JOURNAL OF VIROLOGY, Sept. 2005, p. 11555–11558 Vol. 79, No. 18
0022-538X/05/$08.00+0 doi:10.1128/JVI.79.18.11555–11558.2005

GUEST COMMENTARY

Adaptive Value of High Mutation Rates of RNA Viruses: Separating Causes from Consequences

Santiago F. Elena* and Rafael Sanjuán

Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas-UPV, València, Spain

As a consequence of the lack of proofreading activity of RNA virus polymerases, new viral genetic variants are constantly created. RNA viruses readily adapt to changing environmental conditions. Therefore, the high mutation rate of RNA viruses compared with DNA organisms is responsible for their enormous adaptive capacity.

The above syllogism, with some variation, is deeply rooted in the thinking of many virologists: RNA viruses mutate at the maximum error rate compatible with maintaining the integrity of genetic information (i.e., the error threshold) because this would allow them to quickly find the beneficial mutations needed for adaptation (12, 14, 23, 32). It is an unquestionable fact that RNA virus populations exist as swarms of mutant genotypes (13). Such enormous variability is an unavoidable consequence of the lack of exonuclease proofreading activity of the virus-encoded RNA polymerases (44) with, in some cases, the added contribution of recombination (20, 29, 33). However, the argument that the more mutations are generated, the faster adaptation proceeds is flawed because it ignores the fact that the vast majority of mutations are deleterious, hence hindering adaptation, as shown by recent theoretical developments (25, 34). Therefore, the adaptive value of the RNA virus extreme mutation rate has to be carefully reconsidered, and new alternative explanations, beyond a purely mechanistic level, should be taken into consideration.

WHEN DOES NATURAL SELECTION FAVOR HIGH MUTATION RATES?

The mutation rate itself is a trait that can evolve by natural selection, provided the existence of genetic variation for the character. Given that most mutations have deleterious fitness effects (9, 15, 17, 18, 31, 38), having a too high mutation rate would be prejudicial in the short term simply because (in a first approach) the population equilibrium fitness for a haploid asexual population decreases exponentially with mutation rate (8). In the long term, however, it can be argued that the higher the mutation rate, the more likely it is that beneficial mutations will be produced. An optimal mutation rate, which maximizes the rate of adaptation, is reached when these opposing factors are balanced (3, 25, 27, 34). Hypotheses about the high adaptability of RNA viruses should take into account this trade-off and address why the balance between beneficial and deleterious mutational effects leads to different outcomes in RNA viruses and DNA organisms, including other viruses.

Implicitly, the paradigm that high mutation rates allow for fast adaptation is based on the assumption that, with considerable frequency, the effect of beneficial mutations is strong enough to overcome the burden imposed by deleterious mutations, hence allowing for the hitchhiking of mutator strains with beneficial mutations. Most of the work exploring the evolutionary dynamics of mutator mutants has been done with bacteria, where the spread of some mutator genotypes due to hitchhiking has been experimentally documented (43). In short, mutator mutants must be favored by natural selection if they often face novel environmental conditions (11, 21). Novel conditions are created if (i) environments change rapidly over time, as for viruses coping with host defense mechanisms such as the immune system or RNA silencing, or (ii) environments are heterogeneous and consist of multiple ecological niches, as for viruses infecting different host species or even replicating into different tissues and organs within a single host. Certainly, these two conditions hold for most RNA viruses, but they also hold for DNA viruses. It is possible that in RNA viruses, this hitchhiking of mutator alleles occurs at a particularly high frequency, but there is no experimental evidences either supporting or rejecting this hypothesis, and further research is thus required.

As stated above, a necessary condition for natural selection to optimize the mutation rate is that genetic variability for mutation rate exists in RNA viruses. Indeed, evidence for this variability has been reported. On the one hand, mutator mutants have been isolated, for example, from influenza A virus (45) and human immunodeficiency virus type 1 (HIV-1) (22) populations. On the other hand, antimutator mutants have been repeatedly found associated with genotypes resistant to nucleoside analogues in HIV-1 (26, 35, 52) and poliovirus (36), presumably as a selected strategy to escape from the error catastrophe. Interestingly, increases in the mutant spectrum complexity of vesicular stomatitis virus (VSV) populations obtained by adding chemical mutagens to the environment did not translate into a higher rate of adaptation (30). Therefore, a decrease in mutation rate could come about with little or no loss of adaptive capacity but would benefit the population by slowing the accumulation of deleterious mutations. Indeed, this notion is supported by the observation that HIV-1 clones that became resistant to lamivudine by mutations increasing

* Corresponding author. Mailing address: Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas-UPV, Avenida de los Naranjos s/n, 46022 València, Spain. Phone: 34 963 877 895. Fax: 34 963 877 859. E-mail: sfelena@ibmcp.upv.es.

reverse transcription fidelity did not pay any cost in terms of adaptability (26). Together, the experiments described above failed to prove that changes in mutation rate necessarily translate into changes in the rate of adaptation. It is possible that negative results are a consequence of an insufficient amount of data or that the environments tested were too limited.

DID A HIGH MUTATION RATE EMERGE AS A SIDE EFFECT OF SELECTION FOR FAST REPLICATION?

One simple possible alternative to the adaptive value of the high mutation rate is the existence of a trade-off between replication efficiency and fidelity; i.e., increasing fidelity would come at a cost, resulting in a lower replication rate (10, 24). Biochemical assays of HIV-1 mutants resistant to nucleoside reverse transcriptase inhibitors suggest that this might be the case (1, 35). This hypothesis is in good agreement with RNA virus ecology. RNA viruses represent an extreme case of *r*-selection (i.e., selection for fast replication with poor resource exploitation), in which faster replicators are favored. As expected under *r*-selection, experimental evolution of viral populations faced with constant environments demonstrated that growth rates always increased dramatically (4, 6, 16, 19, 32, 37, 47). Therefore, shorter genomes might be favored by natural selection due to their fast replication, even with a fidelity cost. However, further experimental evidence is needed to support this hypothesis. As a possible experimental design, replacements in the polymerase gene of an RNA virus that cause amino acid changes could be introduced by site-directed mutagenesis and then changes in replication and mutation rates measured. A positive correlation between the effects of the introduced mutation on replication and mutation rates would provide support to the efficiency-fidelity model.

GENOME COMPACTNESS, ANTIROBUSTNESS, AND THE STRENGTH OF PURIFYING SELECTION.

Another promising avenue is to focus on how viral populations manage to deal with the detrimental consequences of a high mutation rate. Krakauer and Plotkin (28) proposed that mutational robustness (i.e., the ability to preserve a constant phenotype despite the genomic mutational load) can be achieved by two distinct mechanisms, each operating at a different range of population sizes. In small populations, such as those typical of most multicellular organisms, robustness evolves by masking the harmful effects of deleterious mutations, often by making certain functions redundant. Examples of such mechanisms include gene duplication, alternative metabolic pathways, or chaperone proteins that buffer against mutation-induced problems in other enzymes. All these mechanisms would produce phenotypes similar or identical to that of the unmutated wild type, such that individuals would have similar chances for survival and reproductive success. By contrast, in viruses or bacteria, which show very large population sizes, robustness might arise through population-level strategies. In the absence of redundancy mechanisms (i.e., genome complexity), individuals would be hypersensitive to deleterious mutations, but those individuals carrying harmful mutations would be readily eliminated from large populations due to the high efficiency of natural selection, thus preserving unmutated genomes in the population. Possible examples of such hypersensitivity include overlapping reading frames, haploidy, and the loss of systems for genome repair (28). In good agreement with this prediction, it has been recently shown that approximately 40% of random point mutations produced by VSV were lethal and that among nonlethal mutations 30% were deleterious, producing, on average, a 25% fitness reduction (38). Furthermore, this fraction of lethal mutants would rise if insertions/deletions are considered. In antiredundant genomes, even a single mutation is likely to cause a significant loss of fitness, but after the initial loss of functionality, further mutations may have a comparatively lesser impact on fitness, suggesting that positive epistasis should be the norm for viral genomes. In good agreement with this expectation, positive epistasis among deleterious mutations has been recently reported for bacteriophage ϕ6 (7), VSV (39), and HIV-1 (2).

SELECTION FOR ROBUSTNESS PUSHES VIRAL POPULATIONS TOWARD NEUTRAL REGIONS OF SEQUENCE SPACE.

Opposite to the antiredundant strategy, but also stemming from population-level selection, is the notion of "survival of the flattest," theoretically postulated by Schuster and Swetina (41) and Wilke (48). When neutral and back mutations are considered, the average equilibrium fitness depends not only on the mutation rate but also on the geometry of the fitness landscape (28, 50). This being the case, another selective pressure comes into play (especially at high mutation rates) that pushes populations towards regions of the landscape where the density of neutral mutations is higher (46, 48). As a consequence, the population has evolved increased robustness against mutations in the average individual genome. This phenomenon of selection for robustness at high mutation rates can be understood either as a pressure for populations to occupy highly connected areas of a neutral network or as a pressure to occupy broad rather than narrow fitness peaks; populations occupying high but narrow peaks are easily mutated to low fitness, whereas populations living in low but broad peaks are less susceptible to deleterious mutations. Although the prediction that the evolutionary fate of a genotype shall determined by its mutational neighborhood is consistent with observations made with bacteriophage ϕ6 (5), direct proof for the "survival of the flattest" theory comes only from digital organisms (51) and simulated RNA-folding evolution (49).

RNA VIRUSES VERSUS DNA VIRUSES

Probably a major challenge for understanding the evolution of mutation rates is the question of why DNA and RNA viruses, which apparently share similar lifestyles, show such different mutation rates. If, as stated by the adaptive value paradigm, RNA viruses have elevated mutation rates because this confers increased adaptability, then why do DNA viruses maintain proofreading mechanisms? It is well known that DNA polymerases can be fully functional without $3' \rightarrow 5'$ exonuclease activity (40), and hence, a DNA virus carrying a loss-of-function mutation should benefit from faster adaptation, eventually displacing strains with proofreading ability. Similarly, the efficiency-fidelity trade-off model is based on selection for fast

replication, which should apply for RNA viruses as well as DNA viruses. Although only partially, this apparent contradiction can be solved by noting that in RNA viruses, genetic information is even more compressed than it is in DNA viruses, because replication and transcription are biochemically equivalent, often catalyzed by a common molecular complex, and, in the case of positive-stranded RNA viruses, based on the same RNA molecule. However, differences between DNA and RNA viruses need to be further explored. For instance, the groups might differ in the intensity with which selection favors antiredundancy, or, alternatively, they might differ in their ability to replicate in neutral spaces, where many mutations can be tolerated. Finally, recent intriguing observations, such as, for example, that some single-stranded DNA viruses can show rates of nucleotide substitution closer to those of RNA viruses than to those of other DNA systems (42), point out the necessity of new hypotheses for the evolution of mutation rates. Genetic hitchhiking, selection for fast replication, antiredundancy selection, and evolution on neutral networks suggest tantalizing new explanations that are worth exploring.

ACKNOWLEDGMENTS

We thank James J. Bull, Fernando García-Arenal, and Claus O. Wilke for fruitful discussions on the adaptive value of virus extreme genetic variability. We also thank two anonymous reviewers for their very constructive comments.

This work has been supported by grants from the Spanish MEC-FEDER (BMC2003-00066 and BFU2004-22607E), the Generalitat Valenciana (GV04B280 and GRUPOS03/064), and the EMBO Young Investigator Program to S.F.E. R.S. is the recipient of a CSIC I3P postdoctoral contract.

REFERENCES

1. **Back, N., M. Nijhuis, W. Keulen, C. Boucher, B. Oude Essink, A. van Kuilenburg, A. van Gennip, and B. Berkhout.** 1996. Reduced replication of 3TC resistant HIV-1 variants in primary cells due to a processivity defect of the reverse transcriptase enzyme. EMBO J. **15:**4040–4049.
2. **Bonhoeffer, S., C. Chappey, N. T. Parkin, J. M. Whitcomb, and C. J. Petropoulos.** 2004. Evidence for positive epistasis in HIV-1. Science **306:**1547–1550.
3. **Bonhoeffer, S., and P. Sniegowski.** 2002. The importance of being erroneous. Nature **420:**367–368.
4. **Bordería, A. V., and S. F. Elena.** 2002. *r*- and *K*-selection in experimental populations of vesicular stomatitis virus. Infect. Genet. Evol. **2:**137–143.
5. **Burch, C. L., and L. Chao.** 2000. Evolvability of an RNA virus is determined by its mutational neighbourhood. Nature **406:**625–628.
6. **Burch, C. L., and L. Chao.** 2001. Evolution by small steps and rugged landscapes in the RNA virus ϕ6. Genetics **151:**921–927.
7. **Burch, C. L., and L. Chao.** 2004. Epistasis and its relationship to canalization in the RNA virus. Genetics **167:**559–567.
8. **Bürger, R.** 2000. The mathematical theory of selection, recombination, and mutation. Wiley, Chichester, United Kingdom.
9. **Chao, L.** 1990. Fitness of RNA virus decreased by Muller's ratchet. Nature **348:**454–455.
10. **Dawson, K. J.** 1998. Evolutionary stable mutation rates. J. Theor. Biol. **194:**143–157.
11. **de Visser, J. A. G. M.** 2002. The fate of microbial mutators. Microbiology **148:**1247–1252.
12. **Domingo, E.** 2000. Viruses at the edge of adaptation. Virology **270:**251–253.
13. **Domingo, E.** 2002. Quasispecies theory in virology. J. Virol. **76:**463–465.
14. **Domingo, E., and J. J. Holland.** 1997. RNA virus mutations and fitness for survival. Annu. Rev. Microbiol. **51:**151–178.
15. **Duarte, E. A., I. S. Novella, S. Ledesma, D. K. Clarke, A. Moya, S. F. Elena, E. Domingo, and J. J. Holland.** 1994. Subclonal components of consensus fitness in an RNA virus clone. J. Virol. **68:**4295–4301.
16. **Elena, S. F., M. Dávila, I. S. Novella, J. J. Holland, E. Domingo, and A. Moya.** 1998. Evolutionary dynamics of fitness recovery from the debilitating effects of Muller's ratchet. Evolution **52:**309–314.
17. **Elena, S. F., and A. Moya.** 1999. Rate of deleterious mutation and the distribution of its effects on fitness in vesicular stomatitis virus. J. Evol. Biol. **12:**1078–1088.
18. **Escarmís, C., M. Dávila, N. Charpentier, A. Bracho, A. Moya, and E. Domingo.** 1996. Genetic lesions associated with Muller's ratchet in an RNA virus. J. Mol. Biol. **264:**255–267.
19. **Escarmís, C., M. Dávila, and E. Domingo.** 1999. Multiple molecular pathways for fitness recovery of an RNA virus debilitated by operation of Muller's ratchet. J. Mol. Biol. **285:**495–505.
20. **Froissart, R., D. Roze, M. Uzest, L. Galibert, S. Blanc, and Y. Michalakis.** 2005. Recombination every day: abundant recombination in a virus during a single multi-cellular host infection. PLoS Biol. **3:**389–395.
21. **Giraud, A., M. Radman, I. Matic, and F. Taddei.** 2001. The raise and fall of mutator bacteria. Curr. Opin. Microbiol. **4:**582–585.
22. **Gutiérrez-Rivas, M., and L. Menéndez-Arias.** 2001. A mutation in the primer grip region of HIV-1 reverse transcriptase that confers reduced fidelity of DNA synthesis. Nucleic Acids Res. **29:**4963–4972.
23. **Holland, J. J., K. Spindler, F. Horodyski, E. Grabau, S. Nichol, and S. VandePol.** 1982. Rapid evolution of RNA genomes. Science **215:**1577–1585.
24. **Hopfield, J. J.** 1974. Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity. Proc. Natl. Acad. Sci. USA **71:**4135–4139.
25. **Johnson, T., and N. H. Barton.** 2002. The effect of deleterious alleles on adaptation in asexual populations. Genetics **162:**395–411.
26. **Keulen, W., A. van Wijk, R. Schuurman, B. Berkhout, and C. A. Boucher.** 1999. Increased polymerase fidelity of lamivudine-resistant HIV-1 variants does not limit their evolutionary potential. AIDS **13:**1349–1349.
27. **Kimura, M.** 1967. On the evolutionary adjustment of spontaneous mutation rates. Genet. Res. **9:**23–34.
28. **Krakauer, D. C., and J. B. Plotkin.** 2002. Redundancy, antiredundancy, and the robustness of genomes. Proc. Natl. Acad. Sci. USA **99:**1405–1409.
29. **Lai, M. M.** 1992. RNA recombination in animal and plant viruses. Microbiol. Rev. **56:**61–79.
30. **Lee, C. H., D. L. Gilbertson, I. S. Novella, R. Huerta, E. Domingo, and J. J. Holland.** 1997. Negative effects of chemical mutagenesis on the adaptive behavior of vesicular stomatitis virus. J. Virol. **71:**3636–3640.
31. **Malpica, J. M., A. Fraile, I. Moreno, C. I. Obies, J. W. Drake, and F. García-Arenal.** 2002. The rate and character of spontaneous mutation in an RNA virus. Genetics **162:**1505–1511.
32. **Novella, I. S., E. A. Duarte, S. F. Elena, A. Moya, E. Domingo, and J. J. Holland.** 1995. Exponential increases of RNA virus fitness during large population transmissions. Proc. Natl. Acad. Sci. USA **92:**5841–5844.
33. **Olsthoorn, R. C. L., A. Bruyere, A. Dzianott, and J. J. Bujarski.** 2002. RNA recombination in brome mosaic virus: effects of strand-specific stem-loop inserts. J. Virol. **76:**12654–12662.
34. **Orr, H. A.** 2000. The rate of adaptation in asexuals. Genetics **155:**961–968.
35. **Pandey, V. N., N. Kaushik, N. Rege, S. G. Sarafianos, P. N. Yadav, and M. J. Modak.** 1996. Role of methionine 184 of human immunodeficiency virus type-1 reverse transcriptase in the polymerase function and fidelity of DNA synthesis. Biochemistry **35:**2168–2179.
36. **Pfeiffer, J. K., and K. Kirkegaard.** 2003. A single mutation in poliovirus RNA-dependent RNA polymerase confers resistance to mutagenic nucleotide analogs via increased fidelity. Proc. Natl. Acad. Sci. USA **100:**7289–7294.
37. **Rokyta, D., M. R. Badgett, I. J. Molineux, and J. J. Bull.** 2002. Experimental genomic evolution: extensive compensation for loss of DNA ligase activity in a virus. Mol. Biol. Evol. **19:**230–238.
38. **Sanjuán, R., A. Moya, and S. F. Elena.** 2004. The distribution of fitness effects caused by single-nucleotide substitutions in an RNA virus. Proc. Natl. Acad. Sci. USA **101:**8396–8401.
39. **Sanjuán, R., A. Moya, and S. F. Elena.** 2004. The contribution of epistasis to the architecture of fitness in an RNA virus. Proc. Natl. Acad. Sci. USA **101:**15376–15379.
40. **Scheuermann, R., S. Tam, P. M. Burgers, C. Lu, and H. Echols.** 1983. Identification of the epsilon-subunit of *Escherichia coli* DNA polymerase III holoenzyme as the *dnaQ* gene product: a fidelity subunit for DNA replication. Proc. Natl. Acad. Sci. USA **80:**7085–7089.
41. **Schuster, P., and J. Swetina.** 1988. Stationary mutant distributions and evolutionary optimization. Bull. Math. Biol. **50:**635–660.
42. **Shackelton, L. A., C. R. Parrish, U. Truyen, and E. C. Holmes.** 2005. High rate of viral evolution associated with the emergence of canine parvovirus. Proc. Natl. Acad. Sci. USA **102:**379–384.
43. **Sniegowski, P. D., P. J. Gerrish, and R. E. Lenski.** 1997. Evolution of high mutation rates in experimental populations of *E. coli.* Nature **387:**703–705.
44. **Steinhauer, D. A., E. Domingo, and J. J. Holland.** 1992. Lack of evidence for proofreading mechanisms associated with an RNA virus polymerase. Gene **122:**281–288.
45. **Suárez, P., J. Valcárcel, and J. Ortín.** 1992. Heterogeneity of the mutation rates of influenza A viruses: isolation of mutator mutants. J. Virol. **66:**2491–2494.
46. **van Nimwegen, E., J. P. Crutchfield, and M. Huynen.** 1999. Neutral evolution of mutational robustness. Proc. Natl. Acad. Sci. USA **96:**9716–9720.
47. **Wichman, H. A., M. R. Badgett, L. A. Scott, C. M. Boulianne, and J. J. Bull.** 1999. Different trajectories of parallel evolution during viral adaptation. Science **285:**422–424.
48. **Wilke, C. O.** 2001. Adaptive evolution on neutral networks. Bull. Math. Biol. **63:**715–730.

49. **Wilke, C. O.** 2001. Selection for fitness versus selection for robustness in RNA secondary structure folding. Evolution **55:**2412–2420.
50. **Wilke, C. O., and C. Adami.** 2003. Evolution of mutational robustness. Mutat. Res. **522:**3–11.
51. **Wilke, C. O., J. L. Wang, C. Ofria, R. E. Lenski, and C. Adami.** 2001. Evolution of digital organisms at high mutation rates leads to survival of the flattest. Nature **412:**331–333.
52. **Wisniewski, M., C. Palaniappan, Z. Fu, S. F. Le Grice, P. Fay, and R. A. Bambara.** 1999. Mutations in the primer grip region of HIV reverse transcriptase can increase replication fidelity. J. Biol. Chem. **274:**28175–28184.

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.