

Bacterial evolution: Jittery genomes

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Recent studies of long-term experimental populations of bacteria have revealed the actual progression of evolutionary change and how rates of phenotypic evolution can be decoupled from rates of genomic evolution.

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A decade ago, Lenski and collaborators [1] began propagating a laboratory strain of the bacterium *Escherichia coli* and, by the time that this article is published, these populations will have quietly surpassed 25,000 generations. Although bacteria, as a group, have been propagating for well over three billion years, they leave virtually no fossil record and evolutionary biologists are resigned to examining the end-products — and deducing the intermediate stages — of this process. But for these long-term populations of *E. coli*, samples were stored every 500 generations, yielding a literally frozen fossil history of the lineages.

Several features make this system an extraordinary resource for examining the process of evolution. Unlike traditional fossils, we are certain that the stored samples of *E. coli* represent an uninterrupted chain between the initial and the present-day populations; and, in addition, bacteria from each stage can be revived and analyzed for virtually any trait. Researchers have already uncovered substantial changes in fitness [2], mutation rates [3], cell size [4] and chromosome structure [5] in the evolved populations; and, by examining these experimental populations over the course of 10,000 generations, Papadopoulos *et al.* [6] have recently obtained insights into the relationship between rates of genomic versus phenotypic evolution.

What is the expected relationship between genotypic and phenotypic change? One might anticipate that neutral genetic changes — those having little or no effect on cell fitness — would show a relatively constant rate of accumulation over time. Predicting rates of phenotypic evolution is much more difficult, however. Evidence from the fossil record, and from natural and experimental populations, has shown that, for certain characters, rates of change are slow and incremental, whereas for others, transformations are quite rapid and are followed by relatively long periods of stasis.

In the case of these evolved populations of *E. coli*, Papadopoulos *et al.* [6] indexed genomic evolution by insertion-sequence (IS) fingerprinting, a technique that uses the positions and abundance of seven small mobile genetic elements (insertion sequences) to establish the genealogical and genetic relationships among individual bacterial clones. IS fingerprints were determined for the ancestral strain, and for seven to twenty clones from two independently evolved populations, each sampled after 500, 1,000, 1,500, 2,000, 5,000, 8,000 and 10,000 generations. In both populations, most clones had the same IS fingerprint as the ancestral strain until generation 2,000; surprisingly, this fingerprint (clone) was never observed in any of the later generations. Other clones arose during the initial 2,000 generations; some became established transiently in the population, but none left descendants that persisted more than 500 generations. Even during the daily serial transfer phase of these experiments, the population sizes never dipped below 10^6 cells, so the observed rise and loss of established lineages were not likely to be due to chance, but to competition between clones harboring different beneficial mutations.

The successive elimination of clones by those harboring new mutations produces a phylogenetic tree with one main trunk, and in which all side branches eventually die out. Along this trunk, there are several 'pivotal' genotypes, which are ancestral to all subsequent clones. These pivotal genotypes are likely to contain beneficial mutations, and the IS fingerprints might serve as a good starting point for recovering new mutations associated with these clones. Although IS elements encode only those genes necessary for their own mobility, transposition events potentially can affect host fitness by disrupting or modulating the expression of a particular sequence. But because these bacteria are strictly asexual, a change in the fitness could be due to IS translocation itself, or to the occurrence of a beneficial mutation anywhere else in the chromosome.

The seven IS elements that constitute the IS fingerprints used as clonal markers by Papadopoulos *et al.* [6] displayed varying levels of stability over the course of the experiment. The ancestral, and all subsequent, strains maintained single copies of IS2, IS4 and IS30 in the same genomic locations. In contrast, the copy numbers and positions of IS150 were highly variable within and between populations; and, overall, there was about a threefold difference in the rate of genotypic evolution, as measured by the mobility of IS elements, between the replicate populations.

The rates of genomic evolution in these long-term experimental populations contrast sharply with the rates of

Table 1

Rates of phenotypic and genomic evolution in long-term experimental populations.

	Initial phase (0–2,000 generations)	Later phase (2,000–10,000 generations)	Ratio of initial/later
Fitness			
Population I	1.16×10^{-4}	2.65×10^{-5}	4.38
Population II	1.10×10^{-4}	3.20×10^{-5}	3.44
Cell size			
Population I	1.15×10^{-4}	8.75×10^{-6}	13.14
Population II	1.03×10^{-4}	1.56×10^{-5}	6.56
IS fingerprint			
Population I	1.88×10^{-4}	1.16×10^{-3}	0.16
Population II	2.15×10^{-3}	2.63×10^{-3}	0.82

Rates are expressed per generation over the elapsed time; therefore, a value > 1 in the last column indicates that evolutionary change has decelerated as the experiment proceeded, and a value < 1 indicates an acceleration. Populations I and II differ only with respect to a neutral phenotypic marker. (For additional details see [6].)

phenotypic evolution, as previously estimated from changes in cell size and fitness [2,4]. For example, after 10,000 generations, the replicate populations, which differed significantly in their rates of genomic evolution, displayed very similar gains in fitness relative to the ancestral strain. But the real differences are observed in the manner in which the phenotypic and genotypic changes accumulate through time (Table 1).

Fitness and cell size were seen to change quickly during the first 2,000 generations, but very little in subsequent generations. In contrast, there is no deceleration in the rates of genotypic evolution; and, in fact, it appears that the opposite trend might occur, as the clones with the ancestral IS fingerprint persist for the first 2,000 generations despite extensive changes in phenotype. Papadopoulos *et al.* [6] suggest that the rapid phenotypic evolution during the early stages of the experiment (and the consecutive takeover by successively fitter strains) would purge much of the existing variation — a process known as ‘periodic selection’ — and that the subsequent decrease in adaptive evolution may, in fact, promote the accumulation of other types of mutation.

The results of this study are clear, but to what extent do they reflect the process of evolution in bacterial populations that, like those in the wild, are not subject to continuous growth under nutrient-rich conditions? Finkel and Kolter [7] addressed this issue by competing strains of *E. coli* grown in aged batch cultures, in which no nutrients were added and from which no cells were removed after their original inoculation. These cultures produced clones with mutations conferring a growth advantage in stationary phase — hence the term ‘GASP’ phenotype — and, even

in refreshed cultures, cells displaying the GASP phenotype were found to outcompete their parental strains.

By holding cultures in stationary phase for progressively longer periods, Finkel and Kolter [7] found that, not only did GASP mutants from 10-day-old cultures outcompete cells in fresh overnight cultures, but cells from 20-day-old cultures outcompeted the mutants from 10-day-old cultures, and cells from 30-day-old cultures outcompeted those from 20-day-old cultures. Although stationary-phase cultures maintain a constant number of viable cells, these experiments show that there is a continual input of new mutations and a constant selection for fitter cells, even under these ‘stationary’ conditions. Hence, in both nutrient-rich and nutrient-limiting conditions, bacterial genomes and populations are highly dynamic.

There is an additional process that promotes adaptive evolution in bacteria — the introduction of genes through horizontal transfer. We have reported that a substantial portion — perhaps 18% — of the genes presently in the *E. coli* chromosome — were acquired from other species [8]. And in another recent paper, Jain *et al.* [9] argue that horizontal transfer has been a rampant and continuous process over the entire history of bacteria. Future research will need to elucidate the relative contributions of mutations and horizontal gene transfer to the rate of phenotypic evolution in bacteria.

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