J. evol. biol. 10 (1997) 743–769
1010–061X/97/050743–27 $ 1.50 + 0.20/0

Journal of Evolutionary Biology

Long-term experimental evolution in *Escherichia coli*. V. Effects of recombination with immigrant genotypes on the rate of bacterial evolution

V. Souza,[1] P. E. Turner[2,3] and R. E. Lenski[2,*]

[1]*Centro de Ecología, Universidad Nacional Autónoma de México, Coyoacán, Ciudad de México, 04510, México*
[2]*Center for Microbial Ecology, Michigan State University, East Lansing MI 48824, USA*
[3]*Current address: Department of Zoology, University of Maryland, College Park MD 20742, USA*

Key words: Complex selection; conjugation; *Escherichia coli*; experimental evolution; hitchhiking; migration; plasmids; recombination.

Abstract

This study builds upon an earlier experiment that examined the dynamics of mean fitness in evolving populations of *Escherichia coli* in which mutations were the sole source of genetic variation. During thousands of generations in a constant environment, the rate of improvement in mean fitness of these asexual populations slowed considerably from an initially rapid pace. In this study, we sought to determine whether sexual recombination with novel genotypes would reaccelerate the rate of adaption in these populations. To that end, treatment populations were propagated for an additional 1000 generations in the same environment as their ancestors, but they were periodically allowed to mate with an immigrant pool of genetically distinct Hfr (high frequency recombination) donors. These donors could transfer genes to the resident populations by conjugation, but the donors themselves could not grow in the experimental environment. Control populations were propagated under identical conditions, but in the absence of sexual recombination with the donors. All twelve control populations retained the ancestral alleles at every locus that was scored. In contrast, the sexual recombination treatment yielded dramatic increases in genetic variation. Thus, there was a profound effect of recombination on the rate of genetic change. However, the increased genetic variation in the treatment populations had no significant effect on the rate of

* Author for correspondence.

adaptive evolution, as measured by changes in mean fitness relative to a common competitor. We then considered three hypotheses that might reconcile these two outcomes: recombination pressure, hitchhiking of recombinant genotypes in association with beneficial mutations, and complex selection dynamics whereby certain genotypes may have a selective advantage only within a particular milieu of competitors. The estimated recombination rate was too low to explain the observed rate of genetic change, either alone or in combination with hitchhiking effects. However, we documented complex ecological interactions among some recombinant genotypes, suggesting that our method for estimating fitness relative to a common competitor might have underestimated the rate of adaptive evolution in the treatment populations.

Introduction

Bacterial reproduction per se is strictly asexual and occurs by binary fission. But if sex is defined as the exchange of genetic material between organisms, then bacteria undergo sexual recombination through the processes of transformation, viral-mediated transduction, and plasmid-mediated conjugation (Levin, 1988; Hopwood and Chater, 1989; Maynard Smith, 1990; Dykhuizen and Green, 1991; Maynard Smith et al., 1993). For example, an F plasmid inserted into a bacterial chromosome converts the bacterium into an Hfr (high frequency recombination) donor strain.

Population genetic studies of bacteria isolated from nature have shown that some species, including *Escherichia coli*, have very high levels of linkage disequilibrium, indicating clonal population structures (Selander and Levin, 1980; Caugant et al., 1981; Whittam et al., 1983; Maynard Smith et al., 1993). However, there is molecular evidence for occasional chromosomal recombination in natural populations of even highly clonal bacteria such as *E. coli* (Milkman and McKane-Bridges, 1990; Maynard Smith, 1990; Bisêrcîc et al., 1991; Dykhuizen and Green, 1991; Maynard Smith et al., 1991; Guttman and Dykhuizen, 1994a, 1994b). In addition, recent analyses of population genetic structure in *Bacillus subtilis* (Istock et al., 1992; see also Roberts and Cohan, 1995), *Rhizobium etli* (Souza et al., 1992, 1994), and *Neisseria gonorrhea* (Maynard Smith et al., 1993) indicate that recombination is more frequent in these species than in *E. coli*. Thus, the prevalence of sexual recombination in bacteria may be quite variable (Maynard Smith, 1990; Maynard Smith et al., 1993; Lenski, 1993; Cohan, 1994a). Moreover, population genetic models indicate that recombination in bacteria may have very different effects on evolutionary divergence depending on whether genetic variants are neutral or adaptive (Cohan, 1994a, 1994b).

Perhaps the most important effect of sexual recombination, along with mutation and migration, is to increase genetic variation. Natural selection may then act on this heritable variation to improve the mean fitness of an evolving population (Fisher, 1930; Muller, 1932). However, an increased supply of genetic variation does not guarantee that adaptive evolution will proceed more quickly. The additional variation may be selectively neutral, in which case the rate of adaptive evolution

should be unchanged (Kimura, 1983); or the genetic variation added by recombination, mutation, or migration may produce a load that actually lowers a population's mean fitness (McNeilly, 1968; Crow and Kimura, 1970; Ford, 1975). The effects of genetic variation on the rate of adaptive evolution may be further complicated by frequency-dependent selection, gene interactions, and environmental change (Frank and Slatkin, 1992).

Bacteria provide excellent experimental models to study evolutionary processes such as mutation, recombination, and natural selection (Luria and Delbrück, 1943; Atwood et al., 1951; Graham and Istock, 1979, 1981; Dykhuizen and Hartl, 1983; Helling et al., 1987; Dykhuizen, 1990; Lenski et al., 1991; Bennett et al., 1992; Lenski, 1992; Cohan et al., 1994; Lenski and Travisano, 1994; Travisano et al., 1995a; Elena et al., 1996). Because of their large population sizes and short generation times, bacteria may be propagated in defined environments for hundreds and even thousands of generations, allowing evolutionary processes to be directly observed. This paper presents a 1000-generation experiment to examine the effects of recombination, coupled with immigration of novel alleles, on the rate of evolution in otherwise asexual populations of *E. coli*.

Experimental overview

The bacterial strains used in this study were isolated from twelve populations of *E. coli* maintained in the laboratory as part of a long-term evolution experiment (Lenski et al., 1991; Lenski and Travisano, 1994). During 10 000 generations of evolution, the mean fitness of these populations, relative to their common ancestor, increased by about 50% on average (Fig. 1). All twelve populations were descended

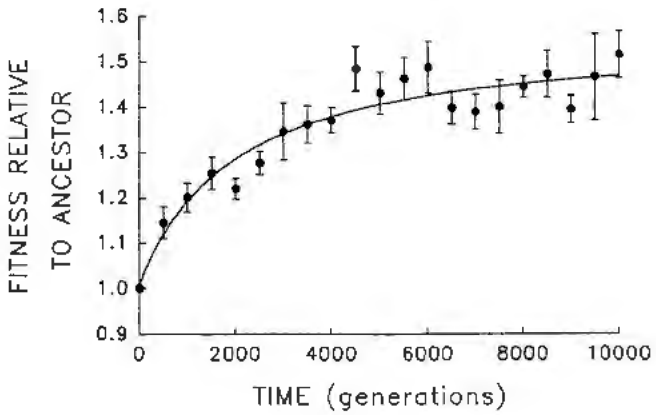

Fig. 1. Grand mean fitness relative to the ancestor for the twelve *E. coli* populations described by Lenski and Travisano (1994). Error bars are 95% confidence intervals. The curve gives the best fit of a hyperbolic model to the grand means, and it shows a significant deceleration in the rate of improvement over time. The ancestral strains in the present study were clonal isolates obtained after 7000 generations of this earlier study.

from a single clone, and mutation was the only source of genetic variation for adaptation by natural selection. Most of the increase in fitness occurred during the first few thousand generations of this experiment. Thus, by generation 5000 or so, the rate of adaptation had slowed to such an extent that a selective plateau was becoming apparent (Fig. 1).

The primary question that we sought to address with our experiment was whether the pace of adaptive evolution (as measured by the rate of increase in mean fitness) could be reaccelerated by giving these populations an additional source of genetic variation; this was achieved by allowing them to undergo sexual recombination with genetically distinct immigrant strains. Whether such an effect is observed will depend critically on whether the immigrant strains carry alleles that are (i) inaccessible to the asexual residents by mutation alone, and (ii) beneficial to the residents in their environment. To that end, a clone was isolated from each of the twelve evolving populations after 7000 generations, by which time the rate of increase in mean fitness had slowed substantially (Fig. 1). Each clone was then used to found a new pair of populations, one of which would experience sexual recombination with immigrant genotypes and the other of which would serve as an asexual control.

Recombination was achieved by periodically adding plasmid-bearing Hfr *E. coli* K12 donor cells to the treatment populations. These donors are genetically quite distinct from the resident *E. coli* B recipients. For example, B and K12 strains produce distinct allozymes at six of twenty loci that were scored electrophoretically by Selander and Levin (1980). [Such differences are not unusual among *E. coli* strains from nature, including those from the same individual host (Caugant et al., 1981).] This degree of evolutionary divergence would seem likely to fulfill condition (i) above, which is that recombination with the immigrant donor strains provides novel genetic variation to the resident populations. Moreover, this divergence allows us to quantify the effect of the recombination treatment at the genetic level by monitoring the movement of several alleles that distinguish the donor and recipient genotypes (Tab. 1).

To prevent the spread of donor genotypes by ecological competition – in contrast to the spread of their genes by recombination and subsequent selection – we used donors that were deficient in growth in the experimental environment owing to mutations in critical metabolic genes. Thus, the immigrant donors could transmit their genes by conjugation, but they could not propagate themselves asexually. The defective alleles used to impair the donors' growth in the selective environment certainly do not fulfill condition (ii) above. However, we emphasize that these deliberate defects affect only a few of the 1000+ genes in *E. coli* (Bachmann, 1987). And because recombination via conjugation is a patchy and irregular process, most recombinant genotypes should be completely free of these deliberate defects. Beyond these few defective alleles, absolutely nothing is known about the potential for particular alleles from *E. coli* K12 to benefit *E. coli* B in the experimental environment. We imagine that at least some of the K12 alleles might be useful to B. However, we cannot reject *a priori* the possibility that every single K12 allele is either selectively neutral or disadvantageous to B in the particular

Table 1. Genetic differences between donor (immigrant) and recipient (resident) strains.

Strain	Physiological loci[a]									Electrophoretic loci[b]					OriT[d]
	Ara	Lac	Tet[c]	Str	Arg	Leu	Ilv	T1X	T6	IDH	6-PGD	ADH	MPI	PEP	
Recipients[e]	+/−	+	*s*	*r*	+	+	+	*s*	*r*	1	2	2	2	1	--
Donors[f]															
REL288	+	+	*r*	*s*	+	+	−	*s*	*s*	2	1	1	1	2	67 ↑
REL291	−	−	*r*	*s*	−	+	+	*r*	*r*	2	1	1	1	2	2 ↓
REL296	+	−	*r*	*s*	−	−	+	*r*	*s*	2	1	1	1	2	12 ↓
REL298	+	−	*r*	*s*	−	−	+	*r*	*r*	2	1	1	1	2	84 ↓

[a] + and − indicate ability and inability, respectively, to grow on sugars L-arabinose (Ara) and lactose (Lac); *s* and *r* indicate sensitivity and resistance, respectively, to antibiotics tetracycline (Tet) and streptomycin (Str); + and − indicate prototrophy and auxotrophy, respectively, for amino acids arginine (Arg), leucine (Leu) and isoleucine-valine (Ilv); *s* and *r* indicate sensitivity and resistance, respectively, to bacteriophages T1X and T6.

[b] Numbers indicate mobility classes for enzymes isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6-PGD), alcohol dehydrogenase (ADH), mannose phosphate isomerase (MPI), and peptidase (PEP).

[c] In each of the donor strains, tetracycline resistance is encoded by a transposon that has been inserted into a gene required for synthesis of an amino acid.

[d] Map position, in minutes, on the *E. coli* K12 chromosome for the origin of transfer of donor genes into recipient cells. ↑ and ↓ indicate transfer in order of ascending and descending map order, repectively.

[e] Except for the Ara phenotype, there were no differences among the twelve ancestral recipient strains. All recipients are *E. coli* B.

[f] The donors were obtained from B. Bachmann, curator of the *E. coli* Genetic Stock Center at Yale University, New Haven, Conn., USA. They were previously designated BW6159, BW6165, BW7261, and BW6169, respectively (Wanner, 1986). All donors are *E. coli* K12.

context of its environment and genetic background. (We did not pursue an alternative approach, in which the independently derived B lines would be allowed to recombine with one another, because these lines lack any mechanism for genetic exchange. Moreover, the replicate lines are still so similar to one another, even after thousands of generations, that they lack suitable genetic markers for monitoring any recombination that might occur if some of them were converted into Hfr donors.)

The twelve pairs of recombination treatment and asexual control populations were then propagated for 1000 generations, in the same environment in which the asexual recipients had been propagated for the previous 7000 generations. Although their environments were identical, the control populations had only mutation as a source of genetic variation, whereas the treatment populations had recombination with the Hfr donors as an additional source of variation. Thus, the experiment sought to address the following questions:

(1) Did alleles from the Hfr donors enter the recipient populations, which would indicate that the recombination treatment had the intended effect of increasing genetic variation?

(2) Did this additional variation promote more rapid adaptive evolution in the treatment populations than in the control populations? That is, did sexual recombination with immigrant genotypes re-accelerate the rate of increase in mean fitness?

If the answers are 'yes' to both questions, then this would provide compelling support for the hypothesis that the additional variation provided by sexual recombination had accelerated the rate of adaptive evolution in these bacterial populations. This outcome would also indicate that certain K12 alleles were useful to *E. coli* B in the experimental environment. If the answers are 'no' to both questions, then this would suggest that the rate of recombination in the treatment populations was too low to have any discernible effect or, alternatively, that none of the K12 alleles were useful to *E. coli* B. A 'no' answer to the first question and a 'yes' to the second would suggest that sexual recombination had accelerated the rate of adaptive evolution, but that the numbers of markers scored were too few to discern its effects at the genetic level.

A 'yes' answer to the first question and a 'no' to the second would require a more complex explanation along one or more of the following lines. According to one hypothesis, the rate of sexual recombination is so high that donor alleles become fixed in the recipient population, even in the absence of any selective utility. According to a second hypothesis, donor alleles may hitchhike to high frequency in association with beneficial *mutations*. The beneficial mutations are presumed to occur at equal rates in treatment and control populations, but their ascent to high frequency is marked by donor alleles that can be readily scored only in the treatment populations. According to a third hypothesis, the methods used to assay the relative fitness of recombinant genotypes are inadequate due to frequency-dependent selection or other complex interactions.

Materials and methods

Bacterial strains

A single clone was isolated at generation 7000 from each of the twelve experimental populations described by Lenski and Travisano (1994). All twelve clones are derivatives of *E. coli* B. Six of the clones can grow on the sugar L-arabinose (Ara^+) and six cannot (Ara^-). Ara^+ and Ara^- strains form white and red colonies, respectively, on tetrazolium-arabinose (TA) indicator plates (Levin et al., 1977). Each clone was used to found one population in the control and recombination treatments described below. Each of these founder, or ancestral, clones was scored for nine physiological and five electrophoretic loci (Tab. 1). There were no differences among the twelve ancestral clones, except for the Ara marker. The ancestral clones were stored in a glycerol-based suspension at −80 °C to allow for direct comparisons between them and their evolved derivatives at a later time.

The donors used in this study were four Hfr strains of *E. coli* K12. Each of these donors has an F plasmid inserted at a different position in its chromosome (Tab. 1).

Thus, each has a different point of origin (OriT site) for conjugative gene transfer. These *E. coli* K12 donors are genetically quite distinct from the *E. coli* B recipients, and they differ at a number of loci (Tab. 1). The genetic differences between K12 donors and B recipients allowed recombinant genotypes to be detected by plating on appropriate media and by allozyme electrophoresis. For example, each donor has a transposon-encoded tetracycline-resistance gene located 10–25 minutes from its OriT site; the transposon itself was inserted into a gene required for synthesis of an amino acid (Wanner, 1986). As a consequence, each donor strain was auxotrophic for at least one amino acid (Tab. 1), so that it could not grow in the minimal medium employed in this experiment. Hence, these Hfr strains could donate genes via recombination but were themselves unable to proliferate and out-compete the prototrophic *E. coli* B recipients.

Culture conditions

The culture medium employed in all experiments was Davis Minimal (DM) broth (Carlton and Brown, 1981) supplemented with 0.002 μg thiamine hydrochloride and 25 μg glucose ml^{-1}. This medium supports a stationary-phase density of $\sim 5 \times 10^7$ cells ml^{-1}. Culture volume was 10 ml, maintained in 50 ml Erlenmeyer flasks that were incubated at 37 °C and shaken at 120 rpm (except as noted otherwise). All cultures were propagated each day by transferring 0.1 ml of each stationary-phase culture into 9.9 ml of fresh medium. The resulting 100-fold re-growth represents ~ 6.64 generations of binary fission each day. The Hfr strains used in this experiment were grown separately in Luria broth (LB), a rich medium that yielded a stationary-phase density of $\sim 2 \times 10^9$ donor cells ml^{-1}.

Recombination treatment

A single ancestral clone was used to initiate each of the twelve populations (six Ara^+ and six Ara^-) in the recombination treatment. Every day, for 150 days, 0.1 ml from the previous day's culture was transferred into 9.9 ml of fresh DM. On day 0, and every fifth day (~ 33 generations) thereafter, we also added 0.01 ml of an equally proportioned volumetric mixture of the four Hfr strains, which had been grown in LB. (The Hfr strains were always freshly grown from the freezer stocks to preclude their further evolution.) This manipulation produced initial densities of about 2×10^6 and 5×10^5 donor and recipient cells ml^{-1}, respectively, for a ratio of about 4:1. On those days when donors were added, the flasks were placed in a non-shaking incubator at 37 °C for one hour to allow uninterrupted mating between donors and recipients. Subsequently, the flasks were transferred to a shaking incubator at 37 °C for 23 hours. On all other days, the flasks were held in a shaking incubator for the full 24 hours. During the next four days, the cultures were propagated by serial transfer. Every 15 days (100 generations), just prior to the addition of donor cells, 10% glycerol was added to a sample from each population, and the samples were placed in a freezer at −80 °C for future study.

Preliminary experiments were performed to make sure that the recombination treatment protocol worked in two important respects: (i) recombinant genotypes (transconjugants) were generated at a measurable frequency, and (ii) the auxotrophic donors died out after their addition to the evolving populations. Figure 2 shows the dynamics occurring in the five-day cycle of the recombination treatment. As we intended, recombinants that possessed the donor's tetracycline resistance (Tet^r) and the recipient's streptomycin resistance (Str^r) were readily detected, and the number of auxotrophic donors declined to <10 cells ml^{-1} by the fifth day (Fig. 2). The frequency of Tet^r Str^r recombinants also declined rapidly, because Tet^r is encoded by a transposon inserted into a gene required for synthesis of an essential amino acid. Thus, most Tet^r Str^r recombinants, like the donors, were auxotropic and hence much less fit than the resident genotype in the experimental environment. However, the fact that these particular recombinant genotypes are poorly adapted does not imply that *all* recombinants are less fit than the resident genotype.

Control treatment

The same twelve clones (six Ara^+ and six Ara^-) used to found the populations in the recombination treatment were also used to initiate the control populations. On day 0, and every fifth day thereafter, each recipient population underwent a sham manipulation to mimic the recombination treatment, but without allowing genetic recombination. In particular, the control populations received 0.01 ml of LB medium in which the Hfr donor strains had been grown to stationary phase, but

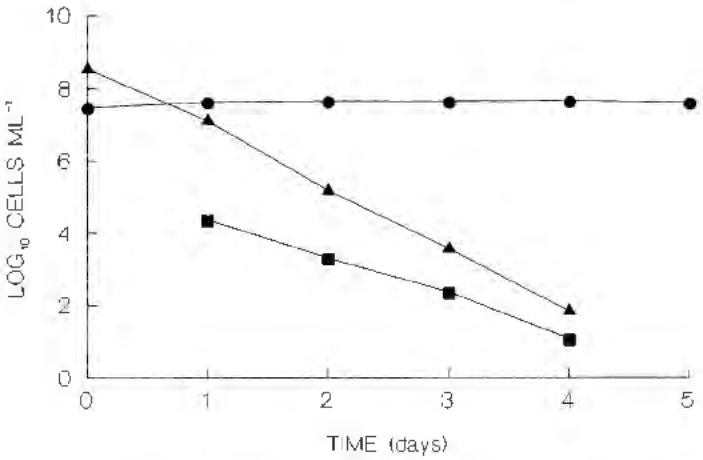

Fig. 2. Dynamics of *E. coli* K12 donors (triangles), *E. coli* B recipients (circles) and recombinant genotypes (squares) during one five-day cycle of the recombination treatment. The number of recombinants is based on inheritance of the donor's tetracycline resistance, which renders the cells auxotrophic, and the recipient's streptomycin resistance. By day five, the density of both auxotrophic donors and recombinants had fallen below the detection limit (10 cells ml^{-1}).

from which all cells had been removed by filtration. Also, for the first hour after receiving this placebo, the control populations were placed in the non-shaking incubator prior to being moved to the shaking incubator. Thus, the control populations experienced the same selective environment as the recombination treatment populations, except for the effects of adding the donor cells themselves.

Fitness assays

To measure relative fitness, two strains were placed in competition under the culture conditions described above, where one competitor was Ara^+ and the other was Ara^-. Each strain was grown separately for one day in DM as a conditioning step to ensure that both competitors were in comparable physiological states. The two competitiors were mixed at a 1:1 volumetric ratio, then diluted 1:100 into fresh DM and allowed to grow and compete for one 24-hour cycle. Initial and final densities of each competitor were estimated by spreading cells on TA plates, which permitted the competitiors to be distinguished by colony color.

Let the initial densities of the Ara^+ and Ara^- strains be $N_1(0)$ and $N_2(0)$, respectively; and let $N_1(1)$ and $N_2(1)$ be their corresponding densities after one day. The average rate of increase (or realized Malthusian parameter), m_i, for either competitor is then calculated as:

$$m_i = \ln[N_i(1)/N_i(0)]/(1 \text{ day}). \quad (1)$$

The fitness of one strain relative to another, W_{ij}, is estimated as the ratio of their Malthusian parameters (Lenski et al., 1991):

$$W_{ij} = m_i/m_j. \quad (2)$$

Screening for recombinant genotypes

Every 100 generations, ten clones were chosen at random from each population in the recombination and control groups. The genotype of each clone was determined using nine physiological loci that could be scored by plating cells on appropriate media: arabinose and lactose utilization, tetracycline and streptomycin resistance, resistance to T1X and T6 bacteriophages, and auxotrophy for amino acids leucine, arginine and isoleucine-valine.

At generation 1000, lysates were prepared for the ten clones from each population. Each clone was grown overnight in 10 ml of LB, and the resulting culture was sonicated using the method of Piñero et al. (1988). These clones were scored for five allozyme loci using cellulose acetate electrophoresis (Hellena Laboratories): alcohol dehydrogenase (ADH), isocitrate dehydrogenase (IDH), mannose phosphate isomerase (MPI), peptidase (PEP) and 6-phosphogluconate dehydrogenase (6-PGD). These five enzymes were chosen (from 16 in preliminary screens) because their mobilities were different for the *E. coli* K12 donors and *E. coli* B recipients used in

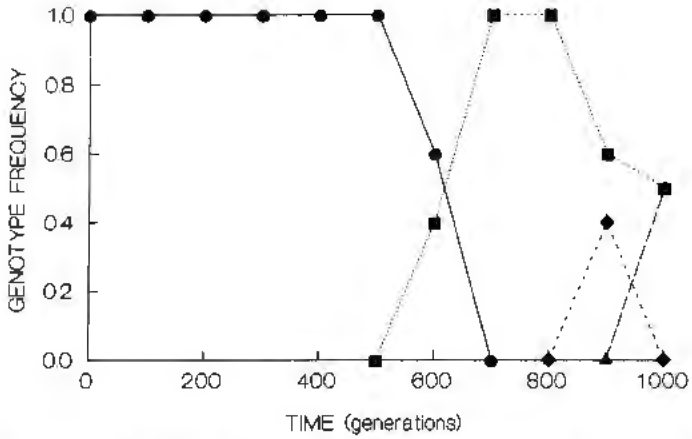

Fig. 3. Evolutionary dynamics in population Ara^{-}1 of the recombination treatment. Samples were obtained every 100 generations, and changes in genotype frequency are based upon the nine physiological loci described in Table 1. The ancestral genotype (circles) and three distinct recombinant genotypes (squares, diamonds, triangles) were detected over the course of 1000 generations.

our experiment (Tab. 1). At least three electrophoretic assays were performed on each clone for each allozyme locus.

We also screened the ten clones sampled from each treatment population at generation 1000 for the presence of extrachromosomal plasmids. We did so because some of these clones were merodiploids, which must have had two copies of certain electrophoretic loci because they expressed both the donor and recipient allozymes. To look for small plasmids, we used alkaline lysis and electrophoresis in a 1% agarose gel with *Hin*dIII Lambda digest as a control (Sambrook et al., 1989). To look for large plasmids, we used the method of Eckhardt (1978; see also Crosa et al., 1994) to lyse cells directly in the well of a vertical gel containing 0.7% agarose. These vertical gels were then subjected to electrophoresis for 1 hour at 8 mA followed by 4 hours at 40 mA, with a 240 kb plasmid, CFN42, serving as a positive control (Quinto et al., 1982).

Results

Genetic changes

Figure 3 shows the genetic dynamics, in one of the recombination treatment populations, that were obtained by scoring nine physiological loci for ten clones at 100-generation intervals. Three different recombinant genotypes were detected in this population, and the ancestral genotype fell below the limit of detection by generation 700. Qualitatively similar dynamics were seen in the other treatment populations (Tab. 2). The median time at which recombinant genotypes were first detected was generation 600. On average, 2.67 different recombinant genotypes

were seen at least once in a treatment populataion over the entire course of the experiment. By contrast, using the same nine loci and sampling scheme, absolutely no variants were detected in any of the asexual control populations (Tab. 2).

The above data must greatly underestimate the extent of genetic variation, and the rate of genetic change, in the recombination treatment populations. Only nine physiological loci were scored; three of these loci (those for amino acid auxotrophy) were subject to strong selection for the resident allele, and no variation was detected at those loci. [However, the Ara$^-$3 treatment population acquired the Tet^r gene, although it did not become auxotrophic (see the Appendix). Evidently, the transposon that encodes the Tet^r gene must have moved from its location in a donor gene required for synthesis of an essential amino acid to some other site.] To further evaluate the effect of the recombination treatment, we also scored ten clones taken from each population at generataion 1000 for the five electrophoretic loci that distinguished the donor and recipient populations.

Table 3 summarizes the variation revealed by combining the data from the electrophoretic and physiological loci at generation 1000. On average, there were 6.08 different genotypes in the recombination treatment populations, which is a remarkably high number given that only ten clones were characterized for each population. The ancestral (recipient) genotype was absent from six of the treatment populations, and the frequency of the ancestral type was $\leq 20\%$ in ten of the twelve populations. The Appendix lists the genotypes and their frequencies in the recombination treatment populations at generation 1000. Again, by contrast, none of the electrophoretic or physiological loci showed any variation whatsoever in the twelve asexual control populations.

Table 2. Summary of temporal dynamics of genetic change in recombination treatment and control populations during 1000 generations. Every 100 generations, ten clones from each population were scored for the nine physiological loci shown in Table 1. The actual dynamics for recombination treatment population Ara$^-$1 are shown in Figure 3.

Population	New genotype first seen	Ancestral type last seen	Number of distinct new genotypes
Recombination treatment			
Ara$^-$1	600 gen.	600 gen.	3
Ara$^-$2	600 gen.	1000 gen.	1
Ara$^-$3	300 gen.	200 gen.	2
Ara$^-$4	200 gen.	1000 gen.	1
Ara$^-$5	500 gen.	500 gen.	5
Ara$^-$6	200 gen.	1000 gen.	3
Ara$^+$1	800 gen.	800 gen.	3
Ara$^+$2	1000 gen.	1000 gen.	1
Ara$^+$3	900 gen.	1000 gen.	2
Ara$^+$4	200 gen.	1000 gen.	5
Ara$^+$5	600 gen.	1000 gen.	4
Ara$^+$6	700 gen.	1000 gen.	2
Control			
All 12	Never	1000 gen.	0

Table 3. Summary of genetic changes in recombination treatment and control populations after 1000 generations. Ten clones from each populations were scored for the nine physiological and five electrophoretic loci shown in Table 1. The Appendix lists the various genotypes and their frequencies in the recombination treatment populations at generation 1000.

Population	Proportion of ancestral type	Number of distinct genotypes, including ancestral type
Recombination treatment		
Ara$^-$1	0%	5
Ara$^-$2	20%	6
Ara$^-$3	0%	6
Ara$^-$4	80%	2
Ara$^-$5	0%	5
Ara$^-$6	0%	6
Ara$^+$1	0%	5
Ara$^+$2	10%	8
Ara$^+$3	10%	9
Ara$^+$4	40%	7
Ara$^+$5	0%	7
Ara$^+$6	20%	7
Control		
All 12	100%	1

Many of the recombinant genotypes were merodiploids for one or more electrophoretic loci; that is, they possessed both donor and recipient alleles. One possible explanation for these merodiploids is that they carry F′ plasmids, which might be generated from unstable Hfr donors. To test this possibility, we screened all 120 clones from the recombination treatment populations for both small and large plasmids. Two isogenic clones from one population (Ara$^-$1) contained a plasmid-like element of ~ 30 kb, which is much smaller than a typical F′ plasmid. These two clones were indeed merodiploids for one locus (IDH), but other clones from the same population that were also merodiploids for the same locus did not contain this element. Moreover, no plasmids were observed in any of the other 118 clones. Thus, it seems that merodiploids arose by recombination events that led to the duplication of chromosomal regions, rather than by the acquisition of extrachromosomal plasmids.

Evidently, the recombination treatment greatly increased the amount of genetic variation that was potentially available for natural selection, and it led to a much faster rate of evolutionary change at the genetic level. Of course, there may well have been genetic changes in the control populations at loci other than the 14 that we could readily score. However, the recombination treatment populations should also be subject to such cryptic changes. Our major conclusion so far is that a substantial fraction of genes have been replaced by donor alleles in the recombination treatment populations, whereas a typical gene has not undergone noticeable change in the control populations.

Changes in fitness

At the end of 1000 generations, we measured the mean fitness of each population in the recombination treatment and control groups relative to a common competitor. This common competitor (REL2543) was the ancestral genotype for recombination treatment and control populations Ara$^-$1 in this study, having been isolated at generation 7000 in the experiment described by Lenski and Travisano (1994). A selectively neutral Ara$^+$ mutant (REL4190) of this clone was also obtained to allow competition experiments with the Ara$^-$ populations. The genetically heterogeneous populations in the control and recombination treatment groups were placed in competition with the commom competitior of the opposite Ara marker state, after each competitor was preconditioned in DM for one day. [The majority of cells in two populations that were initially Ara$^-$ became Ara$^+$ as a consequence of the substitution of donor alleles (see the Appendix). These populations were allowed to compete against the Ara$^-$ common competitor in order to yield accurate fitness estimates.] We also measured the fitness of all twelve ancestral genotypes relative to the common competitor. Fitness assays were replicated four-fold in complete blocks of 36 (corresponding to the twelve ancestral genotypes, the twelve control populations at generation 1000, and the twelve treatment populations at generation 1000). The resulting estimates of mean fitness are shown in Table 4.

We first tested whether the variation provided by mutation alone had allowed the populations in the control group to gain fitness relative to their ancestors. A one-tailed paired comparison between the means for each control population and

Table 4. Mean fitnesses relative to the common competitor for the twelve ancestral genotypes and their derived populations in each treatment group. Each fitness was assayed, with four-fold replication, relative to the common competitor bearing the alternative Ara marker state. Standard errors for the grand means are based on $n = 12$ replicate populations. See text for statistical comparisons.

		Treatment	
	Ancestor	Control	Recombination
Population			
Ara$^-$1	0.984	0.986	1.092
Ara$^-$2	1.017	1.043	1.087
Ara$^-$3	0.967	1.058	0.816
Ara$^-$4	1.000	1.015	0.984
Ara$^-$5	0.881	0.968	0.985
Ara$^-$6	0.954	1.005	1.032
Ara$^+$1	0.965	1.120	1.028
Ara$^+$2	1.016	1.064	1.050
Ara$^+$3	1.104	1.037	1.064
Ara$^+$4	1.049	1.082	1.031
Ara$^+$5	1.042	1.021	1.024
Ara$^+$6	1.009	1.051	1.039
Grand mean (SE)	0.999 (0.016)	1.038 (0.012)	1.019 (0.021)

its ancestor indicates that the average improvement of about 4% is statistically significant ($t_s = 2.349$, df = 11, $p = 0.039$). We then tested whether the added genetic variation provided by sexual recombination with the immigrant donors allowed the twelve populations in the treatment group to gain fitness to a greater extent than their counterparts in the control group. A two-tailed paired comparison between the means for each treatment population and its corresponding control indicates no significant fitness effect due to the recombination treatment ($t_s = -0.801$, df = 11, $p = 0.440$). Because the fitness assays were replicated for all of the populations, we could also perform twelve separate tests of the effect of recombination by comparing the fitness values obtained for each pair of treatment and control populations. In one pair (Ara$^-$1), the treatment population had significantly higher fitness than did its control population ($t_s = 3.377$, df = 6, $p = 0.015$); in another pair (Ara$^-$3), the opposite relationship held ($t_s = -3.193$, df = 6, $p = 0.018$). However, given the fact that we performed twelve tests of the same hypothesis, it is not compelling that two of the tests were significant at the 0.05 level. In fact, applying the Bonferroni criterion (Rice, 1989) to adjust the probabilities to account for these multiple tests renders both differences nonsignificant. Therefore, we conclude that recombination with the genetically distinct donors did not allow the treatment populations to gain fitness more rapidly than the control populations (Tab. 4), despite the fact that the recombination treatment clearly accelerated the rate of genetic change at the marker loci (Tab 2 and 3).

In the Introduction, we suggested three hypotheses that might explain how recombination with immigrant donors could accelerate the rate of genetic change at the marker loci, without causing more rapid adaptive evolution. Given that our evolution experiment yielded those outcomes, we performed two additional experiments in order to shed some light on these alternative hypotheses. The first of these additional experiments sought to quantify the extent of recombination in our main experiment, and the second sought to examine the potential for complex selection dynamics to have obscured adaptive evolution in the main experiment.

Estimation of recombination rate

If the rate of recombination was sufficiently high in our evolution experiment, then recombinant genotypes may have reached a high frequency in the treatment populations even in the absence of any selective utility to donor alleles. This outcome might occur by recombination pressure alone or in association with selective sweeps by beneficial mutations that allowed neutral donor alleles to hitchhike to high frequency. To evaluate these hypotheses, one needs an estimate of the extent of gene flow imposed by the recombination treatment. Therefore, we performed two sets of mating experiments under conditions that were similar to those used every fifth day in the recombination treatment.

In the first set of mating experiments, we used each of the four donor strains separately and monitored the accumulation of recombinants that carried the donor's Tet^r gene and the Str^r allele from the ancestral recipient, REL2543. In all four

Table 5. Proportion of recipients that received the Ara$^+$ allele from two Hfr donors during the standard one-day mating protocol. Each assay was replicated three-fold; mean values are shown below. As described in the text, only about 10% of all recombinants are expected to have gotten any particular donor allele; the proportion of recipients that received any donor alleles at all is thus estimated as 10 times the proportion that acquired Ara$^+$.

Donor strain	Population densities (cells ml^{-1})					Final proportion recombinants T/(R + T)
	Initial		Final			
	D	R	D	R	T	
REL288	9.36×10^6	5.27×10^5	1.67×10^6	5.92×10^7	7.07×10^4	1.25×10^{-3}
REL296	6.85×10^6	7.20×10^5	1.53×10^7	5.69×10^7	1.53×10^4	2.88×10^{-4}

D: donors (Ara$^+$ Str^s); R: recipients (Ara$^-$ Str^r); T: recombinants (Ara$^+$ Str^r).

donors, the transposon-encoded Tet^r gene is located 10–25 minutes from the origin of transfer. These experiments showed that two of the donor strains, REL288 and REL296, were responsible for almost all of the gene transfer, yielding Tet^r Str^r recombinants at rates at least 30-fold higher than the other two donors (data not shown). However, the transposon-encoded Tet^r gene also rendered the resulting recombinants auxotrophic and therefore unfit (recall Fig. 2), which complicated the further use of these data for interpreting effective recombination rates in the evolution experiment.

Therefore, in the second set of mating experiments, we monitored the accumulation of recombinants that had acquired the Ara$^+$ allele from the two most prolific donors, REL288 and REL296, and the Str^r allele from the ancestral recipient, REL2543. (We did not use the other two donors because one, REL291, is Ara$^-$ and the other, REL298, has the Ara$^+$ marker located more than 80 minutes from the origin of transfer, whereas our standard mating protocol lasted only 60 minutes.) Previous studies have shown that Ara$^+$ and Ara$^-$ alleles are selectively neutral in glucose-limited medium in the *E. coli* B genetic background (Lenski, 1988; Bennett et al., 1992; Travisano et al., 1995b). Hence, one can estimate the effective rate of recombination without the confounding effect of strong selection against the recombinant genotypes.

Table 5 summarizes the data from this second set of mating experiments. In each case, only a very small fraction of the recipient population acquired the Ara$^+$ allele. However, we must adjust these frequencies to take into account the fact that we scored the transfer of only one particular gene, whereas we are interested in estimating the fraction of recipients that received any donor genes whatsoever. A recent study has shown that, following an Hfr mating, the average segment integrated into the recipient's chromosome is on the order of ten minutes in length, or about 10% of the chromosome (Lloyd and Buckman, 1995). Therefore, one can estimate that about 90% of all recombination events would *not* have included the Ara$^+$ allele. Hence, the proportion of recipients that received any donor genes would be about 10-fold higher than the proportion that acquired the Ara$^+$ allele.

Even for the most prolific donor, REL288, we estimate that only 1.2% of recipients recieved any donor genes in each mating cycle. For the second most prolific donor, REL296, that proportion falls to 0.3%, and it must be $\ll 0.1\%$ for the other two donors. Considering an equal mixture of all four donors, as was added every fifth day to the recombination treatment, we estimate that only about 0.4% of the recipients received any donor genes during each cycle. In the Discussion, we consider whether this effective rate of recombination might be sufficient to explain the observed rate of change at the marker loci without the need to invoke any selective advantage for recombinant genotypes.

Evidence for complex selection dynamics

In the experiments summarized in Table 4, we always measured the mean fitness of the derived populations relative to a common competitor (the ancestral genotype for one pair of populations). These data gave no indication that the recombination treatment populations had improved to any greater extent than the asexual control populations, despite the many donor alleles that were subsitituted in the treatment populations. However, this method of fitness estimation, by using a common competitor as a yardstick, implicitly assumes that the dynamics of natural selection are frequency-independent and transitive. That is, it assumes that derived genotypes can be ranked relative to one another based on their fitnesses relative to a single competitor.

Evaluating the potential for frequency-dependent effects and nontransitive interactions in our study is challenging, given the large number of replicate populations and distinct genotypes in this study. Therefore, we focused our attention on recombination treatment population Ara$^-$3. After 1000 generations, the fitness of this population appeared to be *below* that of its ancestor (Tab. 4), suggesting that nontransitive interactions might have been involved. Using genotypes from the samples stored at 100-generation intervals, we performed additional competition experiments to look explicitly for nontransitivity. These experiments yielded results consistent with complex selection dynamics, although they did not provide a clear case of nontransitivity. As shown in Figure 4, the dominant genotype at generation 500 had a large *disadvantage* relative to the usual common competitor (an Ara$^+$ mutant of the ancestor for population Ara$^-$1). The same genotype had no disadvantage relative to an Ara$^+$ mutant of its own direct ancestor. The difference in the fitness of this recombinant genotype relative to these two different ancestral competitors is quite large (about 20%) and highly significant ($t_s = 3.612$, $df = 8$, $p = 0.0069$). And yet the two ancestors had virtually identical fitnesses relative to one another, regardless of which one was marked by the Ara$^+$ mutation. Thus, fitnesses in the Ara$^-$3 treatment population may have been significantly underestimated by using the common competitor that we did. Evidently, the fitnesses measured relative to a common competitor were insufficient to capture fully the extent of evolutionary adaptation, at least in this treatment population.

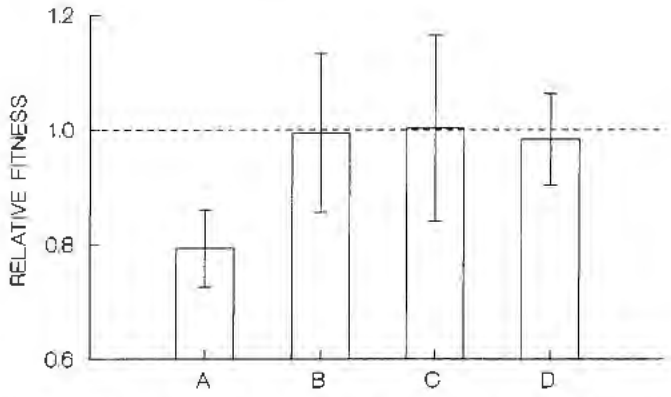

Fig. 4. Complex selection dynamics revealed by pairwise interactions among genotypes. A-3R-500-1 was the dominant genotype in recombination treatment population Ara$^-$3 at generation 500. A-3 is its ancestral genotype, and A-3/+ is identical except for the Ara$^+$ marker. A-1 is the ancestral genotype for another population, and A-1/+ is identical except for the Ara$^+$ marker; A-1 and A-1/+ served as common competitors for the fitness assays summarized in Table 4. (A) A-3R-500-1 is less fit than the common competitor, A-1/+. (B) A-3R-500-1 is of equal fitness to its own ancestor, A-3/+. However, the common competitor and the ancestor are equally fit in direct competition with one another, regardless of the Ara markers: (C) A-1 versus A-3/+; (D) A-3 versus A-1/+. Error bars indicate 95% confidence intervals, based on five-fold replication of each pairwise competition.

Discussion

We sought to examine the effect of sexual recombination on the rate of evolution in a bacterial model system. The populations of *E. coli* that we studied had previously evolved under the same environmental regime for 7000 generations. Twelve populations had been founded by clones of an asexual strain, so that the populations depended entirely on mutation as a source of genetic variation for adaptation by natural selection. Over time, the rate of adaptive evolution in these populations slowed considerably from an initially rapid pace, based on changes in mean fitness (Fig. 1). In this study, we established two new sets of twelve populations each; one set served as controls while the other set underwent sexual recombination. The control populations were propagated for another 1000 generations in the same environment as their ancestors, and they continued to depend on mutation as their sole source of genetic variability. The treatment populations were similarly propagated for 1000 generations in the same environment, but they were also periodically subjected to matings with an immigrant pool of Hfr (high frequency recombination) donors. These donors were genetically distinct from the recipient populations, and the donors themselves could not grow in the experimental environment. However, the donors were able to transfer genes to the resident populations by conjugation. The resulting recombination provided the resident populations with an additional source of variation that might allow them to evolve and adapt more quickly.

Our study can be summarized by two major results. First, the recombination treatment dramatically accelerated the rate of genetic change (Fig. 3, Tab. 2). After 1000 generations, ten individuals from each population were scored at 14 marker loci. On average, the twelve treatment populations contained about six distinct genotypes, with the ancestral genotype representing only about 15% of the total (Tab. 3). But in the twelve control populations, every individual still had the ancestral marker for all 14 loci. Second, the recombination treatment had no measurable effect on the rate of adaptive evolution, based on changes in mean fitness relative to a common competitor. After 1000 generations, the asexual control populations had improved, on average, by a few percent relative to their ancestors; the treatment populations improved by an amount that was statistically indistinguishable (Tab. 4). Thus, the much faster rate of genetic change brought on by sexual recombination did not produce any net advantage with respect to the evolution of mean fitness.

In the following sections, we consider the plausibility of three hypotheses that might reconcile these two outcomes. These alternative hypotheses are not mutually exclusive, and so some combination of the hypothesized effects may explain the experimental results. According to one hypothesis, the recombination treatment was, in some sense, too effective. That is, rather than merely providing an additional source of potentially useful variation, the level of gene flow might have been so high that the recipient populations were faced with a deluge of alleles from the immigrant donors. These donor alleles increased to high frequency by recombination pressure, without the need to invoke any selective benefit favoring the recombinant genotypes. The second hypothesis is a variant of the first hypothesis, insofar as they both assume that the recombinant genotypes do not have any direct selective advantage. But while the first hypothesis presumes that recurrent bouts of recombination alone may drive recombinant genotypes to high frequency, the second hypothesis postulates that donor alleles may have hitchhiked to high frequency in association with beneficial mutations. Under this hypothesis, beneficial mutations are presumed to occur at equal rates in treatment and control populations, but their ascent to high frequency is marked by donor alleles that can be readily scored as recombinant genotypes only in the treatment populations. Thus, certain donor alleles rise to high frequency because, by chance, they have become physically linked to a beneficial mutation, even though the donor alleles themselves are not advantageous. According to the third hypothesis, complex interactions among genotypes might render the estimates of fitness, which were obtained relative to a common competitor, inadequate for evaluating the rate and extent of evolutionary adaptation. For example, if competitive interactions are frequency-dependent or nontransitive, then more rapid adaptive evolution may not lead to higher fitness values relative to an arbitrary competitor.

Recombination pressure

The first hypothesis is that the rate of sexual recombination was so high that donor alleles were fixed in the recipient population, even in the absence of any

selective utility. The fact that, every fifth day, the treatment populations were subject to several donors for every recipient might suggest extreme recombination pressure. However, bacterial conjugation is much more patchy than genetic exchange due to meiosis and fertilization, and it occurs at much lower rates than obligate out-crossing.

To evaluate this hypothesis, we performed mating experiments to estimate the proportion of the resident population that received one or more genes (anywhere in the chromosome) from the Hfr donors during each recombination treatment cycle. Even for the most prolific donor, only about 1% of the resident population received any donor genes per treatment cycle (Tab. 5); and considering that this donor was mixed with three less prolific strains, we estimate that only about 0.4% of the recipients underwent recombination per treatment cycle. Assuming that the recombination events are effectively neutral, then the fraction of residents whose progenitors never received any donor genes, p_0, should decay in an exponential fashion: $p_0 = e^{-ct}$, where $c \cong 0.004$ per mating cycle and t is time expressed in mating cycles. At the end of the experiment (30 mating cycles = 150 days = 1000 generations), the expected proportion of cells receiving at least one donor gene, $1 - p_0$, should have been only about 11% according to this hypothesis. To the extent that some recombinant genotypes were clearly at a selective disadvantage (e.g., those that were auxotrophic) and others might not have received any of the 14 loci that were screened, then we would expect to detect somewhat fewer that 11% recombinants. In fact, however, we found that 85% (102/120) of the clones sampled at the end of the experiment from the treatment populations were recombinant genotypes, and in only one of the twelve populations was this fraction less than 60% (Tab. 3).

Two other lines of evidence concern the dynamics and pattern of genotype replacement. The hypothesis of recombination pressure predicts a more or less constant rate of decay of the ancestral genotype. Also, given the very large population sizes in our experiment ($> 10^6$ cells even during the bottleneck at serial transfer), we would not expect any particular recombinant genotype to reach high frequency during the replacement of the ancestral type; we expect instead that the ancestral type would be replaced by a diverse mixture of recombinant genotypes. But as shown clearly by Figure 3 for one population, the ancestral genotype was replaced quite suddenly between generations 500 and 700 (not gradually) and by only one class of recombinant genotypes (not a diverse mixture). Even more rapid replacements were observed in some other treatment populations (data not shown).

From these analyses, it appears that recombination pressure alone could not have caused the very high frequency of recombinant genotypes that we saw during the evolution experiment. Nonetheless, recombination pressure might have contributed somewhat to the replacement of the ancestral resident by recombinant genotypes that carried alleles from the Hfr donors.

Hitchhiking

The second hypothesis is that alleles from the Hfr donors hitchhiked to high frequency along with beneficial mutations that occurred in the resident genetic

background. Under this hypothesis, substitutions of beneficial mutations are presumed to have occurred with equal frequency in the treatment and control populations, but the ascent of these beneficial mutations to high frequency was marked by alleles that we could screen only in the treatment populations.

This explanation is consistent with the finding that mean fitness increased to a similar degree in the asexual control and recombination treatment populations (Tab. 4). It is also consistent with the dynamics of genotype replacement in the recombination treatment populations (Fig. 3). That is, the hitchhiking hypothesis predicts that the ancestral genotype is replaced suddenly (not gradually) as a rare beneficial mutation sweeps through the population by natural selection (Lenski and Travisano, 1994; Elena et al., 1996); and it predicts that the ancestral type will be replaced by one recombinant genotype (not a diverse mixture) that, by chance, became physically associated with the beneficial mutation. This hitchhiking hypothesis therefore avoids certain problems with the first hypothesis, which relied solely on recombination pressure.

However, the hitchhiking hypothesis is ultimately a special case of genetic drift, in that it presumes that the donor alleles themselves do not confer any selective advantage. As such, the substitution rate of donor alleles depends only on their rate of recombination into the resident population and not on the effective population size (Kimura, 1983), which becomes much smaller due to selective sweeps that purge genetic variation (Levin, 1981). This conclusion obtains because selection for a beneficial mutation is far more likely to eliminate a rare, neutral allele than it is to carry that allele to high frequency in the population. Therefore, the hitchiking hypothesis can better explain the temporal dynamics by which a recombinant genotype achieves high frequency in a *particular* population than could the hypothesis of recombination pressure; but hitchhiking does not provide any better explanation for the unexpectedly high *overall* frequency of recombinant genotypes.

Complex selection dynamics

A third hypothesis to explain the proliferation of recombinant genotypes, without any *apparant* advantage in mean fitness, is that the method used to measure fitness was inadequate due to frequency-dependent selection or other complex ecological interactions. In particular, we measured the fitnesses of the derived populations relative to a common competitor (the ancestral genotype for one pair of populations). These data gave no indication that the recombination treatment populations had improved to any greater extent than the asexual control populations (Tab. 4), despite the many donor alleles present in the treatment populations. However, this method of fitness estimation, by using a common competitor as a yardstick, implicitly assumes that the dynamics of natural selection are frequency-independent and transitive. That is, it assumes that genotypes can be ranked relative to one another based on their fitnesses relative to a single competitor.

In previous experiments using the asexual progenitors of the populations in this study, this assumption was tested in two different ways; in both respects, it seemed

to provide an adequate description of the selection dynamics. First, fitnesses measured relative to the common ancestor increased monotonically in these experi-mental populations (Lenski et al., 1991; Lenski and Travisano, 1994). Second, the magnitude of one derived genotype's advantage relative to another could be accurately predicted from each one's advantage relative to the ancestor (Lenski et al., 1991; Travisano et al., 1995b). However, complex selection dynamics have been demonstrated in several other ecological and evolutionary experiments with microorganisms (Levin, 1972; Chao and Levin, 1981; Paquin and Adams, 1983; Rosenzweig et al., 1994), and it is possible that similar complexities may have arisen in the present experiment.

To examine this possibility, we performed additional competition experiments using genotypes that were isolated from one of the recombination treatment populations. We found evidence that the extent of adaptive evolution had been underestimated in this population as a consequence of using a particular competitor to estimate relative fitness (Fig. 4). Elsewhere, we describe a very strong frequency-dependent interaction between two other genotypes that were also sampled from this same population (Turner et al., 1996). Thus, in this one recombination treatment population, we discovered two interactions (involving two different sets of genotypes) that violated the assumption that the extent of adaptive evolution could be expressed relative to a single yardstick.

Evidently, some of the recombinant genotypes had a selective advantage only in the context of the particular milieu of genotypes in which they arose. Why were violations of this assumption seen in the present study, but not in earlier studies with the progenitor populations? One possibility is that the earlier studies lacked the resolution to see these effects. Relatively small deviations caused by such interactions might have gone undetected in the earlier studies, where fitness differences were usually on the order of 10% or more (Lenski et al., 1991; Lenski and Travisano, 1994; Travisano et al., 1995b). By contrast, in this study, most fitness differences were on the order of only a few percent (Tab. 4), so that subtle effects might have been relatively more important. Alternatively, complex selection dynamics might occur more often among very dissimilar genotypes, such as those created by repeated recombination of alleles from *E. coli* K12 donors and *E. coli* B recipients. It is intriguing in this respect that Levin (1972) described a case of frequency-dependent selection between K12 and B genotypes under culture conditions similar to those in our study.

Acknowledgements

We thank A. Bennett, G. Bush, F. Cohan, L. Eguiarte, L. Ekunwe, D. Fulbright, K. Holsinger, A. Jarosz, P. Johnson, J. Mittler, J. Mongold, R. Redfield, and S. Simpson for valuable discussions, comments on our manuscript, assistance in the laboratory, and logistical support. V. S. was supported by a fellowship from the Universidad Nacional Autónoma de México. P. E. T. and R. E. L. were supported by the U. S. National Science Foundation (DEB-9120006 and DEB-9421237).

References

Atwood, K. C., L. Schneider and F. J. Ryan. 1951. Selective mechanisms in bacteria. Cold Spring Harbor Symp. Quant. Biol. 16: 345–355.

Bachmann, B. J. 1987. Linkage map of *Escherichia coli* K-12, edition 7, pp 807–876. *In* F. C. Neidhardt (Ed.), *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology. American Society for Microbiology, Washington, D.C., USA.

Bennett, A. F, R. E. Lenski and J. E. Mittler. 1992. Evolutionary adaptation to temperature. I. Fitness responses of *Escherichia coli* to changes in its thermal environment. Evolution 46: 16–30.

Bisêrcîc, M., J. Y. Feutrier and P. R. Reeves. 1991. Nucleotide sequences of the *gnd* genes from nine natural isolates of *Escherichia coli*: evidence of intragenic recombination as a contributing factor in the evolution of the polymorphic *gnd* locus. J. Bacteriol. 173: 3894–3900.

Carlton, B. C. and B. J. Brown. 1981. Gene mutation, pp 222–242. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg and G. B. Philips (Eds.), Manual of Methods for General Bacteriology. American Society for Microbiology, Washington, D.C., USA.

Caugant, D. A., B. R. Levin and R. K. Selander. 1981. Genetic diversity and temporal variation in an *E. coli* population of a human host. Genetics 98: 467–490.

Chao, L and B. R. Levin. 1981. Structured habitats and the evolution of anticompetitor toxins in bacteria. Proc. Natl. Acad. Sci. USA 78: 6324–6328.

Cohan, F. M. 1994a. Genetic exchange and evolutionary divergence in prokaryotes. Trends Ecol. Evol. 9: 175–180.

Cohan, F. M. 1994b. The effects of rare but promiscuous genetic exchange on evolutionary divergence in prokaryotes. Am. Nat. 143: 965–986.

Cohan, F. M., E. C. King and P. Zawadzki. 1994. Amelioration of the deleterious pleiotropic effects of an adaptive mutation in *Bacillus subtilis*. Evolution 48: 81–95.

Crosa, J. H., M. E. Tolmasky, L. A. Actis and S. Falkow. 1994. Plasmids, pp 365–386. *In* P. Gerhardt, R. G. E. Murray, W. A. Wood and N. R. Krieg (Eds.), Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington, D.C., USA.

Crow, J. F. and M. Kimura. 1970. An Introduction to Population Genetic Theory. Harper & Row, New York, USA.

Dykhuizen, D. E. 1990. Experimental studies of natural selection in bacteria. Ann. Rev. Ecol. Syst. 21: 373–398.

Dykhuizen, D. E. and L. Green. 1991. Recombination in *Escherichia coli* and the definition of biological species. J. Bacteriol. 22: 7257–7268.

Dykhuizen, D. E. and D. L. Hartl. 1983. Selection in chemostats. Microbiol. Rev. 47: 150–168.

Eckhardt, T. 1978. A rapid method for the identification of plasmid deoxyribonucleic acid in bacteria. Plasmid 1: 584–588.

Elena, S. F., V. S. Cooper and R. E. Lenski. 1996. Punctuated evolution caused by selection of rare beneficial mutations. Science (Washington, D.C.) 272: 1802–1804.

Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Clarendon, Oxford, U.K.

Ford, E. B. 1975. Ecological Genetics, 4th ed. Chapman and Hall, London, U.K.

Frank, S. A. and M. Slatkin. 1992. Fisher's fundamental theorem of natural selection. Trends Ecol. Evol. 7: 92–95.

Graham, J. B and C. A. Istock. 1979. Gene exchange and natural selection cause *Bacillus subtilis* to evolve in soil culture. Science (Washington, D.C.) 204: 637–639.

Graham, J. B. and C. A. Istock. 1981. Parasexuality and microevolution in experimental populations of *Bacillus subtilis*. Evolution 35: 954–963.

Guttman, D. S. and D. E. Dykhuizen. 1994a. Clonal divergence in *Escherichia coli* as a result of recombination, not mutation. Science (Washington, D.C.) 266: 1380–1383.

Guttman, D. S. and D. E. Dykhuizen. 1994b. Detecting selective sweeps in naturally occurring *Escherichia coli*. Genetics 138: 993–1003.

Helling, R. B., C. N. Vargas and J. Adams. 1987. Evolution of *Escherichia coli* during growth in a constant environment. Genetics 116: 349–358.

Hopwood, D. A. and K. F. Chater (Eds.). 1989. Genetics of Bacterial Diversity. Academic Press, London, U.K.

Istock, C. A., K. E. Duncan, N. Ferguson and X. Zhou. 1992. Sexuality in a natural population of bacteria--*Bacillus subtilis* challenges the clonal paradigm. Mol. Ecol. 1: 95–103.

Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge, U.K.

Lenski, R. E. 1988. Experimental studies of pleiotropy and epistasis in *Escherichia coli*. I. Variation in competitive fitness among mutants resistant to virus T4. Evolution 42: 425–432.

Lenski, R. E. 1992. Experimental evolution, pp 125–140. *In* J. Lederberg (Ed.), Encyclopedia of Microbiology, vol. 2. Academic Press, San Diego, USA.

Lenski, R. E. 1993. Assessing the genetic structure of microbial populations. Proc. Natl. Acad. Sci. USA 90: 4334–4336.

Lenski, R. E. and M. Travisano. 1994. Dynamics of adaptation and diversification: a 10000-generation experiment with bacterial populations. Proc. Natl. Acad. Sci. USA 91: 6808–6814.

Lenski, R. E., M. R. Rose, S. C. Simpson and S. C. Tadler. 1991. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2000 generations. Am. Nat. 138: 1315–1341.

Levin, B. R. 1972. Coexistence of two strains on a single resource. Science (Washington, D.C.) 175: 1272–1274.

Levin, B. R. 1981. Periodic selection, infectious gene exchange and the genetic structure of *E. coli* populations. Genetics 99: 1–23.

Levin, B. R. 1988. The evolution of sex in bacteria, pp 194–211. *In* R. E. Michod and B. R. Levin (Eds.), The Evolution of Sex. Sinauer Associates, Sunderland, Mass, USA.

Levin, B. R., F. M. Stewart and L. Chao. 1977. Resource-limited growth, competition, and predation: a model and experimental studies with bacteria and bacteriophage. Am. Nat. 111: 3–24.

Lloyd, R. G. and C. Buckman. 1995. Conjugational recombination in *Escherichia coli*: genetic analysis of recombinant formation in Hfr $\times$ F^- crosses. Genetics 139: 1123–1148.

Luria, S. E. and M. Delbrück. 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics 28: 491–511.

Maynard Smith, J. 1990. The evolution of prokaryotes: does sex matter? Ann. Rev. Ecol. Syst. 21: 1–12

Maynard Smith, J., G. Dowson and B. G. Spratt. 1991. Localized sex in bacteria. Nature (London) 349: 29–31.

Maynard Smith, J., N. H. Smith, M. O'Rourke and B. G. Spratt. 1993. How clonal are bacteria? Proc. Natl. Acad. Sci. USA 90: 4384–4388.

McNeilly, T. 1968. Evolution in closely adjacent plant populations. III. *Agrostis tenuis* on a small copper mine. Heredity 23: 99–108.

Milkman, R. and M. McKane-Bridges. 1990. Molecular evolution of the *Escherichia coli* chromosome. III. Clonal frames. Genetics 126: 505–517.

Muller, H. J. 1932. Some genetic aspects of sex. Am. Nat. 66: 118–138.

Paquin, C. and J. Adams. 1983. Relative fitness decreases in evolving populations of *S. cerevisiae*. Nature (London) 306: 368–371.

Piñero, D., E. Martinez and R. K. Selander. 1988. Genetic diversity and relationships among isolates of *Rhizobium leguminosarum* biovar *phaseoli*. Appl. Environ. Microbiol. 54: 2825–2832.

Quinto, C., H. de la Vega, M. Flores, L. Fernandez, T. Ballado, G. Soberon and R. Palacios. 1982. Reiteration of nitrogen fixation gene sequences in *Rhizobium phaseoli*. Nature (London) 299: 724–728.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43: 223–225.

Roberts, M. S. and F. M. Cohan. 1995. Recombination and migration rates in natural populations of *Bacillus subtilis* and *Bacillus mojavensis*. Evolution 49: 1081–1094.

Rosenzweig, R. F., R. R. Sharp, D. S. Treves and J. Adams. 1994. Microbial evolution in a simple unstructured environment: genetic differentiation in *Escherichia coli*. Genetics 137: 903–917.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Selander, R. K. and B. R. Levin. 1980. Genetic diversity and structure of *Escherichia coli* populations. Science (Washington, D.C.) 210: 545–547.

Souza V., T. T. Nguyen, R. R. Hudson, D. Piñero and R. E. Lenski. 1992. Hierarchical analysis of linkage disequilibrium in *Rhizobium* populations: evidence for sex? Proc. Natl. Acad. Sci. USA 89: 8389–8393.

Souza, V., L. Eguiarte, G. Avila, R. Cappello, C. Gallardo, J. Montoya and D. Piñero. 1994. Genetic structure of *Rhizobium etli* biovar *phaseoli* associated with wild and cultivated bean plants (*Phaseolus vulgaris* and *Phaseolus coccineus*) in Morelos, Mexico. Appl. Environ. Microbiol. 60: 1260–1268.

Travisano, M., J. A. Mongold, A. F. Bennett and R. E. Lenski. 1995a. Experimental tests of the roles of adaptation, chance, and history in evolution. Science (Washington, D.C.) 267: 87–90.

Travisano, M., F. Vasi and R. E. Lenski. 1995b. Long-term experimental evolution in *Escherichia coli*. III. Variation among replicate populations in correlated responses to novel environments. Evolution 49: 189–200.

Turner, P. E., V. Souza and R. E. Lenski. 1996. Tests of ecological mechanisms promoting the stable coexistence of two bacterial genotypes. Ecology 77: 2119–2129.

Wanner, B. L. 1986. Novel regulatory mutants of the phosphate regulon in *Escherichia coli* K12. J. Mol. Biol. 191: 39–58.

Whittam, T. S., H. O. Ochman and R. K. Selander. 1983. Multilocus genetic structure in natural populations of *Escherichia coli*. Proc. Natl. Acad. Sci. USA 80: 1751–1755.

Received 17 September 1996;
revised 4 November 1996;
accepted 7 January 1997.

Appendix.

Genotypes of ten clones from each recombination treatment population at generation 1000.

		Physiological loci[a]						Electrophoretic loci[a]					Genotype frequency
Population[b]	Genotype[c]	Ara	Lac	Tet	Str	T1X	T6	IDH	6-PGD	ADH	MPI	PEP	
Ara⁻1	Rec 1	−	+	*s*	*s*	*r*	*s*	12	2	2	2	1	0.4
	Rec 2	−	+	*s*	*s*	*r*	*s*	12	2	2	2	2	0.1
	Rec 3	−	−	*s*	*s*	*r*	*s*	2	2	2	2	1	0.1
	Rec 4	−	−	*s*	*s*	*r*	*s*	2	2	1	2	1	0.1
	Rec 5	−	−	*s*	*s*	*r*	*s*	1	2	2	2	1	0.3
Ara⁻2	Ancestor	−	+	*s*	*r*	*s*	*r*	1	2	2	2	1	0.2
	Rec 1	−	+	*s*	*r*	*s*	*r*	12	2	2	2	1	0.2
	Rec 2	−	+	*s*	*r*	*s*	*r*	1	2	2	2	12	0.3
	Rec 3	−	+	*s*	*r*	*s*	*r*	12	2	1	2	1	0.1
	Rec 4	+	+	*s*	*r*	*s*	*r*	1	2	1	2	2	0.1
	Rec 5	−	+	*s*	*r*	*s*	*r*	2	2	2	2	2	0.1
Ara⁻3	Rec 1	+	−	*r*	*s*	*r*	*s*	1	2	2	2	1	0.1
	Rec 2	+	−	*r*	*s*	*r*	*s*	2	2	12	2	2	0.3
	Rec 3	+	−	*r*	*s*	*r*	*s*	2	2	2	2	1	0.3
	Rec 4	−	+	*r*	*r*	*s*	*r*	2	2	1	2	2	0.1
	Rec 5	+	+	*r*	*s*	*s*	*r*	2	2	2	2	1	0.1
	Rec 6	+	+	*r*	*s*	*s*	*r*	1	2	1	2	2	0.1
Ara⁻4	Ancestor	−	+	*s*	*r*	*s*	*r*	1	2	2	2	1	0.8
	Rec 1	−	+	*s*	*r*	*s*	*r*	12	2	2	2	1	0.2
Ara⁻5	Rec 1	−	+	*s*	*s*	*s*	*r*	12	2	2	2	2	0.3
	Rec 2	+	+	*s*	*s*	*s*	*s*	12	2	1	2	1	0.1
	Rec 3	+	+	*s*	*s*	*s*	*s*	1	2	2	2	1	0.1
	Rec 4	+	+	*s*	*s*	*s*	*s*	2	2	2	2	12	0.1
	Rec 5	+	+	*s*	*s*	*s*	*s*	12	2	2	2	1	0.4

Appendix. (continued)

Population[b]	Genotype[c]	Physiological loci[a]						Electrophoretic loci[a]					Genotype frequency
		Ara	Lac	Tet	Str	T1X	T6	IDH	6-PGD	ADH	MPI	PEP	
Ara−6	Rec 1	−	−	*s*	*r*	*r*	*s*	12	2	2	2	1	0.3
	Rec 2	−	+	*s*	*s*	*r*	*r*	12	2	2	2	1	0.2
	Rec 3	−	+	*s*	*r*	*s*	*r*	12	2	2	2	1	0.1
	Rec 4	−	+	*s*	*r*	*s*	*r*	12	2	12	2	1	0.2
	Rec 5	−	+	*s*	*r*	*s*	*r*	2	2	2	2	1	0.1
	Rec 6	−	+	*s*	*r*	*s*	*r*	2	2	1	2	2	0.1
Ara+1	Rec 1	−	−	*s*	*r*	*s*	*r*	1	2	2	2	2	0.1
	Rec 2	+	+	*s*	*r*	*s*	*r*	2	2	2	2	2	0.5
	Rec 3	+	+	*s*	*r*	*s*	*r*	12	2	1	2	12	0.1
	Rec 4	+	+	*s*	*r*	*s*	*r*	12	2	2	2	12	0.2
	Rec 5	+	+	*s*	*r*	*s*	*r*	2	2	1	2	1	0.1
Ara+2	Ancestor	+	+	*s*	*r*	*s*	*r*	1	2	2	2	1	0.1
	Rec 1	+	−	*s*	*r*	*s*	*r*	12	2	2	2	2	0.1
	Rec 2	+	+	*s*	*r*	*s*	*r*	1	2	1	2	1	0.1
	Rec 3	+	+	*s*	*r*	*s*	*r*	2	2	1	2	12	0.1
	Rec 4	+	+	*s*	*r*	*s*	*r*	2	2	2	2	1	0.2
	Rec 5	+	+	*s*	*r*	*s*	*r*	2	2	12	2	12	0.2
	Rec 6	+	+	*s*	*r*	*s*	*r*	1	2	12	2	2	0.1
	Rec 7	+	+	*s*	*r*	*s*	*r*	2	2	2	2	2	0.1
Ara+3	Ancestor	+	+	*s*	*r*	*s*	*r*	1	2	2	2	1	0.1
	Rec 1	+	+	*s*	*r*	*s*	*r*	12	2	1	2	12	0.1
	Rec 2	+	+	*s*	*r*	*s*	*r*	2	2	2	2	1	0.1
	Rec 3	+	+	*s*	*r*	*s*	*r*	12	2	12	2	12	0.1
	Rec 4	+	−	*s*	*r*	*s*	*r*	1	2	12	2	12	0.1
	Rec 5	+	−	*s*	*r*	*s*	*r*	1	2	2	2	1	0.1
	Rec 6	+	−	*s*	*r*	*r*	*r*	1	2	2	2	1	0.1
	Rec 7	+	+	*s*	*r*	*s*	*r*	12	2	1	2	1	0.1
	Rec 8	+	+	*s*	*r*	*s*	*r*	12	2	2	2	1	0.2

Appendix. (continued)

Population[b]	Genotype[c]	Physiological loci[a]						Electrophoretic loci[a]					Genotype frequency
		Ara	Lac	Tet	Str	T1X	T6	IDH	6-PGD	ADH	MPI	PEP	
Ara$^+$4	Ancestor	+	+	*s*	*r*	*s*	*r*	1	2	2	2	1	0.4
	Rec 1	+	−	*s*	*r*	*r*	*r*	2	2	2	2	2	0.1
	Rec 2	+	−	*s*	*r*	*r*	*r*	1	2	2	2	2	0.1
	Rec 3	+	+	*s*	*r*	*s*	*r*	12	2	2	2	2	0.1
	Rec 4	+	+	*s*	*r*	*s*	*r*	1	2	1	2	12	0.1
	Rec 5	+	+	*s*	*r*	*s*	*s*	1	2	2	2	2	0.1
	Rec 6	+	+	*s*	*r*	*s*	*s*	1	2	12	2	12	0.1
Ara$^+$5	Rec 1	+	−	*s*	*r*	*r*	*r*	12	2	2	2	2	0.1
	Rec 2	+	−	*s*	*r*	*r*	*r*	12	2	12	2	2	0.1
	Rec 3	+	+	*s*	*r*	*s*	*r*	2	2	2	2	12	0.4
	Rec 4	+	+	*s*	*r*	*s*	*r*	1	2	1	2	1	0.1
	Rec 5	+	+	*s*	*s*	*s*	*r*	1	2	2	2	2	0.1
	Rec 6	+	+	*s*	*s*	*s*	*r*	2	2	2	2	12	0.1
	Rec 7	+	+	*s*	*r*	*s*	*r*	12	2	2	2	1	0.1
Ara$^+$6	Ancestor	+	+	*s*	*r*	*s*	*r*	1	2	2	2	1	0.2
	Rec 1	+	−	*s*	*r*	*s*	*r*	2	2	12	2	12	0.1
	Rec 2	+	−	*s*	*r*	*s*	*r*	12	2	2	2	1	0.1
	Rec 3	+	+	*s*	*r*	*s*	*r*	12	2	12	2	1	0.2
	Rec 4	+	+	*s*	*r*	*s*	*r*	12	2	1	2	12	0.2
	Rec 5	+	+	*s*	*r*	*s*	*r*	2	2	2	2	1	0.1
	Rec 6	+	+	*s*	*r*	*s*	*r*	2	2	12	2	2	0.1

[a] See footnotes to Table 1 for a description of physiological and electrophoretic loci. All genotypes were also Arg$^+$, Leu$^+$, and Ilv$^+$.

[b] Ara$^-$1 through Ara$^-$6 and Ara$^+$1 through Ara$^+$6 are the twelve recombination treatment populations originally composed of Ara$^-$ and Ara$^+$ recipient strains, respectively.

[c] Ancestor denotes a genotype indistinguishable from the original recipient strain. Rec denotes a recombinant genotype with one or more alleles from both donor and recipient strains. Note that some recombinant genotypes are merodiploids for certain electrophoretic loci, having both donor and recipient alleles.