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The role of balancing selection and overdominance in maintaining allozyme polymorphism

Yu. P. Altukhov
Vavilov General Genetics Institute, U.S.S.R. Academy of Sciences, Gubkin Str., 3, 117809 GSP-1 Moscow, U.S.S.R.

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Abstract

Three approaches to the estimation of the role of balancing selection in maintaining allozyme polymorphism are considered: 1) Analysis of the stationary distributions of allelic frequencies in a native subdivided population; 2) Comparison of the genotypic distributions at the early and late developmental stages in successive generations of the same population; 3) Analysis of the 'joint' variability of monogenic and polygenic traits.

The conclusion is drawn that allozyme polymorphism must not be regarded as a transient phase of molecular evolution but as its stationary phase. The mechanisms responsible for supporting such stability are discussed.

Introduction

Biochemical population genetics originated from the contributions of Lewontin and Hubby (1966) 25 years ago. They were the first to use protein electrophoresis for evaluating the amount of genetic variation in the natural populations of *Drosophila*. It was discovered that in many populations polymorphisms are present at more than one third of the enzyme encoding loci surveyed, and that the mean individual heterozygosity at these loci is about 12%. At the same time, similar observations were obtained by Harris (1966) for human populations. Because the electrophoretic analysis reveals only half of all single amino substitutions in a polypeptide chain (Neel *et al.*, 1986), some authors believe that the level of genetic variation might be even greater. Since these initial studies, the number of publications in this field has been continuously growing (see Nevo *et al.*, 1984). The debate as to which of the main factors of micro-evolutionary dynamics, random genetic drift or natural selection, is responsible for maintaining such considerable molecular polymorphism is not yet over. If one assumes that the observed polymorphism of proteins is maintained by an adaptive advantage of heterozygotes or some other form of balancing selection, accepting the multiplicative fitness model, then such a great number of polymorphic loci should produce natural populations carrying a large segregational genetic load (Kimura & Ohta, 1971).

In that context, Kimura (1968) proposed an alternative hypothesis, according to which protein polymorphism is selectively neutral and reflects only a transient phase of molecular evolution due to random genetic drift and the gene mutations ('neutral' theory of molecular evolution, 'neoclassical school' vs. 'balanced school'; Lewontin, 1974; Kimura, 1983). In some cases, the theoretical predictions of Kimura seem to agree with estimates of the actual tempo of nucleotide and amino acid substitutions as well as with the existing levels of protein polymorphism and heterozygosity in natural populations. A detailed analysis of this problem can be found in Kimura's recently published book (Kimura, 1983). However, some problems remain unsolved, in particular the phenomenon of the similarity of allozyme frequencies in spatially and genetically isolated populations.

All of the above questions were considered pre-

viously (Altukhov, 1983) when we described our approach to the analysis of genetic processes in populations. Our approach combines the view of the 'balanced' and 'neoclassical' schools and leads to the conclusion that the genetic differentiation of a species at the molecular level is not only confined to the impact of one or another microevolutionary factor (e.g. natural selection, migration or random genetic drift), but rather determined by their complex interactions. In this paper I address the question concerning the role of balancing selection and overdominance in maintaining allozyme polymorphisms for despite much effort, this problem is still far from being solved. The comments of Lewontin (1974) are appropriate when he says that for demonstrating the effects of overdominance is commonly put forward – i.e. sickle-cell anemia which is connected with the hemoglobin polymorphism observed in some ethnic groups. Is the the lack of more examples of balanced polymorphisms due to the fact that, in nature, selection in favor of heterozygotes for the allozyme genes is a rare phenomenon, or, have too few population-genetic studies been conducted? In an earlier paper, I have argued for the correctness of the second alternative (Altukov, 1983). A review of all respective publications clearly indicates that most researchers have not dealt with real populations as historically formed structures but rather with random samples. This approach widely restricts the possibilities for revealing the effects of selection on allozyme loci.

To demonstrate the effects of selection I will use three approaches which were developed during long-term research work of my laboratory. These approaches are:

1. An analysis of the stationary distributions of allelic frequencies in a native subdivided population;
2. A comparison of the genotypic distributions at the early and late developmental stages in successive generations of the same population;
3. An analysis of the 'joint' variability of monogenic (allozymes) and adaptively significant polygenic traits.

In addition, I will consider the contributions of these three approaches to a new hypothesis which is a compromise between the views of 'neoclassical' and 'balance' schools. I shall try to put forward some additional arguments in favor of the hypothesis for an optimal level of the genetic diversity in a population as a measure for its adaptive maximum (Altukhov, 1983; Altukhov *et al.*, 1986, 1987). The main part of this paper is predominantly based on the results of many years of research in biochemical population genetics of Pacific salmon populations, but I will also use the results of investigations of some other species.

Results

Estimating the selection pressure by the analysis of distributions of allozyme gene frequencies in a subdivided natural population

Many natural populations possess a fine subpopulation structure and are characterized by a systemic organization (Altukhov & Rychkov, 1970). Without a clear understanding of this fine structure and without regular sampling material in space and time, we cannot expect to detect the selection pressure. When all these requirements are met, scientists have a unique opportunity not only for the identification of the type of selection but also for its quantitative estimation. This should be done through approximations of the empirical distributions of subpopulations for allozyme frequencies using the theoretical functions of Sewall Wright (1931, 1969). At present, the most

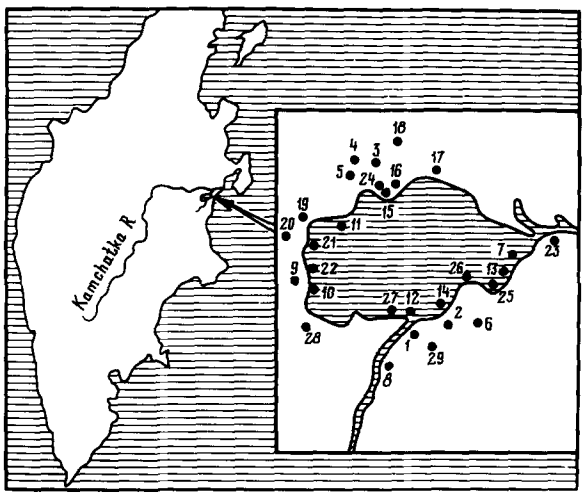

Fig. 1. Location of Lake Azabachye within the system of the Kamchatka River (arrow) and spatial localization of spawning subpopulations (1-28) of sockeye salmon, Oncorhynchus nerka (Walb.).

detailed analysis on this approach was carried out by studying a population of the sockeye, *Oncorhynchus nerka* (Walb.), a species of Pacific salmon which spawns in Lake Azabachye on Kamchatka peninsula (Fig. 1). This native population consists of a system of subpopulations which during spawning are isolated in time and/or space (like other species of Pacific salmons, the sockeyes are monocyclic spawners, i.e., they die soon after spawning). Up to 30-40 such subpopulations may spawn in a lake simultaneously. During several field seasons we studied the genetic structure of 29 subpopulations for the two polymorphic loci, lactate dehydrogenase (Ldh) and phosphoglucomutase (Pgm). Since many of these subpopulations were studied repeatedly, the data on the biological and genetic structure of about 170-180 of such communities were taken. Abundance and sex ratio of fish were determined simultaneously for every spawning ground which provided the data for the estimation of the effective population size (Ne). This value proved to be on the order of about 200 individuals (Altukhov, 1974, 1981; Altukhov *et al.*, 1975 a, b).

Marking experiments carried out by American ichthyologists (Hartmann & Raleigh, 1964) in which sockeyes were marked individually on their spawning grounds of Lake Brooks and Karluk in Alaska make possible the approximative determination of the migration coefficient or, more precisely, the reciprocal value of the coefficient of return to the native spawning grounds. This coefficient was about 2%. Similar estimates were obtained for Kamchatka sockeye populations in lakes Azabachye (Iljyn *et al.*, 1984) and Nachikinskoye (N. V. Varnavskaya, pers. comm.).

Analysis of the variability of sockeyes at the subpopulation level clearly demonstrated a considerable amount of local differentiation of the allele frequencies, in particular at the Ldh locus (Table 1).

Table 1. Main population-genetic parameters for a number of generations of the sockeye population in Lake Azabachye.

Locus	Year	K	N	$\bar{q}$	V_q	Chi-square value for dispersion homogeneity (d.f.)	Chi-square value for population homogeneity
Ldh-B_1	1971	14	737	0.65	0,0074		50.18***
	1972	20	1022	0.61	0,0048		37.53***
	1973	21	1007	0.65	0,0078		65.11***
	1974	14	676	0.66	0,0076		45.59***
	1977	19	934	0.65	0,0084		69.34***
	1978	23	1244	0.65	0,0071	$2{,}69 < X^2_{0{,}05}\ (10) = 18{,}30$	76.72***
	1979	14	846	0.65	0,0091		67.30***
	1980	17	843	0.67	0,0078		63.36***
	1981	11	2239	0.68	0,0073		137.22***
	1982	11	1380	0.67	0,0053		66.74***
	1984	19	1408	0.64	0,0061		79.22***
Total	1971-1984	183	12336	0.66	0,0077		804.49***
Pgm	1971	14	748	0.79	0,0028		22.57**
	1972	20	1004	0.77	0,0019		21.76
	1973	21	981	0.79	0,0041		41.36**
	1974	13	647	0.78	0,0039		45.01**
	1977	19	936	0.81	0,0032	$6.47 < X^2_{0{,}05}\ (9) = 16.90$	39.12**
	1978	23	1241	0.77	0,0025		35.78*
	1979	14	846	0.78	0,0018		17.05
	1980	17	831	0.77	0,0015		14.53
	1982	11	1363	0.79	0,0035		45.94***
	1984	19	1353	0.76	0,0019		25.90**
Total	1971-1984	171	9950	0.78	0,0028		317.34***

Remarks: K is the number of subpopulations; n is the number of fish studied; $\bar{q}$ is the average gene frequency; V_q is the intergroup variance of the gene frequency.

Moreover, the repeated investigation of the same subpopulations year by year revealed a good measure for the temporal variation of their genetic structure. However, if we characterize a system of subpopulations as a whole by estimating the overall mean values of the allele frequencies and their intergroup variances, we will find out that the subdivided population as a whole remains genetically stable, despite the variability of its components (Table 1).

The approximation of the empirical distributions by the corresponding theoretical ones for the case of Wright's 'island' model demonstrates that the stability in the distribution of subpopulation allelic frequencies in time may be adequately explained by the interaction of random genetic drift, migration, as well as selection pressure in favor of heterozygotes. Such pressure is particularly strong for the Pgm locus. The selection coefficients for the Ldh locus were found to be especially high for those subpopulations which breed in the shallow spawning grounds, ponds and streams (Altukhov *et al.*, 1983), although for a subdivided population the process is modelled as a selectively neutral one. The mean frequency of the allele Pgm A in a population system does not actually differ from the equilibrium frequency ($\hat{p}$), predicted by the ratio of the fitness of genotypes (Wi) (Table 2).

In experiments with subdivided populations of *Drosophila melanogaster* over dozens of generations, similar stability was found for the polymorphisms of esterase-6 and α-glycerophosphate dehydrogenase loci (Altukhov & Pobedonostseva, 1978, 1979; Altukhov & Bernashevskaya, 1981; Altukhov *et al.*, 1979a)

Table 2. Estimations of W value for Pgm genotypes in *Oncorhynchus nerka.*

Method of W estimation	Genotypes	W values	Equilibrium allele frequency, $\hat{q}$	Observed mean allele frequency, $\bar{q}$
Using S.Wright's stationary function. Nm parameter is found by the ecological data	AA AB BB	0,942 1,000 0,822	0,756	0,782
Using the ratio of observed and expected numbers of genotypes	AA AB BB	0,943 1,000 0,796	0,782	0,782

as well as over hundreds of generations by means of computer simulation of the joint effects of random genetic drift and gene migration in a system of partially isolated populations (Altukhov *et al.*, 1984). These examples exclude the possibility that protein polymorphism is a transient phase of molecular evolution and testify to its stability both in time and space at least in native natural populations or in their respective models.

In instances where the genetics of a subdivided population is fairly well understood, the rearrangement of the gene pool in the subpopulations does not follow any definite direction but merely reflects the local fluctuations of the gene frequencies which compensate for each other. In this connection the question of the probable number of overdominant genes and, therefore, of the interpretation of the uniformity of the allele frequencies of the protein loci (often seen in vast areas) takes on a new meaning.

Estimation of balancing selection by comparison of the genotypic distributions of allozyme loci at various stages of ontogenesis in successive generations within a population

In the analysis of the genetic structure of the sockeye population, clearly distinguishable differences in the interlocus variance of the allele frequencies can be recognized which persist over the years. Thus, the Ldh locus which is selectively more neutral was found to be more variable when compared to the *Pgm* locus. The *Pgm* locus is under a rather strong selection pressure in favor of heterozygotes. It is not difficult to imagine that by extending the sample size of the enzyme loci being studied, others might be found which may be subjected to even stronger balancing selection pressure. In this case, the interpopulation genetic differences will become less significant and will probably reach a level of a certain spatial uniformity of allozyme gene frequencies. However, if polymorphism for a considerable number of genetic loci is maintained on the basis of overdominance as was already stressed above, the problem of an excessive segregational load will arise. The extent of such a genetic load turns out to

be enormous in accordance with the estimates of the population sizes (viz. 10^7–10^{10} individuals) as were given in the contributions of the representatives of both the 'neoclassical' and 'balance' schools. There are, however, no such native populations in natural habitats. On the contrary, natural populations are subdivided into smaller subpopulations of limited sizes.

We also consider such an approach to be somewhat questionable when evaluating the levels of heterozygosity. The total size of a species is estimated without taking into account its subdivision into a great number of reproductively isolated populations. This approach seems to be possible only if the intensity of gene migration between subdivisions of a species is much greater than that of a spontaneous mutation rate. If these values become comparable (see e.g. *Larson et al.*, 1984), the expected estimates of heterozygosity and those of the effective number of alleles (presuming they are selectively neutral) must be estimated taking into account the size of isolated populations and not of a species as a whole. In such cases, which seem to occur rather often in nature, the problem of a segregational load cannot remain acute. Table 3 shows the results of the respective estimations of the segregational load (value e^L; see Kimura & Ohta, 1971), made on the basis of a realistic evaluation of N, and the coefficients of selection in favor of heterozygotes for various numbers of the diallelic loci whose fitness effects are assumed to be multiplicative. The estimates are carried out for the equilibrium allele frequencies for 'symmetrical' ($\hat{p} = 0.5$) and 'asymmetrical' ($\hat{p} \neq 0.5$) selection. This way the excess of fecundity (e^L) for the fittest individuals turns out to be biologically permissible, especially under asymmetric balancing selection pressure (for details see Altukhov, 1983). Consequently, we cannot reject the hypothesis which emphasizes the important role of overdominance as a mechanism for maintaining the biochemical hereditary variability of populations. The uniformity of the gene frequencies in the areas of native populations of a number of species should be influenced not only by the pressure of the migration of genes but also by the effect of balancing selection. The selection is apparently most significant at early stages of ontogenesis.

Table 3. Excess of fecundity (e^L) of the most adapted genotype.

Number of overdominant loci	Population size		
	1000	10000	100000
	$S_1 = S_2 = 0.05$; $\hat{p} = 0.5$		
500	6.92	9.74	13.11
1000	15.43	25.02	38.07
5000	454.58	1339.23	3422.47
	$S_1 = 0{,}01$; $S_2 = 0{,}09$; $\hat{p} = 0.1$		
500	2.01	2.27	2.52
1000	2.68	3.19	3.71
5000	9.05	13.35	18.72
	$S_1 = S_2 = 0{,}1$; $\hat{p} = 0.5$		
500	48.95	94.97	171.91
1000	238.25	626.24	1449.47
5000	206640	1793529	11713309
	$S_1 = 0{,}01$; $S_2 = 0{,}19$; $\hat{p} = 0.05$		
500	2.09	2.37	2.66
1000	2.83	3.40	3.99
5000	10.23	15.42	22.03

The verification of this hypothesis has become the main task of our investigations in recent years. With this aim in mind we studied the biochemical polymorphism in the populations of the pink salmon, *Oncorhynchus gorbuscha* (Walb.), in the Far East regions of the U.S.S.R. This monocyclic species of salmon spawns in the second year of its lifespan; the fish die after spawning. Accordingly, there are two reproductively isolated lines of generations which spawn in even and odd years respectively. Over a number of years we have studied 19 different spawning stocks which revealed a close relationship to each other with respect to the allele frequencies of nearly all of the loci under investigation (Altukhov, 1990). From the point of view of the 'neutralist' this phenomenon would be explained by migration of genes, thus eliminating interpopulation differences; 'selectionists' would interpret such distributions of allele frequencies of allozyme loci as the effect of a strong balancing selection pressure. Had we data on the reproductive structure of the pink salmon as reliable as those available on the sockeye salmon, the realization of the approach considered above should be quite feasible. Unfortunately, such information is not currently available. Thus the only way to estimate the selective significance of the allozyme polymorphism in *O. gorbuscha* is by comparing the distributions of genotypes at various stages of ontogenesis of this species. The corresponding data were obtained by a compari-

Table 4. Distribution of individual heterozygosity for allozyme loci in pink salmon spawners and larvae.

Groups of fish	Frequency of classes with different number of heterozygote loci per individual			Sample size	X^2 test for heterogeneity of distributions in spawners and larvae (d.f. = 3)
	0	1	2 and more		
Spawners	0.495 ± 0.016	0.391 ± 0.016	0.114 ± 0.015	606	
Larvae	0.620 ± 0.020	0.335 ± 0.020	0.044 ± 0.007	901	38.6***

'Fitness' values of frequency classes in spawners and larvae equal to 0.80 ± 0.04; 1.17 ± 0.08 and 2.57 ± 0.51 in the order of increasing individual heterozygosity.

son between spawners and hatchlings with a large yolk sac. The results (Fig. 2) clearly indicate the effects of selection in favor of heterozygotes from early ontogenetic stages up to adulthood for all the studied allozyme genes. These differences are steadily reproduced from generation to generation and were traced in various populations, both in native ones and those maintained artificially (for details see Altukhov *et al.*, 1987). At still earlier ontogenetic stages the elimination may be directed against either heterozygotes or rare genotypes in such a way that selection acts in a typically balancing and cyclic manner.

The fitness estimated for the interlocus-paired genotypic combinations increases with increasing degree for individual heterozygosity (Table 4). Hence, we cannot exclude the possibility that overdominance may serve as a mechanism for maintaining polymorphism in *O. gorbuscha* for the loci under investigation.

As for the influence of the corresponding genotypes on fitness, these effects seem most likely to be multiplicative, because the respective estimates for the interlocus combinations (W_{ij}) are close to the products of fitnesses of the genotypes of certain loci (W_iW_j), i.e. $W_{ij} \approx W_i \cdot W_j$ (Altukhov *et al.*, 1987).

If these conclusions hold true, and if further investigations exhibit significant prevalence of the selection which is characteristic of that acting in *O. gorbuscha*, then it will not be difficult to explain why random samples of allozyme polymorphism seem to be selectively neutral, whereas selection coefficients at early stages of ontogenesis (including a gametic stage) are clearly non-zero. In previous studies (Altukhov, Dukharev & Zhivotovsky, 1983; Altukhov *et al.*, 1985) such eliminations of new mutations concerning enzyme loci were found. The same mechanism may be equally applied to any rare genotype of allele combinations at various loci (Altukhov, 1980). These data led us to conclude that the optimal heterozygosity (or optimal diversity for a set of independent genes) will be a good measure for the *maximum* adaptation of a population to that environment in which the population was formed and in which it now exists (Altukhov, 1983). Livshits and Kobylyansky (1985) came to similar conclusions.

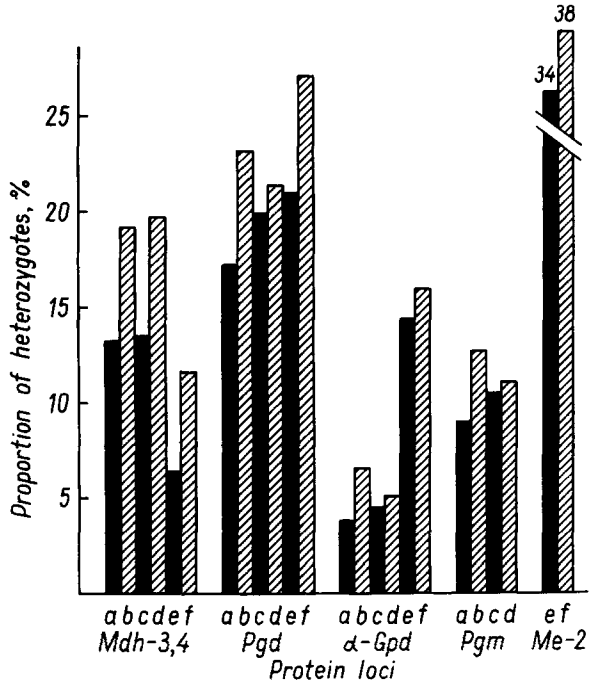

Fig. 2. Heterozygosity (%) observed at several allozyme loci of larvae (black columns) and spawners (hatched columns) in successive generations of the Naiba pink salmon population: a, c, and e – larvae in 1980, 1984, and 1985, respectively; b, d, and f – spawners in 1979, 1981 and 1984, respectively. By the sign-test, the differences between the spawners and the larvae are highly significant. The respective values of total samples of spawners and larvae are 854 and 1,562 individuals.

An important point may be that although a high level of heterozygosity for a set of allozyme loci is advantageous to an individual (if Lerner's homeostasis model is correct), it may be undesirable for the population as a whole since a considerable number of the segregational genotypes will prove to be inadaptive. Lerner's model finds an analogy in information theory according to which both the excess information and its deficit are equally unfavorable for the normally functioning system (Altukhov, 1983). In particular, the unlimited growth of polymorphisms and heterozygosity may result in an enormous increase in the level of genetic recombination which then will block harmonic interlocus interactions. The likelihood of the decay of the cooperativeness of interacting genes must be especially high at early ontogenetic stages because, since the numerous functional systems in a developing organism are still immature, the viability of the organism depends primarily on the most liable links in the chain of metabolic processes ('the rate controlling step' type according to Livshits and Kobylyansky, 1985).

If these assumptions are true, a definite correlation between the level of individual biochemical heterozygosity of parents and viability or variance of viability among offspring is expected to exist. Additionally, there should be a 'concordant' variability of allozyme loci and adaptively significant polygenic morpho-physiological characters. The next section will deal with such relationships.

Detection of the adaptive significance of enzyme polymorphism through the analysis of interrelated variability of monogenic and polygenic traits

Biochemical population genetics has made it possible to fill the gap between Mendelian and quantitative genetics which developed for many years with virtually no overlap. Our approach is characterized by the analysis of the distribution of individual heterozygosity, of various genotypic combinations along the variability curve of a quantitative character, or of a set of such characters which appear to be adaptively significant. Here a very important fact is taken into account: those individuals which approximate the population mean value are shown to have the highest fitness, particularly at early stages of ontogenesis (Bumpus, 1899; McAtee, 1937; Karn & Penrose, 1951 etc.). The regularity of this may be regarded as the effect of stabilizing selection which runs according to a historically formed universal polygenic system evolved under the great influence of heterozygosity and coadaptation of genes (Mather, 1943; Dubinin, 1948; Lerner, 1954). Indeed, in the very first investigations we could already prove the specific character of genotype-distributions of several blood-group loci among morphologically 'intermediate' and 'extreme' phenotypes of humans (Altukhov *et al.*, 1979, 1981; Altukhov, 1980).

The most convincing example of a nonrandom distribution of heterozygosity among groups of individuals which differ in their morpho-physiological characters is the data on various spawning sockeye populations from the lake basins of Kamchatka Peninsula (Altukhov, 1983; Altukhov & Varnavskaya, 1983). In the first part of this paper I have already given examples which provide evidence for a strong heterotic selection at the *Pgm* locus of sockeye which spawn in Lake Azabachye. The differences between the fitness coefficients of the different genotypes were highly significant creating the question of how to explain this differentiation in relation to the characteristics which normally mark the effect of heterosis (e.g. the increased rates of growth and maturation). The sockeye salmon is characterized by sexual dimorphism for body size and complicated reproductive behavior including a system of selective matings. On average, males are larger than females. Besides these large males, a second group of males exists which grow rapidly and mature as small, three-year old individuals who have spent only one year in the sea. As was shown by Hanson and Smith (1967) and McCart (1969), large males possess a selective advantage during the formation of mating pairs by holding a territory in the spawning grounds. As a rule, these large males have spent more than two years in the sea and are 5 to 7 years old by spawning time. However, in years with a water level lower than normal, and in the shallow areas of the spawning grounds, small males will have a reproductive advantage over large males. The mean age of females at spawning varies between 4 and 5 years. Accordingly, with respect to body length at spawning, sockeye

populations exhibit a unimodal frequency distribution for females and a pronounced bimodality for males. The best example for such a clear male dimorphism was found for sockeyes which spawn in Lake Azabachye (Fig. 3). These three groups of fish, which can be easily distinquished during spawning, i.e., were compared with regard to their level of heterozygosity for both the *Ldh* and *Pgm* loci. The experiments were performed with the sockeye salmon of Lakes Blizhnee, Nachikinskoe, and Dal'nee on Kamchatka Peninsula. The data show that females exert an intermediate level of heterozygosity, while small and large males are characterized by maximal and minimal levels, respectively (Fig. 4). Thus, sockeye males and females represent two adaptive systems with the maximal

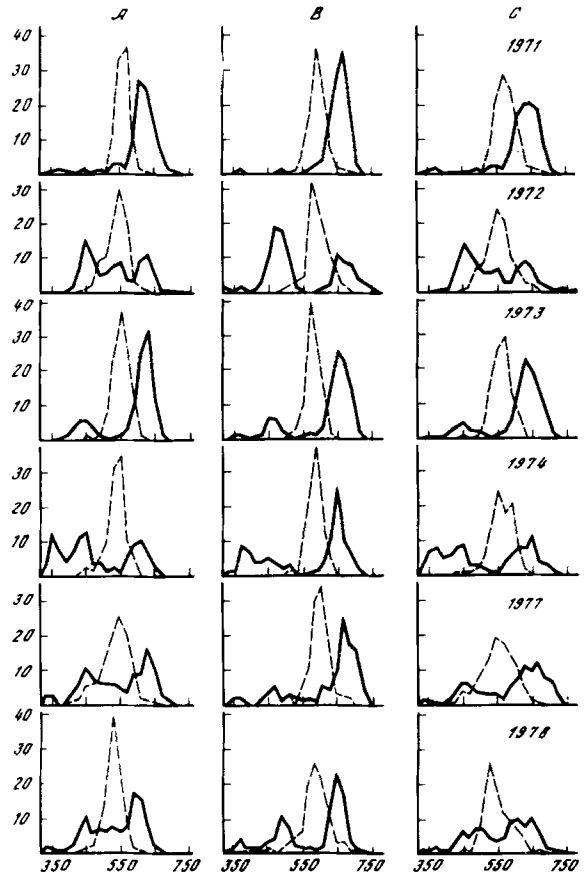

Fig. 3. Distributions of body lengths of males (continuous line) and females (interrupted line) in the spawning portion of the sockeye salmon population system of Lake Azabachye during 1971-1978. Abscissa, body length in mm; ordinate, percent frequency. A, a group of early migrating subpopulations; B, a group of late migrating subpopulations; C, the population system as a whole. The sample size is shown in Table 1.

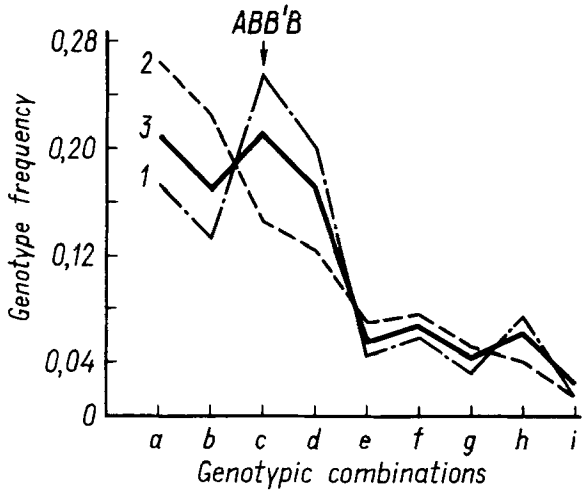

Fig. 4. Frequency distributions of paired genotype combinations at lactate dehydrogenase (B_1B_1, B_1B_1, B_1B_1) and phosphoglucomutase (AA, AB, BB) loci among sockeye salmon spawners of Lake Nachikinskoe, Kamchatka: 1. small males; 2. large males; 3. females. a, B_1B_1AA; b, $B_1B'_1AB$; c, $B_1B'_1AB$; d, B_1B_1BB; e, $B_1B'_1AA$; f, $B_1B'_1BB$; g, $B'_1B'_1AB$; h, $B'_1B'_1AA$; i, $B'_1B'_1BB$.

The differences between large and small males as well as between small males and females are statistically significant ($P < 0.05$).

The genotypic combinations are ranged in the order of decreasing theoretical frequencies.

variance of fitness in males. The high degree of heterozygosity of small males correlates with their increased rate of growth and early maturation.

In a previous section of this paper I demonstrated the effects of selection for allozyme loci in pink salmon. Here I will give our recent results on the viability of hatchlings of this species for various types of crossings, the parents of which had different levels of heterozygosity for the allozyme genes under investigation. Eight combinations of mating pairs were studied characterized by the degree of heterozygosity for the loci of *Mdh*, *Gpd*, *Pgm*, and *Me*. All eggs were artificially fertilized at a hatchery. After hatching, the body length of the hatchlings was determined. There were three series of experiments which allowed the determination of genotypes of 843 larvae. A positive correlation was found between the body length and the degree of individual allozyme heterozygosity (Altukhov, Salmenkova & Kartavtsev, 1991). Minimal losses in progeny, determined by total mortality over the whole period of incubation, were found for larvae produced by parents with intermediate heterozygosity, while the maximum death rate occurred among the progeny of both high and low heterozygous pairs.

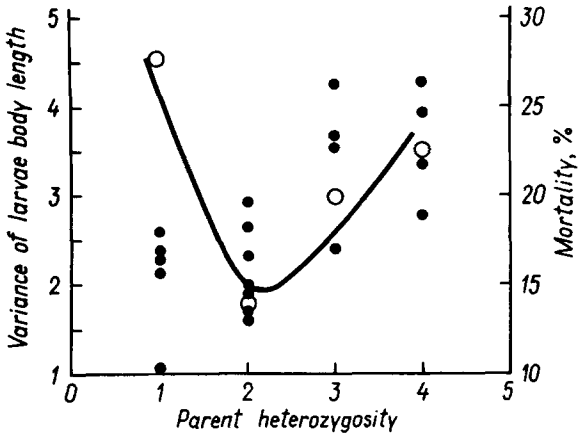

Fig. 5. The connection between the level of individual allozyme heterozygosity of parental pairs (abscissa) and variance of larvae body length (ordinate dark circles); $r = 0.72$; $P < 0.001$; $n = 843$) and death rate (ordinate to the right, open circles; $n = 5184$), throughout incubation of fish eggs and development of premigrating stage in the pink salmon, Oncorhynchus gorbuscha.

The variance of body length was larger in offspring of more heterozygous parents (Fig. 5).

Discussion

During the last decade many papers have been published attempting to analyze the effects of heterosis by investigating allozyme polymorphism in a wide variety of plant and animal species. The results have been recently reviewed by Zouros and Foltz (1987). As these authors note, the study of a correlation between the individual allozyme heterozygosity and adaptively loaded characters has led both to positive and negative results, making it reasonable to summarize these somewhat contradictory findings. One should, however, address the following questions: Why are the connections between the variability of quantitative characters and allozyme polymorphism not always discovered? And why are these links, when discovered, associated with a small number of allozyme loci?

When answering the first question it is very important to regard the 'genotype-environment' interactions. As was noted earlier (Altukhov, 1983), under the same environmental conditions various gene loci are subject to different forms of selection, or are sometimes selectively neutral. Correlations can be estimated only when the conditions are strict enough, i.e. when the uniformly directed selection simultaneously influences a great number of genes. Our recent study of the allozyme polymorphism in Norway spruce (Altukhov *et al.*, 1986) supports this conclusion. We found a positive correlation between the level of individual heterozygosity of the maternal trees (6 loci, i.e. *Idh*-1, *Pdg*-1, *Pdg*-2, *Gpd*, *Got*-3, *Gdh*) and the proportion of unviable seeds produced. The less optimal the conditions for growing, the more pronounced the correlation (Altukhov *et al.*, 1986). In a contemporary study, similar experimental data obtained for another conifer, *Pinus banksiana* (Lamb). The correlation between the allozyme heterozygosity and the rate of growth and the biomass was shown to be strongest under a suboptimal 'stress' environment (Govindaraju & Dancik, 1987 a, b). These results agree very well with a relatively old conclusion concerning the importance of genotype – environment interactions for the expression of heterosis (Griffing & Zsiros, 1971; see also Barlow, 1981).

With regard to the second question, several other factors must be taken into account. For instance, the unstudied but potentially relevant pleiotropic effects of allozyme genes, as well as the fact that the real level of variability in the structural genes may be lower than was believed 25 years ago (for details see Altukhov, 1990) may be of importance.

The data concerning a strong pressure of balancing selection and involvement of a big number of polymorphic genes into this process seem to be of particular interest. On the whole, if my supposition that adaptation is a compromise between the individual and population components of this process holds true, one should expect in nature the existence of several mechanisms promoting the maintenance of the optimal biochemical heterozygosity or, more generally, the optimal genetic diversity. Indeed, the existence of such mechanisms can be proved deductively and illustrated by the corresponding real situations: e.g. assortative matings; subdivision of a population into subpopulations; restriction of free genetic recombination, or selection, which varies in its direction at different stages of ontogeny or in the specimens of different sexes, and adaptive behavior (Ushakov, 1982; Schroder, 1983) directed to maintaining the optimal genotypic structure of a population. The list of examples illustrating the regulation of the optimal

level of genic diversity in natural populations may be easily increased; the necessity for further investigations in this field is quite clear. However, we can state at present that in the case of the overdominance model, the maximum heterozygosity must be concentrated in the tails of the distribution curve of adaptively significant polygenic characters, whereas in other cases maximum heterozygosity may be characteristic of morphologically intermediate phenotypes (see Beardmore & Shami, 1979). Unsolved problems of this kind were recently considered in detail by Chakraborty (1987).

Conclusion

In conclusion, I would like to ask whether the allozyme genes themselves are involved in the process of adaptation, whether they serve as markers of some other vitally important chromosome segments linked together with the corresponding loci of the genome, or whether they accompany these segments by 'hitchhiking'.

1) What explains the fact that selection effects are detectable for practically any of the protein loci when the investigation is especially organized, and that this is not the case if one works in the frame of a formal approach at the level of random samples?

2) How do we explain the adaptive advantage of heterozygotes for several allozyme genes when they are absolutely independent of each other? Note that both in pink and sockeye salmons the studied loci are not linked together.

Taking into account that Pacific salmon have a relatively high number of chromosomes ($2n = 52$; NF $= 100$ in pink salmon and $2n = 56$; NF $= 102$ in sockeye salmon, and that under certain environmental conditions heterosis was seen for all allozyme loci hitherto studied, we have no reason to deny that selection influences the genotypes of the respective loci themselves; therefore no other hypothetic genes or supergenes will exist whose markers they represent.

With the exception of the vitally important monomorphic (Altukhov, 1982, 1990) and polymorphic systems of supergenes which are directly involved in the processes of adaptation at the levels of a population and a species, in all other polymorphic genes the degree of their functional loading (or selective neutrality) varies widely, and therefore it cannot be adequately estimated without accounting for various interactions between genotype and environment. However, this question may be answered only by more investigations of the biochemistry and physiology of adaptations (see Kohen & Hilbish, 1987).

Whatever the final result of the discussion between 'selectionists' and 'neutralists' will be, one important conclusion may be drawn: The widely distributed biochemical polymorphism which was first discovered 25 years ago must not be regarded as a transient phase of molecular evolution, particularly not in natural populations; instead, it seems to represent a steady state. Biochemical polymorphisms can be maintained over a long evolutionary period even by the means of only one fine subpopulation structure with a limited gene flow. Clearly, selection in favour of heterozygotes or some other forms of balancing selection acts as an additional factor to maintain allozyme polymorphisms and thus promote its greater stability.

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