tiated beta cells frequently divided and the capacity to synthesize insulin was increased in the relative absence of proliferating ducts.

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Esterase Heterogeneity: Dynamics of a Polymorphism

Abstract. *In the freshwater fish* Catostomus clarkii, *the frequency of alleles for polymorphic serum esterase varies with latitude. The activity of the allele more frequent in southern populations increases as temperature increases from 0° to 37°C, whereas activity of the allele more frequent in northern populations increases as temperature decreases. Coefficients of selection used to calculate allelic equilibrium within populations are derived from the activity profiles of the genotypes.*

Genetic heterogeneity or phenotypic variation of serum esterase (or both) has been demonstrated in many animal populations (*1*). Variation in serum esterase exists in populations of the Gila mountain sucker *Catostomus clarkii* in past and present tributaries of the Colorado River system, downstream from the Grand Canyon (*2*). This variation is due to the segregation of two codominant alleles, *Es-I*^{a} and *Es-I*^{b}, at a single locus and is manifest as three electrophoretic phenotypes—a fast migrating band of esterase activity, a slow band, and a double band in the heterozygote. There is a cline in the frequency of the *Es-I*^{a} allele which is positively correlated with latitude of the sampled population. The frequencies of the *Es-I*^{a} allele in populations of *C. clarkii* from southern Arizona, central Arizona, southwestern Utah, and northern Nevada are 1.00, 0.84 to 0.92, 0.46 to 0.60, and 0.17, respectively. The maintenance of the cline is dependent upon a spatial gradient in selection associated with changes in the relative fitness of the two homozygotes (*2*). Although temperature was suspected as the selective force in the maintenance of heterogeneity, this was not supported by available evidence. I find temperature is the component of selection, which, according to the enzymatic characteristics of the *Es-I* alleles, must operate through single locus heterosis.

My results were obtained with frozen serums and with fresh serums, and no differences were noted. All individual samples were surveyed with horizontal starch-gel electrophoresis (*2*, *3*), and serums from individuals of the same *Es-I* phenotype were pooled. Serums were vigorously shaken with two volumes of methylene chloride and centrifuged (27,000*g*) at 0°C to remove lipids. Two milliliters of serum standardized to 1.0 percent total protein concentration with a hand refractometer were added to a column (3.2 by 60.0 cm) of G-200 dextran equilibrated with degassed 0.2*M* sodium phosphate buffer. An ascending flow rate of 25 to 30 ml/hour was maintained, and the effluent was collected in 5.0-ml fractions.

Esterase activity was measured colorimetrically on fractionated serums. Individual fractions were adjusted to *p*H 7.0 with tris(hydroxymethyl)aminomethane and maleic acid buffer (*2*). Each fraction was brought to the desired temperature and mixed with an equal amount of substrate solution (1.0 percent α-naphthyl acetate in acetone) at the same temperature. After a 1-hour incubation at the desired temperature (± 0.05°C) with constant agitation, 1.0 ml of 0.2 percent Fast Blue RR was added, and the usual brown precipitate was formed. After 3 minutes, 1.0 ml of 50 percent trichloroacetic acid was added and the colored precipitate was extracted with 5.0 ml of ethyl acetate. Activity, determined at 430 nm, is expressed in arbitrary units of optical density (Fig. 1).

There are significant differences among the three genotypes (Fig. 1) at various reaction temperatures. At 37°C, activity of *Es-I*^{a} was more than ten times that of *Es-I*^{b}, the observed activity of the latter genotype being nearly zero. As might be expected, the activity of the heterozygote was intermediate between the two homozygotes.

Esterase activity observed at 0°C dif-

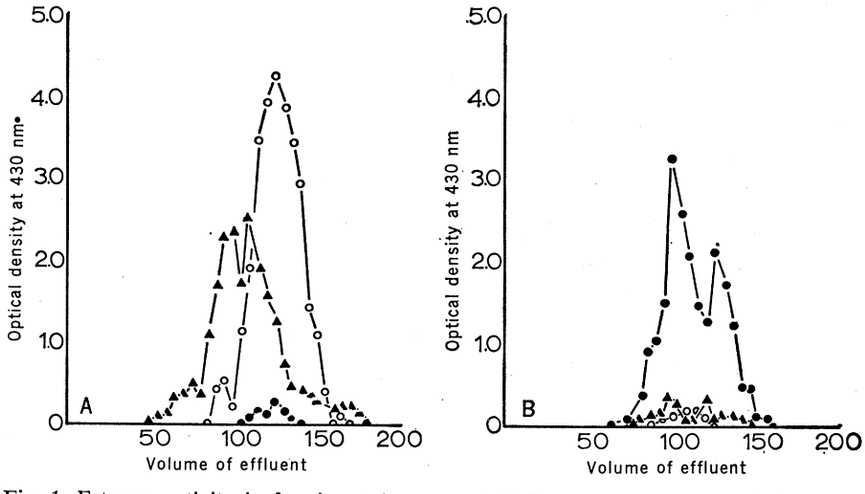

Fig. 1. Esterase activity in fractionated serums. (A) Temperature 37°C; (B) temperature 0°C; ○—○, *Es-I*$^{a/a}$; ▲—▲, *Es-I*$^{a/b}$; ●—●, *Es-I*$^{b/b}$.

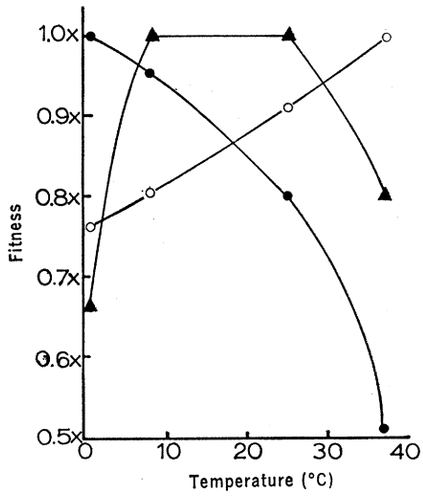

Fig. 2. Relationship between temperature-dependent *Es-I* activities of genotypes and "fitness." ○—○, *Es-I*$^{a/a}$; ▲—▲, *Es-I*$^{a/b}$; ●—●, *Es-I*$^{b/b}$.

fered from that at higher temperatures (Fig. 1B). Populations with *Es-I*^{b} exhibited activity much greater than at 37°C —nearly ten times that of either *Es-I*^{a} or the heterozygote. The observed activity of *Es-I*^{b} is not only greater than that observed for the heterozygote at 37°C, but nearly equal to *Es-I*^{a} at the higher temperature. Activity of the heterozygote was intermediate at upper limits of the tested temperature range, but highest at intermediate temperatures (Fig. 2).

The different temperature optimums of the three genotypes illustrate a functional basis for the observed geographic distribution of the two alleles. The most frequent allele in populations of *C. clarkii* at southern extremes of the range, *Es-I*^{a}, has a higher activity at higher temperatures. The more frequent allele in northern populations, *Es-I*^{b}, has the highest activity at 0°C.

If maximum enzymatic activity is related to maximum genotype fitness at the *Es-I* locus, one can demonstrate a relationship between the temperature dependency of the three genotypes and a selection model explaining the clinal distribution of esterase alleles within these fish populations. Maximum observed activity is equated with "fitness" relative to the slope in change of observed activity over the tested temperature range (Fig. 2). The "fitness" scale so expressed has no absolute meaning, but is merely a means of obtaining relative values for the three genotypes as a function of the slope of individual plots.

In a genetic system where two alleles are selectively maintained by heterozygote advantage, a stable equilibrium exists:

$$\hat{p} = S_2/S_1 + S_2$$

where S_1 is the coefficient of selection against one homozygote (*Es-I*$^{a/a}$), S_2 is the coefficient of selection against the other homozygote (*Es-I*$^{b/b}$), and p is the frequency of the allele represented by the *Es-I*$^{a/a}$ homozygote (*4*).

Arrangement of the temperature-dependent activity profiles of the three genotypes in the manner described above permits the derivation of "fitness" values (x) for each of the genotypes at any specific temperature. Since $S = 1 - W$ (in this case $W = x$), the equilibrium condition $\hat{p}$ can be predicted. As an example, at 10°C maximum activity is exhibited by the heterozygote ($x = 1.0$), whereas *Es-I*^{b} activity is lower ($x = 0.95$; $S_2 = 1 - x$; $S_2 = 0.05$), and *Es-I*^{a} activity is the lowest ($x = 0.81$; $S_1 = 1 - x$; $S_1 = 0.19$). Hence, $\hat{p} = 0.05/0.19 + 0.05 = 0.21$. In other words, an environmental selection temperature of 10°C will produce, by the above scheme, an equilibrium condition where the frequency of the *Es-I*^{a} allele will equal 0.21 (within individuals of that generation). At temperatures lower than approximately 7°C and higher than 30°C a condition of disequilibrium will exist which results in the fixation of *Es-I*^{b} or *Es-I*^{a}, respectively (Fig. 2). Where S_1 equals S_2 (slightly less than 20°C), $\hat{p}$ will equal 0.50.

The functional characteristics of the enzymes demonstrate a somewhat predictable situation: variant allelic products differing in adaptive properties. It is striking, however, that the two enzymes exhibit such markedly different temperature optimums. It seems reasonable to postulate similar functional characteristics for all other heterotically maintained two-allele polymorphisms, although the component of selection need not be temperature, and the functional allelic differences need not be so great. These characteristics would depend on the magnitude of variation in the particular environmental component as well as on the tolerance of the genetic products to the variation.

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Diffusive and Convective Flow Across Membranes: Irreversible Thermodynamic Approach

Abstract. *The experimentally verified hydrodynamic approach to a description of diffusive and convective flow of solute across mutual membrane pathways is compared with the phenomenological equation resulting from the application of irreversible thermodynamics. Inherent nonlinearities in this equation severely, if not absolutely, limit its usefulness.*

In the study of transport across membranes one must frequently find a suitable expression for solute flow when convection (bulk flow of solution) and diffusion are occurring simultaneously. In the classical approach to the problem, one assumes idealized structures for the pores in the membrane and solves the continuity equation after making appropriate mathematical approximations. In another approach developed by Kedem and Katchalsky (*1*) the theory of irreversible thermodynamics is used to obtain a relatively simple linear flow equation for the combined processes. We here examine the consistency of the two approaches for an idealized membrane.

The system to be considered consists of solution *A* in compartment *A* on the left side of the membrane and solution *B* in compartment *B* on the right side. Each solution is dilute, infinitely well stirred, and confined on its side of the membrane by a movable piston. The membrane is homoporous, consisting entirely of right cylindrical pores all having the same radius. The membrane is open to the solute and solvent; that is, it cannot selectively filter