A Molecular Investigation of Genotype by Environment Interactions

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ABSTRACT

The fitnesses conferred by seven lactose operons, which had been transduced into a common genetic background from natural isolates of *Escherichia coli*, were determined during competition for growth rate–limiting quantities of galactosyl-glycerol, a naturally occurring galactoside. The fitnesses of these same operons have been previously determined on lactose and three artificial galactosides, lactulose, methyl-galactoside and galactosyl-arabinose. Analysis suggests that although marked genotype by environment interactions occur, changes in the fitness rankings are rare. The relative activities of the β-galactosidases and the permeases were determined on galactosyl-glycerol, lactose, lactulose and methyl-galactoside. Both enzymes display considerable kinetic variation. The β-galactosidase alleles provide no evidence for genotype by environment interactions at the level of enzyme activity. The permease alleles display genotype by environment interactions with a few causing changes in activity rankings. The contributions to fitness made by the permeases and the β-galactosidases were partitioned using metabolic control analysis. Most of the genotype by environment interaction at the level of fitness is generated by changes in the distribution of control among steps in the pathway, particularly at the permease where large control coefficients ensure that its kinetic variation has marked fitness effects. Indeed, changes in activity rankings at the permease account for the few changes in fitness rankings. In contrast, the control coefficients of the β-galactosidase are sufficiently small that its kinetic variation is in, or close to, the neutral limit. The selection coefficients are larger on the artificial galactosides because the control coefficients of the permease and β-galactosidase are larger. The flux summation theorem requires that control coefficients associated with other steps in the pathway must be reduced, implying that the selection at these steps will be less intense on the artificial galactosides. This suggests that selection intensities need not be greater in novel environments.

HAPLOID clonal species can be highly polymorphic (MILKMAN 1973). That fact eliminates diploidy and sexual reproduction as necessary requirements for maintaining genetic variation in natural populations. This hardly matters when discussing neutral polymorphisms, but it raises questions about the means by which selection protects alleles at other loci. Overdominance and related hypotheses, although sufficient, are restricted to diploids. They do not apply to haploids and cannot be considered general. If such phenomena are, nevertheless, common in diploids, then selection must generally operate in a different way in haploids. There is little evidence to suggest that this is so (resistance to malaria by human $H_b^A H_b^S$ heterozygotes being a rare exception).

The only remaining mechanisms are frequency dependent selection, which requires fitness to decline as the frequency of the allele increases, and variable environments, in which different alleles are favored at different times or places. The first seems unlikely in the absence of plausible means to generate frequency dependence at most loci. As for the second, temporal changes in the direction of selection are incapable of protecting polymorphisms in haploid populations (DEMPSTER 1955; GILLESPIE 1973). However, selection may protect polymorphisms in haploids if different alleles are favored in different demes, with the proviso that migration rates are not so high that the demes coalesce into a single "panmictic" population.

Genotype by environment interactions must be extensive if selection favors different alleles in different environments. Yet despite an extensive body of theory (CHRISTIANSEN and FELDMAN 1975; FELSENSTEIN 1976; HEDRICK 1976; GILLESPIE 1978, 1991; VIA and LANDE 1987; GILLESPIE and TURELLI 1989), there remain lamentably few experimental studies addressing the role of genotype by environment interactions in maintaining polymorphisms. Frequently detected when analyzing quantitative traits (*e.g.*, COMSTOCK and MOLL 1963; GUPTA and LEWONTIN 1982) including fitness components (DOBZHANSKY and SPASSKY 1944; TACHIDA and MUKAI 1985), their occurrence does not guarantee the changes in fitness rankings necessary for protecting polymorphisms (FALCONER 1952; BELL 1990). Changes in the fitness rankings of *Chlamydomonas reinhardtii* genotypes growing clonally in various chemically defined environments (BELL 1991) lack crucial experimental controls demonstrating that periodic selection is absent

(ATWOOD *et al.* 1951a,b; KUBITSCHEK 1974). LYNCH (1987), surveying several loci in isolated populations of Daphnia, detected fluctuations in the frequencies of alleles that were too large to be wholly ascribed to genetic drift. These fluctuations, too, may reflect the hitchhiking effects of periodic selection during clonal growth of Daphnia in summer.

Compelling cases of genotype by environment interaction at single loci are exceedingly rare. One of the few is provided by the lactate dehydrogenase of the common killifish *Fundulus heteroclitus*, which populates a steep cline in temperature from 20 to 40° along the eastern seaboard of North America. The LDH-B$_4^b$ allozyme is the more active below 30°, and the LDH-B$_4^a$ allozyme more active at higher temperatures (PLACE and POWERS 1984). Their activities are correlated with swimming ability and egg hatching time (DIMICHELE and POWERS 1982a,b), both presumably important fitness components in this species.

The goal of this paper is to provide a detailed understanding of how molecular level events give rise to genotype by environment interactions in fitness. The genetic variation is confined solely to natural variants and laboratory mutants of the lactose operon of *Escherichia coli*. Environmental variation is generated by five different galactosides, each of which becomes the sole nutrient limiting growth rates during the competition experiments. Hence, the experiment directly targets the environmental variation at the genetic variation.

The five galactosides differ in the aglycone moieties attached to the galactose. The glucose of lactose is a pyranose (a 6-membered ring), the fructose of lactulose and the arabinose of galactosyl-arabinose equilibrate between pyranose and furanose (5-membered ring) forms and the glycerol of galactosyl-glycerol is a linear three-carbon structure, whereas the methyl moiety of methyl-galactoside is the smallest, a one-carbon structure having a mass < 10% of glucose.

Two of the galactosides occur naturally: lactose (milk sugar) is found in the guts of infant mammals, whereas galactosyl-glycerol is released in large quantities from the monogalactosyldiglycerides of chloroplast membranes by the action of lipases in the guts of plant-eating animals (BOOS 1982). Three galactosides are artificial: lactulose and galactosyl-arabinose (which do not even induce expression of the operon) and methyl-galactoside.

SILVA and DYKHUIZEN (1993) demonstrated that selection on the artificial substrates is more intense than on lactose. The work presented here builds on the foundation they laid by extending the competition experiments to include a second naturally occurring galactoside (galactosyl-glycerol), by investigating the relations between gene expression and fitness and by investigating the kinetics of the β-galactosidase alleles. The con-

tributions to fitness made by alleles of the β-galactosidase and the permease are partitioned using a mechanistic biochemical model of natural selection.

MATERIALS AND METHODS

Media, growth conditions, buffers and substrates: Minimal medium (Davis salts) is 40 mM K_2HPO_4, 15 mM KH_2PO_4, 7.6 mM $(NH_4)_2SO_4$, 1.7 mM trisodium citrate, 1 mM $MgCl_2$ pH 7.3, and complete medium (LB) is 10 g NaCl, 10 g Bacto tryptone and 5 g of Bacto yeast extract per liter. Bacto agar (15 g/liter) is added for plates, and various supplements are added as indicted. Potassium phosphate buffer is 60 mM K_2HPO_4, 40 mM KH_2PO_4, 1 mM $MgCl_2$ pH 7.3. Lactose, lactulose, methyl-galactoside, NAD, galactose dehydrogenase, *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and isopropyl 1-thio-β-D-galactopyranoside (IPTG) were purchased from Sigma Chemical, St. Louis. Galactosyl-glycerol was a kind gift from Dan Dykhuizen. All experiments were conducted at 37° except where specified.

Strains: The strains used in this study are presented in Table 1. The lactose operons of CSH64 (a wild-type K12 operon), JL3300 (a constitutive *lacI⁻* K12 operon that is otherwise wild-type) and six K12 operons carrying mutant β-galactosidase alleles (DEAN *et al.* 1986, 1988) were transduced into DD320, which carries a small deletion of the entire operon and serves as the genetic background for all experiments. Also transduced into this background (DEAN 1989) were the operons of six natural worldwide isolates of the *E. coli* Reference Collection (OCHMANN and SELANDER 1984). The generalized transduction protocol is that of MILLER (1972) using P1 (*cml clr100*) with selection for growth on minimal medium plates supplemented with 0.2 g/liter of lactose.

Competition experiments: Chemostat competition experiments were conducted according to the methods described by DYKHUIZEN and HARTL (1980, 1983) and DEAN (1989). The medium is Davis salts supplemented with 0.01% (w/v) of a carbon source (glucose, lactose, galactosyl-glycerol, lactulose or methyl-galactoside), which serves as the only source of carbon and energy and becomes the sole nutrient limiting growth rates at steady state in the chemostat. The presence of 10 or 100 μM of the nonmetabolizable inducer IPTG assures that all operons are induced to maximal levels during competition for the various galactosides. Control experiments were conducted in the absence of IPTG using glucose as a carbon source.

The relations between gene expression and fitness on each galactoside were investigated by the method of DYKHUIZEN and DAVIES (1980), which uses various levels of IPTG to modulate induction of an inducible operon (TD1) during competition with a constitutive operon (TD2).

The progress of competition between pairs of strains was monitored as follows. A spontaneous mutation (*fhuA*) was isolated from each strain, conferring resistance to the bacteriophage T5. Competition experiments were conducted between a sensitive strain (carrying one lactose operon) and a resistant strain (carrying a different lactose operon). The proportion (R) of the population resistant to T5 was estimated twice a day by counting the number of colonies formed after plating samples in the absence and presence of excess T5 phage on LB plates.

The selection coefficient per hour was estimated by the slope (S) of the regression of $\ln(R/(1 - R))$ against time (DYKHUIZEN and HARTL 1980, 1983). Relative fitness was cal-

culated as $w = 1 - S/D$, where D is the dilution rate of the chemostat (~ 0.33 hr^{-1}). All competition experiments were conducted between TD1 and the various strains. Hence, all the selection coefficients and fitnesses are given with respect to TD1. The effect upon fitness of the *fhuA* mutation was determined by comparing the absolute values of the selection coefficients from two experiments, one in which TD1 was resistant and one in which the competitor was resistant. In no case did the *fhuA* mutation confer a detectable fitness effect. Fitness estimates from each experiment were treated as individual observations when calculating the means and SE reported in the various tables.

Estimating gene expression: Pure cultures of TD1 were grown in chemostats on each galactoside at each of several concentrations of IPTG. To each 0.9 ml of culture sample was added 0.1 ml of a 50 mM solution of ONPG dissolved in Davis salts, the final concentration of 5 mM being sufficient to saturate the permease (DEAN 1989). The rate of change in absorbency at 420 nm is directly proportional to the rate of production of ONP (KOCH 1964) and provides an estimate of the level of expression of the operon when normalized to cell number or cell density. Typically, 10 assays, five for each duplicate chemostat, were conducted at each concentration of IPTG.

Hydrolysis of galactosides: Cells, grown overnight in Davis salts supplemented with 0.2% (w/v) succinate and 100 μM IPTG, were centrifuged, and the pellets resuspended in ice-cold potassium phosphate buffer. After sonication, the cell debris was removed by centrifugation. The amount of protein in each extract was estimated by the method of BRADFORD (1976) from standard curves prepared using immunoglobulin G.

The action of β-galactosidase toward the various substrates was monitored at 340 nm by coupling the production of galactose to the reduction of NAD by galactose dehydrogenase. Ten microliters of a 1:10 dilution of the unfractionated extract was added to 1 ml of a solution containing 2 mM NAD, 150-fold excess galactose dehydrogenase and various concentrations (0.5–32 mM) of lactose or galactosyl-glycerol. Estimates of the kinetic parameters were obtained using the Gauss-Newton method of nonlinear least squares regression. Estimates of the kinetic parameters from each experiment were treated as individual observations when calculating the means and SE reported in the various tables. V_{max} units are μM min^{-1} mg^{-1} cell protein.

Lactulose and methyl-galactoside are contaminated with small amounts of galactose ($<0.2\%$) that seriously compromise estimates of the kinetic parameters of the mutant β-galactosidases. The problem was overcome by incubating reaction mixtures (containing 1–64 mM galactoside) with galactose-dehydrogenase for 10 min before the addition of 10 μl of undiluted extracts.

Several of the mutant enzymes have very high Michaelis constants toward lactulose and methyl-galactoside. Preincubating reaction mixtures containing sufficient substrate to saturate these mutants produces absorbencies in excess of 2, with the consequence that reliable estimates of their V_{max}s and K_ms could not be obtained. Nevertheless, the ratio V_{max}/K_m can still be determined accurately as the slope of a plot of velocities against substrate concentrations when the latter lie far below the K_m.

RESULTS

Estimating selection coefficients: A series of strains (Table 1), genetically identical except for their lactose operons, was constructed using generalized P1 transduction to move operons into DD320, a prototrophic strain with a small deletion of the entire lactose operon. All chemostat competition experiments were conducted between strains carrying the various lactose operons and TD1, which carries the K12 operon. Hence all selection coefficients and fitnesses are given relative to TD1.

Some results are illustrated in Figure 1. The absence of selection during competition for glucose demonstrates that the strong selection observed during competition for galactosyl-glycerol is due to differences between operons and not to mutations accrued in the genetic background during strain construction. The intensity of selection is similar regardless of which strain carries *fhuA*, the genetic marker used to distinguish the strains. Hence, *fhuA* confers no detectable selective effect.

The pooled results (Table 2) provide strong evidence for selection during competition for galactosyl-glycerol, both among operons from the natural isolates and among four mutant β-galactosidase alleles. In no case was selection detected in the control experiments with glucose as the limiting nutrient. Competition experiments show that DD320, which is unable to metabolize galactosyl-glycerol, is washed from the chemostat at a rate indistinguishable from the dilution rate. This demonstrates that galactosyl-glycerol is an essential resource and that neither cross-feeding nor interference competition occur.

During competition for limiting galactosyl-glycerol, strain TD10, which harbors an operon from a natural isolate, is favored at 10 μM and disfavored at 100 μM IPTG. Although IPTG is an artificial inducer of the operon, no differences in expression, as judged by the rate of hydrolysis of ONPG by intact cells, could be detected at the two IPTG levels. Hence, changes in expression of the operon are not responsible for the observed selection. High concentrations of IPTG are toxic to TD10, perhaps because this strain lacks a functional *lacA*-encoded galactoside transacetylase (D. E. DYKHUIZEN, unpublished data), an enzyme which has been implicated in the detoxification of nonmetabolizable galactosides (ANDREWS and LIN 1976). No significant differences in selection coefficients were detected among the other strains at 10 and 100 μM IPTG.

Evidence for genotype by environment interactions: Estimates of the mean fitnesses relative to TD1 on five galactosides, their SEs and the number of replications are presented in Table 3. Lactose (DEAN 1989) and galactosyl-glycerol are naturally occurring substrates, whereas lactulose, methyl-galactoside and galactosyl-arabinose (unpublished summary statistics kindly provided by P. J. N. SILVA and D. E. DYKHUIZEN) are artificial substrates. One-way analyses of variance show that significant differences in fitness exist among the strains within each environment ($P < 0.01$ in all 5

TABLE 1

Strains

Strain	Genotype	*lac* operons transduced into DD320	β-galactosidase mobility	Reference
Natural isolates				
TD1	*lacI*$^+$	CSH64	?	MILLER (1972)
TD9	*lacI*$^+$	ECOR4	7	OCHMANN and SELANDER (1984)
TD10	*lacI*$^+$	ECOR16	2	OCHMANN and SELANDER (1984)
TD11	*lacI*$^+$	ECOR67	7	OCHMANN and SELANDER (1984)
TD13	*lacI*$^+$	ECOR70	2	OCHMANN and SELANDER (1984)
TD14	*lacI*$^+$	ECOR68	5	OCHMANN and SELANDER (1984)
TD63	*lacI*$^+$	ECOR45	5	OCHMANN and SELANDER (1984)
Laboratory mutants				
DD320	$\Delta lacRV$			DYKHUIZEN and DAVIES (1980)
TD2	*lacI*$^-$	JL3300	?	LANGRIDGE (1974)
TD3	*lacI*$^-$	JL1190	?	LANGRIDGE (1974)
TD4	*lacI*$^-$	JL1481	?	LANGRIDGE (1974)
TD10.3	*lacI*$^-$		?	DEAN *et al.* (1986)
TD10.4	*lacI*$^-$		?	DEAN *et al.* (1986)
TD1229.2	*lacI*$^-$		?	DEAN *et al.* (1988)
TD1432.2	*lacI*$^-$		?	DEAN *et al.* (1988)

cases). Hence, the strains differ in fitness from one another, as well as from the control, TD1.

Figure 2 displays the strains ranked by fitness, with bold lines representing significant differences determined using the least significant difference,

$$lsd = t_{(\alpha=0.05)}\sqrt{2MS_{error}\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}.$$

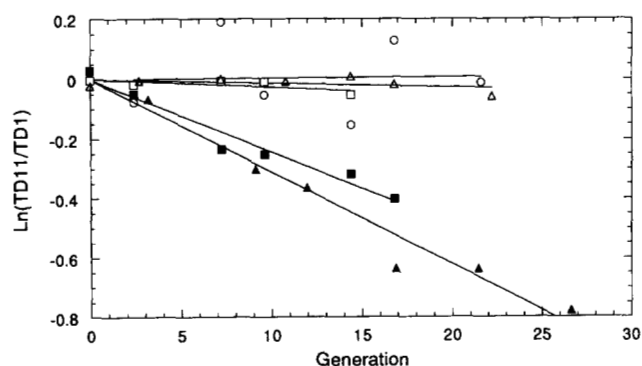TD10 is fittest on lactose, galactosyl-glycerol and methyl-

FIGURE 1.—An example of natural selection between TD1 and TD11. The slopes of the regression lines yield estimates of the selection coefficients. The absence of selection during glucose limitation (open symbols) demonstrates that the selection against TD11 during galactosyl-glycerol limitation (solid symbols) is attributable to differences between the lactose operons. The genetic marker used to distinguish strains confers no detectable selective effect, because competition between TD11 *vs.* TD1.T5R (triangles) and TD11.T5R *vs.* TD1 (squares and circles) yields similar results.

galactoside, but on lactulose and galactosyl-arabinose TD1, TD9 and TD63 are significantly fitter. TD11 is the least fit on methyl-galactoside but is fitter than TD13 and TD14 on galactosyl-glycerol and lactulose.

Of the 210 possible comparisons between pairs of strains in pairs of environments, 40 involve exchanges

TABLE 2

Selection coefficients per generation

Strain	Limiting nutrient	
	Galactosyl-glycerol	Glucose
Natural isolates		
TD1	ND^a	ND
TD9	-0.017 ± 0.002 (5)	-0.003 ± 0.002 (6)
TD10^b	$+0.040 \pm 0.001$ (2)	$+0.000 \pm 0.002$ (5)
TD11	-0.028 ± 0.002 (2)	$+0.000 \pm 0.003$ (4)
TD13	-0.073 ± 0.004 (2)	-0.002 ± 0.002 (4)
TD14	-0.068 ± 0.006 (2)	-0.002 ± 0.003 (2)
TD63	-0.016 ± 0.002 (3)	$+0.001 \pm 0.002$ (4)
Laboratory mutants		
DD320	-0.989 ± 0.016 (1)	ND
TD2	$+0.006 \pm 0.004$ (1)	ND
TD3	-0.100 ± 0.004 (2)	$+0.002 \pm 0.003$ (2)
TD4	-0.328 ± 0.011 (2)	-0.004 ± 0.001 (2)
TD1229.2	-0.048 ± 0.002 (2)	$+0.002 \pm 0.006$ (3)
TD1432.2	-0.061 ± 0.007 (2)	$+0.004 \pm 0.003$ (3)

Values are means $\pm$ SE with number of competition experiments in parentheses.
^{a} Not determined.
^{b} In the presence of 10 μM IPTG only.

TABLE 3

Fitness estimates

Strain	Lactose[a]	Galactosyl-glycerol[b]	Lactulose[c]	Methyl-galactoside[c]	Galactosyl-arabinose[c]
			Limiting nutrient		
TD9	0.967 ± 0.002 (4)	0.983 ± 0.002 (5)	0.956 ± 0.001 (3)	0.950 ± 0.002 (3)	0.959 ± 0.001 (3)
TD10	1.078 ± 0.004 (4)	1.040 ± 0.002 (2)	0.905 ± 0.009 (3)	1.359 ± 0.008 (4)	0.933 ± 0.010 (3)
TD11	0.947 ± 0.002 (5)	0.972 ± 0.002 (2)	0.904 ± 0.009 (3)	0.870 ± 0.004 (3)	0.878 ± 0.004 (3)
TD13	0.946 ± 0.004 (3)	0.927 ± 0.004 (2)	0.846 ± 0.009 (3)	0.947 ± 0.005 (3)	0.877 ± 0.004 (3)
TD14	0.967 ± 0.003 (4)	0.932 ± 0.006 (2)	0.816 ± 0.017 (4)	0.944 ± 0.004 (4)	0.881 ± 0.004 (3)
TD63	1.000 ± 0.004 (3)	0.984 ± 0.002 (3)	0.944 ± 0.006 (4)	0.964 ± 0.004 (3)	0.968 ± 0.001 (3)

Source	d.f.	SS	MS	F	d.f.	SS	MS	F	d.f.	SS	MS	F	d.f.	SS	MS	F	d.f.	SS	MS	F
ANOVAs																				
Strains	5	0.04940	0.00987	247*	5	0.02425	0.004850	194*	5	0.0538	0.0108	27*	5	0.5910	0.1180	1180*	5	0.0270	0.00540	68*
Error	17	0.00062	0.00004		13	0.00022	0.000025		14	0.0054	0.0004		14	0.0014	0.0001		12	0.0009	0.00008	
Total	22	0.05002			16	0.02102			19	0.0592			19	0.5924			17	0.0279		

Values are means ± SE with number of competition experiments in parentheses.
[a] Data from DEAN (1989).
[b] Data from Table 2.
[c] Unpublished summary statistics provided by SILVA and DYKHUIZEN (1993).
* $P < 0.01$.

in fitness ranking, and of these only 24 (11.5%) are "significant" in the sense they occur across *lsds*. The latter are associated with only two of the seven strains, namely TD10 and TD11. Furthermore, the seven strains fall naturally into two groups: all members of the first (TD1, TD9, TD10 and TD63) are fitter than those of the second (TD11, TD13 and TD14).

Estimating genotype by environment interactions: Calculating the simple effects of the linear additive model allows the main effects attributable to strains and environments to be compared to the genotype by environment interactions. However, the calculations were not conducted on estimates of fitness (w), because,

Substrate

Fitness Ranking	Lactose	Galactosyl-glycerol	Lactulose	Methyl-galactoside	Galactosyl-arabinose
1	TD10	TD10	TD1	TD10	TD1
2	TD1	TD1	TD9	TD1	TD63
3	TD63	TD63	TD63	TD63	TD9
4	TD9	TD9	TD10	TD9	TD10
5	TD14	TD11	TD11	TD13	TD14
6	TD13	TD14	TD13	TD14	TD11
7	TD11	TD13	TD14	TD11	TD13

FIGURE 2.—The fitness ranking of strains on each of five galactosides. The bold lines separate strains whose fitnesses exceed a least significant difference. For example, TD11 is fitter than TD13 and TD14 on galactosyl-glycerol and lactulose, less fit on lactose and methyl-galactoside and, within resolution of the techniques, selectively neutral on galactosyl-arabinose.
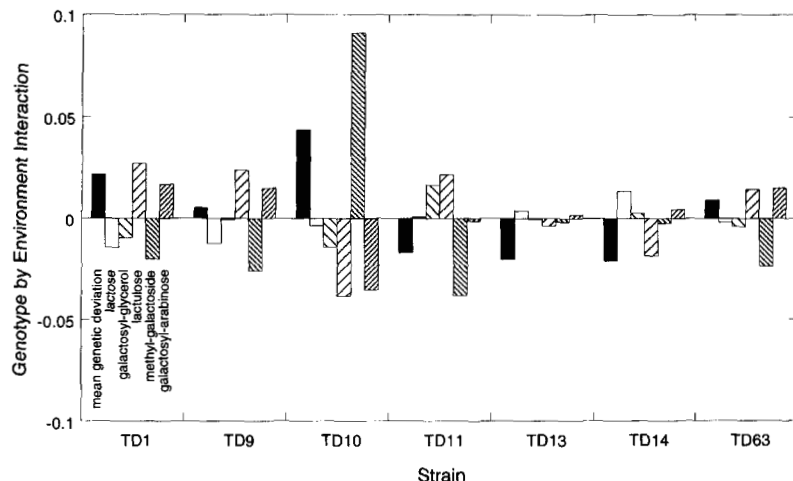
being a ratio of malthusian parameters ($\mu_{isolate}/\mu_{TD1}$), the effects of each strain would be confounded. Taking logarithms transforms fitness onto an additive scale, $\mathrm{Log_e}(w_{isolate}) = \mathrm{Log_e}(\mu_{isolate}) - \mathrm{Log_e}(\mu_{TD1})$, thereby separating the effects. Assume that each has a structure typical of linear additive models, namely $\mathrm{Log_e}(\mu_{isolate \cdot j}) = m + g_i + e_j + ge_{i \cdot j} + \epsilon_{i \cdot j}$ and $\mathrm{Log_e}(\mu_{TD1 \cdot j}) = m + g_{TD1} + e_j + ge_{TD1 \cdot j} + \epsilon_{TD1 \cdot j}$, where m is the mean, g is the genetic contribution, e is the environmental contribution, ge is genotype by environment interaction and ϵ is error, the subscripts defining strain (i) and environment (j). Then $\mathrm{Log_e}(w_{isolate \cdot j}) = g_i + ge_{i \cdot j} - g_{TD1} - ge_{TD1 \cdot j} + (\epsilon_{i \cdot j} - \epsilon_{TD1 \cdot j})$ so that the mean and the environmental components cancel, leaving only the genetic contributions ($g_i - g_{TD1}$), the genotype by environment interactions ($ge_{i \cdot j} - ge_{TD1 \cdot j}$) and an error term ($\epsilon_{i \cdot j} - \epsilon_{TD1 \cdot j}$). The mean of a strain across all environments yields ($g_i - g_{TD1}$). Subtracting the latter from $\mathrm{Log_e}(\mu_{isolate \cdot j})$ yields ($ge_{i \cdot j} - ge_{TD1 \cdot j}$). Hence, the linear additive model ascribes changes in fitness across environments to genotype by environment interactions.

The genotype by environment interactions of the isolate and the control strain can be partitioned. Subtracting the grand mean ($-g_{TD1}$, the genetic contribution to fitness of the control strain, TD1) from the mean of a strain across all environments ($g_i - g_{TD1}$) and from the mean of all strains in a given environment ($-g_{TD1} - ge_{TD1 \cdot j}$) yields g_i (the genetic contribution to fitness of strain i) and $ge_{TD1 \cdot j}$ (the gene by environment interaction of TD1 in environment j). Finally, $\mathrm{Log_e}(w_{isolate \cdot j}) - (g_i - g_{TD1}) - (-g_{TD1} - ge_{TD1 \cdot j}) - g_{TD1} = ge_{i \cdot j}$ (the genotype by environment interaction of strain i in environment j).

The simple effects of the linear additive model are

FIGURE 3.—The simple effects due to genotype by environment interactions ($ge_{i,j}$) and mean genetic contributions (g_i, solid columns) for the seven strains carrying operons from the natural isolates in each of the five environments. The importance of the genotype by environment interactions (hatched columns) to fitness may be judged by comparing their magnitudes to the mean genetic contributions (solid columns).

illustrated in Figure 3. The magnitudes of the interaction terms $ge_{i,j}$ are comparable to, and frequently exceed, those of the mean genetic contributions to fitness g_i. Some strains (notably TD10) appear to be far more sensitive to environmental change than other strains (notably TD13). Evidently, genotype by environment interactions represent major determinants of fitness in some, but not all, strains.

A metabolic model of fitness: An understanding of the causes of these genotype by environment interactions requires a description of fitness in terms of molecular events. The relations between metabolic output (J) and enzyme activities ($E_j = V_{max \cdot j}/K_{m \cdot j}$) for a simple three-step pathway of unsaturated enzymes ending in an irreversible step can be derived by the approach taken by KACSER and BURNS (1973, 1981). When applied to the lactose pathway of *E. coli*, the result is

$$J = \frac{G}{1/E_{wall} + 1/E_{permease} + 1/E_{\beta\text{-}gal}/K_{eq \cdot permease}}, \quad (1)$$

where E_{wall} is the diffusion constant of the galactoside, concentration G, through the porin pores of the outer cell wall, $E_{permease}$ and $E_{\beta\text{-}gal}$ are the activities of the permease and β-galactosidase, and $K_{eq \cdot permease}$ is the apparent equilibrium constant for lactose uptake across the cell membrane. If growth rate (μ) is directly proportional to flux, then fitness of any strain (w_i) is simply

$$w_i = \mu_i/\mu_{TD1} = J_i/J_{TD1} = \frac{1}{C_D^J/d_i + C_P^J/p_i + C_B^J/\beta_i}, \quad (2)$$

where C_D^J, C_P^J and C_B^J are the flux control coefficients (KACSER and BURNS 1973, 1981) or logarithmic gains (SAVAGEAU 1976) of the cell wall, the permease and the β-galactosidase in strain TD1, and d_i, p_i and β_i are the activities at each step in strain i expressed as a proportion of those in TD1. The common genetic background ensures that the cell wall structures are identical so $d_i = 1$ for all strains.

Equation 2 is a biochemical model of pure scramble competition for a growth rate–limiting nutrient. The demonstration that strain DD320, which is unable to metabolize any of the galactosides, has a fitness of 0 on all galactosides (Table 5) demonstrates that interference competition and mutualistic interactions (for example cross-feeding) do not occur. The galactosides are the sole nutrients limiting growth; the population densities in chemostats are directly proportional to the concentration of galactosides entering the growth chamber. Moreover, the model has been experimentally verified for lactose metabolism (DEAN 1989).

Estimating control coefficients: Fitness surfaces were determined using the approach of DYKHUIZEN et al. (1987). First, fitness estimates were obtained for a series of strains carrying mutant β-galactosidase alleles known to differ in activity. Second, competition experiments were conducted between TD1 and TD2 at different levels of IPTG, a nonmetabolizable inducer of the lactose operon. IPTG coordinately modulates the expression of the β-galactosidase and permease of the TD1 operon (JACOB and MONOD 1961), whereas constitutive expression of the TD2 operon, deregulated by a *lacI⁻* mutation, remains unaffected. The expression of the TD1 operon at each level of IPTG was determined using ONPG uptake studies by pure cultures.

Tables 4 and 5 present the activities of the mutant β-galactosidases, relative expression levels of the TD1 operon, together with the fitness estimates. Equation 2 was fitted to these data using the unweighted Gauss-Newton method of nonlinear least squares regression. Estimates of the control coefficients at each step in the pathway of TD1 sum to the theoretical expectation of 1 (Table 6), KACSER and BURNS 1973, 1981), and the model accounts for ≥98.8% of the total variance on each of the substrates.

The open symbols in Figure 4 represent the data used to construct the fitness surfaces. The surfaces obtained

TABLE 4

Kinetic parameters of the β-galactosidases

		Lactose			Galactosyl-glycerol			Lactulose			Methyl-galactoside	
	n	V_{max} (units)[a]	K_m (mM)	n	V_{max} (units)	K_m (mM)	n	V_{max} (units)	K_m (mM)	n	V_{max} (units)	K_m (mM)
Natural isolates												
TD1	8	1.057 ± 0.010	2.422 ± 0.010	4	1.539 ± 0.041	1.446 ± 0.011	3	0.102 ± 0.001	2.733 ± 0.117	4	0.640 ± 0.005	8.580 ± 0.099
TD9	3	0.919 ± 0.007	2.417 ± 0.130	2	1.379 ± 0.021	1.436 ± 0.007	3	0.093 ± 0.002	2.841 ± 0.117	2	0.533 ± 0.025	7.950 ± 0.389
TD10	5	1.057 ± 0.023	2.253 ± 0.077	2	1.834 ± 0.160	1.461 ± 0.054	2	0.097 ± 0.004	2.548 ± 0.034	2	0.688 ± 0.045	8.688 ± 0.075
TD11	5	0.778 ± 0.009	2.405 ± 0.073	2	1.354 ± 0.028	1.529 ± 0.016	2	0.073 ± 0.003	2.316 ± 0.038	2	0.628 ± 0.025	9.449 ± 0.680
TD13	8	0.855 ± 0.012	3.527 ± 0.103	2	1.079 ± 0.041	1.483 ± 0.066	2	0.063 ± 0.002	2.722 ± 0.177	2	0.366 ± 0.034	9.412 ± 0.779
TD14	4	0.682 ± 0.026	2.496 ± 0.071	2	1.146 ± 0.111	1.539 ± 0.034	2	0.067 ± 0.001	2.668 ± 0.027	2	0.446 ± 0.015	9.910 ± 0.831
TD63	4	0.887 ± 0.006	2.352 ± 0.131	2	1.314 ± 0.119	1.370 ± 0.024	3	0.099 ± 0.003	2.848 ± 0.012	2	0.661 ± 0.052	9.528 ± 0.298
Laboratory mutants												
DD320	2	0	ND[b]	2	0	ND	2	0	ND	2	0	ND
TD3	3	0.224 ± 0.012	10.99 ± 1.318	2	0.272 ± 0.007	4.161 ± 0.284	2	0.029 ± 0.002	12.545 ± 2.719	2	0.213 ± 0.004	49.382 ± 0.294
TD4	3	0.266 ± 0.024	28.38 ± 3.629	2	0.162 ± 0.018	12.476 ± 0.287	2	0.033 ± 0.002	31.197 ± 6.083	1	0.264 ± 0.093	146.286 ± 25.253
TD10.3	2	0.926 ± 0.121	2.759 ± 0.282		ND	ND	1	0.080 ± 0.005	2.966 ± 0.163	1	0.643 ± 0.023	11.351 ± 0.227
TD10.4	2	0.926 ± 0.026	1.511 ± 0.246		ND	ND		ND	ND		ND	ND
TD1229.2	3	0.328 ± 0.025	3.495 ± 0.224	3	0.318 ± 0.007	2.156 ± 0.266		ND	ND		ND	ND
TD1432.2	2	0.051 ± 0.006	2.261 ± 0.054	2	0.144 ± 0.004	1.122 ± 0.258		ND	ND		ND	ND
Evolved β-galactosidase[c]												
ebg0		0.011	150		ND	ND		0.010	180		ND	ND
ebgI		0.064 ± 0.007	22.0 ± 1.8		ND	ND		0.003 ± 0.001	34 ± 1.2		ND	ND
ebgII		0.042 ± 0.005	59.0 ± 7.6		ND	ND		0.071 ± 0.007	26 ± 3.7		ND	ND
ebgIV		0.026 ± 0.002	0.824 ± 0.6		ND	ND		0.016 ± 0.001	7.9 ± 0.48		ND	ND
ebgV		0.011	0.69		ND	ND		0.008	6.5		ND	ND

Values are means ± SE. n = no. of independent determinations.

[a] Units, μM of product released/min/mg cell protein.

[b] Not determined.

[c] ebg data from HALL (1984), with maximum velocities expressed per mg cell protein (DEAN et al. 1986).

with the natural substrates, lactose and galactosyl-glycerol, are far less steep around TD1 (position 1,1,1) than those obtained with the artificial substrates, lactulose and methyl-galactoside. An operon, identical to that of TD1 save for a 10% reduction in its expression, would be subject to less intense selection on the natural substrates than the artificial ones. Hence changes in the fitness surfaces, which are generated by changes in the distribution of metabolic control (Table 6), are a potential source of genotype by environment interaction.

A. M. Dean

TABLE 5

Relative activities and relative fitnesses

Strain or IPTG	Lactose			Galactosyl-glycerol			Lactulose			Methyl-galactoside		
	β-Gal	Permease[a]	Fitness	β-Gal	Permease[b]	Fitness	β-Gal	Permease[b]	Fitness	β-Gal	Permease[b]	Fitness
Natural isolates												
TD1	1	1	1	1	1	1	1	1	1	1	1	1
TD9	0.876	0.876	0.967	0.903	(0.783)	0.983	0.877	(0.914)	0.956	0.899	(0.889)	0.950
TD10	1.075	2.16	1.078	1.180	(2.591)	1.040	1.024	(0.793)	0.905	1.062	(3.114)	1.359
TD11	0.809	0.809	0.947	0.832	(0.685)	0.972	0.845	(0.809)	0.904	0.891	(0.741)	0.878
TD13	0.555	0.809	0.946	0.684	(0.441)	0.927	0.620	(0.731)	0.846	0.521	(0.959)	0.947
TD14	0.626	0.86	0.966	0.699	(0.461)	0.932	0.673	(0.671)	0.816	0.603	(0.925)	0.944
TD63	0.864	1	1	0.901	(0.794)	0.984	0.931	(0.882)	0.944	0.930	(0.918)	0.964
Laboratory mutants												
DD320	0	0	0	0	0	0	0	0	0	0	0	0
TD3	0.0467	(1)	0.946	0.061	(1)	0.900	0.062	(1)	0.557	0.058	(1)	0.618
TD4	0.0215	(1)	0.798	0.012	(1)	0.672	0.028	(1)	0.319	0.024	(1)	0.329
TD10.3	0.7691	(1)	0.989	ND[d]	ND	ND	0.723	(1)	0.982	0.759	(1)	0.978
TD10.4	1.4042	(1)	1.006	ND	ND	ND	ND	ND	ND	ND	ND	ND
TD1229.2	0.2150	(1)	0.993	0.139	(1)	0.952	ND	ND	ND	ND	ND	ND
TD1432.2	0.0517	(1)	0.956	0.121	(1)	0.939	ND	ND	ND	ND	ND	ND
Evolved												
β-galactosidase[e]												
ebg0	0.0002	(1)[c]	0	ND	ND	ND	0.001	(1)	0	ND	ND	ND
ebgI	0.0067	(1)	0.563	ND	ND	ND	0.002	(1)	0	ND	ND	ND
ebgII	0.0016	(1)	0.238	ND	ND	ND	0.073	(1)	0.867	ND	ND	ND
ebgIV	0.0032	(1)	0.463	ND	ND	ND	0.054	(1)	0.600	ND	ND	ND
ebgV	0.0012	(1)	0.225	ND	ND	ND	0.033	(1)	0.333	ND	ND	ND
IPTG (μM)[f]												
0		0.117	0.501		0.159	0.746		0	0		0.480	0.700
0.125		ND	ND		0.239	0.811		ND	ND		ND	ND
0.25		ND	ND		0.301	0.853		ND	ND		ND	ND
0.5		0.383	0.715		0.420	0.889		0.181	0.331		ND	ND
1		ND	ND		0.567	0.965		0.612	0.776		0.726	0.840
2		0.608	0.921		0.749	0.983		0.857	0.912		0.871	0.938
5		ND	ND		ND	ND		0.930	0.944		0.940	0.960
10		1	1		1	1		1	1		1	1
60		1	1		ND	ND		ND	ND		ND	ND

[a] Direct kinetic estimates (DEAN 1989).
[b] Estimates based on Equation 4.
[c] Assumed wild-type K12 permease activity.
[d] Not determined.
[e] ebg data from HALL (1984).
[f] Relative expression of K12 operon.

Genotype by environment interactions among β-galactosidases: A second source of genotype by environment interaction resides in the enzyme activities themselves—an enzyme having a high relative activity on one substrate and a low relative activity on another may affect fitness rankings. The kinetic parameters of the β-galactosidases from natural isolates vary considerably (Table 4). For each substrate the range in V_{max} greatly exceeds that for K_m, suggesting that genetic variation primarily affects V_{max} rather than K_m. The environmen-

TABLE 6

Fitness control coefficients

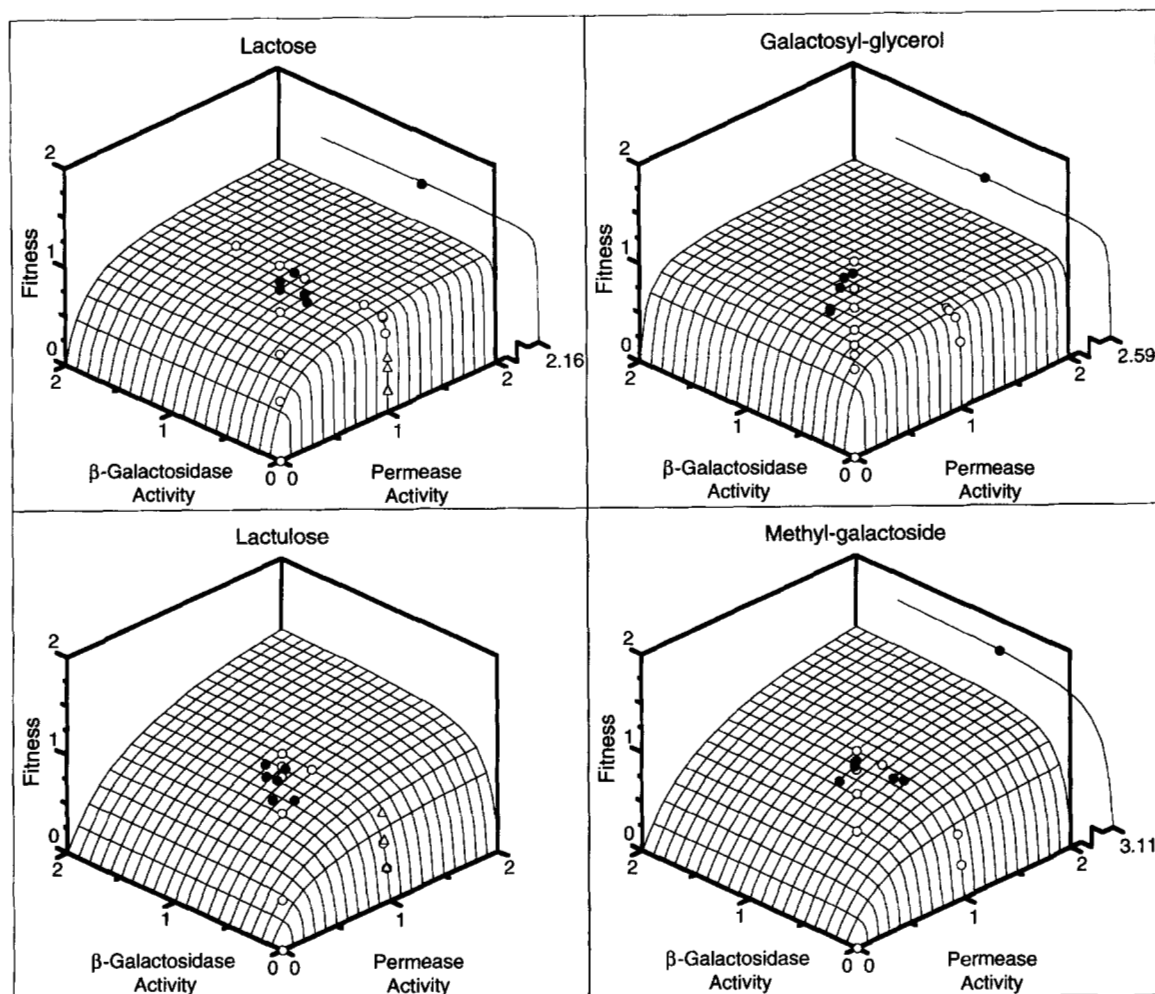| Substrate	C_D^w	C_P^w	C_B^w	Sum
Lactose	0.8404 ± 0.0280	0.1553 ± 0.0199	0.0045 ± 0.0004	1.0002
Galactosyl-glycerol	0.9418 ± 0.0093	0.0607 ± 0.0038	0.0059 ± 0.0004	1.0084
Lactulose	0.5441 ± 0.0719	0.4112 ± 0.0545	0.0531 ± 0.0038	1.0084
Methyl-galactoside	0.5801 ± 0.0429	0.3858 ± 0.0300	0.0433 ± 0.0025	1.0092

Values are means ± SE.

FIGURE 4.—Three-dimensional representations of the fitness surfaces showing the approximate positions of the data points. Open symbols are data from laboratory mutants, alleles of ebg (HALL 1984) and partial induction of the TD1 operon by IPTG. Solid symbols are data from the natural isolates. The surfaces, defined by Equation 3, are essentially a set of CFFs of the type considered by WRIGHT (1934) in his discussion of dominance, HARTL et al. (1985) in their discussion of the limits of adaptation and GILLESPIE (1978, 1991) in his SAS-CFF models.

tal effects are considerable. The V_{max}s toward lactulose are ~15-fold lower than those toward galactosyl-glycerol, whereas the K_ms on methyl-galactoside are typically 6-fold higher than on galactosyl-glycerol.

Changes in activity (V_{max}/K_m) of the β-galactosidases from natural isolates toward one substrate cause proportional changes in activity toward other substrates (Figure 5). The 95% confidence intervals confirm that there is little evidence for genotype by environment interactions among β-galactosidase activities. Clearly, the contributions of genotype by environment interactions to β-galactosidases activities are, at most, weak.

The limited range of β-galactosidases activities from the natural isolates might mask weak genotype by environment interactions. Yet similar results were obtained with laboratory mutants of β-galactosidase and the homologous evolved β-galactosidase (ebg), where the

V_{max}s and K_ms toward each substrate vary over much greater ranges (Table 4). The slopes of the regressions of galactosyl-glycerol and methyl-galactase activities against lactase activities are not significantly different from 1 (Figure 6). Two enzymes, ebgO and ebgV, are relatively more active toward lactulose than lactose. When omitted from the analysis, the slope of the regression of lactulase activities against lactase activities ceases to be significantly different from 1.

The proportional changes in activity toward different substrates, a rather general phenomenon among β-galactosidases regardless of their source, demonstrates that genetic variation need not generate genotype by environment interactions at the molecular level, and that high relative activities toward one substrate do not necessarily imply low relative activities toward others.

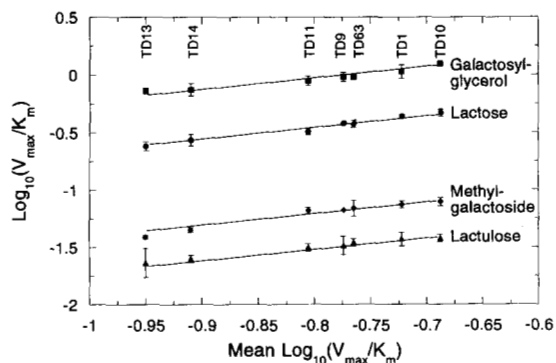**Estimating permease activities from natural iso-**

FIGURE 5.—The norm of reaction of β-galactosidase activity (V_{max}/K_m) for alleles from the natural isolates. Error bars denote 95% confidence intervals. The proportionality of the response on all four galactosides provides little evidence for strong genotype by environment interactions in enzyme activity. Galactosyl-glycerol (■), lactose (●), methyl-galactoside (♦) and lactulose (▲).

lates: Unfortunately, assays for permease activities require the use of radiolabeled galactosides that, with the exception of lactose, are not available commercially. Their syntheses are difficult and prohibitively expensive.

Rearranging Equation 2 to bring relative permease activity to the left hand side yields

$$p_i = \frac{C_P^1}{1/w_i - C_D^1 - C_B^1/\beta_i}. \qquad (3)$$

The permease activities of the naturally occurring operons can now be estimated using the fitnesses (w_i) and relative β-galactosidase activities (β_i) in Table 5
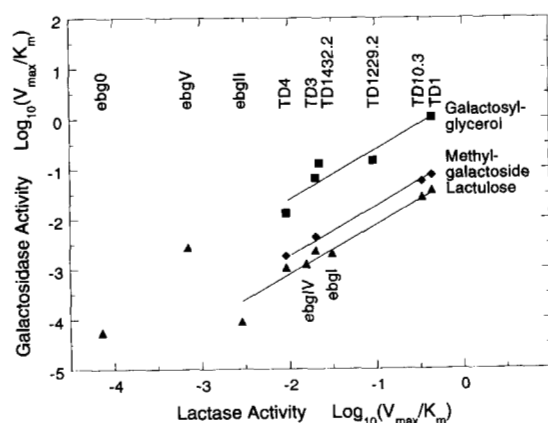
FIGURE 6.—Regression of activities (V_{max}/K_m) toward three galactosides on lactase activities for the laboratory mutants of β-galactosidase and evolved β-galactosidase. With the exception of ebgO and ebgV, which are relatively more active toward lactulose than lactose, there is little evidence for strong genotype by environment interactions among the activities of the laboratory mutants. Galactosyl-glycerol (■), lactose (●), methyl-galactoside (♦) and lactulose (▲).
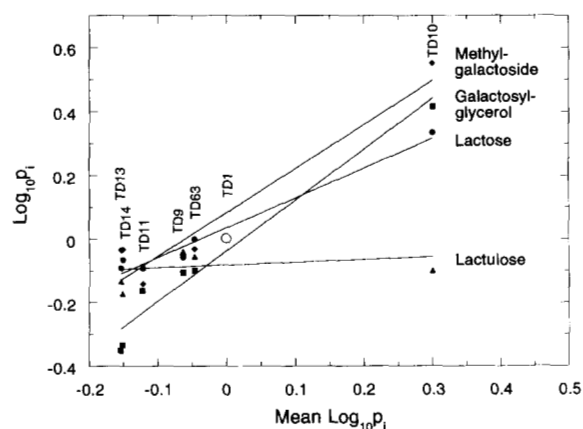

FIGURE 7.—The norm of reaction of relative permease activity (p_i) for alleles from the natural isolates. Here, the lack of proportionality provides evidence of strong genotype by environment interactions among permease activities. Galactosyl-glycerol (■), lactose (●), methyl-galactoside (♦) and lactulose (▲).

and the control coefficients in Table 6. The estimates are presented in Table 5, where they are surrounded by parentheses to denote that the metabolic model was used to estimate them rather than a direct kinetic approach. SEs are not presented because they would be very approximate. First, the control coefficients of Equations 2 and 3 (C_D^1, C_B^1 and C_P^1) were estimated assuming the activities of the β-galactosidase mutants and the levels of expression of the TD1 operon were known with absolute precision. Second, the estimates of the control coefficients are not independent because, theoretically at least, they must sum to 1.

Direct kinetic estimates of the permease activities toward lactose (DEAN 1989) are ~5% higher than those estimated using Equation 4. Such a bias might be attributed to underestimation of the permease control coefficient. Indeed the differences are eliminated when the upper 95% confidence interval of the estimated permease control coefficient is used instead. Both direct and indirect estimates yield the same ranking of activities. If Equation 4 introduces bias, it at least predicts ranking accurately.

Genotype by environment interactions among permeases: Figure 7 shows that changes in relative permease activity toward one substrate do not necessarily cause proportional changes in activity toward other substrates. Apparently, permease alleles show considerable genotype by environment interaction. Some of the interaction might be ascribed to experimental error, some to bias, but some is clearly real. For instance, TD63 is selectively neutral on lactose and selectively inferior on galactosyl-glycerol (Table 5). If the relative permease activity of TD63 remains unchanged in both environments, then the control coefficient of β-galac-

Permease Ranking	Substrate			
	Lactose	Galactosyl-glycerol	Lactulose	Methyl-galactoside
1	TD10	TD10	TD1	TD10
2	TD63	TD1	TD9	TD1
3	TD1	TD63	TD63	TD13
4	TD14	TD9	TD11	TD14
5	TD9	TD11	TD10	TD63
6	TD13	TD14	TD13	TD9
7	TD11	TD13	TD14	TD11

FIGURE 8.—The activity ranking of the permeases from the natural isolates on each of four galactosides. The rankings are remarkably similar to those for fitness (Figure 2). The criterion for grouping strains with bold lines is discussed in the text.

tosidase on galactosyl glycerol is calculated to be 0.15, some 25 times the estimated value of 0.0059 (Table 6). If the control coefficient on galactosyl glycerol is accurate, then the relative activity of β-galactosidase can be calculated to be 0.277, which is far below the estimated value of 0.901 (Table 5). Another example is provided by TD10: on lactose, galactosyl-glycerol and methyl-galactoside this strain is strongly favored, but on lactulose it is markedly inferior (Table 5). The proportionality among β-galactosidase activities (Figure 5) prevents them generating changes in the direction of selection, which instead are ascribed to dramatic changes in relative permease activities. Clearly, strains TD63 and TD10 provide evidence of strong genotype by environment interactions among permease alleles.

The rankings of permease activities on four of the galactosides (Figure 8) are remarkably similar to the fitness rankings. The ratio C_B^I/β_i remains small because the β-galactosidase control coefficients remain small and the range in relative activities is limited. With $C_B^I/\beta_i \ll 1/w_i - C_D^I$, Equation 3 becomes

$$p_i = \frac{C_P^I}{1/w_i - C_D^I - C_B^I/\beta_i} \approx \frac{C_P^I}{1/w_i - C_D^I}. \qquad (4)$$

Consequently, the metabolic model suggests that changes in fitness are dominated by changes in permease activities, which explains the overall similarity in rankings.

Forty-three (34%) of the 126 possible pairwise comparisons involve exchanges in permease ranking, although not all are likely to be significant. The concave relations between fitness and permease activity suggest that errors in the estimation of the control coefficients, whereas affecting estimates and their SE, will not affect permease rankings. Hence, the confidence that a permease allele changes rank can be no greater than the

confidence that its fitness changed rank. Grouping permease rankings by this criterion leaves 18 exchanges (14%) of significance. Of these, nine are associated with strain TD10 dropping rank on lactulose and six with TD11 dropping to the lowest rank on lactose and methyl-galactoside. TD9 and TD13 on methyl-galactoside account for three more exchanges. These occur among strains TD9, TD13, TD14 and TD63, whose fitness and permease rankings differ. This suggests that β-galactosidase alleles influence the fitness rankings on this galactoside. Hence, the latter three exchanges are ascribed with less confidence.

DISCUSSION

The fitness of seven naturally occurring variants of the lactose operon of *E. coli* have been determined on each of five galactosides. Tables 3 and 5 show that the fitness of each strain varies across environments (galactosides). The linear additive model ascribes such changes to genotype by environment interactions. Figure 3 shows that these are commonly of the same magnitude as the mean genetic contributions. The conclusion that genotype by environment interactions are rampant is inescapable.

The selection coefficient of allele i in environment j is approximately $s_{i\cdot j} \approx (E_{i\cdot j} - l) \cdot C_{E\cdot j}^I$, where $E_{i\cdot j}$ is relative enzyme activity and $C_{E\cdot j}^I$ is the control coefficient. Consequently, genotype by environment interactions can arise by two means: changes in control coefficients and changes in relative enzyme activities. Both are present in these data.

Table 6 shows that the control coefficients at each step in the pathway differ among environments. Hence, genotype by environment interactions can be expected at each step. This includes the β-galactosidase, which displays no evidence of interactions at the molecular level (Figure 5). By contrast, the permease displays marked genotype by environment interactions at the molecular level (Figure 7). This constitutes the second mechanism generating genotype by environment interactions in fitness.

Figure 4, which is a three-dimensional representation of the fitness surfaces, provides a ready means to visualize these effects. The surfaces are defined by a series of concave fitness functions (CFFs). The slopes of the tangents to the CFFs passing through TD1 are the control coefficients of the enzymes. Consequently, the topologies of these fitness surfaces are intimately associated with the control coefficients. Merely observing that the surfaces differ warrants the conclusion that extensive genotype by environment interactions are present at both loci. The data from the natural isolates (solid dots) reveal that although the β-galactosidase activities remain fairly constant, those of the permease some-

times change markedly. For example, the activities of all permeases are lower than that of TD1 on lactulose, yet that of TD10 is two to three times higher on the other three galactosides. Variation in the distributions of points across the fitness surfaces illustrates the effects of genotype by environment interactions in enzyme activity.

The surfaces in Figure 4 are representative of a substantial class of similar relations between gene functions and fitness. Similar CFFs have been observed for the branched tryptophan pathway of yeast (NIEDERBERGER et al. 1992). They provide a molecular basis for dominance, including the recessive nature of null alleles in Drosophila, and of inborn errors of metabolism in man (WRIGHT 1934; KACSER and BURNS 1981). Moreover, concave relations between flux and enzyme activity are commonly observed in pathways far more complicated than that studied here (arginine synthesis in Neurospora crassa [FLINT et al. 1980, 1981], sucrose and starch synthesis in Clarkia xantiana [STITT 1990]). Such relations form the basis of a number of modern theories of molecular evolution (GILLESPIE 1978, 1991; HARTL et al. 1985).

Changes in environmental parameters can cause shifts in the distribution of metabolic control (DEAN 1990; GROEN and WESTERHOFF 1990; STITT 1990) that may result in genotype by environment interactions. Such shifts may also expose previously neutral variation to selection. For example, the twofold range in β-galactosidase activities of the natural isolates generates ranges in fitness of $\sim 0.3\%$ on lactose and 3.4% on lactulose. Thus, the selection among β-galactosidase alleles is 10-fold more intense on lactulose than on lactose. Such a shift would be quite sufficient to expose neutral variation ($s < 1/2N_e$) to selection ($s > 1/2N_e$).

The control coefficients in a simple linear pathway must be greater than zero (KACSER and BURNS 1973, 1981). Consequently, changes in the fitness rankings of alleles can only be generated by changes in their activity rankings. The lack of genotype by environment interactions in β-galactosidase activity (Figure 5) prevents exchanges in the fitness rankings of its alleles but does nothing to prevent genotype by environment interactions arising in fitness.

Some allozymes are known to respond differentially to changes in environmental parameters, such as resource abundance, pH, substrate and cofactor concentrations, and temperature and ionic strength (e.g., see PLACE and POWERS 1984). Yet this does not guarantee that genotype by environment interactions at the molecular level will influence fitness. Had β-galactosidase activities displayed exchanges in ranking on lactose and galactosyl-glycerol, they would be of little consequence (Figure 4) because fitness effects are insignificant when

kinetic variation remains within a neutral limit (sensu HARTL et al. 1985).

On the other hand, exchanges in ranking at metabolic steps with large control coefficients are likely to generate genotype by environment interactions in higher phenotypes, such as flux and fitness. The permease alleles illustrate this behavior (Figures 7 and 8). Comparing the activity rankings to those of fitness (Figure 2) reveals that they are remarkably similar, save for exchanges in activity between TD13 and TD9 on methyl-galactoside. That the permease has such a pronounced effect on fitness is not surprising given that both the ranges in activity and the magnitudes of the control coefficients are considerably greater than those of β-galactosidase (Tables 5 and 6, Figure 4). Clearly, permease rankings are the primary, but not the exclusive, determinants of fitness rankings.

Changes in activity ranking at steps with large control coefficients do not guarantee that genotype by environment interactions in fitness are inevitable. This is illustrated by strains TD13 and TD9 on lactose and methyl-galactoside. The beneficial genotype by environment interactions associated with permease activity in TD13 (Table 5) are expected to cause a change in fitness ranking with TD9 on methyl-galactoside. However, the increase in the β-galactosidase control coefficient on methyl-galactoside (Table 6) generated sufficiently strong selection that this did not occur. Clearly, changes in the activity rankings of alleles are necessary, but not sufficient, to generate changes in the direction of selection when two or more loci are considered.

Conversely, changes in the direction of selection can arise in the absence of exchanges in the ranking of enzyme activities. An example of this effect is clearly seen when comparing the fitnesses of the constitutively expressed mutant β-galactosidase of TD3 ($\beta \approx 0.05$, $p = 1$) with the partially induced operon of TD1 ($\beta \approx 0.6$, $p \approx 0.6$) on lactose and on lactulose. The relative activities of both enzymes in each strain are constant, yet on lactose TD3 is fitter than the partially induced TD1 (0.95 vs. 0.92), whereas on lactulose the reverse is true (0.56 and 0.78). Changes in the fitness ranking of genotypes do not require similar changes in ranking among alleles when two or more loci are considered.

Strains TD9 and TD13 on lactose and methyl-galactoside reveal that genotype by environment interactions at one locus may sometimes chancel, even override, the effects predicted at another. The fitnesses of strains TD3 and the partially induced TD1 on lactose and lactulose reveal that an absence of genotype by environment interaction in activity need not prevent changes in the fitness rankings when two or more loci are considered. That both effects are so rare is probably due to the fact that the kinetic variation at the permease has a much greater impact on fitness than that at the β-galactosi-

dase. The situation might change radically if the kinetic variation in the porins, a step with large control coefficients (Table 6), were also considered.

Enzymes have been classified into two loosely defined sets, those that have a single substrate (group I) and those that have multiple substrates (group II) (GILLESPIE and KOJIMA 1968). Electrophoretic surveys over a wide range of species have generally shown that group I enzymes are far less polymorphic than those of group II, the heterozygosities being ~0.05 and 0.18, respectively (SELANDER 1976; GOJOBORI 1982; GILLESPIE 1991).

A possible explanation for the increased heterozygosity of group II enzymes is that a broader range of substrates exposes them to greater environmental heterogeneity, thereby allowing selection to protect a greater proportion of alleles. Such a hypothesis specifically invokes substrate variability as the principle factor in generating genotype by environment interactions. That factor has been subject to direct experimental test.

By any criterion, both β-galactosidase and permease belong in group II. β-galactosidase is highly polymorphic ($H = 0.89$, WHITTAM et al. 1983), hydrolyses a wide variety of β-galactosides and displays considerable kinetic polymorphism toward all four substrates studied here. However, the lack of genotype by environment interaction among β-galactosidase alleles suggests that any environmental variability attributable to the utilization of alternative substrates is unlikely to afford a means to protect this polymorphism.

The permease is also polymorphic (as judged by its kinetic variation) and it transports a wide variety of both α- and β-galactosides. In contrast to β-galactosidase, the permease provides evidence that genotype by environment interactions can generate changes in fitness rankings. On the other hand only 24 of the 210 possible pairwise comparisons of the seven strains in the five environments generate changes in rank across least significant differences. All involve only two strains, TD10 and TD11. The general lack of change in rankings suggests that genotype by environment interactions generated by broad substrate specificity are unlikely to be the principle mechanism by which the permease polymorphism is protected (if indeed it is protected). At best, they are contributing factors.

HARTL and DYKHUIZEN (1981, 1985) introduced the notion of a latent potential for selection, wherein functional differences that are selectively neutral, or at least nearly so, in common environments become strongly selected in rare environments. The idea supposes that mutation generates new alleles with a range of selective effects. Many are deleterious and are rapidly eliminated from the population leaving only those that are selectively neutral or nearly neutral. The phenotypic effects of these need not be expressed in the current environ-

ment, but even if they are, they need not be targets for selection. However, in novel environments they may become important to fitness. Consequently, selection intensities are greater in novel environments. This phenomenon, dubbed the Dykhuizen-Hartl effect by KIMURA (1983), has been proposed to influence the population dynamics of alleles. The idea has been extended to anagenesis (STEBBINS and HARTL 1988) and even macroevolution (KIMURA 1991). A similar effect is predicted from the selectionist arguments of SERVICE and ROSE (1985).

SILVA and DYKHUIZEN (1993) provided evidence for these hypotheses when they demonstrated that the selection on artificial galactosides (lactulose, methyl-galactoside and galactosyl-arabinose) is significantly greater than on the natural substrate, lactose. The results of the competition experiments with a second naturally occurring galactoside (galactosyl-glycerol) also support this observation (Tables 3 and 5).

There is no evidence for gene by environment interactions among the relative activities of the β-galactosidases from the natural isolates. There is evidence for gene by environment interactions among the relative activities of their permeases. However, the variances (after an appropriate logarithmic transformation) show no discernible trend: 0.02 on lactose, 0.07 on galactosyl-glycerol, 0.004 on lactulose and 0.04 on methyl-galactoside. Hence, the increased intensity of selection on the unnatural galactosides can be entirely attributed to the increased control coefficients at these steps.

But the flux control coefficients must sum to 1 (KACSER and BURNS 1973, 1981). The increases in the control coefficients of the permease and β-galactosidase on the novel galactosides must be precisely compensated by a commensurate decrease in the control coefficient of the diffusion step. A hypothetical 10% change in the activity at this step generates selection coefficients ($s \approx -0.1 \cdot C_D^l$) of 8.40% on lactose, 9.42% on galactosyl-glycerol, 5.44% on lactulose and 5.80% on methyl-galactoside. This is precisely the reverse of the Dykhuizen-Hartl effect; the selection on the novel substrates is less intense than on the natural substrates.

The Dykhuizen-Hartl effect ignores the manner in which enzyme activities map onto fitness. The unwitting assumption is that control coefficients remain constant, whereas enzyme activities change across environments. This assumption requires testing whenever the Dykhuizen-Hartl effect is invoked because the flux control summation theorem dictates that half the time changes in control coefficients help generate the appearance of a Dykhuizen-Hartl effect, and half the time they help suppress it. The increased selection among operons on the unnatural substrates, being entirely attributable to increased control coefficients, provides no evidence in support of a Dykhuizen-Hartl effect.

We have just seen how data gathered in support of two hypotheses can be seriously misleading; the general characteristics of the β-galactosidase suggest a typical group II enzyme with its polymorphism maintained by fluctuating fitnesses on different substrates, and the selection coefficients among the operons on unnatural galactosides are increased, just as predicted by the Dykhuizen-Hartl effect. Only by investigating the underlying molecular events has it been possible to demonstrate that the patterns associated with these hypotheses are purely coincidental.

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