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, 1983a; 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, 1983b). 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 \( \ln \left( \frac{R(t)}{S(t)} \right) = \text{const} + st \). 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, 1983a).

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, 1983a), 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\(_2\)HPO\(_4\),
15 mM-KH\(_2\)PO\(_4\), 7.6 mM (NH\(_4\))\(_2\)SO\(_4\), 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 1.96 ± 0.21 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, 1983a; Dykhuizen, de Framond & Hartl, 1984). In each case, one strain was resistant to bacteriophage T5 (T5R), due to the fhuA allele at 4 min on the standard E. coli map, and the other was sensitive to T5 (T5S). The fhuA marker is itself neutral under these conditions (Dykhuizen & Hartl, 1980, 1983a), and it is used to monitor the relative frequencies of the competing strains.

Data were analysed by means of the linear regression \[ \ln \left( \frac{R(t)}{S(t)} \right) = \text{cnst} + st, \] where \( R(t) \) and \( S(t) \) represent the frequency of the T5R and T5S 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 T5R 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, 1983a), which is genotypically $\Delta(eda-edd-zwf)$, $rpsL$. $\Delta$ 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

| 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 |

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, 1983a), 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
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the pentose phosphate shunt. (The map position of \textit{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 \textit{eda} function (in the experiments we have used 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 \textit{eda} and \textit{edd}. Separate competition experiments using first glucuronate–galacturonate and then gluconate thereby provides an assessment of the growth-rate effects of alleles at both the \textit{eda} and \textit{edd} loci.

\textit{Controls.} Previous results with related strains undergoing competition in glucose-limited chemostats have shown that the \textit{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 \textit{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 0.353 ± 0.036 h$^{-1}$ (s.e. among experiments). This value defines the maximum amount of selection that could occur against any \textit{eda} allele. With regard
to \textit{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 \textit{edd} deletion was found to be $0.127 \pm 0.019$ h$^{-1}$ and $0.120 \pm 0.007$ h$^{-1}$. Thus, a selection coefficient of approximately $0.12$ h$^{-1}$ is the upper limit of selection involving \textit{edd} alleles.

| Strains | eda allele | Selection coefficient $\pm$ s.e. | t(D.F.) |
|---------|------------|----------------------------------|----------|
| T5R     | T5S        |                                  |          |
| DD1372  | DD1314     | RM66A RM73C                      | $0.00122 \pm 0.00103$ | 0.1 (12) |
| DD1370  | DD1318     | RM73C RM66A                      | $0.00134 \pm 0.00160$ |          |
| DD1372  | DD1316     | RM66A RM77C                      | $0.00046 \pm 0.00010$ |          |
| DD1371  | DD1320     | RM77C RM66A                      | $-0.00056 \pm 0.00102$ |          |
| DD1372  | DD1318     | RM72B RM66A                      | $0.00061 \pm 0.00118$ |          |
| DD1373  | DD1322     | RM66A RM20                       | $0.00009 \pm 0.00097$ |          |
| DD1372  | DD1322     | RM20 RM66A                       | $-0.00160 \pm 0.00220$ |          |
| DD1373  | DD1318     | RM20 RM66A                       | $-0.00576 \pm 0.00363$ |          |
| DD1374  | DD1325     | RM20 RM182A                      | $-0.00058 \pm 0.00192$ |          |
| DD1405  | DD1322     | RM182A RM20                      | $0.00132 \pm 0.00144$ |          |
| DD1377  | DD1316     | K12 RM77C                        | $-0.00214 \pm 0.00073$ | 4.4 (14)**|
| DD1371  | DD1328     | RM77C K12                        | $0.00416 \pm 0.00124$ |          |

**P < 0.01.

\textit{eda} alleles. Results of chemostat experiments with \textit{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 0, solid lines represent significance. Two tests of significance were carried out. In each experiment, the significance of the regression coefficient was tested by the \textit{F} value in an analysis of variance (data not shown). However, since the experiments are paired (e.g. \textit{eda} (RM73C) T5R versus \textit{eda} (RM66A) T5S in one case and \textit{eda} (RM73C) T5S versus \textit{eda} (RM66A) T5R in the other), a \textit{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 \textit{t} test is more powerful than the \textit{F} tests owing to its greater number of d.f. Fig. 2 represents the finding that all \textit{eda} alleles from natural isolates are selectively neutral. Interestingly, the allele in \textit{E. coli} K12 is not neutral with respect to the others, but is selectively inferior, the selection coefficient against the K12 allele averaging $0.003 \pm 0.001$ h$^{-1}$. Experimental results demonstrating this selection against strains carrying the K12 \textit{eda} allele are shown in Fig. 3. The dashed lines represent the regressions, and the slopes are $0.0042 \pm 0.0012$ and $0.0021 \pm 0.0007$.

\textit{edd} alleles. The situation regarding \textit{edd} is very different from that with \textit{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 \textit{edd} (RM66A) allele relative to \textit{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.

$s = 0.0083 \pm 0.0021$. 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 $s = 0.0009 \pm 0.0010$ and $s = -0.0013 \pm 0.0013$. Altogether, we have identified three classes of edd alleles relative to their selective
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 \( \text{edd(RM66A)} \) versus \( \text{edd(RM72B)} \) and \( \text{edd(RM20)} \) versus \( \text{edd(RM182A)} \). Regarding the \( t \) test as the more powerful, we treat selection as being significant in these cases, and the corresponding lines in Fig. 5 are shown as solid lines. For \( \text{edd(RM66A)} \) versus \( \text{edd(RM72B)} \), the conclusion of significant selective effects is independently confirmed by the unambiguous neutrality between \( \text{edd(RM72B)} \) and \( \text{edd(RM20)} \), on the one hand, and the clear selection between \( \text{edd(RM66A)} \) and \( \text{edd(RM20)} \), on the other. Overall, the selection coefficient against \( \text{edd(RM20)} \)-type alleles, relative to \( \text{edd(RM66A)} \)-type alleles, is \( 0.010 \pm 0.001 \). Selection against \( \text{edd(RM182A)} \) relative to \( \text{edd(RM20)} \) averages \( 0.003 \pm 0.001 \).

**DISCUSSION**

With respect to the alleles from natural isolates, the \( \text{eda} \) locus falls into the pattern previously established for alleles of \( \text{gnd} \), \( \text{pgi} \) and \( \text{zuf} \), which is that alleles from natural isolates are by and large selectively neutral (Dykhuizen & Hartl, 1980, 1983a; Hartl & Dykhuizen, 1981; Dykhuizen, et al. 1984). However, certain \( \text{gnd} \) and \( \text{pgi} \) alleles could be shown to be non-neutral under the appropriate conditions, and this is also the case with the \( \text{eda} \) allele in \( E. \ coli \) K12. In contrast, the \( \text{edd} \) 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 \( \text{eda(K12)} \) allele is selectively inferior to the \( \text{eda(RM77C)} \) allele as
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Table 4. Selection of edd alleles

| Strains | T5R | T5S | edd allele | No. expts. | Selection 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 | 0.18 (17) |
| DD1371  | DD1328 | RM77C | K12  | 2 | -0.00160 ± 0.00151 | **(10)** |
| DD1377  | DD1316 | K12  | RM77C | 2 | -0.00098 ± 0.00272 | 0.24 (10) |
| DD1374  | DD1314 | RM20  | RM73C | 1 | 0.00080 ± 0.00130 | 0.27 (14) |
| DD1370  | DD1322 | RM73C | RM20  | 1 | 0.00026 ± 0.00178 | 0.24 (10) |
| DD1374  | DD1320 | RM20  | RM72B | 2 | 0.00016 ± 0.00160 | 12.2 (24)*** |
| DD1373  | DD1322 | RM72B | RM20  | 2 | -0.00054 ± 0.00216 | 11.9 (14)*** |
| DD1370  | DD1318 | RM73C | RM66A | 2 | -0.00985 ± 0.00078 | **(10)** |
| DD1372  | DD1314 | RM66A | RM73C | 2 | -0.00985 ± 0.00149 | 12.2 (24)*** |
| DD1374  | DD1318 | RM20  | RM66A | 3 | -0.00843 ± 0.00145 | 9.7 (21)*** |
| DD1372  | DD1322 | RM66A | RM20  | 3 | 0.01024 ± 0.00126 | 11.9 (14)*** |
| DD1150  | DD1146 | RM73C | K12  | 1 | -0.01780 ± 0.00186 | 3.2 (14)** |
| DD1159  | DD1137 | K12  | RM73C | 1 | 0.01400 ± 0.00154 | 3.2 (14)** |
| DD1373  | DD1318 | RM72B | RM66A | 2 | -0.00469 ± 0.00206 | 11.9 (14)*** |
| DD1372  | DD1320 | RM66A | RM72B | 2 | 0.00533 ± 0.00263 | 11.9 (14)*** |
| DD1405  | DD1322 | RM182A | RM20  | 1 | 0.00353 ± 0.00162 | **(10)** |
| DD1374  | DD1325 | RM20  | RM182A | 1 | 0.00208 ± 0.00235 | **(10)** |

*, **, ***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.
judged by competition in galacturonate–glucuronate. Selection might also be expected to occur in glucurate, because the _eda_ gene product is used in glucurate metabolism as well. However, these same alleles are selectively neutral in glucurate. One possible explanation of this observation is that the _edd_ enzyme is the limiting step for growth in glucurate, 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 glucurate 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 glucurate, the sole substrate that evokes selection of the _edd_ alleles, is evidently a relatively rare substrate for _E. coli_ in its natural intestinal environment. Glucurate 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 glucurate, 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 glucurate 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 glucurate medium but not in glucose (Hartl & Dykhuizen, 1981). Because of the evident rarity of glucurate 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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