest chromosomal number is 2n and 239 in Acipenser naccari. It is characteristic of Polypterus that the chromosomal size is large. Concerning Holostei, only two species

is correct to express their chromosomal numbers by 2n and 150 ±. In other Carassii, the marker chromosomes, specifically appearing in Kinbuna (C. a. subsp.) and in Ginbuna (C. a. langsdorfii), were not found.

Generally, fish are not differentiated sexually, and there are many fish with unclear sex chromosomes. However, in a swirl of evolution, particularly of fish of the lower group, the presence of the marker chromosomes may show some signs of sex differentiation.

Goldfish long have been known as a variety of Funa (Carassius). Their chromosomal number is 2n and 100 in all species; and their karyotype is the same as that of Gengurobuna (C. a. cuvieri) (Ojima et al., 3). When C-band staining was performed on Wakin, Ryukin, Ranchu, Kurodemekin, Chotengan and Seibungyo, it was clarified that two marker chromosomes appeared in the females and one in the males, similar to the case of Kinbuna (C. a. subsp.) (FIGURE 3.1 a,b). Kometto is a hybrid from a cross between Ryukin and Funa (Carassius). It has been reported that similar hybrids can be obtained by crossing Wakin and Ryukin; Sanshikidemekin and Funa; and Kurodemekin and Funa (Matsui, [illegible]). As proof of these facts, the marker chromosomes appeared using the C-band staining. That is, concerning the female Kometto, some have two marker chromosomes and others have one , but, with regard to the males, one marker chromosome, or none at all were shown. All the above relationships are shown in FIGURE 3.2.

It is said that the original habitat of Goldfish is in South China. Wakin were transferred to Japan for the first time in 1502, and later, Ryukin were also transferred there. At present, we do not know

It is considered that the expansion to the right side of the mode in the distribution of chromosomal numbers of the lower group might be closely related to their being polyploidal.

When comparing the arm numbers of the chromosomes in the lower, intermediate and higher groups (the arm number of chromosomes with the median and the sub-median centromeres is 2; and those with the terminal and sub-terminal centromeres is 1), the arm number in the lower group is 91.5 on the average (the average chromosomal number is 59.6); it is 51.8 in the intermediate group (the average chromosomal number is 46.2); and it is 52.8 in the higher group (the average chromosomal number is 45). It is obvious that the arm number in the lower group is remarkably higher than the same numbers in the other two groups, even in relation to its chromosomal number. Therefore, it is confirmed that there are more chromosomes with median and sub-median centromeres in the lower group than in the higher groups and that there are more chromosomes with terminal and sub-terminal centromeres in the intermediate and higher groups, than in the lower group.

3. Fish hybrids and polyploidy

3.1 Fish polyploidy and evolution

In discussing the evolution of fish, the idea that polyploidy has an important significance can be confirmed by many interesting facts, clarified from studies concerning the chromosomes of certain fish and their hybrids. The polyploidy of fish is a key to solving the riddle of the relationship between the evolution of vertebrates and gene duplication. Polyploidy in men, birds and reptiles is already

have been reported: Amia calva, the chromosomal number of which is 2n and 46; and Lepisosteus productus, of which the chromosomal number is 2n and 68. Concerning the lower group, the smallest chromosomal number is 2n and 22 in Unbra pygmaea; and the greatest is 4n and 206 in Carassius auratus langsdorfii. The mode of this group is 2n and 50 and it occupies 33% of the whole group. Concerning the intermediate group, the smallest number is 2n and 18 in Aphyosemion christyi; and the greatest is 2n and 69 in Poecilia formosa gynogenesis. The mode of this group is 2n and 48 and it occupies 38%. Concerning the higher group, the smallest chromosomal number is 2n and 16 in Sphaerichthys osphromonoides; and the greatest is 2n and 78 in Channa gachua. The mode of this group is 2n and 48 and it occupies 53%.

Looking at the distribution of chromosomes from the point of view of standard deviations, this value is 21.99 in the lower group; 8.38 in the intermediate group; and 4.54 in the higher group. It is obvious that the higher the evolutional position, the more chromosomal numbers converge. Also, which is obvious from these values, there are large differences between the lower group and the intermediate and higher groups. It is most interesting that the distribution is expanded to the right side (>50) of the mode (2n,50) in the lower group (FIGURE 3.3.) The chromosomal number of Carassius of the lower group is basically 2n and 100; however, these polyploidal fish were also confirmed with 150 ± and 200 ±. There is a theory that the chromosomal numbers of Cyprinus and Carassius were originally 2n and 50 but became later 2n and 100 due to their being polyploidal. Such polyploidy is also found in Salmo Oncorhynchus, which belongs to the same lower group as the above.

biwae) with tetraploidy have not been found in the Kanto District. Also, in the Kansai District, the habitat of Shimadojo with tetraploidy is not the same river as the habitat of the Shimadojo with diploidy.

There are many cases of hybrids with polyploidy. Hybrids between Salmo salar (salmon bred in the Atlantic Ocean) and Salmo trutta (brown trout) is a good example of the presence of polyploidy in Salmonidae. From this experiment of 1945, Svädson studied for the first time the aspect of polyploidy being related to the evolution of karyotypes in Salmonidae.

In the case of crossing the female carp (Cyprimus carpio)with the male Gengurobuna (C. a. cuvieri), when the common, edible carp is used as a maternal parent, the chromosomal number of the hybrid is the sum of one half of each of the chromosomal numbers of each of the parents, i.e. 2n and 100 (Ojima et al., 20). However, some carps with supernumerary chromosomes (one or two small fragments of chromosome, called B-chromosomes) were found recently and it was clarified that these chromosomes are frequently found also in Irogoi. Thus, when female Irogoi and male Gengurobuna are bred, hybrids with unexpected polyploidy are sometimes found (TABLE 3.4). In addition to the usual hybrids with diploidy of 2n and 100, hybrids with triploidy of 3n and 150± were obtained. Furthermore, the very interesting result that all hybrids with triploidy are females was recorded.

Ojima et al. (21) obtained a female hybrid of the first generation by crossing a female C. a. curvieri with a male Cyprinus carpio; he then obtained the first generation of the backcross by crossing the above female hybrid and the male C. a. cuvieri. As a result of studies

known. In many cases polyploidy is either lethal (men) or is an unhealthy state. However, in the case of fish, when the fertilized eggs were stimulated physically (Exp. low temperatures) in the developing stages, the cell nucleus reverses during the cell division and fusion occurs between two nuclei. Thus, there is a possibility that triploidy or tetraploidy will occur. This structure was proved experimentally by Makino et al. (16) in 1943.

In Japan, there are interesting fish which have both diploidy and tetraploidy, even in the same species. This phenomenon is observed in the Genus Cobitis, which was reported by Ueno et al. (17). In our country, we have three species of the Genus Cobitis: Cobitis biwae, C. taenia taenia and C. t. striata. As shown in TABLE 3.3, the chromosomal number is 2n and 48 and also 2n and 96 in Cobitis biwae; three systems of 2n and 50, 2n and 86 and 2n and 94 in C. taenia taenia; and 2n and 50 and 2n and 98 in C. t. striata. Subsequent to the karyotype analysis, it is appropriate to consider that, in these cases, the pairing of two homologous chromosomes occurs, rather than the pairing of four homologous chromosomes. Therefore, this is expressed as 2n, rather than 4n. It is also considered that this fact confirms the theory of Ohno (18). That is, there are reports which indicate that: the four homologous chromosomes of the tetraploidy have sufficient possibility to have a chiasma; and there is the possibility that the exchange of genes will occur with great frequency. Therefore, a new evolutional path will develop due to the exchange of genes. Tetraploidy forms two separate bivalent chromosomes, rather than a quadrivalent chromosome, comprised of four homologous chromosomes; therefore, tetraploidy is changed to diploidy. At present, Shimadojo (Cobitis

will grow or not; and whether they will be fertile or not.

For example, an exellent hybrid can be achieved by crossing a beluga (Huso Huso), one ton in weight and bred in the USSR, with a sterlet (Acipenser ruthenus). However, sometimes one may fail to create hybrids by crossings between the same species of Genus Acipenser. Generally, in many cases, hybrids show the intermediate characters of both parents. Also in many cases, hybrids express not entirely new characters, but partially a combination of the superior characters of both parents. As an example, it has been reported that there are three types of electrophoric patterns in fish hybrids: (1) the type with all components derived from the parents; (2) the type with not all components derived from the parents; and (3) the type with the components derived from others than the parents. It is considered that the subject in (3) is related to the heterosis. In fact, it is difficult to confirm heterosis in animals scientifically. (In the case o plants, it is easy to confirm the relationship between the enzyme system and the phenotype). Concerning the growth rate, it is reported that hybrids with a high growth rate have been achieved by crossing different lines of Rainbow trout in the United States. Also, it was reported that, in the USSR, the hybrids between female sterlet (Acipenpenser ruthenus) and male beluga (Huso Huso) were backcrossed with female beluga. As a result, fish were created which grew faster than beluga. By backcrossing, hybrids can be achieved with all or most of certain desirable characters, apart from their other useful characteristics. It has been reported that, in the USSR, the immunity to the red-spot disease in Funa (Carassius) is induced to Carp by backcrossing the female hybrids, obtained from Funa and Carp, with the Carp males.

of the chromosomes in the hybrids of backrossing, it was clarified that, apart from diploidy, the triploidy (3n and 156) appeared, as well as several B-chromosomes. Even more interesting, it was found that all backcross-hybrids with triploidy are females, which is the same phenomenon as in the case of hybrids with triploidy in the first generation of hybrids, as has already been described earlier.

3.2 Fish hybrids and breeding

Breeding fish on a large scale as an important industry has beed developed in many countries, including the main industrial countries, such as Japan, USA, Germany and USSR. Various studies have been carried out concerning freshwater and marine fish, not only in order to utilize fish which exist in nature, but also to create new species, adding new breeds to those already in existence. There are several methods to achieve this. The most modern way is to create hybrids. In this field of creating fish-hybrids, numerous experiments have already been done. For the first time anywhere in the world's records of hybrids, Gesner (22) created a hybrid by crossing Koi (Cyprinus carpio) and Goldfish. Since then and up todate, about 1250 scientists have created hybrids, using 56 fish families, or 1980 species The number of bibliographies concerning these studies grew to reach the number of 1800. Many of these papers deal with the breeding studies from the beginning. Most of them are meant to be only the records of how to establish hybrids. However, as in many cases, hybrids breed well, further thorough studies are required in this field of science in the future. Fish is a suitable material to create hybrids, compared to other animals. However, it is always difficult to foretell whether the creation of hybrids will be successful or not; whether the hybrids

develop and establish a pure line of this species as an experimental aquatic creature. Furthermore, it is expected that this species will play an important role in cancer research or immunological studies in the future. Also, since a cell line of triploidy obtained from Ginbuna (C. a. langsdorfii) keeps the karyotype stability for a longer period (about two years), this species is also useful material for biological studies, a time-consuming process. This aspect may be related to the following. When the culture of the diploidy cell line is continued for many generations, the chromosomal numbers gradually change and become stable at the state of nearly triploidy (lower triploidy or higher triploidy). It is considered that the cells with triploidy genomes are more stable than those with diploidy genomes. However, the reason for this has not been clarified yet.

4.2 Artificial inducement of polyploidy

Concerning the inducement of polyploidy by means of temperature-shock treatment, in 1943, Makino and Ojima (16) clarified cytologically the mechanism of the creation of fish with triploidy, formed by fusion of the sperm-nucleus and the egg with diploidy nucleus, the diploidy of which was induced by refrigeration of the fertilized Carp eggs during the second maturation-division.

Thereafter, in 1969 and 1971, Purdom (23,24) reported his study of inducing polyploidy in Pleuronectes platessa and Platichthys flesus by the low temperature stimulation. According to his studies, the best results can be achieved when the low temperature shock-treatment starts 15 minutes after the fertilization, in order to induce triploidy. In fact, in 1971, it was reported that triploidy was found in nearly

4. Chromosomal engineering

In fish-breeding, the utilization of hybrids belongs to the field of genetic engineering, the meaning of which is a skillful, artificial treatment of fish chromosomes.

4.1 Gynogenesis

Gynogenesis was discovered by researchers studying the frog development about 60 years ago. Recently, Ginbuna (C. a. langsdorfii) widely common in Japanese rivers and lakes, became well known for the gynogenesis. Gynogenesis is an effective means to establish the inbred lines of fish. Many chromosomes of Ginbuna with gynogenesis are triploidal and ±150; the eggs spawned by females are activated by semination and stimulation of the sperm of other species. Unually the diploidal eggs discharge the first and the second polar bodies during the maturation-division, and the chromosomal numbers decrease by one half of the original numbers. However, in this case, after the sperm-semination, the nuclear division, similar to a mitosis, occurs in the eggs and a polar body with ±150 chromosomes is discharged. The head of the invading sperm does not swell and does not form a male prenucleus, but stays as a compact head until the division of cleavage, and it never fuses with the female prenucleus (FIGURE 3.4 a,b).

Eggs of Ginbuna develop by physical or chemical stimulation other than sperm until the stage of discharging polar bodies; but they never develop to the stage of cleavage. Thus, it is considered that the semination of other species is necessary for the division of cleavage. Since all the eggs hatched become females, the entire population comprise f i s h with homogenes. At present, studies are conducted to

lized eggs were brought back to the culture temperature of 32°C and their culture was continued. Valenti also used the ratio of the size of erythrocyte nuclei. As the result, the polyploidy induced in the fingerlings was 0% in the control group, 29% when the shock-treatment was done at 4°C, 75% at 11°C and 10% at 38°C. Although the poly-numbers of polyploidy were not described clearly, since it was observed that there are two kinds of polyploidies (with the ratio of the volume of erythrocyte nuclei being 1.5 - 1.6 times of the normal diploidy; also being about 2.1 times of the normal), there are high possibilities that these polyploidies will be triploidy or tetraploidy.

Concerning the types of polyploidy appearing, the problems remain primarily in relation to the chromosomal numbers, not counted directly, and, secondarily, in relation to the states of eggs after the temperature shock-treatment. In other words, there have been no cytological basic studies, such as studies of the discharge of polar bodies or the period of cleavage division.

Ojima et al.(1978, 26) obtained triploidy by exposing the fertilized eggs of Carps to the low temperature of 0°C for 10 minutes, ten minutes after fertilization (FIGURE 3.5). Ten minutes after fertilization, the second maturation-division is in the developing stage, from the metaphase to the anaphase (FIGURE 3.6 a,b,c,d). When the eggs were exposed at 0°C during this period, the division of the second polar body was stopped due to the low temperature. Then, reversely, one diploidy nucleus is formed by fusing two daughter-chromosomes. By fusing this diploidy and the sperm nucleus, which has already invaded the egg, the triploidy is formed.

100% of fingerlings which were hatched after treating them in the sea-water at about 0°C for three-and-one-half hours, starting 15 minutes after the fertilization. However, it failed to induce the tetraploidy by the low temperature shock-tratment during the division of the first cleavage. These polyploidies were confirmed not by the chromosomes, but by the phenotypes of specific characters. On the assumption that the ratio between the genomes of the eggs with polyploidy, induced by the low temperature shock-treatment, and the normal sperm is 2 : 1, their polyploidies were estimated from the morphological characteristics (such as pigments and vertebral numbers) of Pleuronectes platessa and Platichthys flesus.

That is, if the fingerlings obtained from the eggs of Pleuronectes platessa, fertilized by the sperm of Platichthys flesus and treated by the low temperature shock-treatment, show the intermediate characteristics between the characteristics of the hybrids, resulting from the eggs of Pleuronectes platessa, which were not treated by the low temperature shock-treatment, and the sperm of Platichthys flesus, and the characteristics of Pleuronectes platessa, it is considered that these fish might have triploidy. Furthermore, since the size of nucleus in polyploidy will be larger due to the poly-numbers of polyploidy induced, the polyploidy was judged by comparing the size of the erythrocyte nucleus (generally, the ratio of the nucleus volume between the diploidy and triploidy is 2 : 3). In 1975, Valenti (25) reported the result of the studies of the polyploidy induced by the temperature shock-treatment to the eggs of Tilapea aurea. According to him, after this treatment, at not only low temperatures but also at higher temperatures (4°C, 11°C, 38°C) for 15 minutes and also for 60 minutes, starting after 14 minutes after fertilization, the ferti-

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Also, theoretically, when the fertilized eggs with diploidy were treated by the cold-shock during the division of cleavage, tetraploidies were induced. FIGURE 3.7 shows this mechanism diagram - matically.

It is expected that the breeding techniques of fish by means of chromosomal engineering will be developed further in the future. Anyway, it is considered that the accumulation of cytogenetically based studies of fish will be the key affecting either the rise or fall of the breeding industry of fish in the future.

TABLE 3.1: FISH SPECIES, CHROMOSOMES OF WHICH HAVE BEEN STUDIED

Class	Order	Numbers Reported	Number of species
Cyclostomata	Petromyzoniformes	8	31
	Myxiniformes	3	32
Salachii	Lamna	2	199
	Rajiformes	6	315
	Chimaera	2	25
Dipneusti	Lepidosirenina	1	5
Chondrostei	Polypteriformes	5	11
	Acipenseriformes	8	25
Holostei	Lepisosteiformes	1	7
	Amiiformes	1	1
Lower Group	Kotsuin (Japanese)	9	15
	Clupeiformes	5	295
	Salmo Oncorhynchus	69	508
	Cypriniformes	196	3000
	Anguilliformes	10	603
Intermediate group	Myctophiformes	13	390
	Gadiformes	6	684
	Oryzias	172	827
	Yojiuo (Japanese)	6	200
	Beryciformes	2	143
Higher group	Perciformes	144	6880
	Pleuronectiformes	16	520
	Scorpaeniformes	19	1000
	Gobiesociformes	3	144
	Tetraodontiformes	14	320
	Gasterosteiformes	3	
	Synbranchiformes	1	13
	Lophiiformes	2	215

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TABLE 3.3: CONSTITUTION OF DIPLOIDY AND TETRAPLOIDY IN COBITIS

Species	Chromosomal number	Constitution of chromosomes	
		Median Submedian	Subterminal Terminal
Cobitis biwae	2n=48	21 pairs	3 pairs
	2n=96	43 "	5 "
C. taenia taenia	2n=55	8 "	17 "
	2n=86	31 "	12 "
	2n=94	29 "	18 "
C. t. striata	2n=50	8 "	17 "
	2n=98	20 "	29 "

TABLE 3.2: RELATIONSHIP BETWEEN CHROMOSOMAL NUMBERS AND FISH GROUPS

Group		Cyclostomata, Salachii, Others	Lower Group	Intermediate Group	Higher group
Chromosomes					
Number of species		37	289	199	202
Chromosomal numbers	Lowest number	28	22	18	16
	Highest number	239	206	69	78
	First mode	36 (19%)	50 (33%)	48 (38%)	48 (53%
	Second mode	164 (14%)	48 (9%)	46 (9%)	44 (16%
Standard deviation		51.53	21.99	8.34	4.54

FIGURE 3.1: CHROMOSOMAL PATTERNS BY C-BAND STAINING

a: Kinbuna (Carassius auratus subsp.) ♀ (2n=100)

b: Kinbuna (Carassius auratus subsp.) ♂ (2n=100)

c: Ginbuna (C.a. langsdorfii)

→ shows marker chromosomes, the short arms of which were stained darkly by the C-band staining method

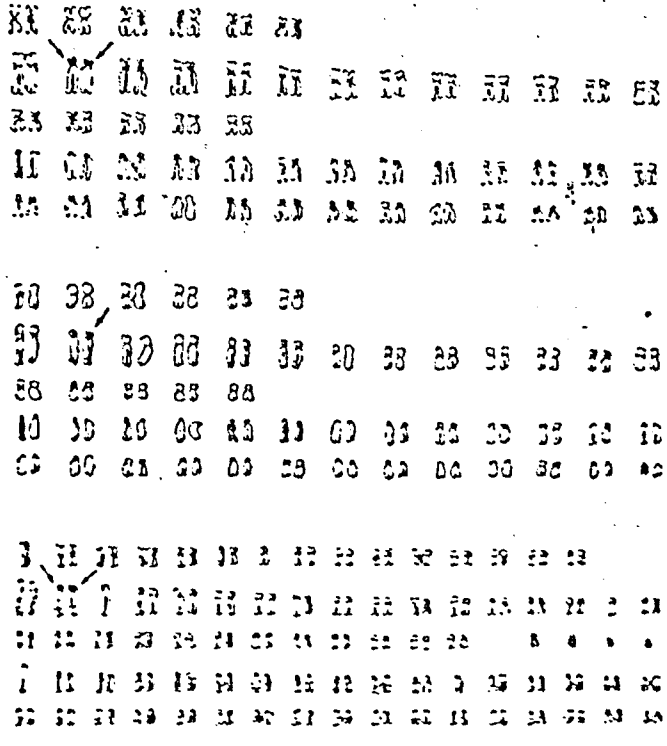


TABLE 3.4: CHROMOSOMES OF TRIPLOIDY (ALL ♀) AND DIPLOIDY WHICH APPEARED IN THE FIRST HYBRID GENERATION OBTAINED BY BREEDING FEMALE CARPS AND MALE FUNA (CARASSIUS)

	94	95	96	97	98	99	100	101	102	103	104	149	150	151	152	153	154	155	156	157	total
1	1		1		2	1	10	23	3	1											42
2				1			6														7
3			1		1	1	2	15	3												23
4			1			1	1	1	5												9
5						1	1	2	20		1										25
6				1			3	12													16
7							4	16	1												21
8		1					4	2													7
9				1			2	12													15
10					2		2	14	1												19
11♀														2	2		6		1		11
12♀												4	1	1	1	7	29	4		1	48
13♀													1	2	2	2	11	2	4		24
14♀													1	2	1	3	12	2	3	1	25
15♀												1				4	1	4	10	1	21
16♀														1	1	1	7	4	3		17
17♀															3		13	1	2	1	20
	94	95	96	97	98	99	100	101	102	103	104	149	150	151	152	153	154	155	156	157	total

FIGURE 3.3: DISTRIBUTION OF CHROMOSOMAL NUMBERS IN THE THREE GROUPS OF TELEOSTEI

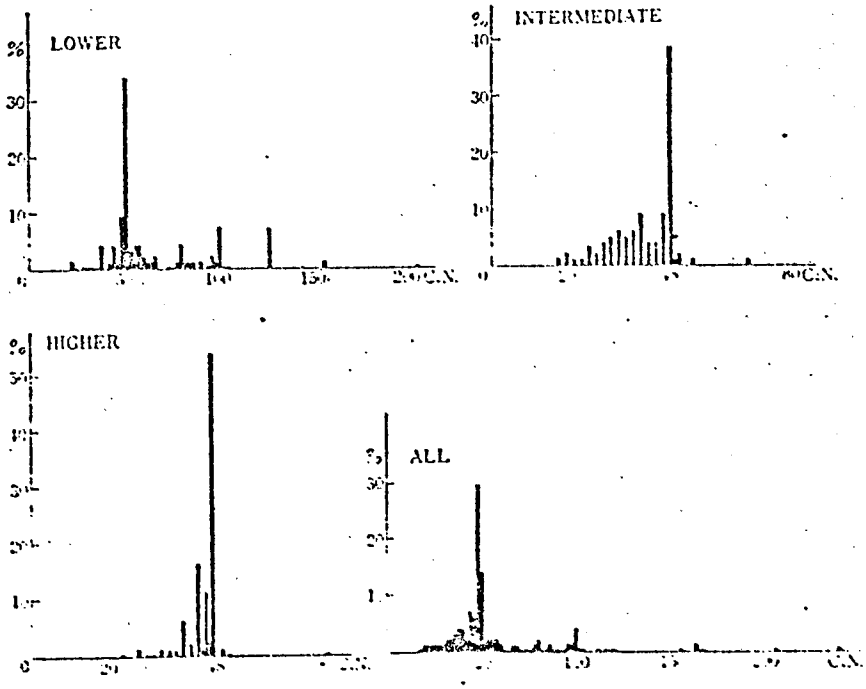


FIGURE 3.2: THREE CASES OF MARKER CHROMOSOMES APPEARING IN KOMETTO

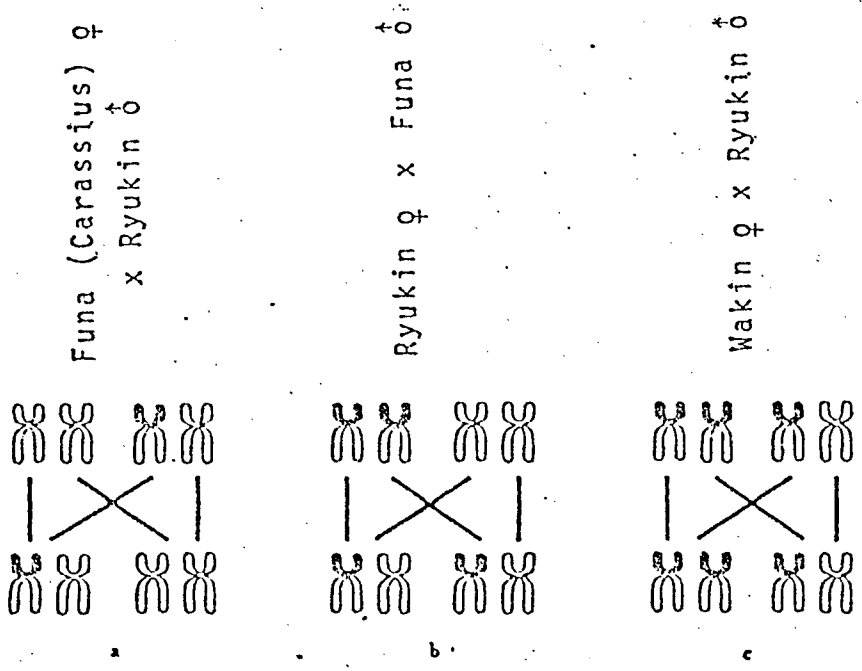


FIGURE 3.5: CARP'S CHROMOSOMES OF TRIPLOIDY FORMED BY THE LOW TEMPERATURE TREATMENT

FIGURE 3.4: CYTOLOGICAL CONFIRMATION OF GYNOGENESIS

a: Insemination by sperm of other species of a Ginbuna (C. a. langsdorfii) egg and the discharge of a polar body

b: the sperm nucleus neither swells nor relates to the fertilization.
It stays without changing during the diyision of cleavage.

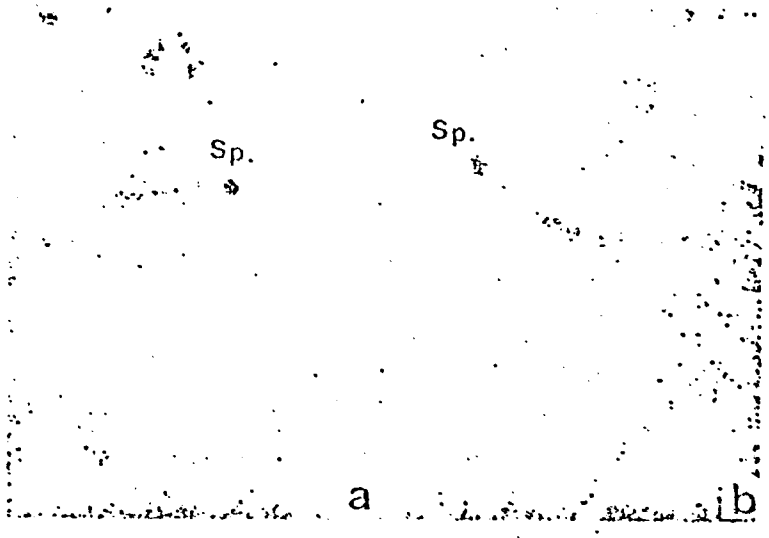


FIGURE 3.7: NORMAL FERTILIZATION OF FISH EGGS AND THE FORMATION OF POLYPLOIDY BY THE LOW TEMPERATURE TREATMENT

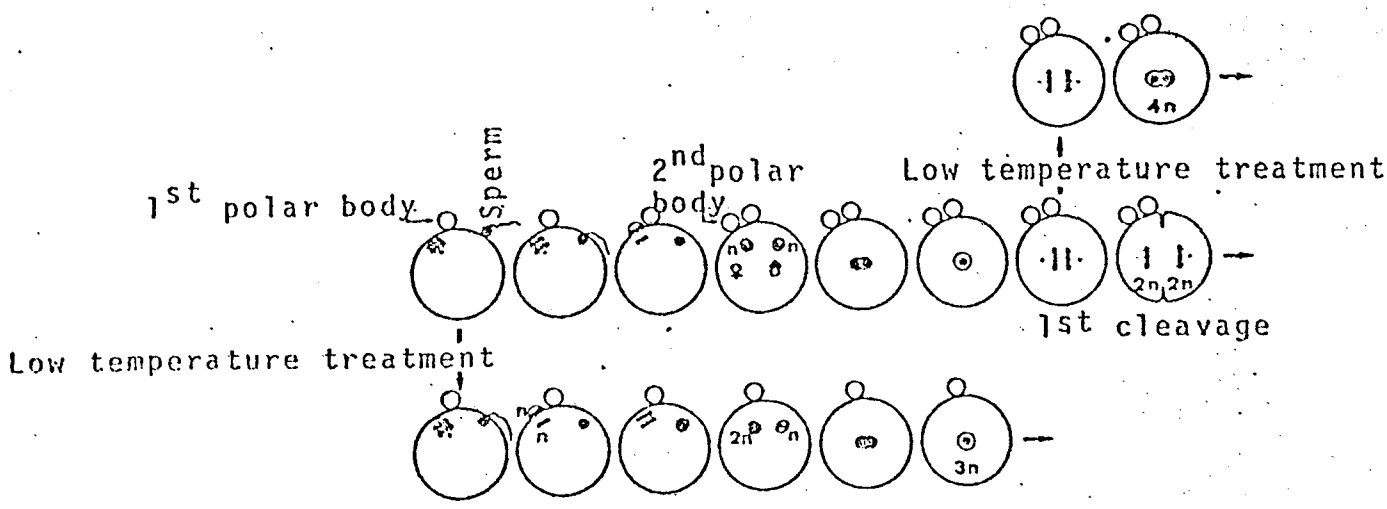


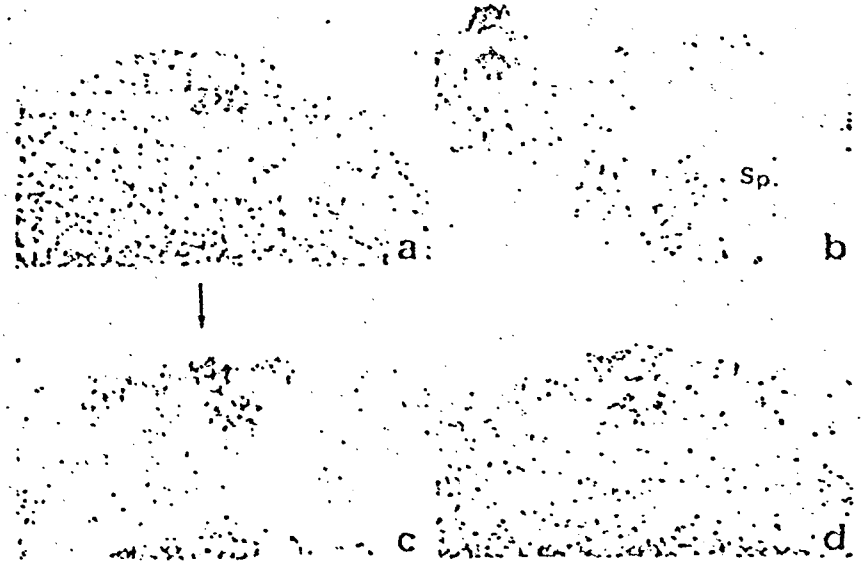
FIGURE 3.6: FORMATION OF POLYPLOIDY NUCLEUS INDUCED TO CARP EGGS BY THE LOW TEMPERATURE TREATMENT

a: Metaphase during the second maturation-division, right after spawning

b: Insemination by sperm and discharge of a polar body (from metaphase to anaphase)

c: Reversal of a polar body, which was discharged, by the low temperature treatment

d: A diploidy nucleus is formed by fusing the polar body reversed, and the egg nucleus



Aquatic environmentalists, investigating contaminant effects on reproduction by non-genetic means (as in physiological and culture experiments), conclude that reproduction seems to be one of the most sensitive measures of chronic sublethal effects clearly meaningful in nature (Sprague [9]; Rosenthal and Alderdice [10]). Fishery biologists concede that generally little is known about even the natural factors in the environment that control mortality of fish eggs and larvae. They emphasize the importance of research on early life stages (NOAA Technical Report [11]). In view of the genetic sensitivity of gametogenesis and cleavage and the importance of regular division of normal chromosomes to continued normal development, it might be expected that cytology and cytogenetics could contribute to understanding the successes and failures in development of fish eggs, both in nature and in laboratory experimentation. By so doing, these subdisciplines of genetics would ultimately help elucidate the result of superimposing detrimental effects of marine contaminants on natural population fluctuations.

This paper describes the adaptation of practical cytological and cytogenetic methods* to the study of embryos of fish eggs collected at sea with other zooplankton. Initial data on Atlantic mackerel, Scomber scombrus, embryos from eggs collected in surface waters of the heavily polluted New York Bight are presented and discussed, along with some results of a second Bight cruise. New methodologies are introduced employing the yolk-sac

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* For a review of the status of the chromosome cytology of the Osteichthyes see Roberts [12] and for USSR work on the embryo cytology of laboratory-spawned fish see Migalovskaya [13].

ARTICLE 4

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CYTOLOGICAL AND CYTOGENETIC STUDY OF FISH EGGS DEVELOPING IN OCEAN SURFACE WATERS AND IN MIGRATING FISH

The life cycles of important commercial species of pelagic fish begin in the surface waters of the ocean with the spawning of yet unfertilized eggs arrested at metaphase or telophase II of meiosis. Gametogenesis is a genetically vulnerable process and this genetic vulnerability is increased further once the externally fertilized fish eggs enter early cleavage (Solberg,1; Muller,2;and Murakami,3). Sensitivity of the eggs of fish to induced mutation appears second only to that of the most sensitive mammalian egg (Donaldson and Foster,4; Polikarpov,5 ; Purdom and Woodhead,6). Heavy metals and chlorinated petroleum hydrocarbons are concentrated in the oceanic microlayer (Duce et al. 7 ; and McIntyre 8). The ocean surface is also directly exposed to atmospheric pollutants. During their incubation, future recruits to the commercial fisheries float passively as buoyant eggs in surface waters, if not in the microlayer itself. The position of fish eggs in the water column maximizes their risk of serious exposure of sensitive early development stages to both cell toxins and weak mutagens (as heavy metals), commonly found in polluted seawater.

* Mackerel studies and methods of development have been supported by the Marine Ecosystems Analysis Program and the Northeast Fisheries Center of the National Oceanic and Atmospheric Administration, US Department of Commerce.

is accomplished with ordinary sewing needles while eggs are viewed under a low-power dissecting microscope. For dissection the egg is pushed into a groove etched onto the edge of a glass microscope slide glued onto a Petri dish used to hold eggs intended for dissection. About 60 embryos can be removed per hour from the approximately 1-mm diameter Atlantic mackerel eggs.

Prior to staining for squashing, embryos are treated post-fixation in 45-60% acetic acid for about 15 minutes. A single intact embryo is squashed under a coverslip on a microscope slide in a few drops of a solution of 19 parts of standard aceto-orcein to which 1 part of proprionic acid has been added. Nearly perfect monolayers of cells are achieved which allow visualization of every mitoses in the embryo (Fig. 2). Usually, four embryos can be squashed onto one slide without monolayer pieces of different embryos becoming mixed. Development stages from early cleavage to the late tail-free embryo stage have been successfully prepared in this manner. Uniform monolayers of the entire embryo are more difficult to achieve at the tail-bud and later stages though such embryos are still useful.

The yolk-sac membrane, which develops from blastoderm cells at gastrulation (see, for example, McEwen [14]), was found to be an excellent source of large mitosing cells from gastrulation through at least the tail-free embryo stage (Figs. 3-5). Large thin ectodermal- and endodermal-derived cells of the sac membrane readily spread out on the slide. Prophase nuclei measure 15 to almost 20 microns across, and metaphase groups 10 to 15 microns across. Telophases measure 20 to 30 microns from spindle pole to spindle pole. Prometaphase chromosomes are often so well spread about the cell as to make almost unnecessary any pretreatment with a c-mitotic agent for the purpose of

membrane of the fish egg, and which display the chromosome configuration of prespawned eggs. The potentials of these methodologies are indicated.

Methods, adaptation and new developments

Fish eggs are collected at sea with other plankton in neuston nets (0.947 mm mesh) towed at the water surface and/or bongo nets (0.333 and 0.505 mm mesh) towed at specified depths. To avoid possible damage to the eggs, tow time is limited to a maximum of 20 minutes and tow speed to 1.5 knots. Collections should be made in a manner similar to what would be followed were eggs to be used for culture. Eggs should remain well aerated as they are towed through the water, and use of a flow meter can record flow of water through nets during each tow. After collection, the unsorted plankton is immediately fixed in a 1:10 dilution of neutralized formalin. The usual paucity of grossly deteriorated eggs is attributed to their falling out of the water column in agreement with the observation of fish culturists that "dying" eggs drop to the bottom of culture containers. Ripe eggs are stripped from fish caught on their spawning grounds. These are also immediately fixed in the dilute neutral formalin.

In the laboratory, characteristic fish eggs are picked out of the plankton for species identification according to standardized procedures for plankton laboratories employing gross egg and larval characteristics, knowledge of fish frequenting the sampling area, and time of year. Eggs of desired species and development stage, from cleavage to late embryo, are then sorted out of the plankton samples. Embryos are dissected away from the egg membranes and stored in vials according to development stage and species (Fig. 1). Dissection

Initial general results of Westward '74* cruise collection of Atlantic mackerel, Scomber scombrus, eggs from the New York Bight

With the first collection at sea of fish eggs (which proved to be almost entirely Scomber scombrus) sampled for cytological study, an effort was made to check all early developmental stages of the mackerel embryo proper for their relative desirability from the cytological-cytogenetic methodologic and informational standpoints. Not all developmental stages of a single species are represented in equal numbers at all sample stations, and at some stations certain stages can be missing. An effort was made to collect data at the gastrula-early embryo stages at most stations where Scomber eggs at these developmental stages were present. At the time this work was done the method for prespawned eggs was not yet developed, and the value of the yolk-sac membrane as an excellent source of mitotic configurations still not recognized.

Even though seemingly intact and normal in the undissected egg, the greatest portion of all cleavage (Stage I) embryos was in various stages of cytological deterioration or cytogenetic disarray irrespective of sample. Still there was station variation. From 0 to 44.4% of these embryos were judged viable and capable of further normal division (see below). Chromosome divisions were easier to visualize in morula (Stage II) embryos and in blastula (Stage III) embryos. Both cytological deterioration and cytogenetic disarray occurred less often at Stages II and III than at Stage I. At gastrulation (Stage IV) there was a large drop in the incidence of disorderly mitoses, the most abnormal embryos presumably not gastrulating.

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* Sailing vessel of the Sailing Education Association, Woods Hole, Massachusetts 02543.

karyotyping. (Karyotyping of fish eggs offers fascinating possibilities in stock identification, as well as in pollution studies.) No mitoses have been observed in the yolk-sac membrane cells of the few hatched yolk-sac larvae thus far examined.

To prepare the sac membrane for cytogenetic study, the entire sac with enclosed yolk is removed from the chorion and the embryo dissected away. The sac with yolk is then flattened onto the slide in the orcein stain, as is the embryo. Storage of large numbers of pre-dissected sacs together presents some problems, and storage in fluids other than formalin is being explored.

Prespawned, unfertilized eggs are torn with a dissecting needle and yolk contents pressed out. The entire chorion is flattened onto a microscope slide in the acid-stain solution. The meiotic metaphase II and earlier chromosome configuration of the fully ripe or ripening eggs is associated with the micropylar opening through which the sperm enters the egg (Fig. 6). First, the microscope slide is scanned for the micropyle, and the area about it then carefully examined at different levels of focus for the relatively small meiotic configuration (Fig. 7). Although these configurations are small, at least in the mackerel egg, they offer the distinct and convenient possibility of detecting in gravid female fish chromosome translocations and chromosome breaks from diakinesis of meiosis through to perhaps telophase II*.

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* Cytological-cytogenetic methodology for the fish eggs is to be written for publication - A. Crosby Longwell, J. Kubinski, and D. Perry.

Later-stage embryos, though retaining the gross appearance of, for example, a tail-bud embryo, might be comprised in large part of cells more reminiscent of a gastrula embryo. This latter condition is attributed to the de-differentiation of cells in seriously stressed embryos. Blastula and morula embryos sometimes contained a single large cleavage cell which had failed to divide further.

In another plankton collection of mackerel eggs from a later (Annandale '77) cruise into the New York Bight (see Discussion) 17,065 mitoses were examined in 304 Stage VI (tail-bud) yolk sacs from 12 sample stations. Not unexpectedly, the state of yolk-sac cells and mitoses corresponded well to the state of the embryo cells and mitoses. Sacs from well-developing mackerel embryos averaged 50-70 mitoses. Mean station percent abnormal mitoses was 6.1-12.0 for all but one station, which had 20.7% of its yolk-sac mitoses abnormal.

Station and area variation in initial study of cytological-cytogenetic development of eggs of the North American Atlantic mackerel in surface waters of the New York Bight

Microscopic data were obtained on 50 of 51 stations on a total of more than 4,030 embryos sampled in surface waters of the New York Bight, May 7-18, 1974. Cytological and cytogenetic measurements were summarized in mortality-moribundity estimates for the different development stages from cleavage (I) through the tail-free embryo stage (VII) at 17 to 35 different stations. Criteria for cleavage (Stage I) viability were that the embryo not be in a state of cytological deterioration; that there be at least one mitosis; and that one-fifth of the mitoses be normal. Criteria for morula and gastrula

Telophase irregularities appear to be the best numerical indicator of cytotoxicity, mutagenicity, and mitotic index in the embryo itself. They are readily observed (Figs. 8 and 9). Particularly at the morula and blastula stages (II and III) there are high incidences (often >50%) of chromosome bridging, indicative of chromosome stickiness due to general cytotoxicity and/or chromosome breakage and translocation. Laggard chromosomes and disoriented chromosomes outside the mitotic spindle are not uncommon at telophase. Incidence of abnormal telophases drops at gastrulation (Stage IV). There appear also to be fewer abnormal telophases at the tail-bud and tail-free embryo stages (VI-VII) than at gastrulation and just after at Stage V (early embryo). The mean of 35 stations and 845 embryos was 12.3% for Stages IV and V combined; for Stages VI and VII the mean of 23 stations and 681 embryos was 5.9%.

Very striking were the wide differences in number of mitosing cells per embryo among stations for all stage embryos available. Complete cessation of division was not uncommon in some embryos. Mean number of telophases per embryo varied from a station low of 0.3 to a high of 93.7 for Stages IV-V for 35 stations and 845 embryos. For Stages VI-VII variation was from 0 to 110 for 23 stations and 681 embryos.

Also striking was the de-differentiated nature of cells in later-stage embryos at certain stations or the faulty premature differentiation of cells in early-stage embryos. (See Fig. 10.) For example, a Stage II (morula) embryo might be characterized by spindle-shaped or otherwise contorted nuclei normally typical of a Stage V (early embryo) with advancing cell differentiation.

between mortality-moribundity for the several development stages and telophase numbers were significant overall. The associations between Stage I mortality-moribundity and other stage estimates were weaker than associations between other stages. (Although technically the most sensitive, Stage I (cleavage) is the most difficult methodologically and also must be most subject to maternal influence - natural and in regard to body contaminant load.)

Samples from the several stations varied widely in egg viability (0 to 100%) as calculated here and also in division rate as indicated above. More stations were represented in Stages IV-V (gastrula-early embryo) mortality-moribundity data than in the case of other stage data. Mortality-moribundity data for Stages IV-V were available on $\frac{35}{49}$ (71.4%) of the stations. For those stations which did not have data for Stages IV-V mortality-moribundity, information from other development stages was used to estimate the quantity*.

In general, variances from the regression equations were larger than those for which direct estimates could be made. To improve overall accuracy Stein estimates were consequently used. This technique is described by Efron and Morris [17], [18], and Dixon [19]. Figure 11 shows graphically the Stein estimates computed for Stages IV-V mortality-moribundity for the several sample stations. Estimates for the several stations over the New York Bight vary widely as seen in Fig. 11. Stations with least calculated mortality-moribundity tend to be either about the periphery of the large

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* This was done by using the regression equation which included the data known for the station as the independent variables. Variance estimates of the estimated percent dead or moribund were obtained using the binomial variance if data were available, and the estimate of variance from the regression if this was used as the estimate (Snedecor and Cochran [16]).

(Stages II and III) viability were that the embryo not be in a state of cytological deterioration; that mitoses over the embryo be orderly; that 50% or more of the telophases be normal; and that there be at least 15 telophases in an embryo. Criteria for gastrula-early embryo (Stages IV-V) and for tail-bud and tail-free embryos (Stages VI-VII) were that the embryo not be in a state of cytological deterioration; that its chromosomes not be in a general state of physiological stickiness over the entire embryo; that it have at least one telophase. When the later stages studied, VI and VII, were all dead or moribund there was an obvious trend for all earlier stages to be dead or moribund. High viability of early stages went along with total viability of Stages VI and VII. Grossly deteriorating fish eggs are not expected to remain in the water column, and the mortality-moribundity estimates presented here are based on the earliest indications of impending embryo death. They are an estimate of differential station characteristics in respect to mackerel embryos. They are not a measurement of total or absolute egg mortality at the stations although they infer that such differs by station or station group. See Table 1 for basic statistics on the data summaries.

Using station-combined data, correlation coefficients were computed for the mortality-moribundity estimates for Stages I, II, III, IV-V, and VI-VII, and for the mean number of telophases per embryo and percent embryos with <15 telophases at Stages IV-V and Stages VI-VII (Table 2)*. The associations

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* All statistical analyses were performed by Dr. T. Holford and M. Johnson, Department of Biometrics, Yale University School of Epidemiology and Public Health, New Haven, Connecticut 06510.

Hudson River Canyon (Area VII) failed to differ significantly from the group of stations in the northeast Bight periphery (Area V) with lowest ranked mortality. Areas I, II, and III, in closest proximities to major dumpsites, as sewage sludge and acid waste, and the Hudson River runoff, did not differ from the station group along New Jersey (Area VI). This and the close match of mortality in Area VIII to the south and east of the dumpsites with that of the dumpsite Areas II and III are in agreement with the general southwesterly circulation of water in the New York Bight (Water Resources Engineers, Inc. [21]).

Discussion

All mackerel spawning in the Bight, which must number in hundreds of thousands, come from the same southern contingent of North American Atlantic mackerel which feed in the relatively clean Gulf of Maine during summer months and live the rest of the year in offshore cleaner waters (Sette [15]). The usual habitat and summer feeding grounds of these mackerel and also their pattern of migration into and through the Bight (Sette [15]) make it likely that stations sampled are uniform in regard to the contaminant body burdens of fish spawning at them. This is probably especially so relative to what must be the sharply differing water qualities at the Bight stations where the mackerel eggs studied here were spawned.

Since completing this first study of Atlantic mackerel eggs collected on the Westward '74 cruise into the New York Bight, a second cruise (Annandale '77) was especially planned and conducted in the Bight for the purpose of taking concomitant samples of mackerel eggs, chemistry samples, and physical oceanographic measurements. The southern contingent of North American Scomber scombrus largely confine their spawning to the New York

area studied or along Long Island, that is, generally most distant from coastal zones and dumpsites. Because of the close correlation of the several measures made on the eggs from the Bight, and the use of other stage data to compute estimates of mortality-moribundity for development Stages IV-V, these estimates on the gastrula-early embryo stages are regarded as reflecting relative overall viability for all the early development stages.

Sample stations were grouped in relation to their geography, proximity to major dumping areas, and general water circulation patterns (Water Resources Engineers, Inc. [21]). A few stations which did not fit into any particular grouping were not included, such as a station at an inlet along Long Island. Differences between the various groups were evaluated using nonparametric tests based on ordering of the Stein estimates of egg mortality-moribundity for Stages IV-V. The Kruskal-Wallis test (see Conover [20]) was used to test whether there were overall differences in mortality-moribundity among the areas. The overall test of differences among the areas was statistically significant ($T = 20.73$, $P<0.05$). The average rank for each area is shown in Table 3. With the exception of the Long Island coastal area along which a current of clean water flows (Water Resource Engineers, Inc. [21]), the four of the eight station groups with least mortality were most distant from coastal zones and from major dumpsites.

All pair-wise tests were performed to investigate which areas seem to contribute to this overall difference. Results are given in Table 4. Only the Long Island coastal group of stations (Area IV) and the group about the

from about Cape Hatteras. Laboratory studies on other fish have demonstrated temperature-salinity optima yielding the highest percentage of viable hatch (for example, Westernhagen et al. [23]; Alderdice and Forrester [24]). However, in nature there must be a physiological adaptation to waters through which fish swim. Grouping of mackerel cytological-cytogenetic or gross abnormality data according to temperature, salinity, or combinations of the two does not appear to offer any ready or complete explanation of the wide variation in cytologically calculated mackerel egg viability or wide variation in any of the several measured factors considered separately. Most likely, temperature and salinity act in nature in synergism and/or antagonism with the contaminants enhancing or ameliorating contaminant effects on the eggs and even the additive and synergistic effects of contaminants with one another. Westernhagen et al. [23] describe the combined effects of cadmium and salinity on development and survival of garpike (Belone belone L.) eggs in the laboratory. The effects of temperature and salinity at the mitotic level are of interest and understanding them may be of use in itself. In field studies of pollution, however, the complicating effects of these natural variables might be desirably removed by considering them in planning sampling strategy.

It is unlikely that either temperature or salinity would act as a mutagen at the narrow temperature extremes occurring in nature. Concentration then on more specific mutagenic events in the chromosomes of the yolk sac, as opposed to the less specific cytology of the embryo proper, could eliminate some problems in interpreting field data. Joined into a membrane three or so layers thick at the most, sac cells, as they encircle the yolk, should be in

Bight (Sette[15]), making impossible the simple comparison of eggs in the generally polluted Bight to some comparatively less-polluted area outside of it. Microlayer and subsurface water and plankton were sampled for heavy metal analyses (analytical chemistry by Dr. G. M. Meaburn, National Marine Fisheries Service Laboratory, Charleston, South Carolina) and for analyses of select paraffinic, chlorinated, and aromatic hydrocarbons (analytical chemistry by Dr. W. MacLeod, National Marine Fisheries Service Labpratory, Seattle, Washington). A total of 3,643 dissected embryos were in the cytological-cytogenetic study and 4,315 embryos in a study of gross developmental abnormalities. As in the '74 collection, mackerel eggs showed wide station variability, with the several cytological and gross developmental factors measured over several early embryo development stages showing good correspondence. Three of approximately fifteen different sets of chemistry samples with much higher contaminant levels than the others corresponded to the three biological samples in which mackerel eggs showed nearly total mortality-moribundity as calculated cytologically and cytogenetically. Two independent statistical studies are currently underway evaluating the correspondence between biological and chemical data sets, and also taking into consideration station temperature and salinity*.

Atlantic mackerel tolerate a rather wide range of temperature and salinity, spawning at a temperature range of about 10-15°C and a salinity of about 26.0-35.6 o/oo (Johnson[22]). North American Atlantic mackerel spawn along the coast and along Long Island as they migrate northward

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* These data, along with the full results of the cytological-cytogenetic study of the Westward '74 cruise, are to be written for publication - A. Crosby Longwell, J. Hughes, and D. Perry.

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intimate proximity to whatever contaminants the egg inbibes and the yolk retains.

While the methods described here were developed because of a need for field data, particularly in the New York Bight, all methodology is directly and even more easily applicable to eggs collected in laboratory experiments with fish and their gametes. A full understanding of the factors governing reproductive success in important commercial species seems possible only through a combination of field data on the commercial species, and laboratory studies on often at least related species. Not only have fish been largely bypassed by modern cytogenetics, the vertebrate egg has until recently been little studied cytologically and cytogenetically due to unavailability of mammalian eggs in large numbers. The fish egg, so well suited to cytogenetic study of its yolk-sac membrane and cytological study of its embryo, offers simultaneously new possibilities for experimental assay and field monitoring of the impact of marine contaminants, and for increasing basic knowledge of the earliest vertebrate reproductive phase. Since the objects of such studies can be important resource species, the chromosomally sensitive early stages of which are incubated in polluted surface waters of the sea and also readily sampled in plankton, the matter is of timely importance and practical use.

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Table 2. Correlation Coefficients of Cytological-Cytogenetic Measures on Atlantic Mackerel Embryos in the New York

	Stage II Morula	Stage III Blastula	Stages IV-V Gastrula-Early Embryo			Stages VI-VII Tail-bud - Tail-free Embryo		
	% Mortality-Moribundity	% Mortality-Moribundity	log % Mortality-Moribundity	No. Telophases	%<15 Telophases	log % Mortality-Moribundity	No. Telophases	%<15 Telophases
Stage I - Cleavage can adv. next stage	-.471	-.587*	-.666**	.599**	-.549*	-.702**	.683**	-.635*
Stage II - Morula % mortality-moribundity		.896***	.877***	-.832***	.836***	.718*	-.478	.494
Stage III - Blastula % mortality-moribundity			.792***	-.904***	.838***	.829***	-.683**	.789***
Stages IV-V - Gastrula-Early Embryo								
log % mortality-moribundity						.772**	-.476	.757**
No. telophases						-.664**	.609**	-.727**
%<15 telophases						.647*	-.354	.719**

*P<0.05; **P<0.01; ***P<0.001.

Table 1. Basic Statistics for Cytological-Cytogenetic Measures on Atlantic Mackerel Embryos in the New York Bight*

Variable**	No. of Stations	Mean	Standard Deviation	Minimum	Maximum
Stage I - Cleavage					
% which could advance to next stage	31	8.9	11.0	0.0	44.4
Stage II - Morula					
% dead or moribund	17	56.8	36.5	0.0	100.0
Stage III - Blastula					
% dead or moribund	24	62.1	34.2	0.0	100.0
Stages IV-V - Gastrula-Early Embryo					
% dead or moribund	35	38.5	32.8	0.0	100.0
mean # telophases	35	34.2	28.3	0.3	93.7
% with<15 telophases	35	49.1	32.9	0.0	100.0
Stages VI-VII - Tail-bud - Tail-free embryo					
% dead or moribund	23	32.8	37.7	0.0	100.0
mean # telophases	23	35.5	37.2	0.0	110.0
% with<15 telophases	23	50.6	39.1	0.0	100.0

*Westward cruise, May 7-18, 1974.

**Sample size - total all stations: Stage I - 1589; Stage II - 374; Stage III - 541; Stages IV-V - 845; Stages VI-VII - 681

Table 4. Pair-wise Comparisons of Stages IV-V Gastrula-Early Embryo Mortality-Moribundity. Kryskal-Wallis Test (T)

Geographic Area*	I	II	III	IV	V	VI	VII
II	.12						
III	.56	.24					
IV	3.76	2.94	4.81**				
V	3.86**	4.50**	5.00**	.80			
VI	.01	.04	1.91	7.18**	5.73**		
VII	3.09	2.88	2.78	.36	2.47	8.47**	
VIII	1.05	.57	.53	4.81**	5.73**	5.59**	3.63

*See Fig. 11 for area subdivision of the studied portions of the New York Bight.

** $P<0.05$.

Table 3. Kruskal-Wallis Test for Differences in Predicted Estimates of Stages IV-V Gastrula-Early Embryo Mortality-Moribundity*

Average Ranked Mortality**							
Geographic Area***							
I	II	III	IV	V	VI	VII	VIII
31.0	30.0	26.4	10.9	5.2	33.4	14.7	23.4

*Overall Test for Differences: T = 20.73 indicates a significant difference between areas. P<.05.

**Large ranks denote higher mortality.

***See Fig. 11 for area subdivision of the studied portions of the New York Bight.

TABLE 6.9: MORTALITY OF THE THIRD GENERATION OF THE PURE BRED LINES OF C. GIGAS BRED IN VARIOUS REGIONS AND THE FIRST GENERATION OF THEIR HYBRIDS

(Imai and Sakai, 1)

Culture Ground	Pure bred line G_1			Hybrid F_1			
	Hokkaido	Miyagi	Hiroshima	Hokkaido x Hiroshima	Miyagi x Hiroshima	Hiroshima x Miyagi	Hiroshima x Hokkaido
Uzu Bay (48. 5-49. 2)	-	19.6	16.9	8.5	6.1	23.6	-
Megawa Bay (48. 4-48. 12)	13.9	20.0	36.7	17.8	16.3	50.3	6.6
Mangokuura Bay (48. 5-48. 12)	12.5	22.4	40.7	10.0	15.3	24.1	21.8
Matoya Bay (48. 4-48. 12)	85.2	88.2	50.0	51.8	46.6	-	46.7

	Pure bred line G_3		Hybrid F_1	
	Hokkaido	Kumamoto	Hokkaido x Kumamoto	Kumamoto x Hokkaido
Oominato Bay	23.7	24.6	19.1	24.5
Megawa Bay	54.7	38.0	42.0	26.0
Mangokuura Bay	48.8	40.8	-	31.8
Hamashima	100.0	65.2	95.4	94.3
Kagami-cho	98.0	71.0	95.3	98.0
Gig Harbor	38.4	44.8	17.2	38.0

TABLE 6.8: GLYCOGEN CONTENTS IN THE FIRST GENERATION OF PURE BRED LINE OF HOKKAIDO BREEDS AND KUMAMOTO BREEDS OF C. GIGAS AND IN THE FIRST GENERATION OF THEIR HYBRIDS (Imai and Sakai, 1)

Culture ground	Pure bred line G_1		Hybrid F_1	
	Hokkaido	Kumamoto	Hokkaido x Kumamoto	Kumamoto x Hokkaido
Oominato Bay	16.3%	6.3%	10.7%	13.2%
Megawa Bay	13.6	8.6	12.5	10.9
Mangokuura Bay	3.2	2.5	-	3.4

TABLE 6.7: SHELL LENGTH OF C. GIGAS BRED IN 4 REGIONS

(...) shows the whole weight, g.
(Imai and Sakai, 1)

	Hokkaido	Miyagi	Hiroshima	Kumamoto
P	210mm 285	128mm 89	68 mm 66	— mm
G_1 (27 months)	121.6±25.6	119.4±25.4	91.6±14.3	—
G_2 (29 months)	133.0±21.2 (271.9±86.0)	118.1±17.3 (179.9±53.9)	109.9±12.0 (105.3±35.0)	—
G_3 (9 months)	89.1±11.1 (93.8±27.8)	80.9±10.9 (63.6±26.2)	58.9±8.4 (32.2±7.7)	—
G_1 (21 months)*	131.8±18.0 (163.6±29.3)	—	89.0± 9.6 (65.9±10.6)	81.4±9.5 (46.6±9.6)

* Cultured at Dominato; others were cultured in Megawa Bay.

P:Parent generation (wild type):

G_1- first generation pure line
G_2- second generation ditto
G_3- third generation ditto

TABLE 6.6: COMPARISON OF CHARACTERS OF C. GIGAS BRED IN 4 DIFFERENT REGIONS (Imai and Sakai,1)

Character	Species			
	Hokkaido	Miyagi	Hiroshima	Kumamoto
Growth	fastest	fast	slow	slowest
Size	largest	large	small	smallest
Shell depth	shallow	intermediate between Hokkaido-Hiroshima	deep	deep
Ratio between flesh and whole weights	smallest	small	largest	large
Degree of smoothness of shell	flat	somewhat wavy	considerably wavy	considerably wavy
Shell color	grayish-white	intermediate between Hokkaido-Hiroshima	blackish-purple	blackish-purple or brown
Mortality	high in the southern area	high in the southern area	high in the northern area	low in all places
Spawning period	early	later than in Hokkaido	later than in Miyagi	earliest (mature eggs are found during the winter)

TABLE 6.5: PHENOTYPE FREQUENCIES AND GENE FREQUENCIES FOUND IN LEUCYL-β-NAPHTHYLAMIDASE OF MYTILUS EDULIS

		Sample group 1	Sample group 2	Total
phenotype (genotype)	frequencies			
	1—1	0 (0.2)	3 (0.4)	3 (0.5)
	1—2	5 (4.2)	2 (4.6)	7 (8.9)
	1—3	3 (3.4)	3 (5.6)	6 (8.8)
	1—4	0 (0.1)	0 (0)	0 (0.1)
	2—2	25(21.5)	24(14.3)	49(35.4)
	2—3	27(34.8)	18(35.0)	45(70.4)
	2—4	1 (1.0)	0 (0)	1 (0.9)
	3—3	18(14.0)	31(21.2)	49(35.0)
	3—4	1(14.8)	0 (0)	1 (0.9)
	4—4	0 (0)	0 (0)	0 (0.0)
Gene	frequencies			
	1	.050	.068	.059
	2	.519	.420	.469
	3	.419	.512	.466
	4	.012	0	.006
	D	−0.37	−0.491	−0.333

Sample Group 1 was collected from the water 25 cm. from the lowest water-line;
Sample Group 2 was collected from the water 1 m. from the lowest water-line;
(...) show the expected values.

TABLE 6.4: GENETIC POLYMORPHISMS OF ENZYMES AND EXCESS OF HOMOZYGOTES IN 2 SPECIES OF CRASSOSTREA, MYTILUS AND ABALONE.

Enzymes	Numbers of allelomorphs	Existence of homozygotes	D	Bibliographies
Crassostrea virginica				
AAT-1	4	+	−	21)
Est-3	5	+	−0.33	21)
LAP-2	5	+	−0.18	21)
PGM-1	6	+	−0.39	21)
GPI	4	+	−0.12	21)
Ostrea lurida				
AAT	3	+	−	41)
GPI	2	+	−	42)
LAP	2	+	−	42)
MP	2	+	−	41)
PGM	2	+	−	42)
Pinctada fucata (Gould)				
LAP	6	+	−0.28	43)
Haliotis discus hannai				
Est-F	3	+	−0.42	44)
Est-S	3	+	−	44)
Strombus luhuanus				
GPI	2+(6)	+	−	45)
SOD	3	+	−	45)

Enzymes: PGM: Phosphoglucomutase

TABLE 6.3: GENETIC POLYMORPHISMS AND EXCESS OF HOMOZYGOTES IN ENZYMES OF C. GIGAS.

Enzymes	Numbers of gene loci	Numbers of allelomorphs	Existence of excess of homozygotes	D	Bibliographies
ATT	2	0	-	-	31)
		3	-	-	31)
ACP	?	?	-	-	37)
AK	1	5	+	-0.26	31)
Cat	1	2	-	-	37)
Est-2	1	5	-	-	31)
Est-F	1	4	-	-	38)
	1	4	-	-	38)
LAP	1	5	+	-0.29	31)
	?	3	-	-	39)
	?	?	-	-	37)
MDH	2	0	-	-	31)
		6	-	-	
	4	?	-	-	37)
MP	2	0	-	-	31)
		2	-	-	
	?	2	-	-	33)
PGM	1	6	-	-	31)
	1	4	-	-	40)
GPI	1	4	+	-0.15	31)
SDH	1	3	+	-0.37	31)
SOD	2	0	-	-	31)
	2	0	-	-	39)
		2	-	-	

Enzymes: ACP : Acid phosphatase
AK : Adenylate kinase
Cat : Catalase
Est : Esterase
MP : Muscle protein
PGM : Phosphoglucomutase
SDH : Sorbitol dehydrogenase

Refer to TABLE 6.2 for other enzymes.

TABLE 6.2: GENETIC VARIATIONS AND EXCESS OF HOMOZYGOTES IN THE ENZYMES OF THREE SPECIES OF MYTILUS.

(..) shows the numbers of allelomorphs, gene frequencies of which are below 0.01.

Enzymes	Gene loci numbers	Numbers of allelomorphs	Existence of excess of homozygotes	D	Bibliographies
Mytilus edulis					
AAT	1	3+(2)	+	−0.28	34)
	1	2	−	−	29), 30)
AP	1	4	−	−	29), 30)
	1	3+(4)	+	−0.05～−0.08	23)
GPI	?	3+(4 6)	−	−	23)
ICD	1	4	−	−	29), 30)
	1	2	−	−	23)
LAP	?	3	+	?	35)
	1	3+(2)	+	−0.13～−0.43	20)
	1	3+(1)	+	−0.33	29), 30)
	1	3+(2)	+	−0.10～−0.20	23)
MDH	2	0	−	−	29), 30)
	?	(2)	−	−	23)
ME	2	2	−	−	29), 30)
		2	−	−	29), 30)
6-PGD	2	2	−	−	29), 30)
	?	2+(3)	−	−	23)
M. californianus					
GPI	?	4+(1)	+	−0.30～ 0.39	19)
LAP	?	6	+	−0.51～ 0.03*	19)
MDH	?	2+(3)	−	−	19)
M. demissus					
SOD	?	3	+	−0.092～0.197**	36)

Enzyme Names:
- AAT : Asparate aminotransferase (Glutamate oxaloacetate transamidase)
- AP : Amino peptidase
- GPI : Glucose phosphate isomerase
- ICD : Isocitrate dehydrogenase
- LAP : Leucyl β-napthylamidase; Leucyl β-napthylamine hydrolase (Leucine aminopeptidase)
- MDH : Malate dehydrogonase, NAD oxidoreductase
- ME : Malic enzyme (Malate dehydrogenase, NADP oxidoreductase)
- 6-PGD : 6-phosphoglucose dehydrogenase
- SOD : Superoxide dismutase (Tetrazolium oxidase) (Tetrazolium reductase)

* Excess of homozygotes found only in young shell groups, but not in mature shell groups.

** Excess of homozygotes only in the smallest group in the upper portion of the tidal interval zones.

TABLE 6.1: COMPARISON OF THE RATES OF GENETIC VARIATIONS BETWEEN MARINE INVERTEBRATES AND A FEW OTHER CREATURES

	Numbers and rates of polymorphic gene loci		Average rate of homozygotes	Bibliographies
Mytilus edulis	7/14	0.50	0.198	29),30)
Crassostrea gigas	11/15	0.73	0.210	31)
Crassostrea virginica	8/17	0.47	$0.095+\alpha$	21)
Homarus americanus	5/44	0.11	0.638	32)
Limulus polyphemus	6/25	0.25	0.661	33)
Drosophila melanogaster	8/19	0.42	0.119	33)
Mus musculus musculus	12/41	0.29	0.091	33)
Human	20/71	0.28	0.067	33)

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seeds, procured otherwise, will be widely adopted, involving various creatures, in order to supply shortages of seeds; in order to induce species of seeds more suitable to the ecological characteristics of the fishing grounds; or in order to improve the genetics of wild populations. Actually, the implantation of oysters and scallops is now being carried out commercially on a large scale. The implantation of C. gigas on the west coast of the USA is a commercially successful venture. However, concerning the implantation of C. gigas in France, there is a high probability to cause the genetic destruction of Portuguese oysters, which produce the F_2 hybrids by crossing easily. Historically famous European oyster grounds have declined one by one, since 1940. However, the strength of C. gigas is due not only to its genetic characters, but also it is due to the seed production techniques used, like the strengthening treatment of seeds. Each species has its own characteristics. It is considered that the invertebrates, which generally are found widely, comprise the characteristic local populations, as is the case of the local populations of C. gigas. All the factors listed above are valuable resources in the genetic breeding studies. When the outbreeding or the artificially procured seeds are used in wild populations, the conservation of wild populations in the artificial or wild conditions has to be conducted at the same time.

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rates and to combine the superior characters by outbreeding, the knowledge of the relationship between the increase of heterozygotes and the growth, the knowledge of the characters governing growth (as shown in AAT in oysters), and the knowledge of the heritability and the genetic gain in each character,are rapidly being accumulated. Also, it is possible to produce seeds with better characters than the wild seeds, depending on the advances in genetic breeding studies and the subsequent development of the artificial seed procurement. In order to adopt the artificial seed procurement, [illegible] of genetic breeding, the program and analysis are prerequisites. It is considered that the widely used artificial seed collection will accompany the development of genetic breeding studies and will be of practical use in the future.

On the other hand, it is considered that in the future, some genetic-breeding studies and the resulting measures will be necessary for the creatures, for which the artificial seed collections have not bee adopted. The reason being the increase in human activities, like settlements of various types of construction in the sea, including equipment for catches, the increased production and culture; also the factor of water pollution, affecting not only the size and the structure of wild populations, but also the genetic constituents and the phenotypes of these populations. Accomplishments in the control and measures of genetic breeding studies of wild populations are necessary in order to advance in the field of having sufficiencies of creatures of economic value.

Implantation of the artificially procured seeds and the wild

methods concerning the imagines. Cultural methods of rearing imagines (pond- and hanging-cultures) have been developed for many species and it is considered that these methods will be used widely in future. On the other hand, however, it is also considered that a method of catching marine invertebrates grown in the wild will be adopted in the future with or without regard to stocking the artificially procured seeds or seeds obtained otherwise.

The quantities of the seeds, procured mainly artificially, must be increased to match the quantities of wild seeds, the supply of which is not constant due to the variable environmental conditions. The higher the increase of the seeds artificially procured, the more involved are their genetic characters and characteristics, the more life activities and the better characters economically can be expected from the seeds; also, m o r e suitable genetic characters for each fishing ground will be required, as well as better methods of culture and of increasing the production.

The increase of homozygotes, caused by the inbreeding, being harmful to marine invertebrates, was confirmed by the cytogenetic studies, the cross test and the analysis of polymorphisms in the wild populations.

An artificial seed procurement on a large scale is an experiment for genetic breeding studies also on a large scale. Differently from the seeds produced in the wild by large parent populations, it is possible to produce genetically very inferior seeds by artificial crossings. As the result of the possibility to increase the survival

their phenotypes were divided into factors by analysis of the variances and were quantified. Heritability is the ratio between the variance due to genetic factors and the entire variance. When it is close to 1, the possibility of a genetic character is large. It is expected that characters with heritability larger than 0.2 can be improved by selective breeding. The effects expected from the selection are shown in the column of expected selective effects (TABLES 6.10 and 6.11). These values show the quantities gained by one generation in the 20% selection of the shells.

Viewing the results obtained, some of them show heritability larger than 1, but it is considered that there may be errors in some figures. However, in general, many characters show higher heritability than expected. If the heritability of each character can be estimated, the genetic characters and the characters affected by the environment, that is, the characters which can be affected only by the improvements of the fishing grounds and cultivation methods, can be distinguished. In this respect, it is expected that the evaluation of heritability will promote not only the breeding studies, but will also provide important guidance for breeding and cultivation studies. Furthermore, it is desirable to organise experimentally on a larger scale these studies, adopting the various theories and methods of analysis.

4. Genetic breeding studies in the future

In order to increase the production, cultivation and eventual use of marine invertebrates, the following are considered to be most important: availability of seeds and the cultivation and control

A remarkable inbreeding effect on Crassostrea virginica was reported (27). In the full-sib cross, 63% of their eggs were not fertilized and only 3% of the fertilized eggs developed normally. While, in the outbreeding, only 13% of the eggs were not fertilized and 70% of the fertilized eggs developed normally. Also, in the full-sib cross lines, many cases of parthenogenesis were found. The occurrence was 10%, but only 0.5% in the control. In another case, it was reported that it was 29%, but only 7% in the control. The survival rate during the swimming period of larvae in the full-sib cross is one sixth of that in the outbreeding lines. This difference increases in the later developing stages of the larvae.

3.3 Genetic and environmental distributions

The environmental conditions of the fishing grounds vary, depending on the years and the sites. Therefore, it cannot easily be clarified how much each character, in particular a quantitative character, is controlled by the genetic factor; how great are the genetic differences between the samples; and also how much are their characters affected by the environment. Recently, a theory of selective breeding, using population genetics, has been developed, with excellent results in animal husbandry. This theory analyzes the genetic and environmental distribution of all characters and provides important guidance for selective breeding.

On the basis of this theory, Lannan (24) and Wada (25) studied C. gigas and Pictada (TABLES 6.10 and 6.11). The procedures were similar for both species (28): after several groups were crossed and their offspring were cultivated in the same conditions, the distributions of

3.2 Inbreeding and outbreeding

Various characteristics of local populations of C. gigas are observed clearly in the 1 - 3 generations of pure-bred lines in the different fishing grounds, and it is obvious that these characteristics are caused by genetic factors. All characters of the first generation (F_1), obtained by crossing, show the intermediate characteristics of both parents (FIGURES 6.4 and 6.5; TABLES 6.8 and 6.9). Similarly, the taste was improved by crossing with oysters bred in Hiroshima. In the first generation (F_1) of the hybrids, the mortality is low when compared with that of the pure-bred lines, except in the case when the Hiroshima breed was used as a maternal parent. Also heterosis was found in this case (TABLE 6.9).

In the second generation (F_2) obtained by crossing F_1, without isolation of any characters, like morphs, a distribution similar to F_1 was demonstrated. This shows that, similar to various quantitative characters known generally, the morphs and glycogen contents are not controlled by only one gene, but are controlled by polygenes.

Concerning many animals, it has been reported that the inbreeding depression, gradually decreasing the life activities, in general, is caused by repetitive inbreeding. Using C. gigas, a full sib-cross was made for three generations, but no obvious lowering of the life activity was found. However, the fourth generation could not be obtained in any of the lines and, when a crossing of different populations of the third generation was made, a tendency to recover the health character was observed.

Both the whole body weights and the soft body-portion weights are heavier in the northern breeds with statistical significance, as demonstrated in all test fishing grounds, from the Tohoku region to the Ariake Sea. The fattening index* is larger in the southern breeds which have deep shells. However, it is smaller in the Kumamoto breeds, which have gonads even during the winter, than that of the Hiroshima breeds. Glycogen contents differ in the test regions. The Hiroshima breeds showed the highest content of all other regions, followed by the Miyagi, Hokkaido and Kumamoto breeds. Concerning taste, the Hiroshima breeds are declared overwhelmingly to be the best, as demonstrated by the panel test; while the Miyagi and Hokkaido breeds are said to be the worst. The Kumamoto breed, with its low glycogen content, occupies second place. However, it is considered that no quantities of glycogen, related to the developmental stages of the storage tissues, affect the taste directly, but that other factors which affect the taste comprise a wide range in the southern breeds. [* According to the maturation (spring-summer), the egg spawning (summer) and the development of storage tissues, the characteristics and the weights of the soft body portion of oysters vary greatly. As an index to show the soft body portion, generally the fattening index (conditioning index) is used, as shown in the following equation:

$$\frac{\text{dry weight of soft body portion}}{\text{volume inside of shell}} \times 100.$$

When measuring the growth, the weight with seasonal variations is not used, but the shell size, like the shell length, is used.]

of eggs and active sperm even in the cold of Megawa Bay during the winter. On the other hand, C. gigas, bred in Hokkaido and Miyagi, mature during the period from early to mid summer. The follicles of mature gonads are full of evenly homologous eggs and sperm in large quantities. This is obviously different from C. gigas bred in southern regions, the reproductive cells of which are very few in some developing stages and are contained in the follicles, spaced in a certain manner.

Since 1961, large quantities of oysters have perished in Matsushima Bay. It was considered that this was caused by a physiological abnormality related to the maturation and the egg spawning in overnourished conditions. However, the mortality of C. gigas bred in Hiroshima was found to be 14%, using the "hanging" test in Matsushima Bay at the same period. This was obviously small, when compared to the 40% mortality of C. gigas, bred in Miyagi. It is thought that this is closely related to the fact that most of the body of C. gigas bred in Miyagi was occupied by mature eggs in large quantities all at once, but C. gigas bred in Hiroshima were not occupied by the mature gonads all at once (25).

The spawning period is the earliest in C. gigas bred in Kumamoto, when cultured in Megawa Bay, followed by those in Hokkaido, Miyagi and Hiroshima (TABLE 6.6). It is considered that this order is not in agreement with the geographical distribution and relates to the tendency of multi-maturation and to the temperatures needed for maturation.

Quantitative characters and economically important characters of marine invertebrates have been studied only by Imai and Sakai (1); also by Lannan (24) concerning C. gigas and Wada (25) concerning Pinctada.

3.1. Local and regional genetic differentiation of population groups

Imai and Sakai (1) clarified the genetic differentiation of local populations of C. gigas by artificial fertilization and artificial collection of seeds. When C. gigas, bred in Hokkaido, Miyagi, Hiroshima and Kumamoto, and 1 - 3 generations of pure-bred shells were cultivated in the same conditions, the shell morphs, the flesh weight, the ratio of the flesh weight to the whole body weight, and the growth showed differences of a certain pattern, coinciding with the geographical distribution of the shells. Also, as the result of the "hanging" test in several fishing grounds, oysters bred in the northem regions, like Hokkaido and Miyagi, adapted well to the fishing grounds, located in cold water at high latitudes, but showed a high mortality rate in the fishing grounds located at lower latitudes. Oysters bred in Hiroshima showed a slightly higher mortality rate in the fishing grounds at higher latitudes. Oysters bred in Kumamoto were healthy, even in the cold water regions.

The quantities of eggs spawned and the fattening index, relating to the production of seed and flesh oysters, showed differences in local populations. C. gigas, bred in southern regions always have young productive cells on their follicle epithelium; they form gonads throughout the year; and tend to spawn eggs many times in one year. In a typical case, C. gigas, bred in Kumamoto, can produce a small quantity

it has also been reported that no AAT - 1 types relate to the heterozygosity of gene loci of the other 4 enzymes.

Results concerning shells have been described so far; reports about shrimps are increasing. Concerning American lobsters (Homarus americanus) bred in eight regions, Tracey (32) analyzed 28-41 gene loci for each sample group and reported that the overdominance of homozygotes was found more in the sample groups obtained offshore, and, especially, in one sample group obtained in Maine, which showed an excess on a significant level. Concerning this fact, although it is considered that the lobster populations are divided by catches, there was no proof of this.

3. Genetics of quantitative characters and breeding

Characters are usually classified into either quantitative or polymorphic characters. Like the variations of enzyme molecules, the variations of several morphs are called polymorphism **; while in the case of variations between individuals, when they are constant and the characters can be measured only by quantity or by numbers, they are called quantitative characters. As quantitative characters, there are varieties covering from morphological characters (like body length, weight, skin color and the patches of domestic animals), the rates of growth and numbers of eggs spawned, to the productive and biochemical characters. Many economically and commercially important characters are quantitative characters. [** Polymorphism. Originally, polymorphism was used to express phenotypes or morphs. Since the population studies using enzyme variations or blood types have advanced, polymorphism is no defined with regard to gene frequencies.]

pulations*. In this case, the rate of genes which will be homozygous and the rate of harmful genes in the homozygotes for every generation must differ greatly, depending on the individuals. As shown on the left side of FIGURE 6.3, in some cases, depending on the individuals, all gene loci are occupied by homozygotes; but in other cases, no homozygotes are present. It is considered that the homo- or hetero-zygosity of individuals, expressed by the sum of the polymorphic enzymes, show the homo- or hetero-zygosities also in many gene groups (genetic background). When the gene groups of a certain individual are more homozygotic, its growth is poor and, generally, it perishes. It is considered that, in this case, the selection does not directly relate to the polymorphism of enzymes, but to genomes. [* Previously, the deviation due to the equilibrium in the numbers of heterozygotes was shown as $D = (Ho - He)/He$. When the inbreeding factor is F, the observed number of heterozygotes is expected to be $2pq(1-F) = 2pq-2Fpq$. Therefore: $D = [2pq(1-F) - 2pq]/2pq = -F$. D is equal to the inbreeding factors when its symbol is changed. D values in TABLES 6.2, 6.3 and 6.4 show $-0.05 \sim -0.55$; however, the inbreeding factors of half-cousin crosses, cousin or sib crosses and the parent-offspring crosses are 0.031, 0.063 and 0.25, respectively.]

On the other hand, there are some cases, when the polymorphism of a single enzyme, like AAT-1, relates to the growth (FIGURE 6.3, right side). Singh and Zouros (21) reported that it is difficult to consider that the AAT - 1^2 ($p = 0.619$), allelomorph with the highest frequency in the population, has a linkage to a harmful gene with complete dominance, on the basis of data from other cases. They also reported that individuals with homozygous AAT - 1^4 grow well. Then,

are (20,21); also that, with lean shells, homozygotes appear with greater frequency than that of heterozygotes (22).

The following are considered to be the causes of the excess of homozygotes: 1) errors due to collection methods; 2) the existence of Null or silent alleles; 3) there are differences between the adapted values of genotypes, therefore, gaps are produced in the genetic equilibrium by selection (23); 4) a population comrises several sub-populations, therefore, there is a Wahlund effect, caused by the mixture of larvae released from parent groups with different gene frequencies (19); 5) inbreeding or selective crossing in the same lines (21).

Recently, Singh and Zouros (21) reported interesting studies of these problems. Concerning C. virginica, collected at a place on Prince Edward Island and cultured for one year; five polymorphic enzymes were analyzed and the results are shown in FIGURES 6.2 and 6.3. As one can see in TABLE 6.4, C. virginica shows a significant excess of homozygotes in 4 of 5 gene loci; excluded is one gene locus, related to AAT - 1. Therefore, the presence of many homozygotes, shown in TABLES 6.2,6.3 and 6.4, cannot be explained by collection errors. Also, it is not correct to assume that the Null or silent alleles appear in various enzymes of only shells or marine invertebrates.

In the case of periphyton, like oysters, even if the Wahlund effect was caused by the settlement of larvae groups as a mass, produced by a cross of the shells settling next to each other or near by, or was caused by the mixture of more than two groups of larvae, it is thought that there are considerable numbers of cases of inbreeding in the po-

which were procured from the coastal wall of Yamashita Park in Yokohama. On the assumption that Hardy Weinberg's Law can be applied to this population, the expected values of all phenotypes were calculated from the gene frequencies obtained, shown in parentheses in TABLE 6.5. When comparing the observed figures with the expected values for each phenotype, the observed figures were larger for homozygotes, as in the cases of 2 - 2 and 3 - 3; but the figures were smaller than the expected values for heterozygotes, as in the cases of 2-3, 1-2 and 1-3. The differences between both figures are significant, when they are checked by the chi-squared test. Therefore, when these diffirences are shown as $D = \Sigma[(Ho - He)/He]$, the values obtained are shown in the bottom line of TABLE 6.5. Ho and He are the observed and expected values of heterozygotes, respectively. When the observed values agree with the expected values, $D = 0$; but in the case of an overdominance of homozygotes, that is, when heterozygotes are fewer than the expected values, D will be negative.

TABLES 6.2, 6.3 and 6.4 show the existence of an overdominance of homozygotes on a significant level and also the values of D. In general, phenotypes, coinciding with the expected values, are found many biological populations, like fish. TABLES 6.2, 6.3 and 6.4 show that there is an excess of homozygotes, but that there are shortages of heterozygotes in the various gene loci of shell populations.

Concerning the excess of homozygotes, it has been known that: 1) an excess of homozygotes is found more often in young shells than in matured shells (19); 2) when shells are studied in the same place, it is found that the better their growth, the more heterozygous they

to unimorphism. The remaining 7 gene loci are unimorphisms. The average rate of heterozygotes is 0.198. This figure is quite high, when compared to other species, similar to the case of C. gigas. It is considered that such high variabilities are related to the differences in environmental conditions and geographical locations, which change radically, for instance, the intertidal zones and other coastal areas. However, since starfish and snails live in deep seas, i.e. in a stable environment, also have a similar variable feature to those in TABLE 6.1, it is hard to explain this variable factor only by environmental causes (17,18).

Concerning bivalves and snails, like Mytilus, Crassostrea ; and abalone, found along the coasts, the enzymes with polymorphisms, the numbers of gene loci related to these enzymes, and the numbers of allelomorphs are summarized in TABLES 6.2, 6.3 and 6.4. FIGURE 6.1 shows the distribution of the numbers of allelomorphs in each gene locus, taken from the above TABLES. Solid lines show the distribution of the total data (excluding allelomorphs with frequencies lower than 0.01, which are shown in parentheses in the TABLES). Broken lines show the distribution represented by the fewest numbers of allelomorphs, with different data concerning the same gene loci. Average numbers of allelomorphs are 3.34 and 3.16 for each case. There are a little over 3 allelomorphs in a polymorphic gene locus.

It is a most remarkable fact in the genetic polymorphisms of shells that the overdominance of homozygotes is found in high frequencies. TABLE 6.5 shows the frequencies of phenotypes (genotypes) and the gene frequencies of Leucyl-ß-naphthylamidase in Mytilus edulis

and fish. Furthermore, these studies have been developed in various other fields, like population genetics and molecular population genetics (16). Studies of marine invertebrates from this aspect were activated suddenly, after 1970, particularly after 1975.

TABLE 6.1 shows the maintenance rate of enzyme variations in each species or population. When summarizing the results of all species studied so far, polymorphisms* were found in nearly 30% of the gene loci, and it is generally considered that the ratio of heterozygotes is 10%, on the average. [* Eventually, harmful genes should be banished by selection from populations. However, sometimes these genes remain in populations with gene frequencies below 0.01, due to the balance between the selection and mutations. The case when more than one allelomorph co-exists with a higher frequency (>0.01) than expected in the balance between the mutation and the wild selection, is called polymorphism. Some scientists consider more than 0.05 is necessary.] That is, in an individual, there are different kinds of allelomorphs (heterozygotes) in 10% of the gene loci. This figure is much higher than expected. The structure which maintains genetic polymorphism with such high frequency is of concern to many scientists, and this has been studied continuously from various aspects and is still studied even at present. From TABLE 6.1, it is clear that marine invertebrates have genetic polymorphisms with high frequency, as generally considered at present, and some species have even higher frequencies than the above figures.

Concerning Mytilus edulis shown in TABLE 6.1, 7 gene loci, or 50% of the total of 14 loci of this species which have been studied, are polymorphic. Among these, it was found that the rate of heterozygotes (allelomorphs) varies from high to around 0.02, which is close

It has been known that in many creatures, like Drosophila, chromosomal variations occur and are maintained in the same species, according to the geographical or seasonal changes of their environment (9). Purpura lapillus, a type of snail, has polymorphism, n = 13 and n = 18, which is related to the environment of their habitats. Also, it has been reported that, since these two types can be crossed, snails with various chromosomal numbers between both types are reproduced and the frequency of their appearances is related to their environmental or geographical distribution (10). Concerning bivalves, polymorphisms in Mytilus edulis and M. californianus have been reported (11). However, no polymorphism has been found in C. virginica, which has been studied rather intensively.

Similarly, no chromosomes of oysters vary between their genera or between their species and individuals; also, their numbers are very stable. However, in C. virginica, bred in Long Island Sound, abnormal chromosomes or abnormal nuclear divisions have been found with great frequency (12). It is considered that these abnormalities may have been caused by poisons due to water pollution.

2. Genetic polymorphisms of enzymes

By skillfully blending together gel-electrophoresis, isolating protein molecules with precision, and the staining methods specific to enzymes, developed in systems-chemistry in 1963, Markert (13), Show et al. (14) and Boyer et al. (15) demostrated clearly the genetic controls of LDH (Lactate dehydrogenase isozymes) and their variations in man and cows. Since then, studies of the variations of enzyme molecules have advanced rapidly with particular regard to mammals (man), Drosophila

population-genetics and breeding-genetics. Here, these studies are summarized and introduced; as well as the future and the meaning of the genetic breeding of marine invertebrates are described.

1. Cytogenetic studies

Cytogenetic studies constitute an important basis for breeding and genetic studies. There are already studies concerning bivalves, like oysters and some snails. Oyster spats are released in the intermediate stage, during the maturation division, between the prometaphase and the metaphase I. Longwell et al. (3,4) developed a method of observing chromosomes clearly and in detail by removing the fats of yolk granules, using the micro-soxhlet extraction method. These scientists reported that the chromosome numbers of Crassostrea virginica are n = 10, and that chiasmata are found in their chromosomes. Thereafter, chiasmata were found also in bivalent chromosomes during spermatogenesis. Then, it was established that males and females cross over during gametogenesis. Oysters cross well between species; also, some oysters, like Crassostrea gigas and C. angulata, easily produce second generation of hybrids. Chromosome numbers of both types of the Genus Crassostrea in the oviparity stage and Genus Ostrea in the larval stage are n = 10. Not only the chromosomal numbers of oyster species, but also their shapes are so similar, that their types and genera cannot be distinguished by their shapes (6,7,8). Menzel (8) studied 4 families of bivalves and reported that all species in these families have the same chromosome numbers. In so far as it is possible to know now, it is considered that less chromosomal differentiation occurs in bivalves at the level of classification below families.

ARTICLE, 6

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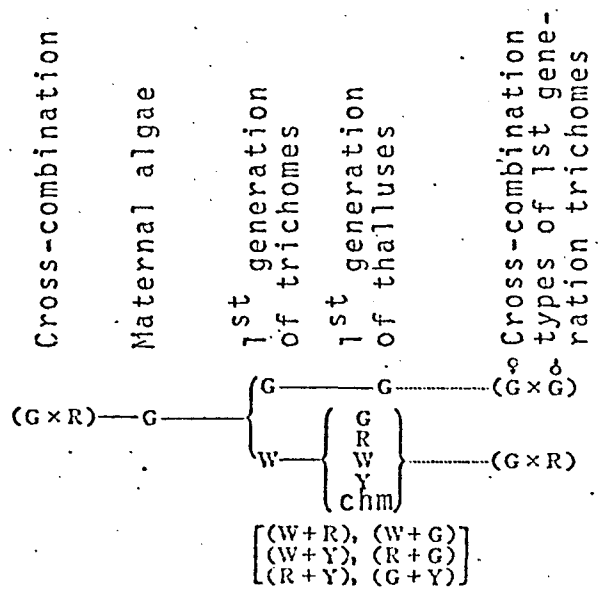
I N V E R T E B R A T A

Marine invertebrates, like oysters, abalones and shrimps, are commercially important everywhere in the world and, therefore, are target creatures for reproduction, cultivation and catches. These marine creatures are found along the coasts and inside the bays. More and more every year, they are being reproduced in cultivation; and their seeds are being supplied by artificial seed collectors - by man. Cultivation of these creatures will be developed even further, using measures to promote the "cultivation fisheries", the objective of which is a better planned reproduction and more effective use of the fishing grounds. However, the breeding and genetic research on aqua-creatures, as well as all other measures involved, are lagging very much behind agriculture and animal husbandry, both of which have been studied for a long time.

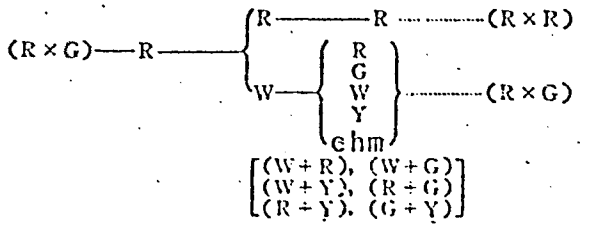
Imai (1) and Loosanoff (2) have studied genetics and the breeding of oysters (C. gigas) since the 1940's. Also, in the last half of the 1960's, and early in the 1970's, C. gigas have been studied intensively with the view to improving cytogenetics, genetic-biochemistry,

FIGURE 5.8: RESULTS OF RECIPROCAL CROSS EXPERIMENTS OF RED AND GREEN TYPES OF SUSABINORI(PORPHYRA YEZOENSIS)

W: wild type R: red type G: green type
Y: yellow type



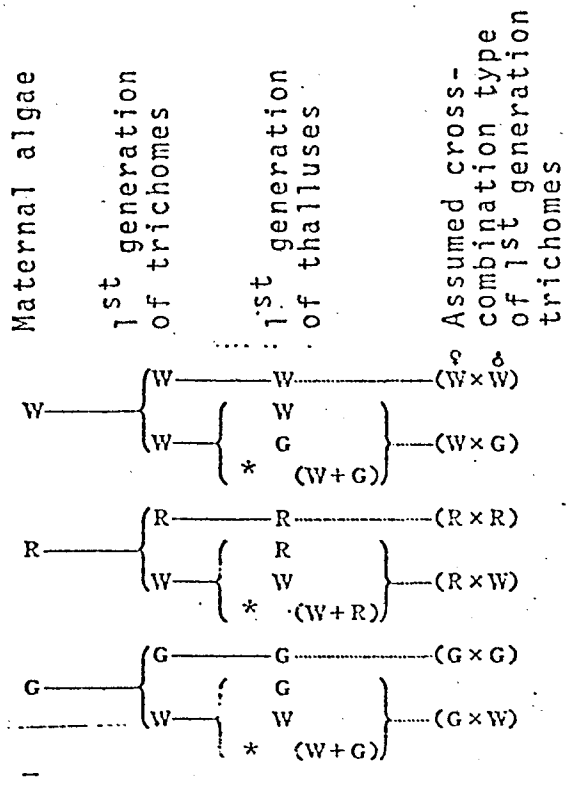
Chimeras with sectional dapples consisting of two color types



Chimeras with sectional dapples consisting of two color types

FIGURE 5.7: RESULTS OF SERIAL CULTIVATION OF WILD, RED AND GREEN TYPES OF SUSABINORI (PORPHYRA YEZOENSIS), FERTILIZED SPONTANEOUSLY

W: wild type R: red type G: green type



* chimeras

FIGURE 5.6: CULTIVATION EXPERIMENT WITH THALLUSES OF THE GREEN TYPE OF SUSABINORI (PORPHYRA YEZOENSIS)

a: maternal algae, thallus with dapples of the green type section (dotted area) and the wild type section.

b: maternal algae pieces, taken from the green type section

c: cultivation method of maternal algae pieces

d: germination of thalluses and trichomes, generated from maternal algae pieces

e: thalluses of the green type generated from maternal algae pieces

f: carpogone (left), antheridium (center) and carpogone generating carpospore (right), formed in thalluses of the green type

g: carpospores

h: trichomes of the green type, forming sporangium

i: shell-spores

j: thalluses of the green type generated from shell-spores.

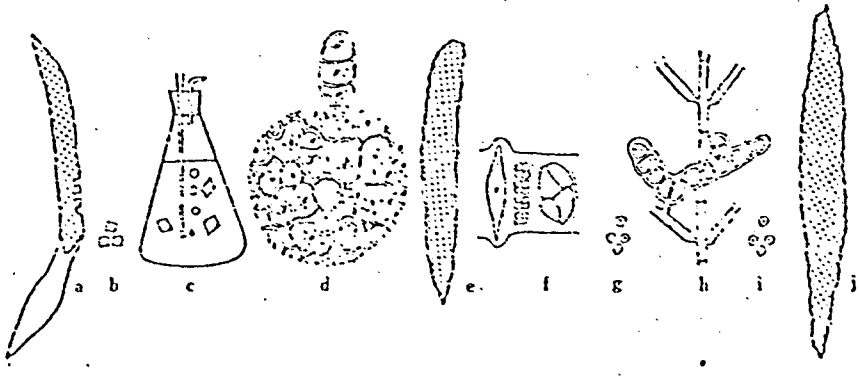


FIGURE 5.5: RESULTS OF SERIAL CULTURE OF CHIMERA THALLUSES WITH SECTIONAL DAPPLES, CONSISTING OF THE WILD TYPE SECTION (BLUE BUD) AND THE RED TYPE SECTION (RED BUD) OF SUSABINORI (PORPHYRA YEZOENSIS)

Maternal algae section	→	1st generation of trichomes	→	1st generation of thalluses	→	2nd generation of trichomes	→	2nd generation thalluses
Blue bud (normal type)	→	Blue bud type	→	Blue bud Red bud	→	Blue bud type Red bud type	→	Blue bud Red bud
Red bud (variant type)	→	Red bud type	→	Red bud Blue bud	→	Red bud type Blue bud type	→	Red bud Blue bud

Spontaneous fertilization — Cytoplasmic inheritance — Nuclear inheritance . Self-fertilization . Pure line

FIGURE 5.4: BIO-VISUAL LIGHT ABSORPTION CURVES OF THE WILD AND VARIANT TYPES OF THE TRICHOMES OF SUSABINORI (PORPHYRA YEZOENSIS)

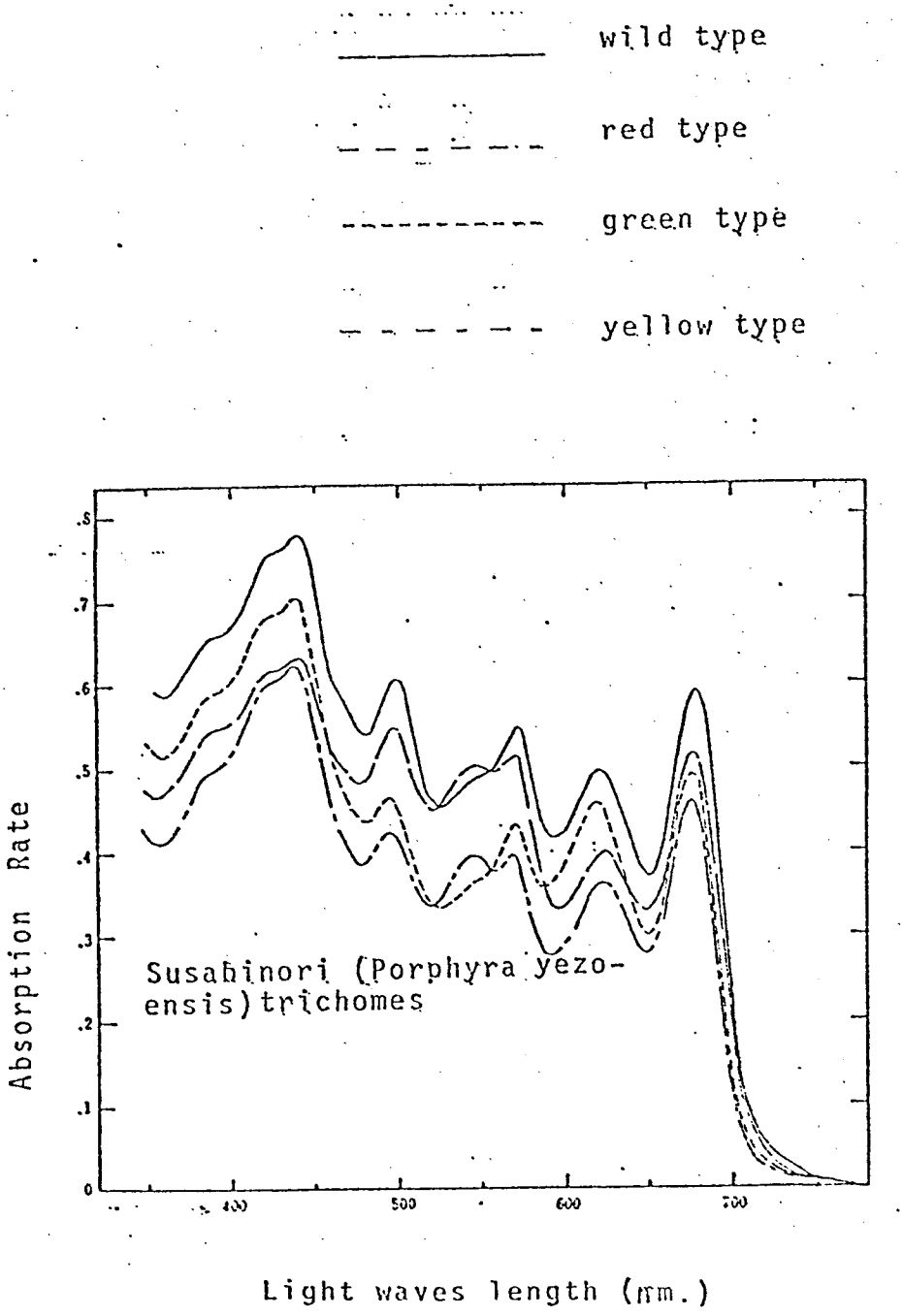


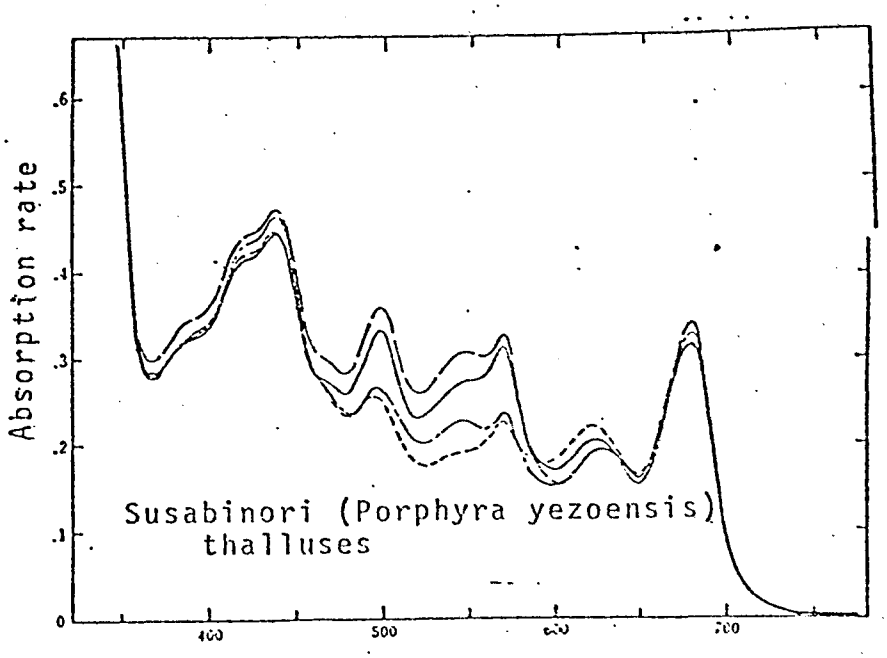
FIGURE 5.3: BIO-VISUAL LIGHT ABSORPTION CURVES OF THE WILD AND VARIANT TYPES OF THE THALLUSES OF SUSABINORI (PORPHYRA YEZOENSIS)

——— wild type

– – – red type

----- green type

—·—·— yellow type



Light waves length (nm.)

FIGURE 5.2: STYLES OF CHIMERA THALLUSES WITH SECTIONAL DAPPLES OF SUSABINORI (PORPHYRA YEZOENSIS)

W: wild type section

R: red type section

G: green type section

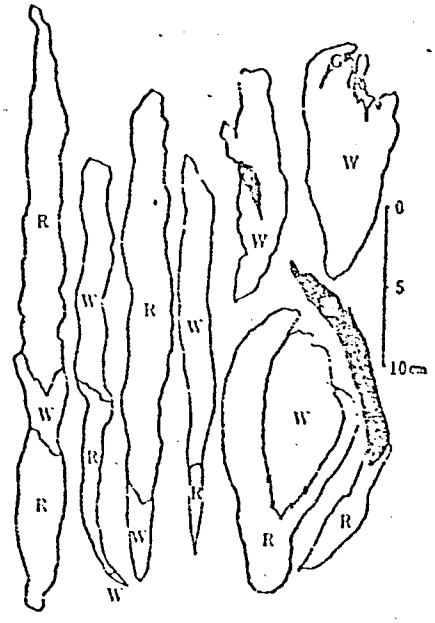
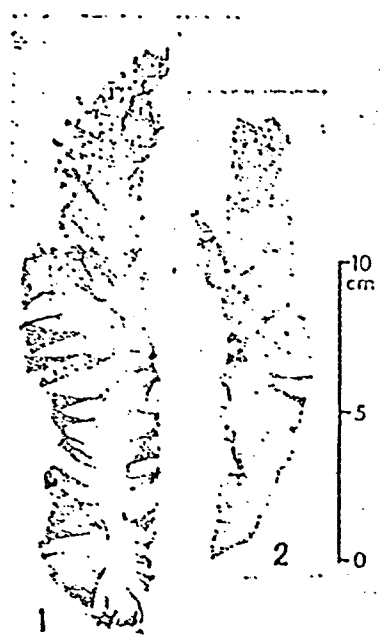


FIGURE 5.1. CHIMERA THALLUSES WITH SECTIONAL DAPPLES OF SUSABINORI (PORPHYRA YEZOENSIS).

W: wild type section

R: red type section

G: green type section



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considered that the red and green types of Susabinori (Porphyra yezoensis) are controlled by a simple, recessive mutant gene with different gene loci. It was experimentally confirmed that the color heredity of Susabinori occurs as a nuclear inheritance throughout the entire life cycle. Also, it was confirmed experimentally that the color types of the chimera-thalluses with sectional dapples are generated from heterozygote-trichomes. Furthermore, a variant of the green type, isolated from the chimera thalluses with dapples of green type sections, already is in cultivation commercially. The characteristics of these variants are: high immunity to the disease of "red-decomposition"; the dry products of this type are highly flavorful; and their sweetness is strong.

It is desirable to develop further the genetic and cultivation studies of the color types, described above, and, at the same time, to create new species by crossing and cultivating on the basis of the studies and achievements made so far.

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heterozygote-trichomes, and these chimera thalluses covered four of the color types isolated in the first generation of thalluses, but each comprised two or three color types. Concerning the chimera thalluses with sectional dapples of two color types, all six combinations of the two color types, predicted theoretically, were observed. Furthermore, it was clarified that the formation of the chimera thalluses with sectional dapples is not heritable.

Miura (4,12) reported that chimera-thalluses with sectional dapples are formed by a mutation of nutritive cells in the thalluses. However, considering the results of the above cross experiment, and the fact that the frequency of these chimera-thalluses reached 80% in cultivated populations, it is not appropriate to assume that the chimera-thalluses with sectional dapples are in fact formed by a mutation of the nutritive cells.

Similarly, with regard to the formation of dappled chimeras (mosaics) of Ogonori (Gracilaria), established by Van der Meer (7), it is considered that, in the case of Susabinori (Porphyra yezoensis), the chimera-thalluses with sectional dapples are formed by the germination of spores with two or four nuclei of different color types, which are produced due to the mitosis without the cytoplasmic fission in the stage of meiosis of the heterozygote-trichomes.

According to the results of the reciprocal cross experiment described, using the red and green types, it was clarified that, in the crossing of the monoecism - Nori, the heterozygote-trichomes (hybrids) can be isolated, using the color variants as markers. It was

these trichomes of the wild types showed the isolation of the color types in the first generation of the thalluses, it is considered that these trichomes are heterozygotes. Among the color types isolated, the red and green types are apparently of the same color type as the parents, and it is considered that the yellow and wild types are recombinations of the red and green types. Since the chimera-thalluses with sectional dapples comprise a combination of the red, green, wild or yellow types, it is considered that the color types of the chimera-thalluses with sectional dapples are showing isolation of the color types similar to the thalluses with one single color type. This isolation of the color type of the parents and of the color type of the recombination into four kinds of color types shows that the red and green types are controlled by a simple gene with different gene loci for each of the colors. Also, since the color types of the heterozygote-trichomes isolating four of the color types, are the wild types, it is considered that the red and green types are recessive; and that the thalluses of the yellow type are a recombination type with genes of both the red and green types; as well as the thalluses of the wild type, which are a recombination type without both the red and green types. The yellow type was a color variant generated and isolated for the first time in these cross-experiments.

Due to the vegetative reproduction of Susabinori (Porphyra yezoensis) liberating monospores (neutral spores) during the plumule period, it is difficult to obtain the isolation ratio of the color types accurately. Therefore, it has not yet been clarified whether the gene loci of the red and green types are in the same chromosome, or in different chromosomes. Also, in this reciprocal cross experiment, the chimera thalluses with sectional dapples were generated from only the

When the red types were used as maternal algae, red and wild types were generated in the first generation of trichomes. In the first generation of thalluses, only the red types were produced from the red types of the trichomes; red, green, wild and yellow types and the chimera with sectional dapples were generated from trichomes of the wild types. Most of the chimeras with sectional dapples have two different color types, having received them from the red, green, wild and yellow types. In some cases, the chimeras have two different color types and the same color type occupies two sections. Also, sometimes the chimeras have three different color types. In these cross experiments, in all the reciprocal cases, trichomes of the wild type were generated in addition to the same color type as the maternal algae. Then, from these trichomes, the wild, red, green and yellow types and chimeras with sectional dapples were generated.

From the results of the above reciprocal cross experiments, it was clarified that, in all reciprocal cross cases, in the first generation of thalluses, there are two types of trichomes: the trichomes which generate only thalluses with the same color type as the color type of the maternal algae in the first generation of the thalluses; and the trichomes which generate the wild, red, green and yellow types and chimeras with sectional dapples of these color types. Since the trichomes which generate thalluses with the same color types as the color types of the maternal algae show the same color types as the maternal algae, it is considered that these trichomes are apparently homozygotes, attributed to self-fertilization. On the other hand, the trichomes which generate the wild, red, green and yellow types and chimeras with sectional dapples show the same color types as the wild types. Since

2.4 Experiment of a reciprocal cross of the red and green types (13,14)

In order to analyze the genes of the color types of Susabinori (Porphyra yezoensis), reciprocal cross experiments of the red and green types were conducted. Nori are fertilized by settling of the sperm, liberated from the maternal body, on the fertilization cone of the carpogone (egg), matured in the maternal body. The fertilized carpogone generates several carpospores. Since not all sperms are capable of movement, they are moved mainly by the flow of seawater. The thalluses of the different color types, which previously had been cultivated separately, were put in the same container when their maturation commenced and were cultivated to maturity. Since Susabinori (Porphyra yezoensis) are monoecismic, if the carpospores are collected from the thalluses of all color types of the cross-combinations and are cultivated for their following generations, the reciprocal cross experiment can be conducted at the same time. In this experiment, they were cultivated from the first generation of the trichomes to the first generation of the thalluses. The results are shown in FIGURE 5.8.

In the case when the green types were used as maternal algae, green and wild types were generated in the first generation of thalluses. Only green types were generated from trichomes of the green types; and the green, red, wild and yellow types and the chimeras with sectional dapples were generated from trichomes of the wild types. Most of the chimeras with sectional dapples have two different color types, having received them from the green, red, wild, or yellow types. In some cases the chimeras have two different color types and the same color occupies two sections.

On the other hand, the trichomes,which generated the same color as that of pe/the maternal algae, color types other than the maternal and the chimeras with sectional dapples in the first generation of thalluses, showed consistently the wild type without any relation to the color type of the maternal algae. It is considered that this result shows that the isolation of color types takes place in the first generation of thalluses. Therefore, it showed that the trichomes of the first generation , which isolated the color types in the first generation of thalluses, were heterozygotes. Also, since all the heterozygote trichomes were the wild types, both the red and green types are recessive to the wild type. From this result, it is considered that both the red and green types are mutants controlled by a simple recessive gene. Furthermore, during this cultivation experiment, the chimeras with sectional dapples were generated only from the heterozygote trichomes. The color types comprising chimeras with sectional dapples matched in color the color types isolated in the first generation of thalluses. It is considered that this fact must be closely related to the structure of the chimera with sectional dapples.

Concerning all the thalluses of the wild, red and green types, fertilized spontaneously, if the isolation mechanism in the first generation of thalluses can be clarified, using their color types as markers, the combination types of the crossing in the first generation of trichomes can be assumed. In this cultivation experiment, only the following crossing combinations were found to be heterozygotes: the wild type X the green type; the red type X the wild type; the green type X the wild type; and the wild type X the red type * . (* not shown in FIGURE 5.7).

the chimera with sectional dapples were generated from trichomes of the wild type. This chimera with sectional dapples consisted of a combination of the wild and green types.

From all these results, it was established that: concerning the first generation of thalluses in this cultivation, in all cases of thalluses of the wild, red, or green types, there are two kinds of trichomes: one kind is trichomes which generate only thalluses of the same color type as the color type of the maternal algae; and the other kind is trichomes which generate the same color as the maternal algae, a different color type from the maternal, and also chimera thalluses with sectional dapples. The trichomes generating only the same color type as the that of the maternal algae in the first generation of thalluses, showed constantly the same color type as had the maternal algae.

Concerning Nori, it is considered that its chromosomal number is 2n in its trichome, but that it is n in its thallus; and that meiosis takes place in the trichome. If this assumption is correct, the result of this cultivation shows that: the isolation of the color types did not take place in the first generation of thalluses, generated from trichomes of the same color type as the color type of the maternal algae; since trichomes of the first generation are of the same color type as the color type of maternal algae and also of the same color as the color type of the first generation of thalluses, these trichomes are homozygotes. Since Susabinori (Porphyra yezoensis) are monoecismic, it is considered that these homozygote trichomes are generated either by self-fertilization or by cross-fertilization with other algae, having the same color types.

2.3 Serial cultivation of thalluses of the wild, red and green types, bred spontaneously. (13)

Thalluses of the wild and green types were collected from a natural population which had them together. The thalluses of the red type were collected from a natural population which comprised wild types, in which the red type was found mixed. Using these thalluses for each color type, a serial cultivation was made from the first generation of trichomes to the first generation of thalluses. It was found that all these thalluses had fertilized spontaneously and matured in the naturally-bred population. The result of the serial cultivation is shown in FIGURE 5.7.

In the case of thalluses of the wild type, in the first generation of trichomes, only the wild type was produced. In the first generation of thalluses, only the wild type was produced from the trichomes of the wild type; also the wild and green types and the chimera thalluses with sectional dapples were generated from trichomes of the wild type. All these chimera-thalluses with sectional dapples consisted of a combination of the wild and green types. In the case of thalluses of the red type, in the first generation of trichomes, the red and wild types were produced. In the first generation of thalluses, only the red type was generated from trichomes of the red type; and the red and wild types; also the chimera with sectional dapples were generated from trichomes of the wild type. This chimera with sectional dapples consisted of a combination of the wild and red types. In the case of the thalluses of the green type. in the first generation of trichomes, the green and wild types were produced. In the first generation of thalluses, only the green type was generated from trichomes of the green type, and the green and wild types, as well as

consisting of the wild and green types. Using this small leaf portion as the maternal algae, a serial culture was made. The procedure and the results of the culture are shown in FIGURE 5.6. This small leaf portion produced thallus and trichome germinations. Thallus must be formed by means of regeneration of nutritive cells of the small leaf portion to a spore (monospore or neutral spore) and its vegetative propagation. Also, it is considered that the trichomes must be the result of germination of the spores (carpospores), which were produced from carpogones, after regeneration of the nutritive cells of the leaf portion to spores and sperm, followed by sexual reproduction between them. Both the thalluses and trichomes, obtained from this small leaf portion, showed the same color types as the green type section of the chimera-thallus with sectional dapples, which were in the maternal algae. Furthermore, the trichomes were generated from the cultured thalluses by self-fertilization. Then, this was followed by generation of thalluses (from the above trichomes). The life cycle of the green type variant was thus completed by cultivation. Throughout all the stages of this life cycle, both the trichomes and thalluses showed a brilliant green color. In this experiment, no dappled chimeras were formed.

From the results of the above described experiments, it was confirmed that the green type is a heritable variation and, therefore, that dapples of the green type are dappled chimeras. Also, for the first time, the green type variant was isolated during this cultivation experiment. Furthermore, it was clarified that the method of isolation of the color types by the culture of a small leaf portion with only nutritive cells corresponds to a type of clonal cell culture, and this method is effective in isolating a pure line of Nori.

color chimeras. Also, a genetic phenomenon of the color types of Susabinori (Porphyra yezoensis) was assumed to be as follows: since the color types of all successive generations after the first generation of thalluses, obtained by self-fertilization, agreed with the color types of the first generation of thalluses, it was considered that a pure line of the color type could be created by self-fertilization. Also, since in the first generation of thalluses, both the same color type as the maternal color type and a different color type from the color type of the maternal algae were obtained, it was considered that a separation of the color types was taking place and, therefore, that the heredity of the color from the trichomes to the thalluses was a nuclear inheritance. On the other hand, since in the first generation of trichomes, only the same color type as that of the maternal algae, was generated consistently, it was thought that the heredity of the color type from the thalluses to the trichomes was a cytoplasmatic inheritance. However, since it was confirmed experimentally that the heredity of the color types was taking place because of the nuclear inheritance throughout the entire life cycle, it was realized that the assumption of the color heredity from the thalluses to the trichomes was a cytoplasmatic inheritance was not correct. Furthermore, in these cultivation experiments, since in the first generation of trichomes, generated from the red type, it was clarified that a wild type was also generated in addition to the red type, it was also concluded that the genetic phenomenon described was an experimental error.

2.2 Serial cultivation of chimera-thalluses, sectionally dappled, consisting of wild and green type portions (15)

A small leaf portion with only nutritive cells was cut off from the green type section of the chimera-thallus with sectional dapples,

2.1 Serial cultivation of chimera-thalluses, sectionally dappled, consisting of the wild and red types, fertilized spontaneously

Spores (carpospores) of the wild and red types were isolated from various parts of the algae and then cultured to obtain a first generation of trichomes. Spores were isolated from this first generation of trichomes and were cultured. Then, a first generation of thalluses was obtained. Since Susabinori(Porphyra yezoensis) is monoecismic, when such a plant is isolated and matured, it can be self-fertilized. Therefore, the first generation of thalluses were isolated in one plant, cultured until maturation and self-fertilized. From this first generation of thalluses, the second generation of trichomes was generated. Then, from the second generation of trichomes, the second generation of thalluses was generated. Thus, serial cultivations were achieved. The results are shown in FIGURE 5.5. Thus , in the first generation of trichomes, the wild type portion generated the wild type and the red type portion generated the red type. In the first generation of thalluses, from this first generation of trichomes, both the wild and red types produced the wild type, the red type and a chimera with sectional dapples. In FIGURE 5.5, the chimeras are not shown. In the second generation of trichomes, generated from the first generation of thalluses, which were self-fertilized, the wild type was generated from the wild type and the red type was generated from the red type. In the second generation of the thalluses, generated from the second generation of trichomes, the wild type generated the wild type only and the red type generated the red type only.

From these results, Miura (4,12) concluded that the portion of the red type was due to genetic mutation and that the dapples were

at the maximum and minimum light absorptions agreed well with the case of the red type; however, the light absorption rate was drastically decreased at the third peak.

Only when these color types are studied from the point of view of the biovisible light absorption curves, is it considered that the red type is a qualitative variation to the wild type; the green type is a quantitative variation to the wild type; and the yellow type is a qualitative variation to the wild type, similarly to the case of the red type, but it is a quantitative variation to the red type.

The above characteristics of the light absorption curves in the thalluses and trichomes are not affected by age, the body-portions, the growing periods, or the growing locations.

2. Genetics of the color types

In the following, the results of the cultivation experiments are described. They were conducted for the analyses of the genetic modes of various types of color;and also analyses of genes which were found in the thalluses and trichomes. The thalluses of Nori generate trichomes by means of sexual reproduction; the trichomes generate the thalluses again by means of asexual reproduction. Since the meiosis occurs when the asexual reprodictive cells are formed in trichomes, a haplophase is found during the period of the thallus, but a diplophase is found during the period of the trichome.

kinds of chimera-thalluses, sectionally dappled, as well as thalluses with various kinds of color types can be found easily in the naturally bred and cultivated populations.

1.3 Bio-visible light absorption curves of color types

Although the color types of Susabinori (Porphyra yezoensis) can be observed easily macroscopically, especially when the samples are grown in the same conditions, this can also be confirmed accurately by recording the bio-visible light absorption curves of the thalluses and trichomes. We used a self-registering spectrophotometer (6). The bio-visible light absorption curves of thalluses and trichomes are shown in FIGURES 5.3 and 5.4, where, from the shorter light-wave side, the first peak was due to chlorophyll-α and β-carotene. The second peak was due to β-carotene and phycoerythrin. The third peak was due to phycoerythrin; the fourth peak was due to phycocyanin; and the fifth peak was due to chlorophyll-α. The differences between the wild type and the variants are found mainly in the third peak, due to phycoerythrin, and also in the fourth peak, due to phycocyanin. That is, concerning the red variant, the maximum light absorption occurred at 568 nm and also at 543 nm in the range of the light wave of the third peak, but the minimum absorption was at 558 nm. In the range of the light waves at the fourth peak, the maximum absorption was at 622 nm, which comprised 3 nm difference from the value of the wild type to the longer wave side. Concerning the green type, the maximum absorption in the range of the light wave lengths at the third and fourth peaks agreed well with those of the wild type. The light absorption rate at the third peak was remarkably low, but became high at the fourth peak. Concerning the yellow type, the light wave lengths

formed at each color portion of the dappled thallus, these cells can be cultured separately in the following generation. As a result, a thallus with the same color type as that of the parents can be obtained. From this, it is considered that color types of dappled thalluses are determined genetically and the dappled thallus is a type of chimera (mosaic). Consequently, these dappled thalluses are called "dappled chimeras". On the other hand, in addition to such dappled chimera thalluses, sometimes spotted or striped, green portions appear sweepingly through the central portion of thalluses of the wild type (14). Therefore, when the different color types appear sectionally in the thalluses, they are called - chimera-thalluses with sectional dapples; and when there are stripes, they are called - chimera-thalluses with striped dapples.

In both the wild and cultured populations, the frequency of chimera-thalluses, sectionally dappled, is high, but the frequency of chimera-thalluses with striped dapples are very low. Most of the chimera-thalluses, sectionally dappled, found in cultivated populations are composed of the wild and red types, but very few of them are of the green type. Presently, while color variants can be bred selectively, the frequencies of various color types in natural conditions are not yet known. However, it has been found that in the cultivated populations, the frequency of thalluses of the red type is high and that of the green type is extremely low. These frequencies corresponded to the frequencies of the chimera-thalluses, sectionally dappled, of the green and red types. The chimera-thalluses with dapples, consisting of the yellow types and also thalluses of the yellow type have not yet been found in wild-bred populations. However, now-a-days, various

tom. The thallus of the green type shows bright green throughout the entire body, also from the top to the bottom, resembling Aosa (Ulva) and Hitoegusa (Monostroma) of Chlorophyceae. However, since the green type is rather purplish, it can be distinguished from the two species of Chlorophyceae. When mature, this type shows a yellow-brown color, specifically in the reproductive cells. The thallus of the yellow type shows a yellow-green color throughout the entire body, from the top to the basal part. The colors of these variants can easily be distinguished in the thallus, if cultivated in the same conditions.

On the other hand, in the case of trichomes, a trichome of the wild type shows generally blackish-purple or brownish-purple colors; the red type shows red or reddish-purple colors; the green type shows a dark green-purple color; and the yellow type shows a yellow-green color . Even in the case when the algae are growing while shells are being drilled*, if all types grow in the same conditions, the differences between them can be recognized easily. (*Trichomes grow while invading by drilling the calcareous portion of shells; however, they also grow without this chemical substance.)

1.2 Thallus with a dappled chimera (3,4)

In addition to the thallus with only one type of color, as described, there are thalluses with dappled colors, consisting of two or three color types. Theoretically, the dappled color type, consisting of four colors, can also be formed; however, this type has not yet been discovered. In most of the dappled thalluses, as shown in FIGURES 5.1 and 5.2, each color portion is divided clearly into upper and lower portions, or cuneiformly. Since the reproductive cells are

to confirm the effects of the crossings. Since the results of these crossings can be confirmed directly, if the genetic variants of the color types are used as markers of the nuclei, it is considered that the color variants of Nori are of great importance for cultivation studies in order to investigate the creation of the variants by crossings and also in order to learn the genetics of populations.

1. Color variants of Susabinori (Porphyra yezoensis)

1.1 Color types

Yuda (11) and Miura (11,12,13) have reported that in the color types of Susabinori (Porphyra yezoensis), there are red, green and yellow color-type variants; and also a wild type. Among these variants, the red and green types are due to spontaneous mutation. The yellow type is a recombinant variant, derived from the trichome of the heterozygote (hybrid), generated by crossing the red and green types. The experimental materials and the isolation of these variants are described in the paragraph on genetics.

In Nori, there are two states: a thallus state, which can be seen macroscopically, and a trichome state, which is like a mold and is microscopically small. Its life cycle is completed by generating the trichome from the thallus and then generating the thallus from the trichome states. The wild, red, green and yellow types are observed not only in the thallus but also in the trichome states.

The thallus of the wild type shows a greenish-brown-purple color; its basal part is greenest. In contrast, the thallus of the red type shows red throughout the entire body from the top to the bot-

Oobaasakusanori and Narawasusabinori were obtained by selection and cultivation of algae with a higher ratio between the length and width of the leaves, after isolating the large size algae with low sterility, a feature which appears accidentally in Asakusanori and Susabinori, by cultivating their trichomes. It is characteristic that both species grow fast in a straight line and are high yielding.

In recent years, as mentioned above, the results of studies of breeding of useful marine algae have been yielding data steadily. In this paper, the author describes studies of the color variants and genetic studies of the color types in Susabinori (Porphyra yezoensis), as a part of cultivation studies concerning Nori and conducted by the author and others.

Van der Meer (7) reported excellent genetic studies of a mutant with the color type of marine algae. This author and others (6,7, 8) conducted genetic analyses of spontaneous mutants of the green type and artificially-created mutants of the red type in two kinds of Ogonori(Gracilaria). He established that both types are formed due to simple recessive genes, occupying different gene loci. Furthermore, the authors clarified genetically the structure of the dappled chimera (mosaic),the gynandromorphism, and the mosaic between sporophytes and gametophytes, using the color variants as markers. The authors and other scientists are greatly indebted to these authors for the results of their studies.

In the studies concerning the crossing of Nori, although there are pilot studies by Suto (9) and Yuda (10), indirect methods were used

ARTICLE 5 (Part II)

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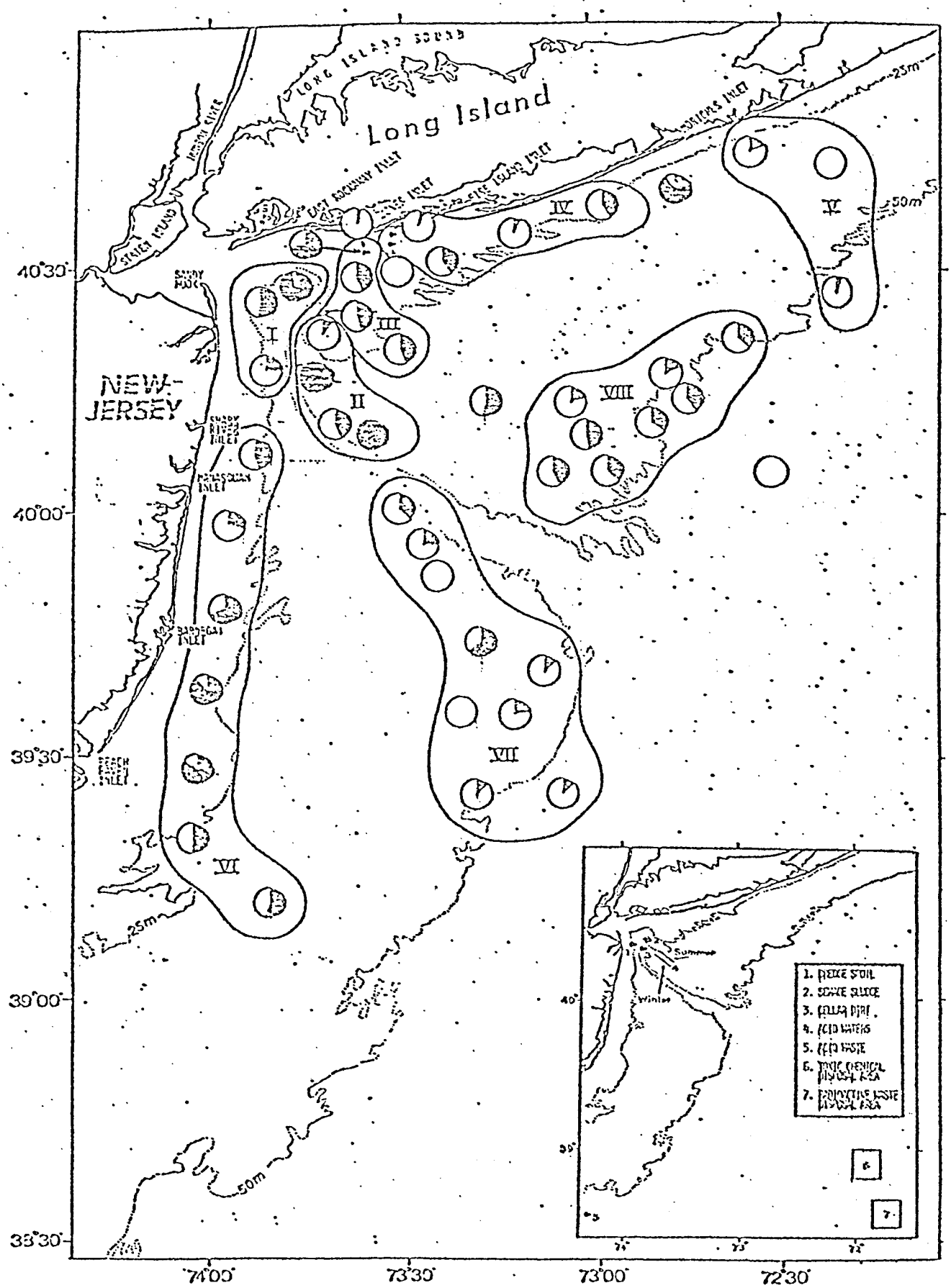
PRESENT ASPECTS OF AQUACULTURE AND ITS FUTURE

Among useful marine algae, the following species have been cultivated and used commercially: Laminaria No.860 and No.1170 (1), procured from China; Chondrus T4 from Canada; and Oobaasakusanori (Porphyra) and Narawasusabinori (Porphyra yezoensis) from Japan (3,4,5). Laminaria in China and Nori in Japan are used as edible marine algae, while Chondrus in Canada is used as a source to extract polysaccharide, called carrageenin. Carrageenin is a type of paste and is used widely as a food additive. But the two species of Laminaria were created by selective culturing from a wild population. The line of Laminaria No.1170 was selected by X-ray treatment from a line already established. Compared with a wild population, it is characteristic that Laminaria No.860 grows faster and larger at high temperatures; therefore, it is high yielding and the content of Thyroid is higher. The No.1170 species has similar characteristics to No.860, but its water content is low.

The Chondrus T4 is a sterile gametophyte and was selected from a wild population. Since its content of Kappa-Carrageenin is high, its vegetative reproduction is good, and it grows well in a tank, it is a suitable species for cultivation.


Long Island
NEW-
JERSEY
I
II
III
IV
V
VI
VII
VIII
25m
50m
40°30'
40°00'
39°30'
39°00'
38°30'
74°00'
73°30'
73°00'
72°30'

-135-

Figure 11. Graphic representation of Stein estimates of cytologically-cytogenetically appraised mortality-moribundity of gastrula-early embryo (Stages IV-V) eggs of Atlantic mackerel in the New York Bight. Circles indicate station positions. Shaded portions of circles show the percent embryos dead or moribund. Stations are grouped I-VIII for the purpose of statistical treatment (see Tables 3 and 4). All stations were sampled on the May 7-18, 1974 cruise of the sailing vessel Westward.

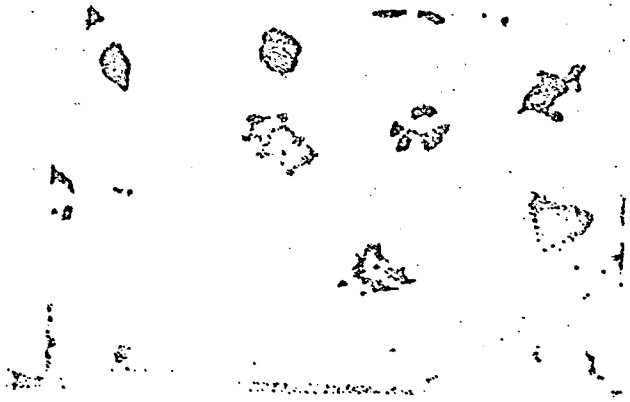
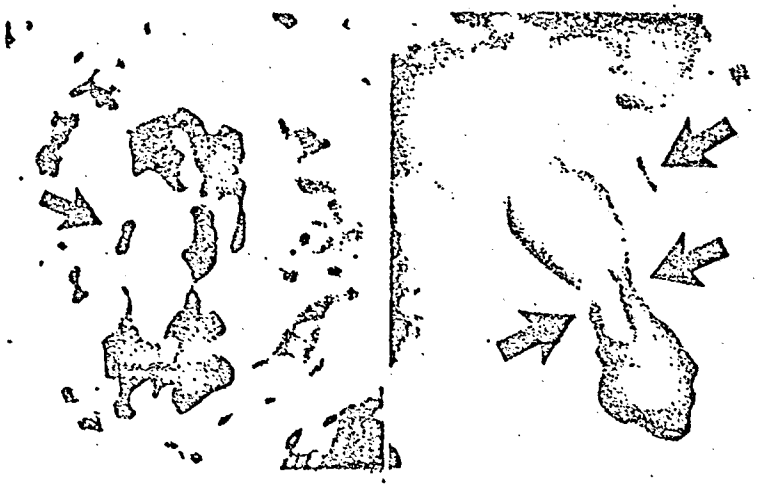


Figure 10. Division-arrested, abnormally differentiated, monolayered cells of a blastula embryo of an Atlantic mackerel egg from plankton. 63X light objective.



Figures 8 and 9. Abnormal telophases in gastrula embryos of Atlantic mackerel eggs from plankton showing displaced and laggard chromosomes, and chromatin bridges. 63X light objective.

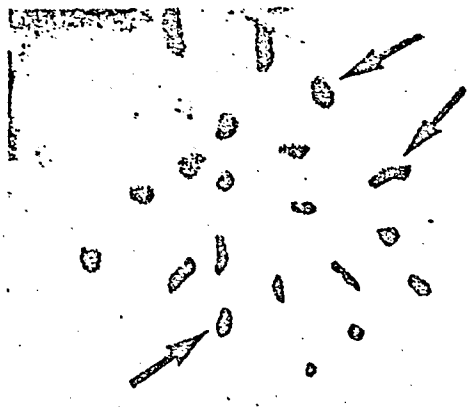


Figure 7. Metaphase II chromosomes of a ripe pre-spawned egg stripped from an Atlantic mackerel on its spawning migration to the New York Bight. 63X light objective.

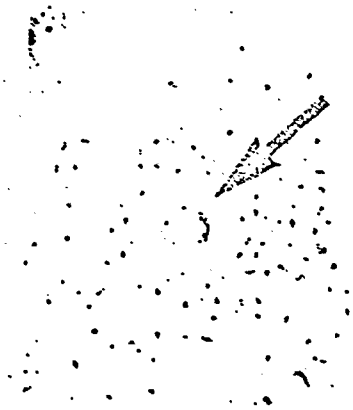


Figure 6. Portion of the chorion of a pre-spawned Atlantic mackerel egg showing the micropyle. Egg was stripped from a ripe fish on its spawning migration. 63X light objective.

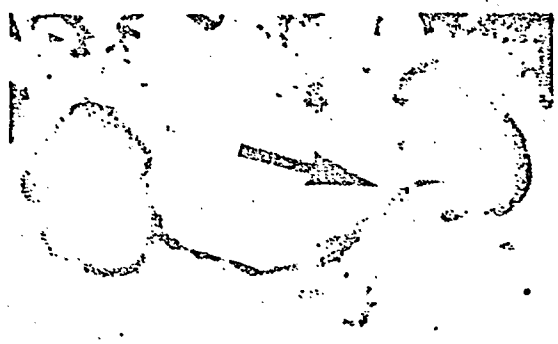


Figure 5. Abnormal telophase with a chromosome bridge in the yolk-sac membrane of an Atlantic mackerel egg from plankton. 63X light objective.

Figure-4. Abnormal metaphase in the yolk-sac membrane of Atlantic mackerel egg from plankton. Two chromosomes have failed to orient on the mitotic spindle. 63X light objective.

Figure 3. Prometaphase chromosomes in the yolk-sac membrane of an Atlantic mackerel egg from plankton. Not pretreated with any c-mitotic agent. Largest chromosomes about 6 μ in length. 63X light objective.

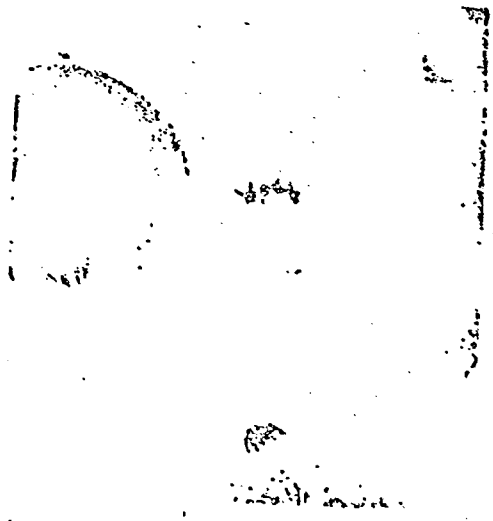


Figure 2. Normally mitosing cells in monolayer spread of a blastula embryo of Atlantic mackerel egg from plankton. 63X light objective.

Figure 1. Undissected Atlantic mackerel egg from plankton at morula stage of development at upper right. Oil globule to top of egg, embryo to bottom. At lower left at arrow is a morula embryo dissected free from its egg. Intact egg about 1 mm in diameter.

TABLE 7.3: F_1 HYBRIDS OF SALMONIDS WITH HETEROSIS

Combinations	Names	Survival ability	Growth
Kawamasu ♀ x Iwana ♂	Kawaiwa	Higher than both parents	Faster than Iwana
ditto x Sakuramasu ♂	Kawasakura	Higher than both parents after fingerling period; lower hatching rate	Same as Sakuramasu
ditto x Himemasu ♂	Kawahime	same as above	Intermediate between parents
Brown trout ♀ x Iwana ♂	Braiwa	same as above	Faster than Iwana
ditto x Kawamasu ♂	Tiger trout	Higher than both parents	Same as Brown trout
ditto x Sakuramasu ♂	Bra sakura	Higher than both parents after fingerling period; lower hatching rate	Faster than Sakuramasu
ditto x Amago ♂	Bra amago	Same as Brown trout up to one year of age (*)	Faster than both paren after two years of age
Biwamasu ♀ x Iwana ♂	Biwaiwa	Higher than both parents after fingerling period; lower hatching rate	Same as parents
Sakuramasu ♀ x Biwamasu ♂	Sakubiwa	Higher than both parents	Faster than both paren

(*) Higher than parent species after two years of age.

TABLE 7.2: THE SIZE OF MATERNAL PARENTS AND THE NUMBERS OF EGGS SPAWNED DURING THE SPAWNING PERIOD OF SELECTED PARENT GROUPS OF RAINBOW TROUT

(Donaldson, 40)

Year of egg collection	Ages	Numbers of parent fish, eggs of which were collected	Fish length (cm)		Numbers of eggs collected	
			Average	Largest	Average	Largest
1944	2	12	36.3	39.0	1,653	2,121
1946	2	39	41.9	48.0	2,011	2,932
1948	2	78	41.2	50.0	1,958	3,094
1950	2	129	35.5	45.5	1,315	2,097
1952	2	51	40.9	45.0	2,145	3,810
1953	2	27	38.9	43.5	2,032	3,631
1954	2	47	44.6	51.5	2,377	3,960
	3	36	50.5	57.0	4,042	6,105
1955	2	23	50.7	56.5	3,824	5,123
	3	28	59.6	67.0	5,029	8,850
1956	2	84	49.0	53.5	3,231	5,915
	3	9	59.2	65.0	6,149	7,311
1957	2	58	46.4	55.0	3,161	9,639
	3	14	67.4	72.0	7,117	11,475
1958	2	57	57.6	66.0	5,617	9,077
	3	2	73.5	74.0	15,767	16,839
1959	2	39	60.0	63.0	5,224	6,960
	3	6	70.2	73.0	8,689	11,580
1960	2	63	59.6	68.0	5,132	8,263
	3	6	70.8	72.0	9,030	11,186
1961	2	29	52.9	59.0	4,809	6,833
	3	5	67.1	71.0	9,092	13,515
1962	2	73	57.8	68.5	6,080	13,407
	3	20	66.1	72.0	9,176	16,482
1963	2	83	52.5	63.0	...	...
	3	8	67.6	70.5	...	...
1964	2	48	59.1	66.5	6,091	8,345
	3	12	64.0	71.0	9,763	13,634
1965	2	81	58.5	65.5	6,274	11,525
	3	30	68.3	78.0	11,556	16,160
1966	2	131	57.0	63.0	7,793	15,872
	3	32	68.7	74.5	13,301	29,922
1967	2	36	60.7	67.0	7,335	13,707
	3	29	67.2	73.5	11,099	23,502
1968	2	70	60.4	66.0	9,259	13,144
	3	5	68.1	72.0	11,718	21,253

TABLE 7.1: RESULTS OF CULTIVATION OF VARIOUS SPECIES OF CARP AND F_1 HYBRIDS WHICH SHOWED HETEROSIS, FOR TEN MONTHS (25°C ± 1°C)

Species of parents and F_1 hybrids		Survival rate (%)	Weight increase (%)	Average food effect (%)	Degree of heterosis
Mirror carp		72.5	603.5	82.1	
German-scaled carp		76.3	495.7	78.4	
Yamato carp		80.0	481.7	79.8	
Big-belly carp		90.8	405.8	74.3	
Wild carp		42.2	82.4	50.5	
Yamato carp ♀					
x Mirror carp	♀×♂	84.4	855.2	85.8	+5.1
x German-scaled carp	♀×♂	77.7	737.9	84.3	+28.3
x Big-belly carp	♀×♂	90.6	650.2	83.4	+5.4
x Wild carp	♀×♂	73.3	590.0	78.7	+1.5

FIGURE 7.3: SURVIVAL RATE OF OFFSPRING OF HYBRIDS BETWEEN KAWAMASU (Sf) AND IWANA (Sp)

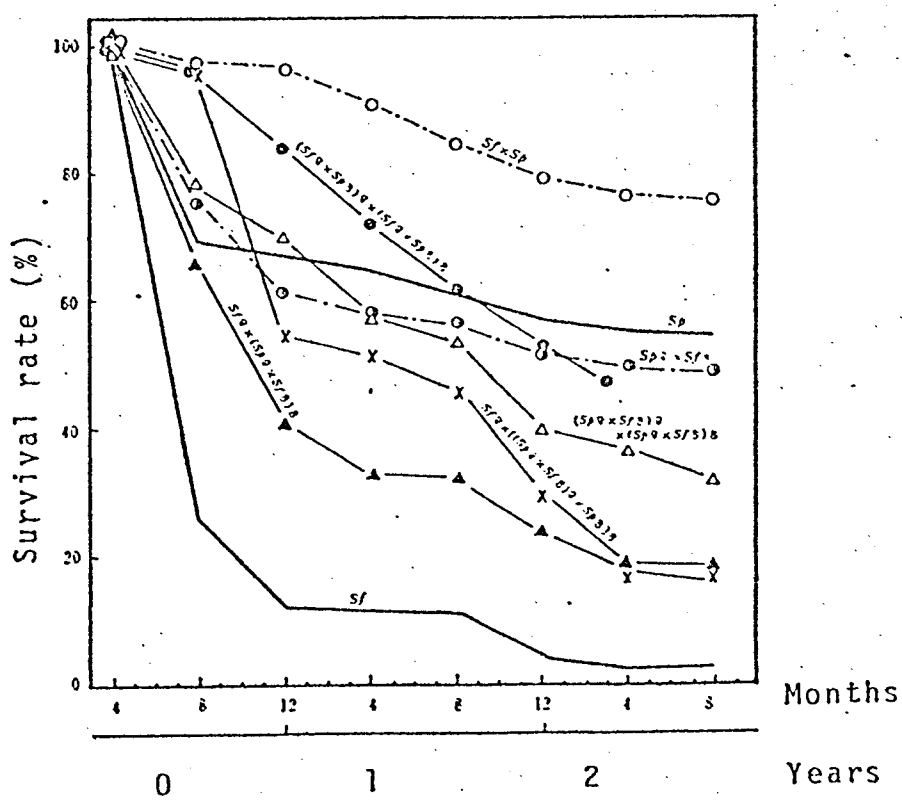


FIGURE 7.2: GROWTH CURVES OF YAMATO-MIRROR F_1 (YAMATO ♀ x MIRROR ♂) AND PARENT SPECIES

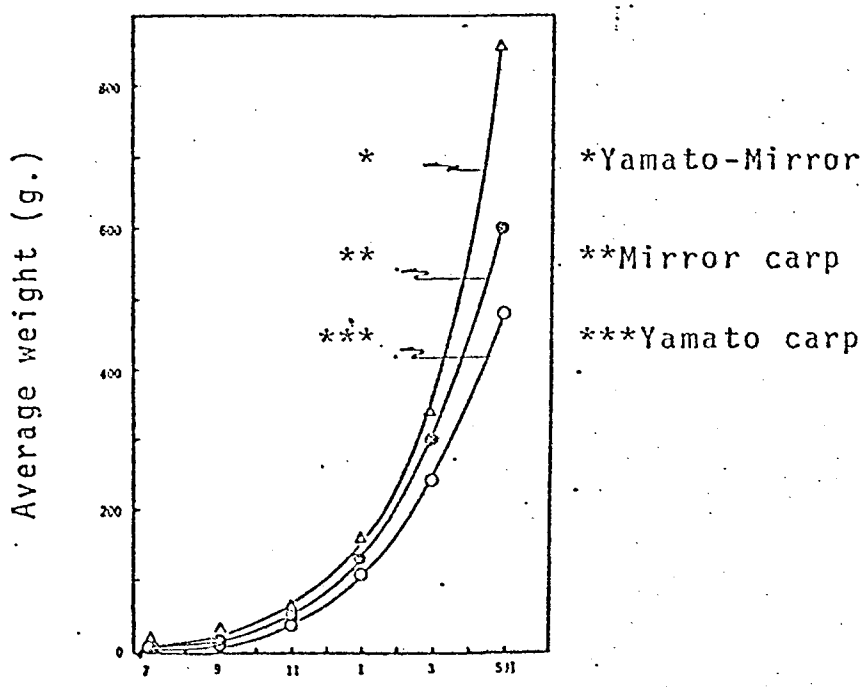


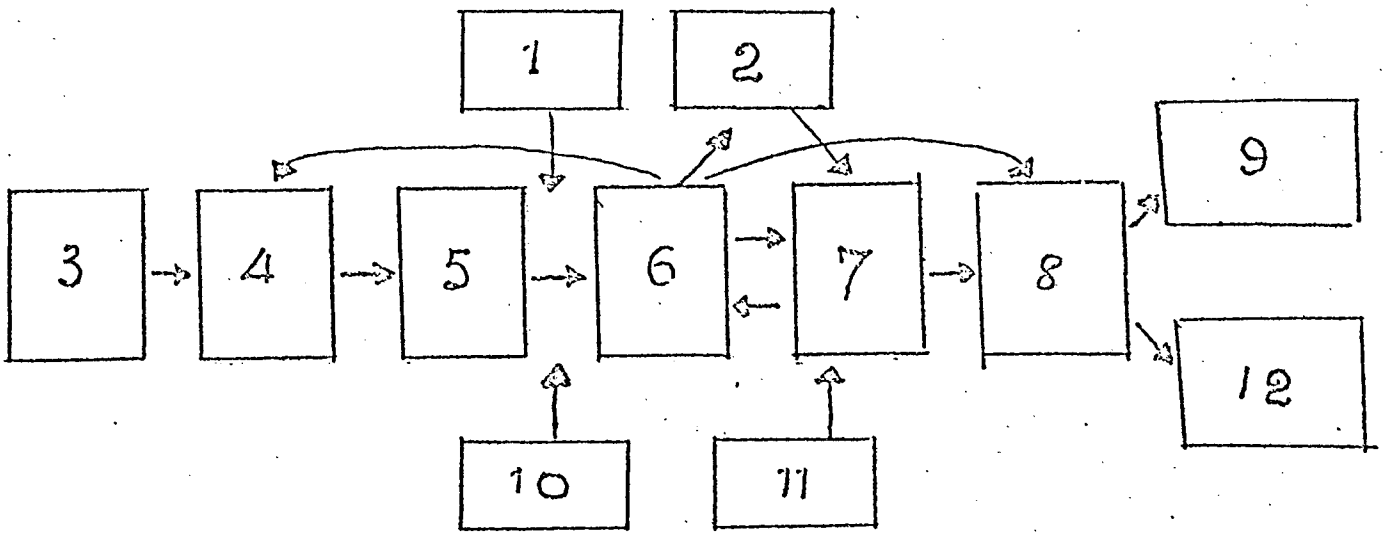
FIGURE 7.1: BREEDING METHOD AND BREEDING FISH SPECIES IMPORTANT TO AQUACULTURE

———— : nearly completed, or with substantial effect

— . — . — . : under study and with a partial effect

---------- : study commences; no effect yet

(...) : yield per year (in tons), "Agricultural Statistics" of 1976"



1. Estimation of heritability
2. Preservation of species
3. Rearing of wild species
4. Establishment of reproduction techniques
5. Study of genetic polymorphisms
6. Selection
7. Crossing
8. Cultivation of commercial species
9. Cultivation as business
10. Inducing mutations artificially
11. Permanent preservation of sperm
12. Transplanting

———— Hamachi (Yellow tail) (101,800)

———— Koi (Carp) (26,300)

———— Unagi (Eels) (26,300)

———— Salmonids (16,800)

———— Tai (6,600)

———— Ayu (5,700)

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using X-rays or Gamma-rays and they are already in practical use (87). However, concerning fish, the effect of radiation on the formation of their morphs or on the survival rates are still under investigation (88-90). The studies of these aspects concerning fish should be developed more in the future.

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other hand,, not only water temperature, but also light periodicity is related to the maturation and spawning of fish, the habitat of which is in the colder water zones. Thus, for this type of fish, it is necessary to control both the water temperature and the light periodicity.

With regard to domestic animals and agricultural crops, there are organizations for preservation, for breeding and for distribution of new lines, in addition to the lines which are already in existence, including the original species. But concerning fish, there are almost none of such organizations anywhere. Particularly, concerning the marine creatures, since their medium is the sea and it is more difficult to work in this medium and more care is required, when controlling them every day. Unless, fish lines are preserved and controlled properly, even if new populations with excellent characters can be created, they will vanish without becoming of value commercially.

If reproduction cells (sperm) can be preserved permanently a state ready for fertilization, this is more desirable than preservation of the parent fish, in order to advance breeding. Recently,also concerning fish, studies of preservation by freezing sperm have been advanced (83-86). The effects of these studies are expected soon. Also, it is considered that the development of this technique is useful not only in a cultured population, but as one of the measures for the genetic variations of wild populations, caused by changes in the environmental conditions due to artificial interference.

In the case of agricultural crops, some new species with excellent characters have already been created by inducing mutarts,

Efficient production is always demanded of all species and all the time; however, many other desirable characters vary according to the species in demand, or the changing times. Therefore, it will be important in the future to create a new population with some desirable characters on the basis of the initial data procured by world-wide analyses of genetic polymorphisms in many populations.

In order to advance breeding, subsequent generations should be checked. Generally, many fish need a longer period to reach their maturity in comparison with domestic animals or agricultural crops in the natural conditions of temperate or polar zones. This fact is responsible for considerable delays in the development of breeding studies of fish. For example, carps usually need at least 3-4 years for their maturation; thus, it takes at least 9 years to complete experiments which would cover three generations. Also, since the medium of fish cultivation is water, it is very difficult to control their cultivation throughout long periods of time. Therefore, in the study of breeding, it is very important to shorten the period of maturation. From the results of the experiments by the author, concerning carps, the following was clarified: it became possible for both male and female carps to spawn and fertilize their eggs within ten months after hatching by serial cultures at 26°C; also, mature carps can spawn eggs throughout the year by control of the temperature (not in print yet). From these results, in order to develop the breeding of fish, the habitat of which is in warm water, it is necessary to provide facilities, where water temperature, as well as spawning, can be controlled and where the young fish can be brought up to the parent stage in a shorter period of time. On the

phisms of wild populations have recently commenced. It is a prerequisite to accumulate data procured by basic study and, in the future, fish resources must be controlled in the right way.

Among the culture-populations, carps have been cultivated throughout history and are already reared as are domestic animals. Concerning rainbow trout, breeding studies recently have been developed extensively. However, concerning most of the other important species in aquaculture, only the wild species are being cultivated. Only recently, has it become possible to reproduce these species in artificial conditions.

Breeding studies have not yet commenced. In artificial conditions, it is obvious that the wild species are at a disadvantage, when compared to species selected for cultivation, as in the case of carps. Therefore, it is important to establish reproduction techniques for these species as soon as possible; then, to develop analyses of genetic polymorphisms, to improve their selection and crossing, and, finally, to create a species which can be produced effectively, even in artificial conditions. Also, more efficient reproduction will be possible by selection, even if sufficiently developed reproduction techniques are not now available.

In the case of carps, as mentioned previously, there are cultivated species with excellent genetic characters. Since these species were selected accidentally, their numbers are limited. It is presumed that fish with excellent genetic characters, but not yet discovered, must exist somewhere in nature. Thus, it is also important to keep searching for them.

breeding are present for a longer period, they tend to overpopulate and their body sizes are still small at the harvesting period. Thus this phenomenon is one of the problems to be solved in aquaculture. 100% or nearly 100% of the F_1 hybrids, resulting from crossings of different species of Tilapia, are males. It is considered that this fact is very convenient for the control of fish density in a culture-pond (60-62, 80,81). Among the various hybrids, mentioned previously by Suzuki and Fukuda (47,69,70), most of them between the Genera were sterile, or sexually neutral. Only a few fish matured. However, since their gonads were small, the numbers of eggs spawned were extremely low and the quantities of sperm were very small; and, even if their eggs were fertilized, they could not survive after the eyed-period. In these sterile hybrids, the ratio of the weight of the edible portion (the flesh without the internal organs) to the weight of the fish body is remarkably larger than the same ratio of parent species (TABLE 7.4). Also, their silvering continues throughout their entire lives and their flesh color shows a deeper pink than that of the parent species. They survive for over 10 years and their body weight reaches more than 7 kg. (82). Therefore, the first generation hybrids are more desirable as edible fish than the existing species. Furthermore, even if they are stocked back to nature, it is considered that there is no danger similar to that in the case of the hybrids between species, as has already been mentioned earlier.

5. Development in the future

Concerning fish, it is important to develop not only the breeding of cultivated populations, but also the genetic control of wild populations. Studies relative to the analyses of genetic polymor-

the hybrids of these species are nearly intermediate to both parents. However, in appearance, it is difficult to distinguish these hybrids from the parent species, from the F_2 hybrids or from the hybrids obtained by backcross breeding (70,76).

Concerning other salmonid fish, there are many reports that the survival rate of the hybrids F_2 or the hybrids by backcross breeding is much lower in the earlier period than that of the parent species (48,49,77-79). Thus, when transplanting fish, it is necessary to acquire sufficient data concerning the existence of closely related species, which may form undesirable natural hybrids, and also concerning the existence of their spawning areas.

If the F_1 hybrids of Yamato and mirror carps (Yamato-mirror), already mentioned, are stocked because of their faster growth rate and their resistance to diseases, the mirror carps with irregular scales, unpalatable to Japanese, are also produced in the wild. The following fact is even more undesirable than the case of mirror carps, viz.: carps with irregular scales, introduced to Japan in 1905, and considered to have undesirable factor N, have been stocked not only in the ponds for cultivation, but also in brooks. Subsequently, they spread everywhere in Japan. This can be called gene pollution.

4. Commercial value of sterile or monogenic hybrids

When crossing different species, depending on the combinations, sterile or monogenic hybrids F_1 can be produced. When Tilapia are cultivated in tropical areas, where water temperatures suitable for their

gametes of different species having few opportunities to link with each other because of the different distribution of closely related species and also because of their different spawning periods and spawning habits; even if fertilization takes place between the different species, the hybrids F_1 cannot survive or, if they do, they are sterile (68-70). However, if the ecological segregation systems between different species are broken by artificial causes, such as the transplantation of closely related species with different distribution, the construction of dams, or the destruction of the banks of rivers and lakes from natural causes, sometimes, hybridization can take place in nature (71,72). In Japan, such hybridization in nature took place due to a breakdown of the segregation system; this has been reported between salmonid fish; Salvelinus pluvius x Salvelinus fontinalis (73); Yamame x Amago (74); and Salvelinus pluvius x Yamame (75). The F_1 hybrids obtained by the artificial crossing of Kawamasu (brook trout) and Iwana (S. fontinalis) and also between Yamame (Cherry trout) and Amago (Biwa trout), as already described, can be bred well and produce F_2 and also hybrids by backcrossing. The hatching rates of these hybrids are much lower than those of F_1 and the parent species, and also their survival rates, after the fingerlings period, are lower than those of the parent species, as shown in FIGURE 7.3 (70).

Hybrids F_1, which show heterosis, have advantages when cultured in ponds. Stocking in nature of these hybrids is not desirable as their offspring, with a lower survival rate than the present species, might be produced in nature in the future. It is also considered not desirable not only to stock the hybrids, but also to stock brook trout in the water territory of Iwana (S. pluvius), or transplant Amago (Biwa trout) to the water territory of Yamame (Cherry trout). Morphologically,

economic advantages have been created by crossing sterlet and beluga, both of which belong to acipenser (54-57). Sterlet live in fresh water. Beluga live in the sea and return to fresh water streams for spawning. Their hybrids can adapt and live either in freshwater or salt water. Their growth rate is higher than that of sterlet and they grow well even in ponds which have poor nutrition. Female beluga take 16 years to mature; and male beluga take 12 years. However, it takes 4 years for male beluga hybrids to mature and 6-7 years for female beluga-hybrids.

With regard to other fish, the seeds of which can be collected artificially, many outbreeding experiments have been conducted. Concerning sunfish (58,59), tilapia (60-62), buffalo (63), channel catfish (64) and Moroko (65), it has been reported that the hybrids F_1 showed heterosis and that they grow faster and larger than the parent species. Purdom (66) obtained triploids by subjecting the hybrid eggs between Hirame and Karei to low temperatures. Their growth rate was faster than that of diploids.

3. Transplantation, stocking and wild-hybrids

The increase in the yield of Ayu and Salmonids is attributed to their transplantation. However, sometimes hybrids are formed in the wild by transplantation and they affect wild populations undesirably. It is usual in the wild for there to be geographical or breeding segregation systems among the different species of fish. Thus, it is not natural for them to have hybrids (67). Also, in the case of fish, there are obstacles to the formation of natural hybrids due to the

Terao (48) crossed salmon and Himemasu and released their F_1 into the Shkotsu Lake. The F_1 grew faster than Himemasu, but the hatching rate and the floating rate of F_2 and also of the hybrids obtained by back-crossings were much lower than those of F_1, or their parents. It takes 5-6 years for lake trout in Canada to mature. During this period, they are subject to great damage by Petromyzoniformes. Splake trout, a hybrid between lake trout and speckled trout (brook trout) which have a shorter maturation period, but mature in three years when they are cultured in ponds. They grow well in lakes and are recommended in place of lake trout (49,50).

2.4 Other fish

Yellow tail, eels, Ayu (Plecoglossus) and Tai are cultivated in large numbers in Japan and they are important species for aquaculture. Among these, the reproduction techniques for Ayu and Tai have been developed in recent years. With regard to Yellow tail and Eels, the yields of which are the largest or next to being the largest, the techniques for their reproduction have not been developed yet (FIGURE 7.1), and only the wild types, caught in their natural surroundings, have been cultured. For these fish, the breeding is presently impossible. Yields of Funa (Carassius) are rather small compared to those of other fish; however, several variants are used at present, such as Kinbuna (C. auratus subsp.), Ginbuna (C. a. langsdorfii) and Gengorobuna (C. a. cuvieri) (53).

Recently, in the USSR, the natural environments of some fish have been destroyed by the construction of power plants. In order to obtain new species which can adapt to new environments, hybrids with

fully observed. In recent years, the demands for species originally bred in Japan have increased. When these species are cultivated in ponds, the growth rate is very low compared to that of rainbow trout at large, and also they are susceptible to diseases. Suzuki and Fukuda (46,47) crossed 62 combinations of Salmonids, bred in Japan and abroad, in order to improve their characters genetically. Hybrid F_1 by these crossings were cultivated until they reached 3 years and 9 months of age, in constant conditions of water temperature, flow speed of water, oxygen content, amount of food for consumption and the density of fish. At least 9 combinations of these crossings showed heterosis and were less susceptible to specific diseases; also, their survival rates were higher than those of any parent species, after the period of fingerlings (TABLE 7.3). The growth rate was either faster than that of the parents or equal. For example, Iwana (Salvelinus pluvius) bred in Japan, has wild characters, is difficult to adapt to artificial feeding and their growth rate is slow. But they are resistant to diseases and their survival rate is relatively high. On the other hand, Kawamasu (brook trout which are similar to Iwana by classification and morphologically, adapt easily to artificial feeding, grow faster than Iwana, but are susceptible to diseases. Hybrids F_1 obtained by crossing Iwana and Kawamasu showed remarkable resistance to diseases. Their survival rate was higher than that of both parents, they adapted easily to artificial feeding and they grew much faster than Iwana. The fact that the heterozygous fish show a stronger resistance to specific diseases suggests that the susceptibility to diseases is a recessive inheritance, similar to the case of the hybrids of carps, previously described. These hybrids F_1 may have more advantages when cultivated in ponds, compared to the species already in existence.

Ever since rainbow trout were imported for the first time to Japan in 1907, the spawning time has become earlier. At the Freshwater Regional Fishery Research Laboratory at Nikko, the spawning showed the maximum at the end of March in the year of 1911, but it showed the maximum at the beginning of December in the year of 1964 (41). A simila phenomenon has been observed at the Ooizumi Experimental Laboratory of the Tokyo University of Fisheries (42). This change is probably due to the result of selection, when fish obtained from eggs, spawned early every year, were used as the parental fish for the following generation. Also, since among rainbow trout of the same age, the fish of larger size mature earlier (43), their eggs, spawned earlier, hatch earlier than those spawned at a later period. The fingerlings start their feeding earlier and have grown larger by the time they reach their spawning period. Thus, their spawning period commences earlier.

Considering these observations, the phenomenon of spawning at an earlier time is due to environmental factors which affect the growth of trout, such as the types and availability of food, the quality of the water and the water temperature, in addition to the genetic factors. A similar phenomenon - an early maturation - is known to occur to Masunosuke, by crossing the fish which matured early (44).

Crossings between different species of Salmonid have been performed in Europe, USA and Japan (Suzuki, 45). However, there have been only a few extensive studies of the cases when F_1 and their offspring have been kept under the same conditions as the parent species for a long period of time and when the economically efficient characters such as the growth rate and the resistance to diseases, have been care-

of Salmonids became possible. However, their breeding is still in a primitive stage compared with that of carp, even in the case of rainbow trout, which are being cultivated most extensively at present.

Concerning rainbow trout, the characters required in them have been until now: an increase in the egg numbers spawned, earlier maturation, earlier collection of eggs, better metabolic efficiency and a higher growth rate. Lewis (37), by selection from the ordinary rainbow trout, obtained a line which spawns in November [this trout normally spawns early in the spring]. At the 2nd year of age, the spawning rate of this new line was 98% compared to 53% for the parental line. Also, at the second year of age, the number of eggs spawned increased to 1,693 from 723 in the parental line. Millenbach (38) also obtained a line which showed a higher spawning rate at the second year of age. He observed that, when these trout were released into streams, the time of maturation overlapped the start of the fishing season and the color of these fish turned blackish; however, anglers prefer rather immature fish but with the silver-white color. For 23 years, Donaldson and Olson (39) and Donaldson (40) have been selecting rainbow trout which come up the river inside the campus of the University of Washington. In 1944, the average length of these two years old trout was 36.3 cm., the largest being 39.0 cm.; the numbers of eggs spawned was 1,653, the largest number being 2,121. The length of the fish and the numbers of eggs spawned increased every year. In 1968, the length of two years old trout reached 60.4 cm. on the average (the largest being 66.0 cm.); and the number of eggs spawned increased to 9,259 on the average (the largest number being 18,144) (TABLE 7.2).

they are unattractive to Japanese because of their irregular lining of scales; they are difficult to cook because of their greater body height; they are easy to catch but have low pulling strength, etc.(36).

Assuming that, among the various species of carps, species A has a favorable character of dominant inheritance and an unfavorable character of recessive inheritance; also assuming that species B has a different favorable character of dominant inheritance and a different character of recessive inheritance, by crossing A and B, it may be possible to produce hybrids F_1 with both favorable characters and without both unfavorable characters. On this assumption, Suzuki and Yamaguchi (34) tried 9 crossings among the species described above. Hybrid F_1 (Yamato x Mirror) between female Yamato carp and male mirror carp; also hybrid F_1 (Yamato x German-scaled carp) between female Yamato and male German-scaled carp, showed heterosis. In particular, heterosis was remarkable in the first crossing. This hybrid shows a better gain from food consumption and a higher growth rate than not only Yamoto carp but also the mirror carp (TABLE 7.1, FIGURE 7.2). Also, this hybrid is resistant to specific diseases and has regular scales. Thus, this species can be recommended for cultivation in the future. F_2 of this hybrid has a similar growth rate, comparable to F_1, but is more susceptible to diseases and has lower metabolic efficiency. Also, F_2 is divided into scaled carp and mirror carp in the ratio of 3 to 1. Therefore, only F_1 is desirable for use as food.

2.3 Salmonids

Compared to carps, the cultivation of Salmonids has a rather short history. It was only after the 17th century that, the breeding

hybrid is adopted in many culture grounds. When ropsha carps are inbred, their resistance to disease is decreased; but when they are crossed with carps bred in the Ukraine, a higher growth rate and strong resistance to diseases are shown as the result; the survival rate is then also higher.

Studies concerning selection and crossings have mainly been directed to growth rates or immunity to diseases. However, no research appears to have been done on the food efficiency. Culturing carps in Japan is effected mostly through feeding and the aspects of their high rate of growthand metabolic efficiency are of importance. Yamato-carps and Asagi-carps, both of which are Japanese breeds; the wild and scaled carps, which are German breeds; and the mirror carps were cultivated in various environments and compared by Suzuki and Yamaguchi (34) and by Suzuki et al. (35). In all environments, both the metabolic efficiency and the growth rates were in the following order: mirror carp > scaled carp > Yamato carp > Asagi carp > wild carp. The mirror carps are the best species with regard to the metabolic efficiency and growth rates. On the other hand, wild carps, considered to be the original species of carps, showed the lowest values in both rates. When cultivated in running water at 25°C, the increase in the weight of the mirror carp was 7.3 times that of the wild carps and the metabolic efficiency was 1.6 times that of wild carps (TABLE 7.1). Also, the survival rate of wild carps is low under artificial conditions and, generally, it is difficult to spawn them. These facts show that, when cultivating fish in artificial environments, the wild types have a greater disadvantage than the other selected species. However, the mirror carps have also many disadvantages, i.e.: their susceptibility to diseases (35)

was not selected, was 30 - 81%; while the mortality of the offspring of the selected group was only 2 - 15%. Prokhorchik (20), by selection of cultured carps bred in the Beloruss Republic, obtained carp lines with higher growth rates, higher hibernation rates and higher yields per hectare than those of the originally cultured carps; also higher than those of mirror carps.

When fewer numbers of parents are used in repetitive selections, more of the offspring become homozygous. Therefore, it is considered that there is a risk that inferior, unwanted characters will become phenotypes. In the second generation of offspring from a pair of carp parents, the increase in weight is lower by 10-20% than that of the firs generation; also, the survival rate is lower, but the abnormally-shaped carps increase. However, there are cases when heterosis is caused by outbreeding (21,22). Carps have intermuscular bones. These bones are disliked by man. Recently, using X-rays, the relationship between the numbers of intermuscular bones and the fish variations, also between the numbers of back bones and the intermuscular bones have been studied (23,24). However, the creation of species with fewer bones has not yet been achieved.

There have been many cases of heterosis in the growth or in the resistance to disease, when carps of different species have been crossed frequently (25-33). Kirpichnikov and his colleagues (29,30) reported that: the F_1 hybrid (ropsha carp) between a mirror carp bred in Poland and a wild carp bred in the Amur River (a strong river with low temperatures) shows a high growth rate under severe weather conditions in Russia, as well as high resistance to "Bauchwassersucht". Thus, this

they grew faster than the Japanese breeds. Also, it has been reported that the hybrids F_1 resulting from crossing the German and Japanese breeds, grew excellently (14, 15). Thereafter, hybrids of these two breeds were distributed everywhere and, even now, the characteristics of the leather carps, mirror carps and line carps are found in the edible carps and Nishiki carps. Such carps with irregular scales are not favored as food by Japanese, who have thought since the olden times that carps should have scales. It is doubtful whether the carps which were transplanted and called leather carps and mirror carps, were the same species as the carps described previously. However, since both the leather and line carps are found at present, it is certain that the species with the N factor, disadvantageous to cultivation, must have been transplanted.

The body heights of the cultured species bred in Europe are higher in relation to their body lengths, than is the case with other species. This phenomenon is considered a genetic character (16,17). However, since the body height is easily affected by environmental factors and there is no correlationship between the body height and the growth rate, it is considered a risk to use the body height as a selection-indicator (18,19).

Apart from breeding carps by actual experience, the selection studies of crossings have also been followed intensely, particularly since World War II. Schaperclaus (18) injected into carps the live virus of "Bauchwassersucht", which was causing great damage to carps in the European culture-grounds every year; he then selected the carps which were not infected by this disease. The mortality of the group,which

the weight, the heritability became extremely low (0.10-0.15). It is said that mirror carps will not be affected now by selection (7,8).

In Europe, carps with fewer scales have been preferred for cooking and selection has been made for this purpose. Carps are divided into the following 4 groups, according to the types of scales they have. The first group is a scaled carp with scales over its whole body; the second group is a line carp with one line of scales along each side laterally and one line of scales on both sides of the dorsal fin; the third group is a mirror carp with large-sized scales only on the basal part of its dorsal fins and on a portion of its tail base; and the fourth group is a leather carp which scarcely has any scales on its body surface. These types are controlled by two different factors. If we assume that the factor related to the appearance of scales is S and the factor related to the lack of scales is N; then S is dominant to N and is inherited according to Mendel's Law (9). Factoors of a scaled carp comprise nnSS or nnSs; in the case of a line carp, NnSS or NnSs is true; in the case of a leather carp, Nnss; and in the case of a mirror carp, nnss. The NNSS, NNSs, NNss are lethal and fish with these scales cannot survive.

Of the above four types, the mirror carp and the scaled carp show the highest growth and survival rates. Mirror carps particularly are recommended as species for cultivation in Europe (10,11,12,13).

In 1905, 4 female leather carps and 1 male mirror carp, bred in the Eisch River in Germany, were transplanted to Japan for the first time. These carps with irregular scales were called "German carps" and

mercial species are created in the above manner. Today, these methods have been systematized due to well developed scholastic studies, even in the field of agriculture. Many of the present species used in cultivation and breeding were created by our ancestors, who gained their expererience by repetitious selection and crossings, quickly detecting spontaneous mutants (an accidental product) by careful daily observations, and then culturing these mutants. Recently, in the field of agriculture, the following were also considered to be important breeding methods: after inducing mutations artificially, populations with desirable characters are created from many variants; sperm from populations with specific characters are preserved permanently and used when required. Using such methods, the breeding of cultured populations of fish is now reviewed in the following.

2.2 Carps

In the past, for people who lived inland and could not obtain marine products easily, carps were an important source of animal protein. These fish have been cultivated since ancient times and, among fish, the method of breeding them has advanced most. The origin of carps is in Central Asia. It is said that, in China, carps were cultivated 2,400 years ago. At first, wild carps were caught and cultivated, and the reproduction techniques have also been long established. In the long history of breeding, our ancestors developed the breeding of fish species by repeated selection, as they did with domestic animals. At present, throughout the entire world, many local variants of species are known.

Among these, the breeding of mirror carp has been advanced most. As the result of the selective policy for populations to increase

Thus, each genetic polymorphism of populations has to be analyzed from all points of view. Then, on the basis of the data of these analyses, creation of a new population with the desired characters, responding to requirements, is the objective of actual breeding cultured populations.

The methods of breeding such populations do not differ greatly from the methods which have produced excellent results in the field of agriculture. As shown in FIGURE 7.1, the start of culturing wild creatures is followed by establishment of the reproduction techniques which are suitable for them in the artificial environment. The genetic polymorphisms of each culture-population are analyzed and a new population with desirable characters is created by selective breeding. This new population could become a commercially valuable species directly. Furthermore, two different populations are crossed in order to give the required characters of both populations to their offspring; or, in order not to have the undesirable characters in their offspring, but presently found either in both populations or in one of them. If these goals can be achieved successfully, commercially useful species can be created. Also, sometimes, hybrids F_1 of different species may be either sterile or monogenic. These phenomena are sometimes adopted in the commercial fields. When new populations with desirable characters are created by crossing, they are distributed immediately; the individuals with every possible combination of the various genetic structures, isolated from the F_2 generation, are then cultured.

Most of them are heterozygotic. From them, fish with desirable characters are selected and the F_3 lines are established. Then, the genes become homozygous, caused by continued selective breeding. Com-

1. Genetic control of wild and semi-wild populations

The fish yield from wild populations, which are not controlled, is far greater than the yield from cultured populations. Therefore, it is necessary to increase wild populations with specific genetic characters by protecting them or transplanting them; while it is also necessary to increase the catches of fish populations with disadvantageous characters. Once a wild population is controlled by man, it is not a purely wild population anymore. However, for convenience, such populations are distinguished from cultured populations, which are controlled by man throughout almost their entire life.

Since early in this century, in order to identify wild populations and local groups and also in order to clarify the reproduction resources, mostly morphological and ecological studies have been developed on the basis of data on the resources. However, studies of wild populations are fewer, when compared with studies of cultured populations. Recently genetic studies have been developed of the populations of: tuna (1), cod (2,3,4) and salmon (5,6). Due to the advancement in biochemical techniques, evaluations of genes or genotypes of wild populations are being made intensively, using isozymes.

2. Breeding of cultured populations

2.1 Purpose and methods of breeding

Breeding is the creation of a biological population with genetic characters which are desirable to man, who then grows and increases it. Therefore, breeding is closely related to industry. The required characters vary, depending on the kinds of creatures and the changing times.

ARTICLE 7

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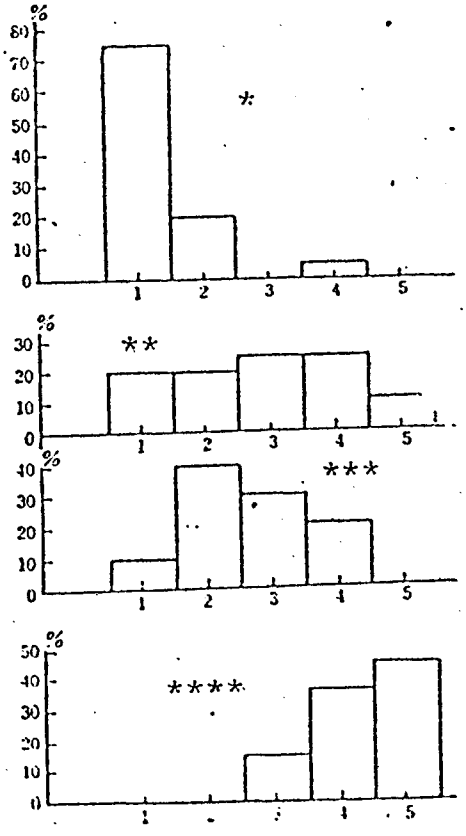
F I S H

At the time when "civilization" had not been developed, it was never thought that man could control fish, except specific species, like carps. Today, fish are in demand not only for man's food, but also as food for domestic animals or as food for culturing fish. Fish have been overcaught due to the great progress in fishing techniques and the natural breeding grounds of fish have been destroyed due to the environmental deterioration. Therefore, the natural resources for fish are decreasing. Having in mind the varieties of food habits, this also applies to sport-fishing, a stabilized production of fish by cultivating various species is indeed needed. Therefore, as fish resources are being depleted, it is necessary to develop a systematic method of control, based on a genetic analysis of wild populations. Moreover, in order to produce fish populations with utmost efficiency, using cultivation and not just trying to improve its techniques, it is important to create new populations with all the advantages of genetic improvement of the species. Consequently, the present state of fish breeding and the problems involved with fish studies for the future are reviewed and discussed in this paper on the basis of the aspects referred to above.

FIGURE 6.5 SHELL COLOR OF THE FIRST GENERATION OF PURE-BRED LINES OF C. GIGAS, BRED IN HOKKAIDO AND KUMAMOTO, AND THE FIRST GENERATION OF THEIR HYBRIDS

(Imai and Sakai,1)

1: nearly whole shell is white
2: partially black
3: about one half is black
4: mostly black
5: whole shell is black



* Hokkaido G_1

** Hokkaido x Kumamoto F_1

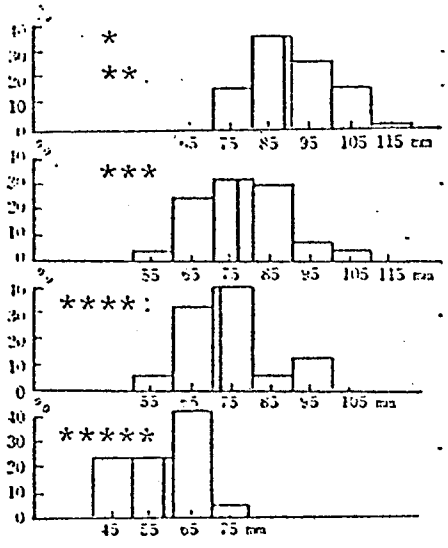
*** Kumamoto x Hokkaido F_1

**** Kumamoto G_1

Shell color

FIGURE 6.4: DISTRIBUTION OF THE SHELL LENGTHS OF THE THIRD GENERATION OF PURE-BRED LINES OF C. GIGAS, BRED IN HOKKAIDO AND HIROSHIMA, AND THE FIRST GENERATION OF THE HYBRIDS BETWEEN THE HOKKAIDO AND HIROSHIMA BREEDS

(Imai and Sakai, 1)



. * Hokkaido G_3
. **Average value
. ***F_1 Hokkaido x Hiroshim
. ****F_1 Hiroshima x Hokkai
. *****Hiroshima G_3

FIGURE 6.3: GENOTYPE AND WHOLE BODY WEIGHT OF C. VIRGINICA

Left figures show the average weight of shells with 1,2,3 or 4 heterozygotes in 4 gene loci, relating to Est - 3, PGI, LAP and PGM.

Right figures show the genotype of AAT-1 and the weight.

L: Groups, the whole body weight of which is below 1 g.

H: Groups, the whole body weight of which is over 4 g.

Numbers show the numbers of shells

(Singh and Zouros, 21)

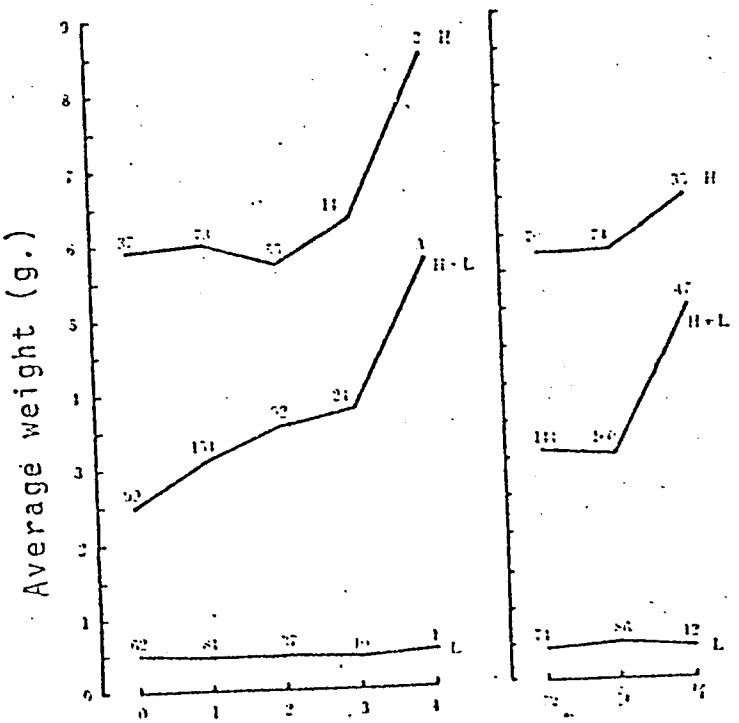


FIGURE 6.2: DEVIATIONS OBTAINED FROM THE EXPECTED VALUES OF HETEROZYGOTE FREQUENCIES IN C. VIRGINICA, DIVIDED INTO 4 GROUPS (<1 g., 4-6 g., 6-8 g., >8 g.), ACCORDING TO THE WHOLE BODY WEIGHT

(Singh and Zouros, 21)

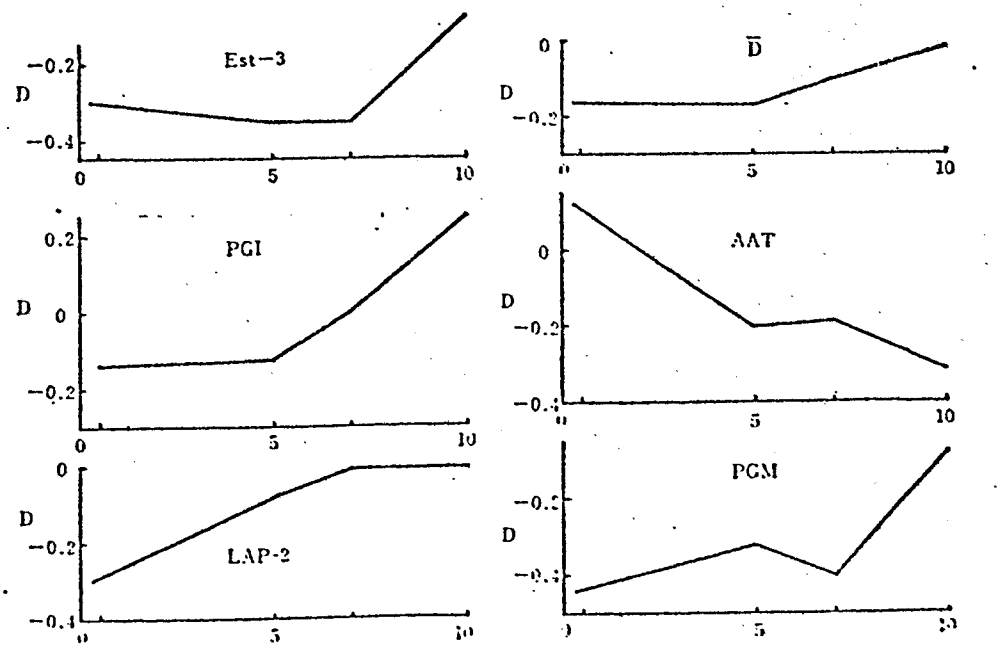


FIGURE 6.1: DISTRIBUTION OF ALLELOMORPHS IN THE POLYMORPHIC GENE LOCI RELATING TO THE ENZYMES OF BIVALVES AND SNAILS FOUND ALONG THE COASTS.

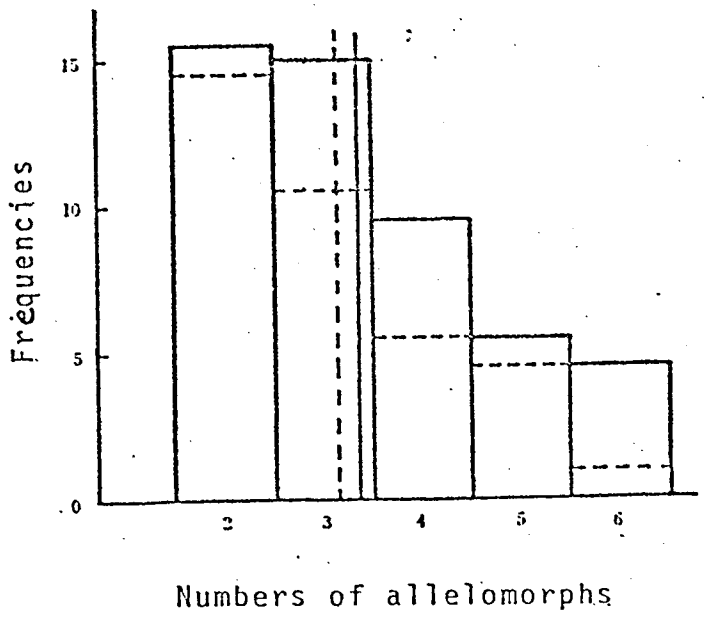


TABLE 6.11: EXPECTED VALUES OF HERITABILITY AND THE EXPECTED SELECTIVE EFFECT OF SHELL CHARACTER OF PINCTADA BY ANALYSIS OF VARIANCES

(Wada, 25)

Characters	Years	Heritability ± standard error (1)	Expected selective effect (2)	Average phenotype values	Rate of expected effect (%)
Shell height (SH)	1	.32±.29	1.25mm	24.4mm	5.1
"	2	1.05±.76	8.25mm	43.3mm	19.1
Hinge line length (HL)	1	.05±.08	.20mm	28.7mm	.7
"	2	.63±.47	4.41mm	46.0mm	9.6
Shell width (SW)	1	.36±.32	.52mm	8.1mm	6.4
"	2	.81±.52	2.13mm	16.9mm	12.6
SH+HL+SW	1	.15±.16	1.28mm	61.1mm	2.1
"	2	.90±.52	15.00mm	106.2mm	14.1
SH/(SH+HL+SW)	1	.58±.43	.015	.400	3.8
"	2	.70±.49	.014	.403	3.4
HL/(SH+HL+SW)	1	.57±.43	.016	.469	3.4
"	2	.57±.46	.014	.432	3.2
SW/(SH+HL+SW)	1	.49±.39	.006	.132	4.5
"	2	.42±.39	.004	.160	2.5
Wet weight of shell		1.09±.52	3.86g	8.12g	47.5
Dry weight of shell		1.07±.52	3.07g	6.82g	45.0
Color of pearly layer (C.I.E.)	x	.63±.48	.018	.304	5.9
	y	.22±.26	.007	.308	2.3

TABLE 6.10: EXPECTED VALUES OF HERITABILITY AND THE EXPECTED SELECTIVE EFFECT OF CHARACTERS OF C. GIGAS BY ANALYSIS OF VARIANCE

(Lannan, 24)

	Heritability ± standard error	Expected selective effect	Parent generation, Average phenotype value	Rate of expected effect
Shell width (W)	.81±.07	11.1mm	33.7mm	33%
Shell height(H)	1.17±.05	8.7mm	10.7mm	81
Shell length(L)	.81±.27	18.9mm	52.1mm	36
L+W+H.....	.93±.28	36.3mm	96.5mm	38
L/H.....	.31±.18	0.76	.674	11
Whole body weight (TW)	.33±.19	7.9g	29.7g	27
Soft body weight (MW)	.37±.20	2.9g	6.3g	32
MW/TW.....	.46±.22	0.22	.210	10
Larvae survival rate	.31±.06	.31%	3.51%	9
Settling rate	.09±.08	.02%	4.45%	0.5

TABLE 7.4: COMPARISON OF EDIBLE PORTIONS OF F_1 HYBRIDS OF SALMONIDS

Parent species and F_1	Number of fish	Average body weight (g.)	Average weight of internal organs (g.)	Average weight of reproductive gonads (g.)	(Body weight - weight of internal organs)/body weight (%)
Kawamasu.....	f 23*	813.9±85.4**	186.0±17.9**	157.8±16.1**	76.9±1.6**
Iwana.....	f 21	476.9±39.2	99.7±11.2	85.6±11.4	79.0±1.9
Brown trout.....	f 25	434.7±30.8	89.2±8.2	76.1±7.8	79.5±1.2
Sakura trout.....	f 25	311.6±30.5	57.5±4.2	51.2±3.8	81.1±1.1
Hime trout.....	f 19	562.8±25.0	146.3±10.6	91.8±5.2	74.0±1.4
Kawa-sakura.....	f 23	321.2±58.2	23.6±4.7	5.1±3.1	92.4±1.1 (P<0.001)
Kawa-hime.....	f 16	458.4±79.0	35.6±9.1	7.9±8.6	92.1±1.5 (P<0.001)
	fm 2	649.0	45.2	0	93.0
Tiger trout.....	f 24	505.4±46.1	34.4±3.3	0.5±0.1	93.2±0.2 (P<0.001)
Bra-iwa.....	f 20	451.1±22.8	34.0±3.8	0.4±0.1	92.5±0.6 (P<0.001)
Bra-sakura.....	f 19	436.8±73.2	29.1±5.8	2.6±2.1	93.5±0.4 (P<0.001)
	fm 6	261.5±138.2	16.2±7.5	0	93.8±1.8

* f is female
fm is neutral

** 95% reliable limit

QUESTIONS, ANSWERS AND GENERAL DISCUSSION

Chairmen: S. Sudo
Tohoku University, Department of Agriculture
Y. Nose
Tokyo University , Department of Agriculture
K. Fujino
Kitazato University, Department of Fisheries

Y. Taniguchi (University of Kochi, Department of Agriculture):
According to data on carps by Moav, selections, performed in order to increase the growth rate, showed negative results. What was the cause of these results? Was it caused by inbreeding?

K. Wada (Pearl Research Laboratory):
As the result of natural selection during the evolutionary process and also as the result of the artificial selection in the breeding process throughout the long history in Europe, the increase in the growth rate reached its limit. The manner of breeding was devised not to have inbreeding. However, it is not certain whether inbreeding was actually avoided or not.

K. Suzuki (Freshwater District Fisheries Research Laboratory):
The chromosomal numbers of carps or Carassius vary depending on the use of either Osmic Acid or tissue culture methods. How does it concern the cases of other species of fish?

Y. Ojima (Kansaigakuin University, Department of Science):
Some showed the same numbers, using both methods, but others showed a little difference. (1)Chromosomes were observed, using mashing way. Why air-dry method was not used? (2) It is necessary to show the normal karyotype of fish, used for the experiments, as a control. What do you think about this? (3) Is pollution in New York Bay still getting worse? What about the values of COD and BOD?

Sudo (Tohoku University, Department of Agriculture): (1) Is the quantity of phycoerythrin, contained in the green and yellow types of Nori, less than that in the wild type, or there is none at all?

(2): Also, did you observe the absorbance of phycoerythrin, extracted and purified?

Miura:
(1) The quantity of phycoerythrin, contained in these types of Nori, is small. (2) Mr. Ashida of Tokyo University is now working on these absorbances.

Y. Nose (Tokyo University, Department of Agriculture):
When you obtain the seeds of Nori, how much attention do you give to the resistance of Nori, as mentioned previously?

Miura:
Presently, this is not of great interest. However, this problem is actually being studied in some districts.

K. Fujino: (Kitazato University, Department of Fisheries):
The day before yesterday (October 7), Mr. Sudo mentioned the differences of the gene frequencies of the isozymes, depending on the regions where Nori were produced. Are there similar differences among the color polymorphisms, which Mr. Miura studied?
Sudo: We now have a plan to study this and the result is expected soon.
Miura:
Concerning the biochemical field, presently Mr. Ashida of Tokyo University is studying this. We can get accurate results later.

Sudo:
How to breed agar which are diploid and have many chromosomes?. (Such as Wakame, excluding Nori).

Miura:
At present, selective breeding might be the most effective. On the other hand, since the exchange of combinations and crossings takesplace in

Nori, which is half-diploid and with few chromosomes, it is possible to create mutants by crossing them.

Fujino:
Please explain the causes of the excess of homozygotes in many gene loci of various aquacreatures, as shown by the slide-pictures.

K. Numachi (Tokyo University, Institute of Oceanography):
The following causes are considered: (1) errors at the collection stage; (2) existence of zero, or Null, genes ; (3) selection based on the difference of adaptive values among the genotypes; (4) Wahlund effect due to the structure of sub-populations; and (5) inbreeding.

Nose:
As is the case of abalone, studied by Fujino, heterozygotes of isozyme polymorphisms of oysters showed high growth and survival rates. Is it easy to create many young shells with these genetic constituents?

Numachi:
In order to discover the enzymes related to the growth and survival rates, such as AAT-I in oysters, there is no other way but by repeated experiments. Having accumulated the data of basic studies, the following generations are then checked.

Fujino:
When the characteristics of polymorphisms of specific enzymes, such as the heterozygote dominance, are clarified, young shells with these genotypes can be easily created in abundance. For example, presently, in the case of Ezoawabi, in order to analyze the polymorphisms of enzymes, it is impossible to get small pieces of body tissues without sacrificing the animals used for experiments.
However, the following manner can be recommended. For instance, the frequency of polymorphisms of a wild population, bred in Ekiko area of Iwate Prefecture, has been clarified. Therefore, when males and females are obtained from this wild population at random, the probability of creation of desirable combinations can be predicted.
From the analyses of the offspring, produced by these combinations, the

types of parent species used for crossings can be found. Then, the seeds, which will produce undesirable combinations, can be eliminated, but the crossings of desirable combinations will then be repeated. Also, a better way to develop the technique of collecting tissue pieces is to use electric knives and anesthesia, without sacrificing animals.

Suzuki:
There are some cases when chromosomal numbers vary in the same species of oysters. Are their phenotypes different, depending on their chromosomal numbers?

Numachi:
Chromosomal polymorphisms are found not in oysters, but in Purpura lapillus. Their forms vary, depending on the chromosomal numbers.

Fujino:
(1) can F_1 hybrid, obtained between species, be used in nature?

(2) Generally, is it difficult to succeed in creating F_2 hybrids, obtained between species, and hybrids by back-crossing? For example, in Sendai Bay, hybrids between Ishi-garei and Kawa-garei can be found, but inducing of genes of Ishi-garei to the population of Kawa-garei can scarcely be found.

Suzuki:
(1) Transplantation and stocking is useful in the water districts without the species which have already existed and can create wild hybrids, such as lakes and rivers without any areas for spawning.
(2) Generally, since in many cases the F_1 hybrids, obtained between different species, are infertile, or their F_2 cannot survive, their *ever offspring/are hardly *created. However, in the case of Kawamasu (river trout) and Iwana, it is possible to produce them. Many fish, which are considered to be the hybrids between both of these, are found in the rivers in Japan.

T. Harada (Kinki University, Department of Agriculture):
There was a mention that no breeding studies of Hamachi (yellow tail) have been made. However, the F_1 hybrids were produced by us by crossing

Hamachi and Hiramasa; Hamachi and Kanpachi; and Kanpachi and Hiramasa. Also, the F_2 and hybrids by back-crossing were obtained. Similar experiments, using Madai and Ishidai, are being developed. Some results have been reported at the Meetings of the Japan Fishery Society.

Kato (Freshwater Region Fisheries, Research Laboratory):
Selection of useful characters of rainbow trout was performed and their inbreeding was reported throughout 2-3 generations. It is considered that the effectiveness of selection was proved. At present, concerning the egg numbers and the diameter of eggs, no inbreeding depression was shown. However, since there is no theoretical basis for these selections, it is considered improper to repeat the inbreeding, in order to keep these characters improved. When repeating the inbreeding of only excellent individuals, the inbreeding depression might be prevented.

Fujino:
It is considered that the inbreeding depression is due to the appearance of heterozygotes between the inferior, harmful genes and the superior, normal genes; they act as carriers (phenotype is normal) in populations; and the appearance of heterozygotes is caused by the increase of homozygotes due to inbreeding.
Concerning specific characters, if no inferior harmful genes, causing inbreeding depression, exist originally in the population, or if they are eliminated by artificial selection, no inbreeding depression takes place in this specific character. A book concerning artificial inbreeding as a breeding technique has been written. As an actual example, please refer to a series of reports concerning inbreeding and selection using rainbow trout (Institute of Fish Genetics, State Department, USA) (28,29).

Wada:
Some animals (mice) are strong with regard to inbreeding, but others (chickens) are weak. These mechanisms are not clarified. In actual breeding, this phenomenon has to be considered.

Fujino:
As mentioned previously, the appearance or non-appearance of the inbreeding depression concerning specific characters depend upon whether:

inferior,harmful genes, which are allelomorphs related to the appearance of these characters, are included in the population or not. Their existence can be predicted on the basis of prior investigations of the "foundation stock" of breeding resources.

The polymorphisms of enzymes are used differently, depending upon whether it is the case with adaptive values or a neutral case. Therefore, concerning any species of creatures, is there a general way to classify and organize all enzymes into either types?

Whether the genetic variations, including polymorphisms of enzymes show the neutral or adaptive values, depends on the correlation between the genetic background of the population and the environmental conditions of the area where this particular population is distributed. Whether or not the adaptive values are present in a specific polymorphism can be judged from the relative data on specific wild populations and on artificial populations. However, concerning the same polymorphism, same studies have to be done of each wild population. It is impossible to predict the types of polymorphism of different enzymes. Presently, the volume of data on this aspect is insufficient to develop general methods of classification and organization.

When reviewing the speeches, presented today, questions, answers and general discussion, the main field of genetics and breeding studies of aquaculture are divided into the following two parts. The first part is genetic control of the creatures, the seeds of which can be produced by aquaculture. The second part covers the genetic control of wild populations. In the first case, species, used for aquaculture, are studied. The theory and manner, which have been developed for flowers, trees, agricultural crops and husbandry animals, are mostly being adopted. Since the numbers of species of aquatic creatures are large and their evolutionary degrees vary, it is important to organize the subjects and to establish the correct goals for the studies. These relate to either the "Resources of breeding" or "Breeding techniques".

In order to accomplish this task, theories and methods are needed for the following: morphology of creatures with important performance traits; quantitative genetics; studies of physiological characteristics; population genetics; studies of biochemical characteristics; and cytogenetics.

Concerning the second case, the study by A.C. Longwell about the effects on wild populations by water pollution, dealing with the aquatic resources, is quite unique. Also, as pointed out by K. Suzuki, the possibility of effects on the species already in existence by transplantations and stocking have to be taken into consideration.

At this Meeting, the problems concerning preservation of genetic resources were scarcely presented or discussed. It is clear that these problems have to be studied in conjunction with advances in the breeding studies and their applications. Concerning this subject, the aquatic creatures have been divided into three groups:

(1) creatures useful for the culture;
(2) wild or semi-wild creatures; and
(3) creatures used for experiments.

The problems concerning these creatures have already been organized*, and the discussion about them is expected in the future.

[*K. Fujino: Preservation method of genetic resources concerning aquatic creatures. Aquaculture, 3, 17-21 (1978).]