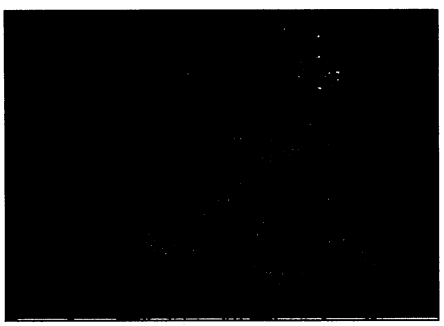
Ecological recovery after the Mount St. Helens eruption in 1980 has proceeded with minimal human interference and with notable success; however, the recovery of tropical marine ecosystems such as coral reefs after large natural disturbances "is almost invariably confounded by anthropogenic stresses." The idea that *Pseudomonas aeruginosa* combines the genetic strategies of mutation and recombination, evolving hypermutable and hyperrecombinant strains, to adapt to the complex and harsh environment in the lungs of cystic fibrosis patients is discussed. And the relative contributions of nongovernment and government scientists, researchers in the biotechnology industry, and policy-makers to the agricultural biotechnology debate are examined.

Confounding Factors in Coral Reef Recovery

The effects of the Mount St. Helens eruption on the surrounding forest and the processes affecting recovery from this major disturbance, which Franklin and MacMahon discuss in their Perspective "Messages from a mountain" (*Science*'s Compass, 19 May, p. 1183), are similar to the effects of hurricanes on coral reefs. For both the forest and the reef, major natural disturbances leave most of the ecosystem structure intact, and recovery can begin from surviving organisms (or, in some cases, portions of organisms) (*1*). Both the species composition and the physical structure of these ecosystems influence the course of recovery.



Buck Island Reef National Monument, St. Croix, after Hurricane Hugo in 1989.

In the case of Mount St. Helens, the recovery process has had little interference from humans. In contrast, the recovery of coral reefs from hurricanes is almost invariably confounded by anthropogenic stresses, most notably fishing. Overfishing can delay reef recovery by removing herbivorous fishes that keep algal growth in check. If grazing is not intense enough, algae (particularly "fleshy" seaweeds) can inhibit both growth of adult coral colonies and settlement or survival of coral recruits (*2*). Humans have now degraded tropical marine ecosystems to the point where our ability to evaluate ecological theories about succession and effects of disturbance has been compromised.

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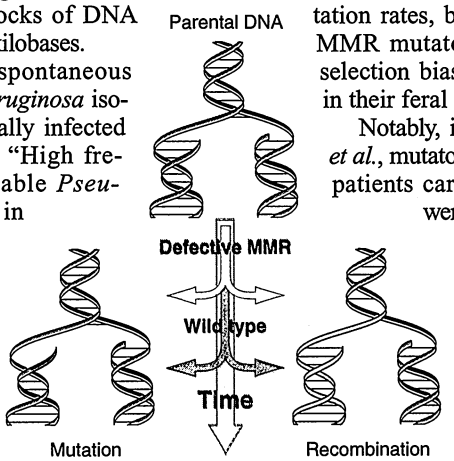
Pseudomonas Survival Strategies in Cystic Fibrosis

Pseudomonas aeruginosa is a versatile species exhibiting variable colony morphologies, multiple ecological niches, and diverse disease consequences. Several laboratories have observed this adaptability at the molecular level, especially when the cystic fibrosis (CF) lung is a reservoir for chronic infection by the organism. Römling *et al.*, for example, compared 18 CF isolates of clonal strain C of *P. aeruginosa* and showed that the chromosome of each isolate had a hypervariable structure (*1*). The complex pattern of DNA rearrangements in the chromosome included large inversions and acquisition of blocks of DNA ranging from 1 to 214 kilobases.

In their study of spontaneous mutation rates of *P. aeruginosa* isolates from 30 chronically infected CF patients (Report, "High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection," 19 May, p. 1251), Oliver and colleagues found that the lungs of 11 out of the 30 CF patients were colonized by a hypermutable (mutator) strain of the organism. In contrast, a mutator phenotype was not found in 75 non-CF patients acutely infected with *P. aeruginosa*. Oliver *et al.* and Rainey and Moxon, authors of the accompanying Perspective "When being hyper keeps you fit" (*Science*'s Compass, p. 1186), ascribe the evolution of these mutator phenotypes to the hitchhiking of mutator alleles within the adaptive mutants they spawned—seemingly, an inherent consequence of being a haploid organism. Moreover, Oliver *et al.* suggest that selection pressure (the unfavorable environment within the lungs of CF patients subjected to antibiotic challenge and other undefined stresses) culls and enriches mutators of *P. aeruginosa*, analogous to the specific selection schemes used for in vitro enrichment of *Escherichia coli* (*2*) and *Salmonella enterica* (*3*) mutators.

The studies by Oliver *et al.* complement the molecular and genetic studies highlighted above. Specifically, their results suggest that, in addition to the hypermutable phenotype, promiscuous recombination in mutator strains might be a significant source for adaptive change in *P. aeruginosa*. When we reported on the high incidence of mutators found among pathogenic strains of *E. coli* and *S. enterica* (*4*), we proposed that the hyper-recombinagenic nature of particular mutators contributed to the evolution of these enteric pathogens, indicating that horizontal transfer and homeologous recombination (recombination between diverged DNA sequences) helped frame the genetic diversity of these populations. All mutators found (predominantly with mutations in the *mutS* gene) in our studies were deficient in methyl-directed mismatch repair (MMR), a pathway that plays a central role in blocking the recombination of mismatched DNA. We reasoned, therefore, that it was not (solely) the hypermutable phenotype, because defects in any of 25 genetic loci in *E. coli* increase spontaneous mutation rates, but the promiscuity of MMR mutators that provided the selection bias for mutator bacteria in their feral settings.



Mutation and recombination occur on a shortened time frame in MMR-deficient bacteria.

Notably, in the report by Oliver *et al.*, mutators from at least 4 of 11 patients carrying mutator strains were defective in the *mutS* gene. The *mutS* mutator, unlike any other mutator alleles, plays an exacting and singular role in homeologous recombination (*5*). These MMR mutator strains could be the mixing pools where chromosomal changes—from ei-

ther mutation or recombination—are spawned and sorted, because the pathways and payoffs of the MMR mutator are manifold. Such strains can (i) promote diversification, precipitating adaptive mutations such as resistance to antibiotics; (ii) rapidly accrue multiple independent changes, making an unlikely event (such as successful infection) possible; or (iii) assemble multiple mutations from different chromosomes into one by recombination. Thus, MMR mutators (in particular, the *mutS* mutators) may underpin the compelling evidence showing interchange of DNA among *P. aeruginosa* during chronic infections.

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Response

LeClerc and Cebula propose that mechanisms leading to high mutation frequencies (mainly in the *mutS* gene) that we observed in *P. aeruginosa* strains from CF patients may also explain the hypervariable chromosomal structure observed in different *P. aeruginosa* isolates (*1, 2*). Both adaptive strategies, leading to hypermutator and hyperrecombinant phenotypes in *E. coli* and *Salmonella* MMR-defective strains, have been described (*3*).

We considered the hypothesis of a combined hypermutable and hyperrecombinant phenotype. This possibility is not entirely obvious because MMR deficiency increases the recombination rate only for homeologous sequences, but not for homologous (identical) sequences (*4*), and because most CF patients are infected only with a single *P. aeruginosa* clone (*5*). Therefore, despite the possibility of a high recombination rate, only very similar (or identical) sequences can be shared by *P. aeruginosa* individuals living in a CF lung, and thus the probability of acquiring new or innovative DNA blocks is very low. The problem is that gene sequences in *P. aeruginosa* (including strains from CF patients) are, apparently, less polymorphic than the corresponding macrorestriction patterns, suggesting that DNA rearrangements, insertions, and deletions are the main cause of *P. aeruginosa* chromosomal diversity (*1, 2*).

Bacteria have two main strategies to accelerate evolution to adapt to new environments: mutation and recombination. Mutations may be important when a population is confronted with critical, abrupt, and unspecific changes in the environment, eventually permitting rapid, but not always optimal, adaptation. Recombination may adapt more habitat-specialized populations to comparatively small but more specific fluctuations in the environment. In general, the adaptive biology of bacteria tends to be more mutation-based, because there is a strong risk of exposure of the organism to quite different environments, and because strong environmental changes are more frequent in simpler habitats. The exception is bacteria able to reach high specialization in essentially constant and unique habitats (such as *Helicobacter pylori, Neisseria meningitidis,* or *Streptococcus pneumoniae*) in which recombination becomes the major driving adaptive strategy (*6*).

The position of *P. aeruginosa* in this conceptual frame is paradoxical. *P. aeruginosa* resembles a large-environmental-spectrum organism, but its main adaptive strategy appears to be recombination (*1, 2*). This species has a high metabolic versatility, including the ability to adapt to virtually all aquatic mesophilic habitats (*2*). In the case of the CF lung environment, *P. aeruginosa* has a particularly complex challenge, requiring simultaneous adaptation to dehydration, iron starvation, leukocyte influx, antibacterial peptides, and frequently changing, aggressive, and prolonged antibiotic therapy. In this case, perhaps, its metabolic versatility is not enough to allow a rapid adaptation to this complex habitat. In the absence of innovative related DNA (because the population has a clonal structure), hypermutation may arise as the only available strategy to accelerate adaptation.

A combined strategy using both mutation and recombination (when possible) would have been favored by natural selection. As LeClerc and Cebula suggest, the deficiency in the MMR system provides the potential for the use of both strategies. In the lungs of CF patients, and after a certain (long) period of time, several lineages of MMR mutators are expected to be selected by the hitchhiking effect of different adaptive mutations, thus increasing the genetic divergence. At this point, hyperrecombination between mutationally adapted lineages may occur, and a number of DNA interchanges among *P. aeruginosa* variants ensures the progression toward an optimum in the bacterial adaptation to the lung environment. That would result in a second-order genome diversification: large-scale chromosomal rearrangements have been found in *P. aeruginosa* isolates from CF patients (*1, 7*). This strategy would also tend to minimize the frequency of deleterious mutations acquired by mutators.

We consider that the hypothesis of a combination of hypermutation and hyperrecombination strategies in *P. aeruginosa* cannot be ruled out. On the contrary, it should be tested appropriately; for instance, by studying variations in both nucleotide sequences and large DNA fragments among sequential isolates from single patients. However, we suggest that this combination may occur in this particular environment in a sequential way; that is, genomic rearrangements should be preceded by mutations to be fully effective, because only when a certain degree of variation in the population has been generated by mutation can MMR deficiency have the opportunity to increase variation by increasing the recombination rate.

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Scientists Have Not Been Silent

In his Editorial "Opportunity for agricultural biotechnology" (28 Apr., p. 615), Richard J. Mahoney accuses the scientific community of being "missing in action" on the agricultural biotechnology public debate. Few would disagree that more needs to be heard from agricultural and food scientists in both public and private sectors. They have not been silent, however. In 1996, 11 scientific societies representing some 80,000 scientists united their efforts to articulate the scientific concerns about regulatory policy for agricultural biotechnology. The consortium decried regulation based on process rather than product and declared it "scientifically indefensible to regulate the inherited traits of plants for pest and disease resistance under statutes developed specifically for chemical pesticides applied externally to plants" (*1*). Process-based regulation remains the cornerstone of the Environmental Protection Agency's (EPA) policy.

Repeatedly, in testimonies to Congress,