

Contingency Loci, Mutator Alleles, and Their Interactions

Synergistic Strategies for Microbial Evolution and Adaptation in Pathogenesis^a

DAWN FIELD,^b MARCELO O. MAGNASCO,^c E. RICHARD MOXON,^d
DAVID METZGAR,^b MARK M. TANAKA,^e CHRISTOPHER WILLS,^b
AND DAVID S. THALER^{f,g}

^b*University of California, San Diego, La Jolla, California 92093-0116, USA*

^c*Center for Studies in Physics and Biology, Rockefeller University, New York, New York 10021, USA*

^d*Institute of Molecular Medicine, University of Oxford, UK*

^e*Department of Biological Sciences, Stanford University, Stanford, California 94305-5020, USA*

^f*Sackler Laboratory of Molecular Genetics and Informatics, Rockefeller University, 1230 York Ave., New York, New York 10021, USA*

EVOLUTION OF MUTATION RATES

The classic view of evolution is a series of alternating and distinct steps. The first step is the generation of diversity through mutation; the second, natural selection. There is a growing awareness that in some cases these steps conjoin; for instance, the classic view breaks down when mutational changes alter the very machinery that generates diversity, giving rise to qualitatively different and interesting phenomena. The evolution and differentiation of a variety of mutation rates among individual genes in a genome and individual members of a population play key roles in the evolution of adaptive strategies and genomes.¹⁻³ The spectra of mutations that arise are indirectly selected through conflicts between the requirement for producing enough useful genetic variability and the necessity to minimize the accumulation of deleterious mutations. The spectrum generators of genetic variability continually evolve under indirect selection based on phenotypic selection by the environments and the dynamics of change from one environment to another. In a changing environment involving recurring, "predictable" selection (e.g., the infective cycle of a pathogen), this phenomenon is clearly expressed in phase variation strategies (e.g., phenotypic switching of surface antigens) involving "contingency loci".¹ In contrast, rapidly changing environments that do not have a historic component can favor global mutators. We suggest the existence also of important evolutionary contexts in which the interactions of contingency loci with global mutators in a single genome will be favored or be disfavored by selection.

^aThis work was supported by the Sloan Foundation (to M.M. and D.S.T.), a National Science Foundation Doctoral Improvement Grant (to D.F.), and the Lucille P. Markey Charitable Trust.

^gCorresponding author. E-mail, thalerd@rockvax.rockefeller.edu

DISTINCT EVOLVABLE STRATEGIES FOR GENERATING GENETIC VARIATION

Bacteria use at least two evolvable mechanisms to change their rates of mutation. Contingency loci and mutator alleles are both evolvable strategies employed to facilitate adaptation to novel environments. A number of hypervariable loci that encode virulence factors determining host-pathogen interactions have been discovered in bacteria.¹ The term “contingency” locus has been coined to emphasize their role in facilitating adaptation to the differing microenvironments within and between hosts.^{1,2} Among the best studied examples of specific contingency loci with high mutation rates are the 14 known or putative hypermutable virulence factors in the genome of *Hemophilus influenzae*.⁴ These genes encode proteins that directly interact with the host, and they all contain hypermutable iterations of short repeats within their reading frames. The hypermutability of these iterations, through slippage-mutation, results in the ability of *H. influenzae* to switch its phenotype rapidly through altered transcription or frameshifting, thus helping it to evade host immune defenses and to colonize successive host microenvironments.

By contrast, the alleles of genes of DNA metabolism, for example, those of mismatch repair, affect the global mutation rate and are called “mutator alleles” which give rise to strains called “mutators.” Global mutators have been shown to be important in adaptive microbial evolution in both theoretic and empiric studies.^{3,5,6} Global mutators produce high mutation rates throughout the genome, and different mutator loci produce different spectra of mutations. Global mutators also lower the sequence stringency required for homologous recombination.⁷ These two adaptive strategies therefore provide a “specialist” and a “generalist” approach to producing sufficient genetic variation for survival. These differences between contingency loci and global mutators are fundamental to understanding their distinct roles in genomic and organismal evolution. Little is known about if or with what frequency these two strategies coexist in a single genome, and if they do, what kinds of interactions between them might occur. We focus here on the implications of the evolution of these two strategies on aspects of host-pathogen interactions.

Host-pathogen interactions involve strong selective pressure on both host and pathogen. Pathogenic bacteria must evolve to survive the host’s constantly evolving immune defenses (one example of the Red Queen race). Pathogens must also adapt, by physiology or by evolution, to new microenvironments as they invade and colonize different compartments (e.g., nasal mucosa, lungs, and blood) within the host. These challenges (mode and tempo) are predicted to shape both the short- and long-term evolution of the genomes of pathogenic bacteria. Understanding microbial strategies for evolutionary adaptation is therefore key to understanding pathogenesis.

ONE WAY TO THINK ABOUT CONTINGENCY LOCI AND GLOBAL MUTATORS

Local and global mutators have both similarities and differences (TABLE 1). Each has been documented separately, but they have not yet been found in the same cells among natural isolates of bacteria. Neither have these constructions been created and studied in the laboratory. What properties and selective scenarios might be anticipated from evolving global and local mutators in the same cells?

TABLE 1. A Summary of Important Distinctions between Contingency Loci and Mutator Alleles^a*Contingency loci*

increase locus-specific mutation rates
 "specialist" strategy
 best suited to frequent bouts of "predictable" selection
 involves the evolution of an inherently unstable DNA sequence at a specific locus (e.g., a run of mono-, di-, tri-, or tetra-nucleotide repeats which produce frequent frameshift mutations)
 result in a limited repertoire of variation at each locus (on, off [quantitative modulation of expression is possible if the repeat run is in the gene control region], or alternative frame)
 can produce reversible mutations (e.g., slippage at an iterated repeat)

Mutators

increase global mutation rates (though not uniformly)
 "generalist" strategy
 best suited to novel selection challenges that may be frequent or infrequent
 involves the ability of a potential mutator gene to acquire alleles that lead to alterations in genomic mutation rate
 results in a virtually unlimited repertoire of variation throughout the genome
 catalyze both reversible mutations (point mutations and frameshifts, chromosomal duplications) and others that are irreversible (deletions, recombination between divergent sequences)

^aDespite differences, these two strategies are similar in that (1) both increase genetic variation by increasing mutation rates above background rates; (2) both are evolvable and inheritable; and (3) both are more advantageous in variable environments than in stable environments.

We consider four basic combinations of local and global mutators, (- -), (+ -), (- +), and (+ +), as follows. FIGURE 1 indicates the state of each mutator, "+" or "-", as a node on a graph. The combinations of both mutators is shown as an edge on the same graph. Along these edges we have indicated the selective scenarios we believe may be associated with each combination. Brief justification for these statements follows. (- -) is a state of maximum genetic stability. This state is most favored in a constant environment or at least one in which physiologic adaptation is sufficient to accommodate stresses that occur. (+ -) are cells that have "local mutators" such as contingency loci, but do not have "global mutators" such as *mutS* or *mutL*. This is the scenario associated with iterative, historically repeated rounds of selection. This is the situation faced by a pathogen as it follows a stereotypic course of invasion from one host compartment to another. (- +) indicates bacteria that have no contingency loci or other local mutators, but that are global mutators. This is apparently the condition of 1-3% of natural isolates of *E. coli* and *S. typhimurium*.^{8,9} Because of the general nature of global mutators, they will be most able to cope with novel selections that have no historic component. The introduction of new antibiotics provides novel selection, and the emergence of resistance to new antibiotics may be an evolutionary adaptation most attainable by global mutators. Global mutators also enhance interspecies recombination and intrachromosomal recombination between divergent sequences.⁷

The (++) combination of local and global mutators has not yet been documented either in nature or in the laboratory, but we predict the discovery of such strains. We consider that this genetic makeup is likely to give rise to newly emergent clones and strains (or perhaps

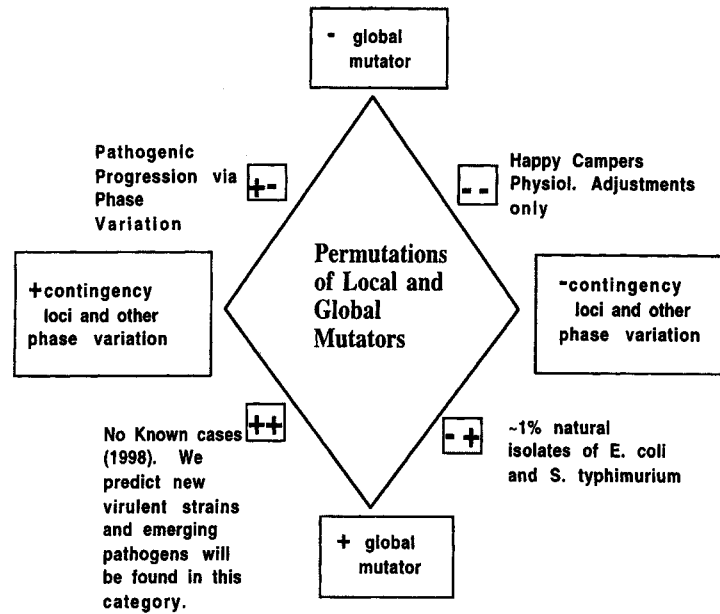


FIGURE 1. Selective scenarios and the genomic, evolutionary, and ecological consequences of the four possible combinations of the presence or absence of contingency loci (+) and mutators (+) in a single genome. Host-pathogen interactions provide a potential example of selective pressures strong enough to drive the evolution of both contingency loci and mutator alleles. Note that a particular strain can be anywhere along an edge, that is, it may have only a slightly elevated global mutation rate but great instability at contingency loci.

even species) of microbial pathogens. Combining contingency loci and global mutators may be synergistic. For example, *mutS* and *mutL* are especially prone to slippage at interative sequences such as the ones driving the evolution of genetic variability at contingency loci in *H. influenzae* and other pathogenic species. Thus, the likelihood exists that the rate of frameshifting at contingency loci will rise in a global mutator by a greater amount than will the mutation rate at other noncontingency loci in the same genome, and this may be key to adaptation in extremely short time periods (e.g., hours during the course of infection of a new host) and under extremely intense selective pressures. In addition, passing through a stage as a global mutator with dysregulated mismatch repair might facilitate the de novo evolution (or "fine-tuning" of the length of the iteration of existing) contingency loci. A special case of both theoretic and experimental interest is the one which global mutators are themselves contingency loci. High rates of frameshift mutations in (A)8 and (C)8 tracts within the hMSH3 and hMSH6 DNA of human mismatch repair genes have been found in both hereditary and spontaneous colon cancer.¹⁰

Ninio^{11,12} has proposed that "transient mutators" are behind much of what is called spontaneous mutation. Ninio proposed that transient mutators arise through the mistranscription or mistranslation of genes whose products are involved in the fidelity of DNA replication. The cell is a mutator for as long as the aberrant protein exists. When a result-

ing mutant cell is examined, it will no longer be a mutator. So far, we and others have discussed contingency loci only in terms of repeated sequences that are prone to frameshift mutation at the DNA level. Another class of contingency loci is possible; consider a hypothetical class of "contingency loci type B" or "Ninio-effect prone alleles" that are particularly susceptible to transcriptional and/or translation frameshifting. Strains with these hypothetical Ninio-effect prone alleles in DNA fidelity genes are predicted to frequently give rise to transient mutators. Ninio's transient mutators could be identical to the population fraction posited to be in a "hypermutable state."¹³⁻¹⁵ By hypothesis, cellular physiology could alter the probability of a contingency locus type B manifesting its transient mutator quality. Physiologic modulation of the Ninio effect would be another mechanism for "inducible evolution."^{16,17} Contingency loci type B offer another way to evolve mutation rates.

REFERENCES

1. MOXON, E.R., P.B. RAINEY, M.A. NOWAK & R.E. LENSKI. 1994. Adaptive evolution of highly mutable loci in pathogenic bacteria. *Curr. Biol.* **4**: 24-33.
2. MOXON, E.R. & D.S. THALER. 1997. The tinkerer's evolving toolbox. *Nature* **387**: 659-662.
3. MAGNASCO, M. & D.S. THALER. 1996. Changing the pace of evolution. *Physics Lett. A* **221**: 287-292.
4. HOOD, D.W., M.E. DEADMAN, M.P. JENNINGS. *et al.*, 1996. DNA repeats identify novel virulence genes in *Haemophilus influenzae*. *Proc. Natl. Acad. Sci. USA* **93**: 11121-11125.
5. TADDEI, F., M. RADMAN, J. MAYNARD-SMITH, B. TOUPANCE & P.H. B.G. GUYON. 1997. Role of mutator alleles in adaptive evolution. *Nature* **387**: 700-702.
6. SNIEGOWSKI, P.D., P.J. GERRISH & R.E. LENSKI. 1997. Evolution of high mutation rates in experimental populations of *E. coli*. *Nature* **387**: 703-705.
7. RAYSSIGUIER, C., D.S. THALER & M. RADMAN. 1989. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature* **342**: 396-401.
8. LEClerc, J.E., B. LI, W.L. PAYNE & T.A. CEBULA. 1996. High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science* **274**: 1208-1211.
9. MATIC, I, M. RADMAN, F. TADDEI *et al.* 1997. Highly variable mutation rates in commensal and pathogenic *Escherichia coli*. *Science* **277**: 1833-1834.
10. YAMAMOTO, H., H. SAWAI, T.K. WEBER, M.A. RODRIGUEZ-BIGAS & M. PERUCHO. 1998. Somatic frameshift mutations in DNA mismatch repair and proapoptosis genes in hereditary nonpolyposis colorectal cancer. *Cancer Res.* **58**: 997-1003.
11. NINIO, J. 1991. Connections between translation, transcription and replication error-rates. *Biochimie* **73**: 1517-1523.
12. NINIO, J. 1991. Translation and transcription errors on mutation rates. *Genetics* **129**: 957-962.
13. HALL, B.G. 1990. Spontaneous point mutations that occur more often when advantageous than when neutral. *Genetics* **126**: 5-16.
14. TORKELSON, J., R.S. HARRIS, M.J. LOMBARDO, J. NAGENDRAN, C. THULIN & S.M. ROSENBERG. 1997. Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation. *EMBO J.* **16**: 3303-3311.
15. FOSTER, P.L. 1997. Nonadaptive mutations occur on the F' episome during adaptive mutation conditions in *Escherichia coli*. *J. Bacteriol.* **179**: 1550-1554.
16. RADMAN, M. 1977. Inducible pathways in deoxyribonucleic acid repair, mutagenesis and carcinogenesis. *Biochem. Soc. Trans.* **5**: 1194-1199.
17. ECHOLS, H. 1981. SOS functions, cancer and inducible evolution. *Cell* **25**: 1-2.