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Potential for hitchhiking in the *eda-edd-zwf* gene cluster of *Escherichia coli*

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SUMMARY

The loci *eda*, *edd* and *zwf* form a tightly linked cluster in *E. coli* that functions in the metabolism of galacturonate, gluconate and glucose. This cluster has been transferred from six natural isolates into the genetic background of *E. coli* K12 and examined with regard to effects on growth rate in chemostats. Although the naturally occurring *eda* and *zwf* alleles are selectively neutral, the *edd* alleles are not. The *edd* alleles fall into three functional classes distinguished by their effects on growth rate in gluconate medium, the most common classes differing in fitness by approximately 1 % per hour. This extensive non-neutral genetic variation of *edd* is discussed in light of the evident rarity of gluconate as a natural substrate. We propose that gluconate selection is intermittent in space or time, providing the population an opportunity to accumulate non-neutral genetic variants during periods of relaxed selection. Such genetic variants will eventually be sorted out by the intermittent periods of gluconate selection, and during these periods the linked *eda* and *zwf* alleles will experience pronounced hitchhiking effects.

INTRODUCTION

The experiments described in this paper provide a detailed analysis of the effects of specific environmental factors on the evolutionary potential of a block of tightly linked genes. Linkage often creates ambiguities in the interpretation of experiments in population genetics, particularly in eukaryotes, because an observed change in the frequency of any particular allele may result from its hitchhiking with a linked selected locus. In prokaryotes, hitchhiking creates problems that are, in principle, as difficult as those in eukaryotes, in spite of the relative simplicity of prokaryotic genomes. As a practical matter, the extensive genetic map and knowledge of correlated metabolic functions in such organisms as *E. coli* provide significant improvements in the ability to detect or eliminate hitchhiking effects (Dykhuizen & Hartl, 1980, 1983*a*; Hartl & Dykhuizen, 1981; Dykhuizen, de Framond & Hartl, 1984).

We have studied naturally occurring alleles of three tightly linked loci in the region of 41 min on the standard *E. coli* linkage map (Bachmann, 1983). The *zwf*

locus codes for glucose-6-phosphate dehydrogenase (EC 1 . 1 . 1 . 49), the *edd* locus codes for phosphogluconate dehydratase (EC 4 . 2 . 1 . 12), and *eda* codes for phospho-2-keto-3-deoxy-gluconate aldolase (EC 4 . 1 . 2 . 14). The clockwise map order of the genes is *eda-edd-zwf*. The *zwf* alleles were studied previously and found to be selectively neutral (Dykhuizen *et al.* 1984).

The strategy of our approach is to study the relative growth rate of otherwise isogenic strains undergoing competition in continuous culture. The growth rate of the cells is limited by the availability of substrate of either *edd* or *eda*, and also by the relative ability of the competing strains to utilize the substrate for growth and cell division. Under such conditions competition for substrate is unusually intense, and any physiologically significant differences between the allozymes would be expected to be reflected as differences in growth rate. Appropriate choice of substrate allows the selective effects of *edd* and *eda* alleles to be identified individually. The particular continuous-culture device we use is a bacterial chemostat, in which fresh nutrient medium is continuously added to a growing culture of cells as an equal volume of the culture is removed by means of a siphon (reviewed in Dykhuizen & Hartl, 1983*b*). When competition for nutrient is the sole factor limiting to growth, then the dynamics of chemostat competition is very simple. If $R(t)$ and $S(t)$ represent the relative frequencies of two competing strains, R and S, in the chemostat at a time t hours after inoculation, then the selection coefficient, s, of the R strain relative to the S strain is given by the slope of the regression equation $\ln [R(t)/S(t)] = cnst + st$. The selection coefficient estimated by this method corresponds to the malthusian parameter, which is appropriate for populations with overlapping generations (Crow & Kimura, 1970). Under standard conditions we can detect selection coefficients as small as approximately 0·002 h^{-1} between the competing strains (Dykhuizen & Hartl, 1983*a*).

We have studied six naturally occurring alleles of *eda* and six of *edd*, along with the allele at each locus normally present in *E. coli* K12. Our principal finding is that the six naturally occurring alleles at the *eda* locus are selectively neutral relative to each other, but all of them are selectively superior to the allele in *E. coli* K12. On the other hand, the *edd* alleles fall into three distinct classes with selective neutrality among alleles in the same class but non-neutrality among alleles in different classes. The non-neutrality of *edd* alleles thus provides a potential for hitchhiking at both the *zwf* and *eda* loci.

MATERIALS AND METHODS

Genetic manipulations. Strains were constructed by means of bacteriophage P1-mediated transduction using P1 (*cml clr100*) according to the methods of Miller (1972). The recipient strain was DD725 (Dykhuizen & Hartl, 1983*a*), which carries an *eda-edd-zwf* deletion along with *rpsL* (streptomycin resistance). In the transductions, selection was for growth on minimal medium containing glucuronate, which selects for Eda^+ function. The entire *eda-edd-zwf* block of genes is selected by this method owing to the deletion in the recipient.

Chemostats. Chemostat medium consists of Davis salts (40 mM-K_2HPO_4, 15 mM-KH_2PO_4, 7·6 mM $(NH_4)_2SO_4$, 1·7 mM sodium citrate and 0·8 mM-$MgSO_4$)

plus one of three limiting carbon sources: (1) glucose at 0·1 g/l, (2) gluconate at 0·1 g/l, or (3) galacturonate (0·05 g/l) plus glucuronate (0·05 g/l). The first of these requires Zwf^+ for optimal growth, the second both Eda^+ and Edd^+, and the third Eda^+. The flow rate of fresh medium into the chemostat was adjusted to give a doubling time of $1{\cdot}96 \pm 0{\cdot}21$ h (S.E. among experiments).

Table 1. *Naturally occurring isolates*

Natural isolate	Origin	G6PD electromorph
RM73C	Orangutan, female	2
RM77C	Human, female	3
RM66A	Human, male	4
RM72B	Gorilla, female	4
RM20	Red wolf, female	5
RM182A	Rabbit	5

Pairs of strains were inoculated and sampled periodically as described (Dykhuizen & Hartl, 1983*a*; Dykhuizen, de Framond & Hartl, 1984). In each case, one strain was resistant to bacteriophage T5 ($T5^R$), due to the *fhuA* allele at 4 min on the standard *E. coli* map, and the other was sensitive to T5 ($T5^S$). The *fhuA* marker is itself neutral under these conditions (Dykhuizen & Hartl, 1980, 1983*a*), and it is used to monitor the relative frequencies of the competing strains.

Data were analysed by means of the linear regression $\ln [R(t)/S(t)] = cnst + st$, where $R(t)$ and $S(t)$ represent the frequency of the $T5^R$ and $T5^s$ strains at time t, and the slope s is an estimate of the relative fitness (growth rate) of the competing strains. Significance of s was tested by means of analysis of variance of the regression. In most cases the experiments were paired by interchanging the $T5^R$ marker between competing strains. Such pairing produces two slopes corresponding to the two experiments, and the significance of the difference between these slopes was determined by means of the appropriate t test (Snedecor & Cochran, 1967). Slopes of replicate experiments were pooled as described in Snedecor & Cochran (1967).

RESULTS

Isolates of *E. coli* from natural sources were from the Milkman (1973) collection and were provided by B. R. Levin. Table 1 provides the strain designations, their origin, and the G6PD (glucose-6-phosphate dehydrogenase) electromorph present in each isolate as determined by Selander & Levin (1980). In accordance with convention, the wild-type allele from a natural isolate is designated by the symbol of the locus in question followed in parentheses by the name of the original isolate. For example, *zwf* (RM73C) designates the *zwf* allele originally present in strain RM73C. Likewise, the *eda* and *edd* alleles from this strain are designated *eda* (RM73C) and *edd* (RM73C). The alleles from *E. coli* K12 used in the experiments derive from strain DF1071 (Fraenkel, 1968).

Isogenic strains for chemostat competition were produced according to the method outlined in Table 2 for RM73C. The genetic background of the final strains

is that of DD725 (Dykhuizen & Hartl, 1983*a*), which is genotypically *Δ(eda-edd-zwf)*, *rpsL*. *Δ* represents a deletion that includes the three indicated loci, and *rpsL* is a mutation affecting a ribosomal subunit protein that confers resistance to streptomycin. As outlined in Table 2, the *eda-edd-zwf* region from each natural isolate was transferred into the genetic background of DD725 by means of

Table 2. *Method of strain construction*

Strain	Relevant genotype	Source
RM73C	*zwf*(RM73C)	Natural isolate
DD938	*zwf*(RM73C), *rpsL*	P1 from RM73C → DD725, Eda^+ selection
DD1121	*zwf*(RM73C), *rpsL*	P1 from DD938 → DD725, Eda^+ selection
DD1137	*zwf*(RM73C), *rpsL*	P1 from DD1121 → DD725, Eda^+ selection
DD1314	*zwf*(RM73C), *rpsL*	P1 from DD1137 → DD725, Eda^+ selection
DD1370	*zwf*(RM73C), *rpsL*, *fhuA*	Spontaneous $T5^R$ in DD1314

transduction. Four consecutive transductions were performed in order to minimize the amount of extraneous linked genetic material introduced into DD725. In each transduction, selection was on minimal glucuronate, which selects for Eda^+, although the entire *eda-edd-zwf* region is incorporated into the recipient strain because of the deletion in DD725. As the final step in strain construction, a spontaneous *fhuA* (bacteriophage T5 resistance) mutation was isolated in each strain. The *eda-edd-zwf* region from each natural isolate is therefore represented by a matched pair of strains, one sensitive to phage T5 ($T5^S$) and the other one resistant ($T5^R$). Since *fhuA* is a selectively neutral marker under our conditions (Dykhuizen & Hartl 1980, 1983*a*), the experiments can be carried out in matched pairs, for example *eda* (RM73C) $T5^R$ versus *eda* (RM66A) $T5^S$ in one case, and *eda* (RM73C) $T5^S$ versus *eda* (RM66A) $T5^R$ in the other. This pairing strategy serves to replicate each competition experiment and also provides an independent check of possible effects that might be due to unrecognized differences affecting growth rate in the genetic background. Since the $T5^R$ marker is always the one monitored, the slopes in the paired experiments are expected to have opposite sign but to have the same magnitude, provided the *fhuA* allele is itself neutral. The difference between the slopes provides an estimate of two times the selective difference attributable to the alleles in question.

The metabolic roles of *eda* and *edd* are outlined in Fig. 1. The genes whose enzyme products are represented by the unlabeled arrows have all been mapped, and their map positions all lie at least 2 min distant from the *eda-edd-zwf* cluster (Ritzenthaler, Blanco & Mata-Gilsinger, 1983). Consequently, all genes affecting the metabolism of the growth substrates inscribed in rectangles must be identical in the strains being tested, because 2 min of chromosome is the maximum that can be cotransduced with bacteriophage P1.

The key point of Fig. 1 is that *eda* function, but not *edd* function, is necessary for growth on glucuronate and galacturonate. On the other hand, growth on gluconate requires both *eda* and *edd* function. Indeed, *edd* is specifically induced by gluconate, and the enzyme serves as one branch of gluconate metabolism, the alternative branch being catalysed by the product of the *gnd* locus and leading to

the pentose phosphate shunt. (The map position of *gnd* is 44 min (Bachman, 1983), and it codes for 6PGD = 6-phospho-D-gluconate: $NAD(P)^+$ 2-oxidoreductase, EC 1.1.1.43.) Consequently, the relative growth rate of cells on glucuronate or galacturonate serves as an assay of *eda* function (in the experiments we have used

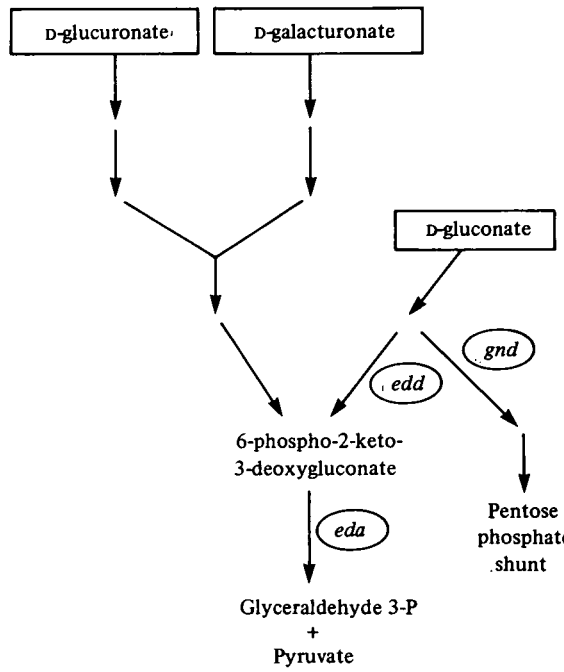

Fig. 1. Pathways of metabolism of glucuronate, galacturonate and gluconate in *E. coli*.

a mixture of equal amounts of both substrates), whereas the relative growth rate of cells on gluconate provides an assay of the joint functional effects of *eda* and *edd*. Separate competition experiments using first glucoronate–galacturonate and then gluconate thereby provides an assessment of the growth-rate effects of alleles at both the *eda* and *edd* loci.

Controls. Previous results with related strains undergoing competition in glucose-limited chemostats have shown that the *zwf* alleles in the strains in Table 1 are selectively neutral (Dykhuizen, de Framond & Hartl, 1984). These results also serve as negative controls for the present experiments because the finding of selective neutrality excludes the possibility of extraneous genetic background effects on growth rate. In competition experiments to determine the effects of specific alleles on fitness, it is also necessary to have positive controls in order to assess the selective effects of known enzyme-inactivating mutations. With regard to *eda*, mutations that inactivate the enzyme are unable to grow on glucuronate or galacturonate. In terms of selection in chemostats, this implies that the selection coefficient should equal the dilution rate, which in the experiments reported here averages $0{\cdot}353 \pm 0{\cdot}036\ h^{-1}$ (S.E. among experiments). This value defines the maximum amount of selection that could occur against any *eda* allele. With regard

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to *edd*, selection against an inactive allele is substantially less than the theoretical maximum because the pentose shunt provides an alternative metabolic route for gluconate. In two gluconate-limited chemostats, selection against an *edd* deletion was found to be $0{\cdot}127 \pm 0{\cdot}019$ h^{-1} and $0{\cdot}120 \pm 0{\cdot}007$ h^{-1}. Thus, a selection coefficient of approximately $0{\cdot}12$ h^{-1} is the upper limit of selection involving *edd* alleles.

Table 3. *Selection of* eda *alleles*

Strains		*eda* allele		Selection coefficient ± S.E.	*t*(D.F.)
$T5^R$	$T5^S$	$T5^R$	$T5^S$		
DD1372	DD1314	RM66A	RM73C	$0{\cdot}001\,22 \pm 0{\cdot}001\,03$	0·1 (12)
DD1370	DD1318	RM73C	RM66A	$0{\cdot}001\,34 \pm 0{\cdot}001\,60$	
DD1372	DD1316	RM66A	RM77C	$0{\cdot}000\,46 \pm 0{\cdot}000\,10$	0·7 (14)
DD1371	DD1318	RM77C	RM66A	$-0{\cdot}000\,56 \pm 0{\cdot}001\,02$	
DD1372	DD1320	RM66A	RM72B	$0{\cdot}000\,61 \pm 0{\cdot}001\,18$	0·2 (10)
DD1373	DD1318	RM72B	RM66A	$0{\cdot}000\,09 \pm 0{\cdot}000\,97$	
DD1372	DD1322	RM66A	RM20	$-0{\cdot}001\,60 \pm 0{\cdot}002\,20$	1·0 (12)
DD1373	DD1318	RM20	RM66A	$-0{\cdot}000\,76 \pm 0{\cdot}003\,63$	
DD1374	DD1325	RM20	RM182A	$-0{\cdot}000\,58 \pm 0{\cdot}001\,92$	0·8 (12)
DD1405	DD1322	RM182A	RM20	$0{\cdot}001\,32 \pm 0{\cdot}001\,44$	
DD1377	DD1316	K12	RM77C	$-0{\cdot}002\,14 \pm 0{\cdot}000\,73$	4·4 (14)**
DD1371	DD1328	RM77C	K12	$0{\cdot}004\,16 \pm 0{\cdot}001\,24$	

**$P < 0{\cdot}01$.

eda alleles. Results of chemostat experiments with *eda* alleles are shown in Table 3, and a summary of the overall pattern of effects is given in Fig. 2. In this diagram, each line represents a chemostat. Dashed lines represent experiments in which the selection coefficient was not significantly different from *O*, solid lines represent significance. Two tests of significance were carried out. In each experiment, the significance of the regression coefficient was tested by the *F* value in an analysis of variance (data not shown). However, since the experiments are paired (e.g. *eda* (RM73C) $T5^R$ versus *eda* (RM66A) $T5^S$ in one case and *eda* (RM73C) $T5^S$ versus *eda* (RM66A) $T5^R$ in the other), a *t* test of the difference between slopes is also appropriate because this difference estimates two times the selection coefficient of the one strain relative to the other. Indeed, the *t* test is more powerful than the *F* tests owing to its greater number of D.F. Fig. 2 represents the finding that all *eda* alleles from natural isolates are selectively neutral. Interestingly, the allele in *E. coli* K12 is not neutral with respect to the others, but is selectively inferior, the selection coefficient against the K12 allele averaging $0{\cdot}003 \pm 0{\cdot}001$ h^{-1}. Experimental results demonstrating this selection against strains carrying the K12 *eda* allele are shown in Fig. 3. The dashed lines represent the regressions, and the slopes are $0{\cdot}0042 \pm 0{\cdot}0012$ and $0{\cdot}0021 \pm 0{\cdot}0007$.

edd alleles. The situation regarding *edd* is very different from that with *eda*, in that significant selective differences occur among the naturally occurring alleles. Details are provided in Table 4, and a specific example is shown in Fig. 4. When cells are grown in competition in gluconate (circles), selection in favour of the *edd* (RM66A) allele relative to *edd* (RM72B) is evident, the slope of the regression being

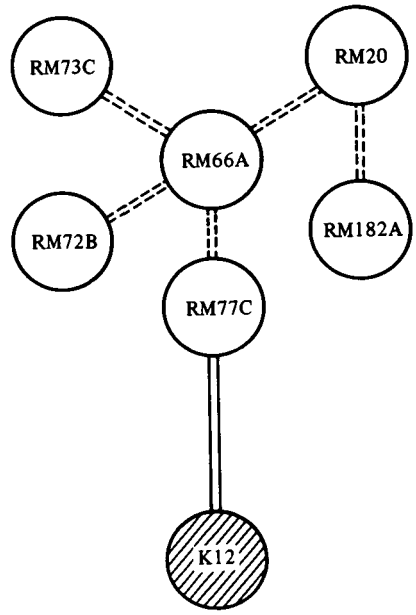

Fig. 2. Patterns of selection involving *eda* alleles. Each line represents a chemostat in which strains carrying the indicated alleles were in competition in galacturonate–glucuronate medium. Dashed lines indicate nonsignificance of selection coefficient, solid lines indicate significance.

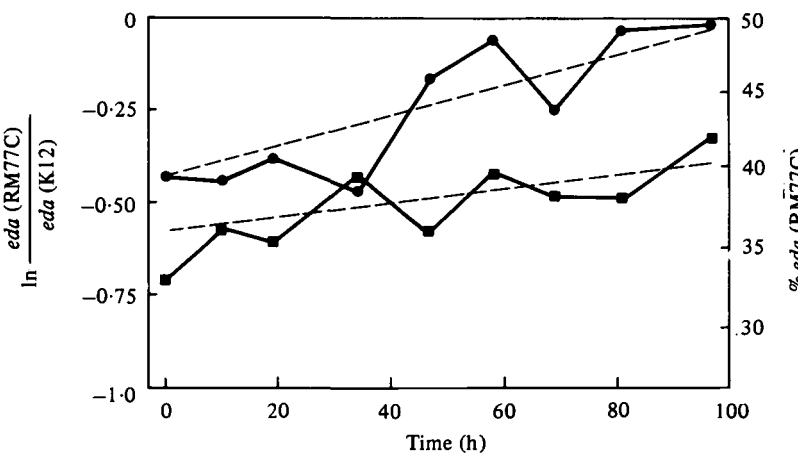

Fig. 3. Selection against *eda*(K12) relative to *eda*(RM77C) in galacturonate-glucuronate medium. Dashed lines are regression lines. Specific strains were ●, DD1371 versus DD1328 and ■, DD1377 versus DD1316.

$s = 0{\cdot}0083 \pm 0{\cdot}0021$. That the selection is indeed attributable to the *edd* alleles is demonstrated by the selective neutrality of the same strains when in competition in chemostats limited for glucose (triangles) or glucuronate–galacturonate (squares), the corresponding slopes being $s = 0{\cdot}0009 \pm 0{\cdot}0010$ and $s = -0{\cdot}0013 \pm 0{\cdot}0013$. Altogether, we have identified three classes of *edd* alleles relative to their selective

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effects. These classes are represented in Fig. 5 by different types of shading. In two cases, the paired experiments had individually nonsignificant F values but the t test for the difference between the slopes was significant. Details are provided in Table 4. The cases in question are *edd*(RM66A) versus *edd*(RM72B) and *edd*(RM20) versus *edd*(RM182A). Regarding the t test as the more powerful, we treat selection

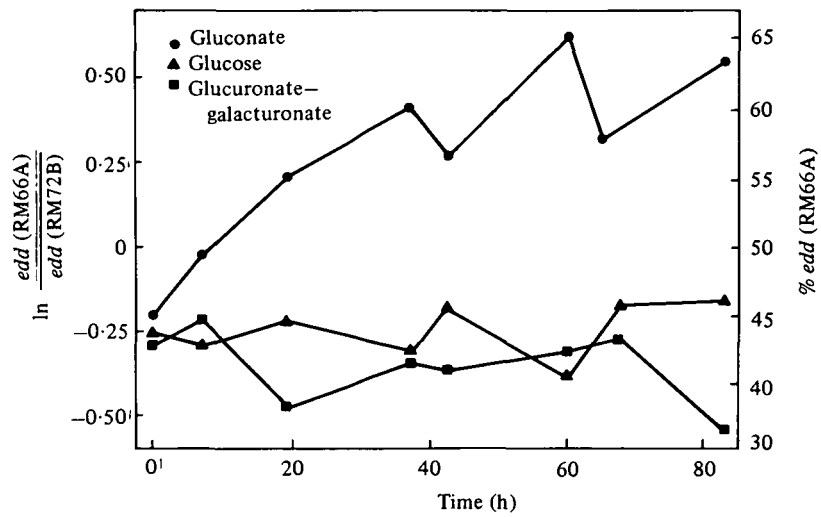

Fig. 4. Selection against *edd*(RM72B) relative to *edd*(RM66A) as evidenced by significant selection in gluconate medium (●) but nonsignificance in glucose (▲) and galacturonate–glucuronate (■). Specific strains were DD1372 versus DD1320.

as being significant in these cases, and the corresponding lines in Fig. 5 are shown as solid lines. For *edd*(RM66A) versus *edd*(RM72B), the conclusion of significant selective effects is independently confirmed by the unambiguous neutrality between *edd* (RM72B) and *edd*(RM20), on the one hand, and the clear selection between *edd*(RM66A) and *edd*(RM20), on the other. Overall, the selection coefficient against *edd*(RM20)-type alleles, relative to *edd*(RM66A)-type alleles, is $0{\cdot}010 \pm 0{\cdot}001$. Selection against *edd*(RM182A) relative to *edd*(RM20) averages $0{\cdot}003 \pm 0{\cdot}001$.

DISCUSSION

With respect to the alleles from natural isolates, the *eda* locus falls into the pattern previously established for alleles of *gnd*, *pgi* and *zwf*, which is that alleles from natural isolates are by and large selectively neutral (Dykhuizen & Hartl, 1980, 1983*a*; Hartl & Dykhuizen, 1981; Dykhuizen, *et al.* 1984). However, certain *gnd* and *pgi* alleles could be shown to be non-neutral under the appropriate conditions, and this is also the case with the *eda* allele in *E. coli* K12. In contrast, the *edd* locus stands out from loci previously studied in that it is highly polymorphic for alleles that have significant effects on fitness.

There is an apparent discrepancy in the data that warrants some discussion. In particular, the *eda*(K12) allele is selectively inferior to the *eda*(RM77C) allele as

Table 4. *Selection of* edd *alleles*

Strains		*edd* allele		No.	Selection	
$T5^R$	$T5^S$	$T5^R$	$T5^S$	expts.	coefficient ± S.E.	*t* (D.F.)
DD1371	DD1318	RM77C	RM66A	2	0·00102 ± 0·00187	0·09 (22)
DD1372	DD1316	RM66A	RM77C	2	0·00126 ± 0·00126	
DD1371	DD1328	RM77C	K12	2	−0·00160 ± 0·00151	0·18 (17)
DD1377	DD1316	K12	RM77C	2	−0·00098 ± 0·00272	
DD1374	DD1314	RM20	RM73C	1	0·00080 ± 0·00130	0·24 (10)
DD1370	DD1322	RM73C	RM20	1	0·00026 ± 0·00178	
DD1374	DD1320	RM20	RM72B	2	0·00016 ± 0·00160	0·27 (14)
DD1373	DD1322	RM72B	RM20	2	−0·00054 ± 0·00216	
DD1370	DD1318	RM73C	RM66A	2	−0·00985 ± 0·00078	12·2 (24)***
DD1372	DD1314	RM66A	RM73C	2	0·00985 ± 0·00149	
DD1374	DD1318	RM20	RM66A	3	−0·00843 ± 0·00145	9·7 (21)***
DD1372	DD1322	RM66A	RM20	3	0·01024 ± 0·00126	
DD1150	DD1146	RM73C	K12	1	−0·01780 ± 0·00186	11·9 (14)***
DD1159	DD1137	K12	RM73C	1	0·01400 ± 0·00154	
DD1373	DD1318	RM72B	RM66A	2	−0·00469 ± 0·00206	3·2 (14)**
DD1372	DD1320	RM66A	RM72B	2	0·00533 ± 0·00263	
DD1405	DD1322	RM182A	RM20	1	−0·00353 ± 0·00162	2·0 (10)*
DD1374	DD1325	RM20	RM182A	1	0·00208 ± 0·00235	

*, **, ***$P < 0·05$, 0·01 or 0·001, respectively.

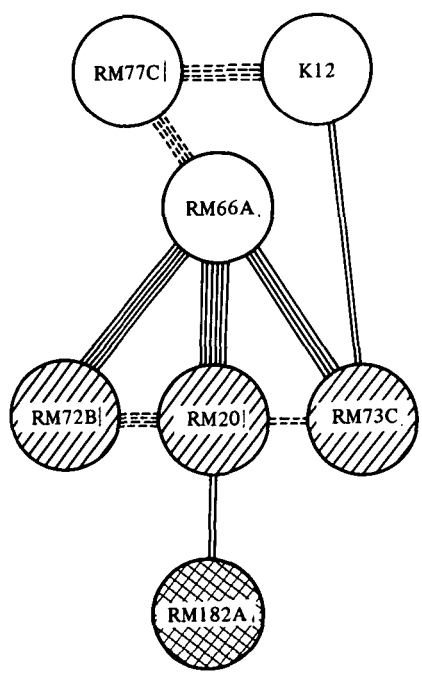

Fig. 5. Pattern of selection of *edd* alleles. Each line represents a chemostat, with solid lines representing individually significant slopes or slopes of paired experiments with significant divergence. Dashed lines indicate nonsignificance.

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judged by competition in galacturonate–glucuronate. Selection might also be expected to occur in gluconate, because the *eda* gene product is used in gluconate metabolism as well. However, these same alleles are selectively neutral in gluconate. One possible explanation of this observation is that the *edd* enzyme is the limiting step for growth in gluconate, so minor functional differences in *eda* would not be expressed as differences in fitness with this substrate. In the terminology of Kacser & Burns (1973, 1979), the sensitivity coefficient of the *eda* enzyme might be low in the gluconate pathway, so a small difference in the activity of the *eda* enzyme would bring about a negligible change in flux through the entire metabolic pathway. This phenomenon is related to one previously observed with *gnd* alleles, which was called metabolic compensation (Dykhuizen & Hartl, 1980).

Because of the selective effects of the *edd* alleles, the *zwf-edd-eda* gene cluster has the potential for significant hitchhiking effects occurring both at the *zwf* and *eda* loci, even though the naturally occurring *zwf* and *eda* alleles that we have studied are selectively neutral. However, it is necessary to emphasize that gluconate, the sole substrate that evokes selection of the *edd* alleles, is evidently a relatively rare substrate for *E. coli* in its natural intestinal environment. Gluconate is conspicuous by its absence in compilations of carbohydrates found abundantly or in trace amounts in typical foodstuffs (Schaffer, 1972; Shallenberger, 1974). Since the *edd* locus is specialized for utilization of gluconate, the locus is evidently one that is not continuously essential in the organism's economy. Consequently, for perhaps substantial periods of time corresponding to the absence of gluconate in the environment, alleles at the *edd* locus may not be subject to natural selection in spite of being functionally different. Loci like *edd* that are specialized for rare substrates will be able to accumulate a diversity of functionally distinct alleles by means of mutation, random genetic drift and hitchhiking with other loci, owing to the periods when the alleles are not subject to selection on their own merits. Following this reasoning, one would conclude that loci of lesser importance in an organism's economy would be the very ones that could maintain polymorphisms of non-neutral alleles, and precisely this argument has been invoked to explain the great variability in nutritional versatility and other characters employed in bacterial biotyping (Mason & Richardson, 1981). We have previously observed a similar situation with two *gnd* alleles, *gnd*(RM77C) and *gnd*(RM215C), which are detrimental to fitness in gluconate medium but not in glucose (Hartl & Dykhuizen, 1981). Because of the evident rarity of gluconate as a limiting growth substrate in nature, hitchhiking of *eda* and *zwf* alleles with *edd* alleles may be a relatively rare occurrence in natural populations. If so, then, in spite of the potential for hitchhiking, the block of linked genes would, in the short run, be affected mainly by founder effects, random genetic drift and hitchhiking with other selected loci. Over evolutionarily significant periods of time, of course, the potential for hitchhiking would be expected to be important in influencing the evolution of the entire block of genes.

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