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Metabolic Flux and Fitness

Daniel E. Dykhuizen, Antony M. Dean and Daniel L. Hartl

Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110-1095

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ABSTRACT

Studies of *Escherichia coli* under competition for lactose in chemostat cultures have been used to determine the selective effects of variation in the level of the β-galactoside permease and the β-galactosidase enzyme. The results determine the adaptive topography of these gene products relative to growth in limiting lactose and enable predictions concerning the selective effects of genetic variants found in natural populations. In the terms of metabolic control theory, the β-galactosidase enzyme at wild-type-induced levels has a small control coefficient with respect to fitness ($C = 0.018$), and hence genetic variants resulting in minor changes in enzyme activity have disproportionately small effects on fitness. However, the apparent control coefficient of the β-galactoside permease at wild-type-induced levels is large ($C = 0.551$), and hence even minor changes in activity affect fitness. Therefore, we predict that genetic polymorphisms in the *lacZ* gene are subject to less effective selection in natural populations than are those in the *lacY* gene. The β-galactoside permease is also less efficient than might be expected, and possible forces resulting in selection for an intermediate optimum level of permease activity are considered. The selective forces that maintain the lactose operon in a regulated state in natural populations are also discussed.

IN a 1983 paper entitled "Polymorphism and Enzyme Function," KOEHN, ZERA and HALL (1983) ask, "To what degree is it possible to describe a molecular mechanism for fitness differences among enzyme variants?" The question is profound, for it is a challenge to describe fitness in terms of some specific function of enzyme activity and, thus, to unite population genetics with physiology. How, indeed, do changes nested deep in the biochemistry of cells affect the fluxes and substrate pools of metabolic pathways, and how, in turn, do these changes affect reproductive success? KOEHN, ZERA and HALL (1983) note several cases in which the biochemical properties of an allozyme may serve as an adaptation to a particular environmental circumstance, such as high temperature or low salinity, but they emphasize that these parallelisms represent *potential* mechanisms of adaptation, not proven ones.

Many naturally occurring enzyme variants demonstrate differences in their physiological consequences, but it is not known how, or whether, these differences affect fitness. For example, BARNES and LAURIE-AHLBERG (1986) have studied the effects of allozymes of *sn*-glycerol-3-phosphate dehydrogenase (*Gpdh*) on the mechanical power output of the flight muscles in *Drosophila melanogaster*. They found a small but significant, or marginally significant, effect of *Gpdh* allozymes, which depended on both rearing and flight temperatures. Overall, they show that the *Gpdh* polymorphism is best regarded as a minor polygene affecting quantitative variation in power output during flight, with an important component in the effect resulting from genotype-by-environment interaction. However, BARNES and LAURIE-AHLBERG (1986) also conclude that "it is not clear whether such small effects (on the order of a few percent) have any important consequences in terms of the forces that control the allozyme frequencies in nature." This conclusion is consistent with that of KOEHN, ZERA and HALL (1983), who, in answer to the question as to whether it is possible to describe a molecular mechanism for fitness differences among enzyme genotypes, finally provide an unequivocal negative.

To relate population genetics with physiology using enzyme variants that occur in natural populations of eukaryotes is a very ambitious undertaking. Substantial progress has been made in the face of at least three formidable obstacles. The first obstacle is that of phenotypic complexity. In eukaryotes, it is hard to imagine an example in which enzyme activity and fitness are closely, causally and unambiguously related in a clear and simple manner. In virtually all instances, the activity of each individual enzyme is but one component in a vastly complex metabolic network, and each enzyme variant may account for only a small proportion of the total genotypic variance of classical components of fitness, such as viability, mating success, fertility or longevity. This complexity can, in some cases, be overcome by means of targeted selection experiments, in which particular growth conditions are imposed in order to identify physiological differences resulting from enzyme variants (VAN DELDEN, BOERMA and KAMPING 1978; DE JONG and SCHARLOO 1976; EANES *et al.* 1985).

A second problem results from the observation that differences in enzyme characteristics among naturally occurring enzyme variants are usually rather small. Small differences in enzyme characteristics might often be expected to result in small differences in fitness, and in many cases they may remain undetected. For example, EANES and HEY (1986) have studied 11 rare electrophoretic variants of glucose-6-phosphate dehydrogenase in *D. melanogaster*. Most of the variants exhibit apparent *in vivo* activities which are similar to one or the other of the common variants, and the *in vivo* activities of the variants are not well correlated with the *in vitro* estimates of specific activity. A similar situation regarding alcohol dehydrogenase activity and survival in ethanol has been reported by MIDDLETON and KACSER (1983).

A third major problem in uniting physiology with population genetics in studies of natural populations results from linkage disequilibrium. Selective effects that result from the presence of a particular allele may be difficult to prove because of possible undetected linkage disequilibrium, and conversely, selective effects that result from a block of genes in linkage disequilibrium may incorrectly be attributed to the presence of a particular allele contained within the block of genes. In experimental systems, this problem can be overcome by the use of isogenic strains.

Many of the experimental difficulties in the study of the molecular mechanisms of selection can be overcome or minimized by the use of continuous culture of prokaryotic organisms such as *E. coli* (HARTL and DYKHUIZEN 1985). This system offers advantages in terms of simplicity of life history, large effective population size and sample size, availability of mutants or methods of obtaining mutants that cover a large range of enzyme activities, and control over genetic background and strain isogenicity.

In a previous paper (DEAN, DYKHUIZEN and HARTL 1986), we have studied the fitness effects of laboratory mutations in chemostats in order to determine the relation between fitness and enzyme activity of β-galactosidase in *E. coli* cultured in chemostats in which the limiting nutrient is lactose. We have now extended the approach to be able to include the effects of variation in activity of the β-galactosidase permease. These results empirically define the adaptive topography of the two major genes of the lactose operon of *E. coli* relative to growth in limiting lactose. The results suggest that genetic variants with small differences from the wild-type-induced levels of β-galactosidase activity may be subject to much weaker selection than minor variants in the activity of the β-galactoside permease. Thus, the β-galactoside permease appears to demonstrate the greater potential for natural selection to act on small differences in activity.

MATERIALS AND METHODS

Estimates of the fitness of inducible strains, relative to a *lacI* constitutive strain in lactose-limited chemostats, were obtained as described in DYKHUIZEN and DAVIES (1980) for various levels of induction of the *lac* operon. The dilution rate of the chemostats was set at a generation time of 2.0 hr. The limiting nutrient was lactose (0.1 gm/liter), and the growth medium also contained one of four concentrations of the gratuitous inducer isopropyl-β-D-thiogalactoside (IPTG); namely, 0.0, 0.6, 2.0 or 60.0 μM/liter. Corresponding levels of induction of the *lac* operon were determined by enzyme assays as described (DYKHUIZEN and DAVIES 1980). Direct estimates of permease activity under chemostat growth conditions were not carried out. However, the level of permease activity has been calculated based on the relative levels of induction of β-galactosidase, using the fact that *lacZ* and *lacY* are induced coordinately by IPTG (JACOB and MONOD 1961). Since the function of the β-galactoside transacetylase is still obscure (INGRAHAM, MAALØE and NEIDHARDT 1983), its role in chemostat growth cannot be assessed at present. Therefore, the control coefficient with regard to fitness that we ascribe to the permease should in reality be interpreted as an apparent control coefficient because it is based on calculated permease levels and also includes both the permease and a contribution, if any, from the transacetylase.

Estimates of the fitness of β-galactosidase missense mutants, relative to a *lacI* constitutive strain, were obtained as described in DEAN, DYKHUIZEN and HARTL (1986). Missense mutations with small effects on enzyme activity were obtained by the reversion of nonsense mutations. To estimate the selection coefficients of strains with low levels of β-galactosidase activity, we used data of HALL (1984). HALL's strains are all *E. coli* K12 derivatives; thus, they contain the same *lacY* permease as in the other experiments.

THEORY OF METABOLIC FLUX

KACSER and BURNS (1973, 1979 and 1981) have shown that the flux (F) through a linear metabolic pathway is related to the activities of its constituent enzymes as follows:

$$F = \frac{S_0 - S_n/K_{0,n}}{\sum_{i=0}^{n-1} \left(\frac{K_m(i)}{V_{max}(i)}\right)\left(\frac{1}{K_{0,i}}\right)}. \quad (1)$$

In this model, each enzyme is assumed to be unsaturated and to catalyze a reaction which is, in principle, reversible. S_0 and S_n are the concentrations of the first and final substrates in the pathway, and the $K_m(i)$ and $V_{max}(i)$ are the Michaelis constants and maximal velocities of the forward reactions. $K_{0,i}$ is the equilibrium constant between the first and ith substrates and is equal to the product of the equilibrium constants across the individual intervening steps.

In a chemostat, competition for lactose as the sole resource limiting the rate of growth ensures that the external concentration of lactose (S_0) is small enough that the enzymes used in its metabolism are unsaturated (DYKHUIZEN and HARTL 1983). Also, $K_{0,n}$ will typically be rather large for many catabolic pathways, such as lactose metabolism and glycolysis, because the Gibbs free energies associated with catabolism of sugars to pyruvate are generally rather large. Thus, $S_n/K_{0,n}$ will be rather small compared with S_0, so that

the numerator is essentially equal to S_0. Furthermore, because this flux limits the rate of growth of the strains, one expects the growth rate (μ) to be directly proportional to the flux (F) across the pathway. Consequently, (1) may be rewritten to a good approximation as

$$\mu = \frac{S_0}{\sum_{i=0}^{n-1} \left(\frac{K_m(i)}{V_{\max}(i)}\right)\left(\frac{1}{K_{0,i}}\right)} = \frac{S_0}{\sum_{i=0}^{n-1} \frac{1}{B_i}}, \qquad (2)$$

where $B_i = (V_{\max}(i))\,(K_{0,i})/K_m(i)$. Let θ_i be the fraction of activity that a mutant enzyme possesses, relative to wildtype, so that

$$\theta_i = \frac{V'_{\max}(i)K_m(i)}{V_{\max}(i)K'_m(i)}, \qquad (3)$$

where the primes represent values pertaining to the mutant. Substituting into (2), the growth rate of a mutant strain can be written as

$$\mu' = \frac{S_0}{\sum_{i=0}^{n-1} \frac{1}{B_i\theta_i}}. \qquad (4)$$

The relative growth rate (relative fitness) of a mutant relative to wild type in lactose-limited chemostats is therefore

$$w = \frac{\mu'}{\mu} = \frac{\sum_{i=0}^{n-1} \frac{1}{B_i}}{\sum_{i=0}^{n-1} \frac{1}{B_i\theta_i}} = \frac{A}{\frac{1}{B_0\theta_0} + \frac{1}{B_1\theta_1} + C}, \qquad (5)$$

where, on the left-hand side, w represents the fitness of a mutant relative to wild type, and, on the right-hand side, the first two terms of the summation in the denominator have been expanded because they represent the effect of mutant permease and β-galactosidase, respectively. The constants in (5) are

$$A = \sum_{i=0}^{n-1} (1/B_i)$$

and

$$C = \sum_{i=2}^{n-1} (1/B_i).$$

Inverting (5) results in the expression

$$\frac{1}{w} = \frac{a}{\theta_0} + \frac{b}{\theta_1} + c, \qquad (6)$$

where $a = 1/(B_0A)$, $b = 1/(B_1A)$ and $c = C/A$.

The fitness surface as a function of θ_0 and θ_1 (relative activities of permease and β-galactosidase) may therefore be estimated from (6) by means of multiple linear regression using the reciprocals of the observed fitnesses and enzyme activities. This is analogous to the double-reciprocal Lineweaver-Burk plot used in enzyme kinetics.

RESULTS

The total metabolic context of the lactose operon in *E. coli* is discussed in MILLER and REZNIKOFF (1978). Estimates of relative fitness of strains in chemostats and assays of enzyme activity were carried out as described in DYKHUIZEN and DAVIES (1980) and DEAN, DYKHUIZEN and HARTL (1986). The data are presented in Table 1, where both the growth rates and activities are expressed as proportions of those obtained for the constitutive expression of wild-type (*E. coli* K12) enzymes of the *lac* operon. The upper part of Table 1 contains data from chemostats in which the *lac* operon was partially or completely induced by IPTG (data from DYKHUIZEN and DAVIES 1980). The middle part pertains to strains of *E. coli* K12 containing various constitutive *lacZ* missense mutations (data from DEAN, DYKHUIZEN and HARTL 1986). Both sets of experiments used isogenic strains and an identical genetic background. The lower part of the table pertains to strains containing various types of alleles of *ebg* (evolved β-galactosidase; data from HALL 1984). In the IPTG induction experiments, the level of permease was assumed to vary in proportion to that of β-galactosidase, because the proteins are coordinately controlled. In the other experiments, the *lacZ* and *ebg* alleles were in genetic backgrounds containing a wild-type permease.

Multiple linear regression as described by SNEDECOR and COCHRAN (1967) was used to estimate the parameters a, b and c in equation (6) and their standard errors. The effects of sampling variance in the observed fitnesses have not been taken into account in the regression. The resulting parameter estimates and

TABLE 1

Relative fitness and enzyme activities

	Fitness	Lactase activity	Permease[a] activity	Predicted fitness
IPTG (μM/liter)				
60.0	1	1	(1)	1
2.0	0.921	0.608	(0.608)	0.921
0.6	0.715	0.383	(0.383)	0.823
0.0	0.501	0.117	(0.117)	0.497
TD1/TD2	1	1	(1)	1
TD10.3	0.989	0.746	(1)	0.999
TD10.4	1.006	1.361	(1)	1.001
TD3	0.946	0.053	(1)	0.929
TD4	0.798	0.011	(1)	0.723
K12	1	1	(1)	1
ebgI	0.563	0.007	(1)	0.613
ebgII	0.238	0.002	(1)	0.279
ebgIV	0.463	0.003	(1)	0.429
ebgV	0.225	0.001	(1)	0.219

[a] Inferred relative values (see MATERIALS AND METHODS).

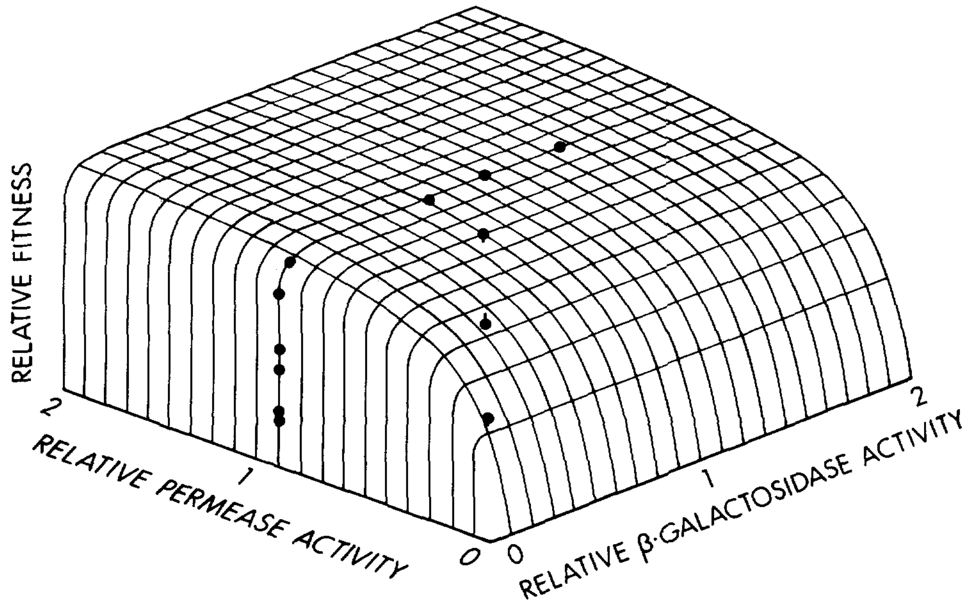

FIGURE 1.—Three-dimensional representation of the fitness surface. Lines are placed at 0.1 intervals of enzyme activity, relative to a value of one for the constitutive level of expression. On this scale, the wild-type-induced level of expression is at $x = y = 0.117$. The dots are the data points used in estimation of the parameters.

their standard errors were as follows:

$$\hat{a} = 0.12934 \pm 0.03174$$
$$\hat{b} = 0.00425 \pm 0.00024$$
$$\hat{c} = 0.86641 \pm 0.10169$$

The model accounts for 96.6% of the total variance. Although different three-parameter regression equations might also fit the data, the high proportion of the variance explained by this particular equation would seem to imply that the metabolic-control model is quite adequate. The final column in Table 1 gives the predicted values of the fitnesses, calculated from (6). The average 95% confidence limits are approximately 15% above and below the estimated values. With the exception of ebgII and egbIV, all observed values lie within the 95% confidence intervals of the estimated values. The errant values for ebg may simply reflect the imprecision in estimating low levels of lactase activity.

Figure 1 is a three-dimensional representation of the fitness surface showing the approximate positions of the data points used in the model. The precipitous cliff on the left-hand side indicates that small differences at low lactase activities usually cause dramatic changes in fitness. The more shallow slope on the right-hand side indicates that larger differences at low relative permease activities are necessary to generate similar fitness changes. Yet, as activities of both enzymes increase, the growth rate moves away from the adaptive valley and comes to reside on a fitness plateau. Here, even relatively large changes in the activities of either enzyme may result in negligible changes in fitness.

Further consideration of (6) gives insight into the interaction of enzyme activities in determining fitness. The control coefficient at step i in a linear metabolic pathway is defined in BURNS *et al.* (1985) as

$$C_i = \frac{\partial F/F}{\partial \theta_i/\theta_i} \tag{7}$$

For small absolute changes, the control coefficient is approximately equal to the proportional change in flux divided by the proportional change in activity (KACSER and BURNS 1973).

At inducible levels of the *lac* operon proteins, any small increase in lactase activity has a negligible effect on fitness in chemostats, as illustrated by the slope of the curve near *relative enzyme activity* = 1 in Figure 2. In terms of metabolic theory, β-galactosidase has such a small control coefficient ($C_1 = 0.018$) that minor increases in lactase activity have little effect on fitness. The obvious prediction is that most *lacZ* alleles that are polymorphic in natural populations may be selectively neutral, or nearly neutral, provided that their enzyme activity is not too greatly different from that of wild type.

However, in contrast to β-galactosidase, the wild-type-induced level of permease is not on or near a plateau, but rather is on the shoulder of the curve (Figure 2). In terms of metabolic theory, the permease has a large apparent control coefficient ($C_0 = 0.551$). In fact, over 95% of the doubling in fitness gained by a strain becoming constitutive in a lactose-limited chemostat is due solely to increased permease activity. In the case of permease, alleles found to be polymorphic in natural populations may be subject to

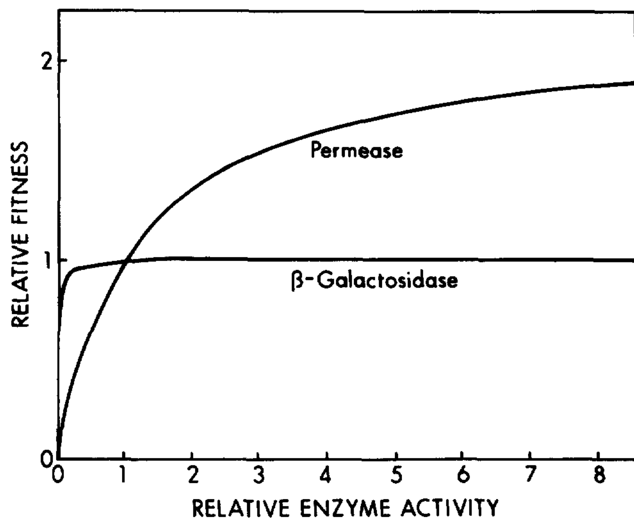

FIGURE 2.—The effect on fitness of changing the activity of one enzyme while holding the other constant at a value of one. Both fitness and enzyme activities are presented relative to the values for the wild-type-induced level of activity. The constitutive level of activity corresponds to the extreme right in the figure.

selection, even though their effects on permease activity may be relatively small.

DISCUSSION

Studies of laboratory mutations undergoing selection in lactose-limited chemostats have defined the adaptive topography of the β-galactosidase and permease genes in *E. coli*. Consideration of the adaptive topography leads to several predictions regarding the adaptive effects of naturally occurring genetic polymorphisms in these genes. First, the control coefficient of the β-galactosidase enzyme with regard to fitness is quite small ($C = 0.018$); therefore, genetic variants demonstrating small differences in enzyme activity from wild type may be selectively neutral or nearly neutral. In contrast, the apparent control coefficient of the β-galactoside permease is quite large ($C = 0.551$); consequently, genetic variants that mildly alter permease activity may be subject to selection. Whether permease systems tend to have large control coefficients in general is not known, but SALTER, KNOWLES and POGSON (1986) have also found that the control coefficients of aromatic amino acid transport systems in rats are relatively large at induced levels of activity. However, these control coefficients are with regard to flux rather than fitness and are thus not directly comparable to those reported in this paper.

Genetic polymorphism of β-galactosidase is extensive and well documented. In one electrophoretic study of 109 clones from 91 sources throughout North America, 18 distinct electromorphs of β-galactosidase were observed (SELANDER and LEVIN 1980). Studies of the enzymatic and fitness effects of these naturally occurring alleles are in progress in our laboratory. In addition to the electromorphs, one clone was found to contain a null allele for β-galactosidase, which is of course lethal when lactose is the only available source of carbon. Unfortunately, owing to the lack of an assay system suitable for screening, the level of genetic polymorphism of the β-galactoside permease is unknown.

The chemostat system is noteworthy for the very strong selection pressure it imposes for the efficient acquisition and utilization of the limiting substrate. In the case of lactose limitation, the selection is so intense that mutations which produce the enzymes constitutively will regularly and predictably take over in the chemostat in the course of a few days (HORIUCHI, TOMIZAWA and NOVICK 1962; SILVER and MATELES 1969; DYKHUIZEN and HARTL 1978). The evolution of constitutive expression decreases the apparent control coefficient of the permease, consistent with our previously proposed model of enzyme evolution (HARTL, DYKHUIZEN and DEAN 1985). Such mutations are not selected in natural environments, in which lactose concentrations fluctuate and lactose is not the sole carbon source. Fluctuation between limiting and excess lactose selects against constitutive mutants. This is demonstrated by the finding that, when constitutive mutants that have been selected in the chemostat are removed from the chemostat and plated on medium containing excess lactose, the cells die owing to the excessive transport of lactose and consequent loss of membrane potential (DYKHUIZEN and HARTL 1978; WILSON, PUTZRATH and HASTINGS WILSON 1981; GHAZI, THERISOD and SHECHTER 1983). Lactose-constitutive mutants are also disfavored in the absence of lactose (NOVICK and WEINER 1957; ANDREWS and HEGEMAN 1976; KOCH 1983). These, and perhaps other, forces are sufficient to maintain the regulated state of the lactose operon in natural populations.

One of the key results of the metabolic control theory of KACSER and BURNS (1973) is the summation theorem, which states that the sum of all control coefficients in a linear pathway with fixed input must equal unity. If one enzyme in the pathway is "inefficient," then it will have a large control coefficient. But then, as a consequence of the summation theorem, other steps in the same pathway must have very small control coefficients, so that small variation in the activity of these steps will have a negligible effect on flux. Any substantial increase in the activity of the inefficient enzyme would reduce its control coefficient, but at the same time it would increase the control coefficients of one or more other enzymes along the way. In this manner, genetic variation in the enzyme activity of the other steps in the pathway, which formerly had negligible effects, would come to influence flux significantly. Hence, the selective ef-

fects of genetic variation in enzyme activity at one step in a pathway is conditioned by the activities of other enzymes in the pathway. Genes that modify the fitness effects of other genes may often be nothing more than alleles affecting other steps in the same metabolic pathway.

The conditional nature of selection coefficients may be illustrated using the adaptive topography of the lactose operon. As demonstrated, at the normal level of induction of the lactose operon, the permease has a large apparent control coefficient and the β-galactosidase has a small one. Were the activity of the permease to increase, say 20-fold, then its control coefficient would decrease (from $C = 0.551$ to 0.067); however, this increase in permease activity would also approximately double the control coefficient of the β-galactosidase (from $C = 0.018$ to 0.038). It is of interest to note that, at induced wild-type levels, the sum of the apparent control coefficients of β-galactosidase and permease is 0.569, leaving 0.431 for the other steps in lactose metabolism; whereas with the hypothetical 20-fold increase, the sum is 0.105, leaving 0.895 for the other steps and thus rendering them relatively more important for fitness.

Other things being equal, one would expect natural selection to favor permease variants which increase the efficiency of the permease and thereby decrease its control coefficient. However, the observed high control coefficient of the β-galactoside permease suggests that other forces countervail and act to maintain permease efficiency at an optimum. Perhaps the effects of lactose permeation on membrane potential are also involved at this level of selection.

The general implication of this work in the evaluation of the selective effects of polymorphic alleles is to suggest a shift of perspective in which the control coefficients of the gene products become paramount (HARTL, DYKHUIZEN and DEAN 1985). In this approach, selection or selective neutrality are not intrinsic and unalterable characteristics of the alleles alone, but rather are conditional phenomena which depend in part on the aggregate effects of other enzymes and which can be quantified by means of control coefficients. However, it is important to emphasize that we refer to the control coefficients with respect to *fitness*, defined as the proportionate change in fitness resulting from a small proportionate change in flux, in analogy with equation (7). In nutrient-limited chemostats, in which growth rate is proportional to the rate at which the limiting nutrient is metabolized, fitness is proportional to flux, and the relation between enzymatic control coefficients and fitness control coefficients is straightforward. This cannot always be expected to be the case, particularly in complex natural environments, as demonstrated in the present example by the fact that constitutive strains are detrimental in nature. Nevertheless, the experimental determination of the adaptive topography under controlled environmental conditions can provide important insight into the types of polymorphisms in natural populations and the mechanisms by which they are maintained.

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LITERATURE CITED

ANDREWS, K. J. and G. D. HEGEMAN, 1976 Selective disadvantage of nonfunctional protein synthesis in *Escherichia coli*. J. Mol. Evol. **8:** 317–328.

BARNES, P. T. and C. C. LAURIE-AHLBERG, 1986 Genetic variability of flight metabolism in *Drosophila melanogaster*. III. Effects of *Gpdh* allozymes and environmental temperature on power output. Genetics **112:** 267–294.

BURNS, J. A., A. CORNISH-BOWDEN, A. K. GROEN, R. HEINRICH, H. KACSER, J. W. PORTEOUS, S. M. RAPOPORT, T. A. RAPOPORT, J. W. STUCKI, J. M. TAGER, R. J. A. WANDERS and H. V. WESTERHOF, 1985 Control analysis of metabolic systems. Trends Biochem. Sci. **10:** 16.

DEAN, A. M., D. E. DYKHUIZEN and D. L. HARTL, 1986 Fitness as a function of β-galactosidase activity in *Escherichia coli*. Genet. Res. **48:** 1–8.

DE JONG, G. and W. SCHARLOO, 1976 Environmental determination of selective significance or neutrality of amylase variants in *Drosophila melanogaster*. Genetics **84:** 77–94.

DYKHUIZEN, D. E. and M. DAVIES, 1980 An experimental model: bacterial specialists and generalists competing in chemostats. Ecology **61:** 1213–1227.

DYKHUIZEN, D. E. and D. L. HARTL, 1978 Transport by the lactose permease of *Escherichia coli* as the basis of lactose killing. J. Bacteriol. **135:** 876–882.

DYKHUIZEN, D. E. and D. L. HARTL, 1983 Selection in chemostats. Microbiol. Rev. **47:** 150–168.

EANES, W. F., B. BINGHAM, J. HEY and D. HOULE, 1985 Targeted selection experiments and enzyme polymorphisms: negative evidence for octanoate selection at the G6PD locus in *Drosophila melanogaster*. Genetics **109:** 379–391.

EANES, W. F. and J. HEY, 1986 *In vivo* function of rare *Gbpd* variants from natural populations of *Drosophila melanogaster*. Genetics **113:** 679–693.

GHAZI, A., H. THERISOD and E. SHECHTER, 1983 Comparison of lactose uptake in resting and energized *Escherichia coli* cells: high rates of respiration inactivate the *lac* carrier. J. Bacteriol. **154:** 92–103.

HALL, B. G., 1984 The evolved β-galactosidase system of *Escherichia coli*. pp. 165–185. In: *Microorganisms as Model Systems for Studying Evolution*, Edited by R. P. MORTLOCK, Plenum, New York.

HARTL, D. L. and D. E. DYKHUIZEN, 1985 The neutral theory and the molecular basis of preadaptation. pp. 107–124. In: *Population Genetics and Molecular Evolution*, Edited by T. OHTA and K. AOKI. Japan Scientific Societies Press, Tokyo.

HARTL, D. L., D. E. DYKHUIZEN and A. M. DEAN, 1985 Limits of adaptation: the evolution of selective neutrality. Genetics **111:** 655–674.

HORIUCHI, T., J.-I. TOMIZAWA and A. NOVICK, 1962 Isolation and properties of bacteria capable of high rates of β-galactosidase synthesis. Biochim. Biophys. Acta **55:** 152–153.

JACOB, F. and J. MONOD, 1961 On the regulation of gene activity. Cold Spring Harbor Symp. Quant. Biol. **26:** 193–211.

INGRAHAM, J. L., O. MAALØE and F. C. NEIDHARDT, 1983 *Growth of the Bacterial Cell.* Sinauer Associates, Sunderland, Massachusetts.

KACSER, H. and J. A. BURNS, 1973 The control of flux. Symp. Soc. Exp. Biol. **32:** 65–104.

KACSER, H. and J. A. BURNS, 1979 Molecular democracy: who shares the controls. Biochem. Soc. Trans. **7:** 1149–1160.

KACSER, H. and J. A. BURNS, 1981 The molecular basis of dominance. Genetics **97:** 639–666.

KOCH, A. L., 1983 The protein burden of *lac* operon products. J. Mol. Evol. **19:** 455–462.

KOEHN, R. K., A. J. ZERA and J. G. HALL, 1983 Enzyme polymorphism and natural selection. pp. 115–136. In: *Evolution of Genes and Proteins,* Edited by M. NEI and R. K. KOEHN. Sinauer Associates, Sunderland, Massachusetts.

MIDDLETON, R. J. and H. KACSER, 1983 Enzyme variation, metabolic flux and fitness: alcohol dehydrogenase in *Drosophila melanogaster*. Genetics **105:** 633–650.

MILLER, J. H. and W. S. REZNIKOFF (eds.), 1978 *The Operon.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

NOVICK, A. and M. WEINER, 1957 Enzyme induction as an all-or-none phenomenon. Proc. Natl. Acad. Sci. USA **43:** 553–566.

SALTER, M., R. G. KNOWLES and C. I. POGSON, 1986 Quantification of the importance of individual steps in the control of aromatic amino acid metabolism. Biochem. J. **234:** 635–647.

SELANDER, R. K. and B. R. LEVIN, 1980 Genetic diversity and structure in *Escherichia coli* populations. Science **210:** 545–547.

SILVER, R. S. and R. I. MATELES, 1969 Control of mixed-substrate utilization in continuous cultures of *Escherichia coli.* J. Bacteriol. **97:** 535–543.

SNEDECOR, G. W. and W. G. COCHRAN, 1967 *Statistical Methods.* Iowa State University Press, Ames, Iowa.

VAN DELDEN, W., A. C. BOERMA and A. KAMPING, 1978 The alcohol dehydrogenase polymorphism in populations of *Drosophila melanogaster*. I. Selection in different environments. Genetics **90:** 161–191.

WILSON, D. M., R. M. PUTZRATH and T. HASTINGS WILSON, 1981 Inhibition of growth of *Escherichia coli* by lactose and other galactosides. Biochim. Biophys. Acta **649:** 377–384.

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