# Bacteria are different: Observations, interpretations, speculations, and opinions about the mechanisms of adaptive evolution in prokaryotes

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**To some extent, the genetic theory of adaptive evolution in bacteria is a simple extension of that developed for sexually reproducing eukaryotes. In other, fundamental ways, the process of adaptive evolution in bacteria is quantitatively and qualitatively different from that of organisms for which recombination is an integral part of the reproduction process. In this speculative and opinionated discussion, we explore these differences. In particular, we consider (*i*) how, as a consequence of the low rates of recombination, ''ordinary'' chromosomal gene evolution in bacteria is different from that in organisms where recombination is frequent and (*ii*) the fundamental role of the horizontal transmission of genes and accessory genetic elements as sources of variation in bacteria. We conclude with speculations about the evolution of accessory elements and their role in the adaptive evolution of bacteria.**

For the most part, the genetic theory of adaptive evolution was developed by sexually reproducing eukaryotes, for sexually reproducing eukaryotes. Although there have been some wonderful theoretical studies of population genetics and evolution of bacteria—many of which are cited herein—the formal (mathematical) and informal (verbal) theory of the mechanisms of adaptive evolution in bacteria is modest in its volume, breadth, and level of integration.

Bacteria are haploid, reproduce clonally, and rarely subject their genomes to the confusion of recombination. Thus, one might believe (and both of us once did believe) that the genetic basis of adaptive evolution in these prokaryotes would be simpler than that of so-called higher organisms and that the theory to account for it would be straightforward extensions of that already developed for sexually reproducing eukaryotes. As we shall try to convince the reader, in this personal (read, opinionated) discussion, this situation is by no means simple. Bacteria are different. The mechanisms of adaptive evolution in the prokaryotic world raise a number of delicious theoretical and empirical questions that have only begun to be addressed.

## Observations

**Adaptation by the Acquisition of Genes and Accessory Genetic Elements from Without.** The most striking feature of retrospective studies of genetic variation and molecular evolution in bacteria is the extent to which these organisms are chimeras. Much of the DNA of bacteria classified as *Escherichia coli* has been acquired relatively recently: more than 17% of the open reading frames of the *E. coli* K-12 genome was acquired in the last 100 or so million years from organisms with G + C ratios and codon usage patterns distinguishable from those of other strains of *E. coli* and closely related Enterobacteriacae (1). Moreover, a substantial amount of the variation in bacteria is not in their chromosomal genes. Bacteria commonly carry arrays of active and retired accessory genetic elements (plasmids, prophages, transposons, and integrons), the composition of which also varies widely among members of the same bacterial species. Although, at any given time, some of these elements, such as insertion sequences and cryptic plasmids, may not carry genes that code for specific host-expressed phenotypes, others are responsible for the more interesting adaptations of bacteria to their environment. For example, many of the genes coding for the adhesins, toxins, and other characters responsible for the pathogenicity of bacteria, ''virulence factors,'' either are present in clusters known as ''pathogenicity islands'' (2–5), which almost certainly had former lives as accessory elements or as parts thereof, or are borne on functional accessory elements, such as plasmids and prophages of temperate viruses. Some of these islands and virulence-encoding accessory elements may have had a long history with one or a few specific pathogenic species of bacteria (6). On the other hand, many are distributed among bacteria that, based on their less mobile chromosomal loci, are phylogenetically quite different (7). Plasmids and their running dogs, transposons, also bear many of the genes responsible for antibiotic resistance and, all too commonly, multiple antibiotic resistance (8), and many of the other characters that make the lives of prokaryotic organisms as interesting and exciting as they are. Included among these plasmid-borne genes are those that code for the fermentation of exotic carbon sources, the detoxification of heavy metals, and the production of allelopathic agents, such as bacteriocins (9). Bacteria also commonly carry integrons, elements that acquire, accumulate, and control the expression of genes acquired from external sources (10–13).

All of these islands and accessory genetic elements and even some seemingly ordinary chromosomal genes, like those for the resistant forms of the penicillin-binding proteins of *Streptococcus pneumoniae* (14), were acquired from other organisms (primarily, but possibly not exclusively, other bacteria). They were picked up in a number of ways: as free DNA (transformation), through bacteriophage (transduction), or by intimate contact with other bacteria (conjugation). Although genetic exchange may occur less frequently in bacteria than in sexual eukaryotes (for which recombination is an integral part of the reproductive process), the phylogenetic range across which genetic exchange can occur in bacteria is far broader than that in extant eukaryotes. From a prokaryotic perspective, sexual eukaryotes like ourselves are incestuous nymphomaniacs: we do ''it'' too far often and almost exclusively with partners that, from a phylogenetic perspective, are essentially identical to ourselves. To be sure, genes acquired by horizontal transfer can be a source of variation for adaptive evolution in ''higher'' eukaryotes, up to

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and including those high enough to publish (15, 16). However, for at least contemporary eukaryotes, it is sufficient (and sufficiently problematic) to develop a comprehensive genetic theory of adaptive evolution based on variation generated from within by mutation (broadly defined to include transposition and chromosomal rearrangement) and recombination among members of the same species. By contrast, in contemporary as well as ancient bacteria, the horizontal transfer of genes (and accessory genetic elements) from other species is a major source of variation and is fundamental to the genetic theory of adaptive evolution in these prokaryotes.

The extent to which genes and accessory genetic elements are exchanged among phylogenetically distant populations raises a number of issues beyond those of the genetic mechanisms of adaptive evolution addressed herein. Perhaps the most fundamental of these other issues are the classical ones of the definition of species and the remarkable discreetness of the genomic clusters of organisms we classify as members of the same species of bacteria. For interesting and appealing considerations of these issues, see refs. 17–20.

## Interpretations, Speculations, and Opinions

**Adaptive Evolution by Mutation and Selection of Chromosomal Genes.** Although genes and accessory elements acquired from external sources are responsible for many of the interesting adaptations of bacteria to their environments and their seemingly saltational evolution, the mundane processes of mutation and selection at chromosomal loci are by no means inconsequential for the long-term as well as day-to-day evolution of bacteria. On the other hand, at least quantitatively and possibly qualitatively, the chromosomal population genetics of adaptive evolution in bacteria are different from those of sexually reproducing eukaryotes.

**Adaptive Evolution When There Is Little or No Chromosomal Gene Recombination.** The primary differences between the chromosomal population genetics of bacteria and those of sexually reproducing eukaryotes arise as a consequence of the low rates of chromosomal gene recombination in bacterial populations. To be sure, if one looks hard enough, some natural mechanism of chromosomal gene recombination can probably be demonstrated to occur in most species of bacteria. And collectively, the chromosomal genomes of some species of bacteria may even be at or near linkage equilibrium (21, 22). Nonetheless, because bacteria reproduce clonally, by binary fission, any given population will be composed of relatively few genetically distinct lineages, with recombination between them occurring only on rare occasions. We postulate that for most natural populations of bacteria, the probability of a gene in one lineage (clone) being replaced by homologous recombination with a gene from another lineage of its own or another species is on the order of the mutation rate or lower (refs. 23–25; see also ref. 26). The low rates of recombination in bacterial populations have at least four important (and interesting) ramifications for at least the local (short-term) population genetics of adaptive evolution in bacterial populations.

*Sequential evolution.* Within each bacterial lineage, adaptive evolution will proceed by the sequential accumulation of favorable mutations, rather than by recombinational generation of gene combinations; in this respect, bacterial evolution will be similar to that depicted in the top portion of Muller's famous diagram of evolution in asexual and sexual populations (27). The evolution of a better genotype *ABC* from its less fit ancestor *abc* will proceed in stages, one gene at a time, with the order and rate of evolution depending primarily on the fitness of the intermediates (*aBc*, *aBC*, etc.). If, individually and collectively, the intermediates have a selective advantage over the *abc* ancestor, this evolution can proceed quite rapidly (28). If, on the other hand, the intermediates are not favored, it may take a great deal of time before the best genotype, *ABC*, is assembled. Moreover, the best genotype will most likely arise by mutation from an intermediate form, rather than through the recombinational merger of different intermediate forms; for a more extensive and formal (mathematical) consideration of the process of mutation and selection in bacteria, see ref. 29.

*Compensatory evolution.* In bacteria, adaptation to the effects of deleterious genes that have become fixed (because of their being favored in another environment or for other reasons) is likely to be through amelioration of those effects by the ascent of compensatory mutations at other loci, rather than by the evolution of more fit variants at the deleterious loci (30, 31). In sexual eukaryotes as well as bacteria, these compensatory mutations are likely to be the first to arise when, as is probable, there are more ways to improve fitness of the organism than mutation (reversion) at the deleterious locus. If recombination is common, however, these compensatory mutations and the genes whose deleterious effect they ameliorate will rapidly become separated, especially if these loci are not closely linked. Because of the low rate of recombination in bacterial populations and the fact that, when recombination does occur, only a small fraction of the genome is replaced, the deleterious and compensatory genes will remain in linkage disequilibrium for extensive periods, no matter how far apart they lie on the chromosome. As the frequency of the compensatory mutants increases in the population, the intensity of selection for better alleles at the deleterious locus will drop off. Moreover, if the compensatory mutations become fixed, adaptive valleys may be established and effectively preclude the ascent of even more fit mutations at the deleterious locus itself (32).

*Low effective population size.* Although the total sizes of bacterial populations may be enormous, their genetically effective sizes can be quite low as a consequence of periodic selection, i.e., selective sweeps of better-adapted mutants (23, 33, 34) or as a result of bottlenecks, contractions in population size associated with transmission to new hosts or microhabitats. These low effective population sizes and selective sweeps have at least two major ramifications for adaptive evolution. One is to purge variation accumulating in the population by selection as well as by genetic drift. Another is to make a given population less fit than it would be if it did not have to deal with the stochastic trials and tribulations of periodic selection and bottlenecks. Although, on Equilibrium Day, the most fit genotypes will ascend to their rightful places, on the days before, those best types may well be lost as the population passes through bottlenecks (35, 36).

*The evolution of genes that augment the rate at which variation is generated.* Because low rates of recombination allow modifier loci and the genes whose effects they modify to remain together for extensive periods, natural selection can favor modifying traits that are advantageous in the long run despite their short-term disadvantages (37, 38). One example of such a situation is the evolution of mutators, genes that increase the rate at which variation is generated by mutation. Because they generate deleterious mutations and possibly for other reasons as well, mutator genes, which are commonly defective mismatch repair loci, are anticipated to be at a selective disadvantage. However, continuous changes or heterogeneities in the selective environment can cause bacteria bearing these mutators to ascend by hitchhiking with the beneficial mutants they generate (39, 40). As anticipated by this theory, in long-term experimental (41, 42) and natural populations of bacteria (43) adapting to new and/or changing environments, one can find relatively high frequencies of mutator genes.

**It's Not the Rate That Counts.** Although in any given bacterial population, the absolute rate of chromosomal gene change by recombination may be as low or lower than that of mutation, the

consequences of recombination for adaptive evolution in bacteria can be far more profound than those of mutation. By legitimate (homologous) or illegitimate (nonhomologous) recombination, bacteria can acquire new genes that have evolved in other often phylogenetically and ecologically distant populations. These genes can code for phenotypes that can expand or alter the ecological niche of their host bacterium (44), and if they are favored in the recipient population, they will ascend. Given sufficient time and sufficiently intense selection, bacteria will acquire whatever genes they need either directly or indirectly through intermediate species. And, as we have learned from the evolution of antibiotic resistance, "sufficient time" need not be very long. For this reason, bacterial ecosystems have been characterized as a "global gene pool" (45). From the perspective of adaptive evolution, it is generally not the rate of recombination that is important but rather the existence of mechanisms for gene exchange, the range of "species" with which a populations of bacteria can (and do) exchange genes, and the intensity of selection for those genes.

**Adaptive Evolution by the Transmission of Accessory Genetic Elements.** From one perspective, the accessory genetic elements of bacteria are parasites and symbionts, and their population and evolutionary biology can be—and has been—treated in that context with little or no reference to their role as sources of variation for their host bacteria. However, as we have discussed above, much of the real "action" in adaptive evolution in bacteria is through genes borne on, transmitted by, and sequestered from these elements. And, from this perspective, the population and evolutionary dynamics of these elements form an integral part of the process of adaptive evolution in bacteria.

**Variations on a Single Theme.** Traditionally, we classify the accessory genetic elements of bacteria into functional, rather than phylogenetic groups: primarily as plasmids, transposons, and temperate phages. Furthermore, we typically draw a distinction between these peregrine elements and those with less mobile and autonomous life styles such as islands and integrons. However, the distinction between plasmids, phages, and transposons is somewhat artificial even on functional grounds. For example, when they are in bacteria, the prophage of the phage P1 is a plasmid and that of Mu is a transposon. Moreover, although some plasmids and a few transposons code for the machinery needed for their own infectious transfer by conjugation, the mobility of many plasmids and of most transposons, integrons, islands, and even ordinary genes is by hitchhiking on conjugative plasmids or phages or by being picked up as free DNA by hosts with transformation mechanisms. Although the modes of replication and transmission are critical for considerations of the population dynamics, existence conditions, and ecology of these different classes of accessory elements (46–51), they are of only secondary import for a general consideration of adaptive evolution in bacteria. From this perspective, the most important factor is the extent to which these elements are mobile and the range of bacterial hosts they infect. In this sense, the accessory bacterial genetic elements can be seen as arrayed along a continuum from phages, plasmids, and transposons to pathogenicity (and nicer) islands, integrons, and even stay-at-home chromosomal genes.

**Why Be a Vagabond When You Can Stay at Home?** Accessory elements at the most mobile end of the continuum may be maintained as "genetic parasites," spreading by infectious transfer alone without bearing genes that augment the fitness of their host bacteria. A parasitic existence is almost certainly the case for purely lytic (virulent) phages (52) and possibly for many temperate phages as well (50). Although a formal possibility, we believe that it is unlikely that plasmids and transposons are purely parasitic (53) and even less likely that islands and integrons are maintained without at least occasionally paying for their dinner. The rates of infectious transfer of these elements are almost certainly not great enough to overcome the fitness burden their carriage imposes on their host bacteria (54–56) and their losses by vegetative segregation. If this assumption is correct, then these elements must bear genes that are at least sometimes beneficial to their host bacteria. After all, these accessory elements are vertically transmitted (in the course of cell division); thus, it would be to their advantage to carry genes that augment the fitness of their hosts. But can this "niceness" account for the maintenance of accessory genetic elements? If accessory element-borne genes provide a selective advantage to bacteria and the accessory elements themselves are either costly or unstable, why are those genes not sequestered by the host chromosome?

In a recent investigation, we, along with Marc Lipsitch (57), addressed this question from a slightly different angle, that of the existence conditions for plasmids. We demonstrated that, under broad conditions, if host-expressed genes have higher fitness when carried on the chromosome than on an infectiously transmitted plasmid, then those genes will be sequestered eventually by the chromosome. These usurpings of genes from the plasmid by the chromosome will be the case even when selection for those genes is intermittent, as is the case for antibiotic resistance and most other plasmid-encoded characters. A broader interpretation of this result is that, *if the mobility of host-adaptive genes has a cost, that mobility will be lost eventually*. Plasmids, transposons, and temperate phages, or the genes they carry will give up their vagabond lifestyle and become islands.

On the other side, using primarily simulation methods, we demonstrated two seemingly realistic situations under which those genes can be maintained for extended periods on infectiously transmitted accessory elements. The first of these is the continuous entry into that population of lineages that are more fit than existing ones. By being infectiously transmitted, those favored (or occasionally favored) genes will be able to make their way to the rising stars rather than being lost along with their has-been hosts. The second situation involves movement among two or more distinct bacterial ecotypes in an ecologically heterogeneous environment. Although the accessory element may be lost from any particular ecotype at any particular time, it can return via horizontal transfer, and in the long term, the host-beneficial genes can persist on accessory elements.

In this interpretation, genes originally carried on accessory elements (and even the elements themselves) are in a continuous state of flux with respect to their mobility and within-host stability. As the habitat of a bacterial population becomes more stable and/or the opportunities for its accessory elements to move to uninfected populations decline, selection will favor the incorporation of those elements or the favored genes they carry into the chromosome. The opposite will occur in more interesting times and places. Selection will favor the mobility of accessory elements, and broadly favored but narrowly available genes and former elements will be seduced back into a vagabond lifestyle. A corollary of this interpretation, if interpretations are allowed to have corollaries, is that genes that become widely popular for some environmental reason—such as the genes for antibiotic resistance after the human use of antibiotics—will be borne initially by more mobile accessory elements. Whether the mobile elements bearing these genes will be phages, conjugative or nonconjugative plasmids, or transposons or whether those genes would be acquired by transformation will depend on a variety of historical, genetic, and ecological factors that are specific to the bacteria and habitats involved.

There are other conditions that may favor the infectious transfer of accessory elements as well. For example, a number of

characters expressed by bacteria of one lineage augment the fitness of bacteria of other lineages in their vicinity whether the beneficiaries carry those genes that code for that character or not. Examples include the production of secreted agents that kill competing bacterial species (e.g., antibiotics and bacteriocins) and somatic cells (e.g., toxins) and those that detoxify or condition the local environment for bacterial growth (e.g., $\beta$-lactamases and other enzymes that denature antibiotics exogenously; ref. 58). One consequence is that bacteria not carrying these genes can free-ride on the efforts of those that do. In such cases, infectious gene transfer would provide a way to convert these "cheaters" into good citizens that share the burden as well as the advantages of carrying and expressing these genes (J. Smith, unpublished work).

**Evolving to Evolve: The Evolution of Infectious Gene Transfer.** Thus far, we have considered the mechanisms that can *maintain* infectiously transmitted genetic elements over evolutionary time. But how did these mechanisms and the capacity for acquisition of genes from without evolve in the first place? We believe that, for accessory genetic elements, the most parsimonious (and possibly even correct) answers to these questions are those that treat the individual genetic elements, rather than their hosts, as the objects of natural selection.

**Infectious Gene Transfer as a Product of Coincidental Evolution?** A number of years ago, Richard Lenski and B.R.L. (24, 25) considered the mechanisms responsible for conjugative plasmids and phages to serve as vehicles (vectors) for the infectious transfer of host genes: plasmid-mediated conjugation and phage-mediated transduction. They postulated that these forms of bacterial sex are coincidental to the infectious transfer of the elements themselves and to the presence of recombination repair enzymes in their host bacteria. Although we are now in a new and doubtless more enlightened millennium, this coincidental evolution hypothesis remains plausible and parsimonious, albeit still not formally tested. Clearly, generalized transduction and plasmid-mediated recombination (such as that by the F+ plasmid of *E. coli* K-12) are to the disadvantage of the phage and plasmid, respectively. Coincidental evolution also seems a reasonable hypothesis for the propensity of conjugative plasmids and bacteriophages to pick up hitchhiking accessory elements, such as mobilizable nonconjugative plasmids. However, the ability to hitchhike (mobilizability) may well be an evolved character of the hitchhiking element.

Can coincidental evolution also explain transformation and transformability (competence)? The mechanisms that naturally transforming bacteria have for picking up free DNA from the environment, protecting it from destruction by restriction enzymes, and incorporating it into their genomes are complex and highly evolved. A number of hypotheses have been presented for the selective pressures responsible for the evolution of transformation. One of these is consistent with a coincidental evolution hypothesis. In accord with this hypothesis, transformation evolved as a mechanism for acquiring food (nucleotides) from the external environment, and recombination is a coincidental side effect of DNA entering the cell (59). Alternatively, it has been proposed that transformation evolved specifically as a mechanism to acquire genes from without as templates to repair double-stranded breaks (refs. 60 and 61, but see also refs. 62 and 63). To these hypotheses, we would like to add the following, not mutually exclusive alternative. We postulate that the ability to acquire genes from other organisms as a source of variation for adaptive evolution is the selective pressure responsible for the evolution and maintenance of transformation. In this interpretation, transformation evolved and is maintained through processes similar to the one proposed for the evolution of mutators (39, 40), but in this case, the source of variation is external rather than internal. An analogous mechanism may also favor the evolution and maintenance of transposons, integrons, and other elements or processes that, once acquired, augment the rate at which variability is generated. (J. Smith, personal communication).

## Conclusion

We have argued that the mechanisms of adaptive evolution are quantitatively and qualitatively different in bacteria than they are in sexual eukaryotes. This difference is primarily a consequence of the frequency of homologous gene recombination being low in bacteria and high in sexual eukaryotes and of the phylogenetic range of gene exchange being broad in bacteria and narrow in contemporary eukaryotes. Also contributing to this difference is the prominent role of viruses, plasmids, and other infectiously transmitted accessory genetic elements as bearers and vectors of genes responsible for adaptive evolution and their seemingly negligible role in this capacity in contemporary eukaryotes.

Clearly, both of these regimes of recombination and horizontal gene transfer are associated with groups of organisms that have been successful. To be sure, there may well be bacteria in which horizontal gene transfer plays little role in adaptive evolution (*Mycobacterium tuberculosis*, perhaps) and higher eukaryotes that rarely, if ever, engage in homologous gene recombination (64). There may also be species of bacteria in which recombination occurs at high rates and species of contemporary eukaryotes in which infectiously transmitted viruses and other accessory elements play a prominent role as sources of variation for adaptive evolution. We propose, however, that these are the exceptions. We postulate that evolutionary success of bacteria as a group is a consequence of their capacity to acquire and express genes from a vast and phylogenetically diverse array of species and that the success of eukaryotes as a group relies on the high rates at which genes are shuffled by recombination.

**Coda.** In this report, we have focused on how the mechanisms of adaptive evolution in bacteria are different from those of sexually reproducing eukaryotes. We have not considered how they are similar, nor have we covered the wonderful studies in which bacteria have been used as a model system, a tool, for studying evolution experimentally and actually testing, rather than just championing, general hypotheses about the ecological, genetic, biochemical, and molecular basis of evolution. For an excursion into that literature, we refer the reader to the work of Julian Adams, Jim Bull, Allan Campbell, Lin Chao, Patricia Clark, Fred Cohan, Tony Dean, Dan Dykhuizen, David Gordon, Barry Hall, Dan Hartl, Ryzard Korona, Richard Lenski, E. C. Lin, Rick Michod, Judy Mongold, Robert Mortlock, Rosie Redfield, Peg Riley, Paul Sneigowski, Francois Taddei, Michael Travisano, Paul Turner, Holly Wichman, Miro Radman, Lone Simonsen, and others, who will, doubtless, reprimand us for our unintentional transgression of not including their names in this list.

This article is dedicated to the memory of Ralph V. Evans (1958–1990). Ralph had been doing theoretical and experimental studies of adaptive evolution in bacteria. If not for his premature death by cancer, his name and contributions would have had a prominent role in this discussion.

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