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Letters to the Editor

Is the Quasispecies Concept Relevant to RNA Viruses?

The study of RNA virus evolution has blossomed over the last 20 years. Despite the emergence of this new discipline, there has been little active debate over perhaps the most fundamental question of all. Do RNA viruses evolve in a manner that is qualitatively different from other life forms? For some workers, two essential facets of RNA viruses—their tiny genomes and their high mutation rates—mean that these organisms are subject to different evolutionary rules than DNA-based life forms with much larger genomes and lower mutation rates. Central to this world view is the concept of the quasispecies, originally developed by Eigen and colleagues as an evolutionary model of the first RNA replicators (10) and applied to RNA viruses by Domingo, Holland, and coworkers (5, 6, 11). Such is the success of the quasispecies that it is frequently cited whenever sequence polymorphism is encountered in viral populations and used to explain an enormous array of evolutionary observations (5, 6, 11, 22). The counter position, though rarely stated, is that while RNA viruses might evolve extremely rapidly, they are subject to the very same evolutionary processes as other organisms, which can be explained by the concepts of mutation, genetic drift, and natural selection commonly used in population genetics (13, 16, 17).

The aim of our letter is to review the evidence for the quasispecies as a viable evolutionary model for RNA viruses. We claim that the quasispecies is at best an unnecessary and at worse a misleading description of RNA virus evolution and argue in favor of a population genetic approach, thereby placing the study of RNA viruses on the same footing as other organisms. It is our desire to stimulate debate and set down an agenda for future research.

The quasispecies defined. One of the most notable aspects of the quasispecies is that although rigorous definitions exist, most workers simply use the term as a surrogate for intrapopulation genetic variation, ignoring its true evolutionary meaning. While this usage does little harm, it is frustrating that the essence of the quasispecies is often unappreciated. The lack of rigor over definitions of the quasispecies has also been shown by Eigen (8).

In simple terms, the quasispecies refers to an equilibrium process of mutation and natural selection which generates a population of variable genomes. These genetic variants are organized around one or a set of genotypes of highest fitness known as master sequences (7, 8, 9, 10, 18). A critical element of quasispecies theory, and one which is not usually found in population genetic models, is that the frequency of any individual virus in the quasispecies is a function of both its own replication rate and the probability that it will arise by the erroneous replication of other members of the population. Consequently, viruses are not independent entities in the quasispecies but are linked by mutational couplings, so that the entire population forms a cooperative structure that evolves as a single unit. The consequence of this population structure is that natural selection is no longer directed toward the single fittest variant, as in most population genetic models, but instead acts on the whole mutant distribution—the quasispecies in its entirety—which will then evolve to maximize its average replication rate (7, 8, 9).

Another element of the quasispecies which distinguishes it from population genetic models is that the random sampling process of genetic drift is redundant, because the small genomes, large population sizes, and high mutation rates of RNA viruses mean that the sequence space (i.e., all possible allelic combinations) surrounding the master sequence will be completely explored, thereby preventing any drift from taking place (7, 9). Crucially, it is the absence of drift which produces the mutational coupling, in turn allowing natural selection to act on the quasispecies as a whole. This sits in contrast to population genetic models in which genetic drift would occur at neutrally evolving genomic sites, even in large populations. Furthermore, the lack of genetic drift means that although the master sequence will generate mutant genomes upon replication, thereby producing the quasispecies distribution, it will maintain a stable frequency in the population through time.

Do RNA viruses form quasispecies? Although the quasispecies is frequently cited, there have been few critical studies of whether it applies to populations of RNA viruses. The crucial question here is not whether the quasispecies concept is flawed, because both theoretical and simulation studies show that this is an important and viable model of sequence evolution given certain key assumptions, but whether the population structure it defines exists in nature.

The most basic evidence presented in favor of the quasispecies is that populations of RNA viruses are highly variable. While this is undoubtedly the case (although some have suggested that sequence diversity may be artificially elevated because of *Taq* polymerase error [21]), it is not in itself definitive evidence for the existence of a quasispecies. Extensive genetic variability can also be derived from a model based on mutation and selection acting exclusively on single, independently evolving viral genomes, so long as mutation rates are high enough. The assumption that RNA viruses are in mutation-selection equilibrium can also be questioned. Although some viral populations will undoubtedly be in such a state of balance, for those subject to powerful immune selection, such as human immunodeficiency virus and hepatitis C virus, which are often described as quasispecies, populations are more likely to be in a state of flux as mutants rise and fall in the population depending on their respective fitness.

At face value, much better evidence for the quasispecies is provided by the stability of consensus sequences through time. If the consensus sequence is assumed to represent the genome of highest fitness (i.e., the master sequence), then the observation that this remains intact despite high mutation rates could mean that the viral population forms a cooperative structure. The first evidence that this might be the case in RNA viruses occurred with laboratory populations of Qβ phage where an equilibrium distribution of closely related mutants was observed, with a stable consensus sequence maintained throughout passage history (4). Similar experiments on vesicular stomatitis virus (VSV) also revealed a stable consensus sequence over multiple passages in cell culture (23), which was again taken as evidence that this virus formed a quasispecies.

However, this view of viral evolution ignores the critical effects that neutral sites (i.e., those nucleotide positions where all possible alleles have equal fitness) have on population structure. Specifically, if an RNA virus genome contains neutral

sites, then the number of sequences close to maximum fitness may exceed the population size of the virus itself (13). This would prevent the formation of a quasispecies because the viral population could not occupy all the sequence space around the master sequence and so would be subject to genetic drift, which in turn would prevent the mutational coupling necessary for natural selection to act on the entire mutant distribution. Since every neutral site increases the size of the neutral space by a factor of four, only a small number are required for the number of possible genetic combinations to be far greater than realistic viral population sizes. For example, 50 neutral sites in an entire viral genome would be enough for the neutral space to exceed a population size of 10^{30}. Crucially, even with genetic drift operating in this manner, a stable consensus sequence can be found, as long as this sequence has the highest fitness and so is subject to stabilizing selection (13). Consequently, the stability of consensus sequences does not represent hard evidence for the existence of a quasispecies.

Unfortunately, little is known about how many truly neutral sites exist in viral genomes. In particular, it is likely that many synonymous sites, the most likely candidates for neutrally evolving positions, are in reality subject to selective constraints imposed by codon usage bias and RNA secondary structure. It is also possible that complex epistatic interactions exist between neutral sites, so that they do not act independently, and that the adaptive landscape is characterized by sharp peaks of high fitness separated by low fitness valleys. If the latter is true, and such a complex fitness landscape has been documented for the bacteriophage ϕ6 (1), then viral populations may become trapped in small regions of sequence space so that genetic drift is prevented and the population forms a quasispecies. Hence, determinations of the fitness of individual mutants and the nature of adaptive landscapes are key areas for future research (2), although these are clearly two of the most challenging tasks in evolutionary genetics. Despite these considerations, analyses of some viruses have revealed sufficient neutral sites for extensive drift, thereby preventing quasispecies formation (13).

One way in which proponents of quasispecies theory have sidestepped the problem of genetic drift at neutral sites is by redefining the quasispecies in terms of phenotypic space, perhaps reflecting a set of essential amino acid residues (20). As this reformation removes neutral sites, genetic drift is no longer a problem. Although perhaps a reasonable extension to the model, it evidently removes much of the distinction between quasispecies and population genetic models. Furthermore, the only genetic variants which would then be part of the quasispecies are those which occur at the relevant selected sites, perhaps a tiny minority of the total genetic variation seen in populations of RNA viruses, so that the revised theory has little explanatory power.

The discovery that genomes of bacteriophage ϕ6 surrounded by a sequence space of deleterious mutations evolved to lower fitness than genomes whose neighbors were of higher average fitness has also been cited as evidence for the quasispecies (2). Although important, this observation does not unambiguously demonstrate a quasispecies, especially if the lower fitness genomes had intrinsically higher deleterious mutation rates or if equilibrium conditions had yet to be reached. A more powerful proof of the quasispecies would be if genotypes (or phenotypes) of lower individual fitness were able to directly outcompete those of higher fitness because they were linked, by mutational coupling, to a set of high fitness genotypes with which they evolved in a concerted fashion. This would be evidence that the whole viral population is the unit of selection. Intriguingly, such an observation has been made for VSV, where a high fitness variant only came to dominate the population if it was introduced above a threshold level (3). This was taken to mean that high fitness variants were suppressed by interactions within the quasispecies, as the theory predicts. However, this observation can be equally well explained by population genetic models in at least two ways. The simplest explanation again involves genetic drift. Specifically, under genetic drift the probability that a high fitness variant achieves fixation is partially dependent on its initial frequency. Hence, most rare variants will be lost by drift in small populations, despite having superior fitness, and any allele, even if advantageous, is subject to the vagaries of drift when it is at low frequency. Since the population sizes in the VSV experiments were low, the sampling effects of genetic drift should be given careful consideration. A second explanation invokes clonal inference, which has recently been demonstrated in RNA viruses (14). Under this model, beneficial mutations that become transiently common but do not achieve ultimate fixation because of interfering beneficial mutations are relatively abundant. Furthermore, the probability of fixation of a beneficial mutation decreases with both population size and mutation rate.

The final piece of evidence in favor of the quasispecies, and perhaps the most controversial, is that viral populations may harbor a molecular memory of their evolutionary history which can be replayed if past selective pressures reappear (19). This notion stems from experiments with foot-and-mouth disease virus in which the mutations that accumulated during cell culture reflected the previous passage history of the viral population. Although this observation is presented as a form of evolutionary memory, in reality, however, the authors have merely demonstrated that, because of their high mutation rates, viral populations often harbor low frequency variants that can rapidly respond to changing selection pressures. Indeed, the repeatability of evolution in the face of common selection pressures is well documented in natural systems (12).

The power of population genetics. Although built on a firm theoretical basis, it is our contention that quasispecies models may be unrealistic descriptions of the evolution of RNA viruses in nature. In particular, we believe that there is a lack of clear-cut empirical evidence that populations of RNA viruses form quasispecies, particularly outside the laboratory environment, and that all the observations used to support the quasispecies theory can be equally well explained by classical population genetic models.

We also believe that there has been a general misunderstanding and misrepresentation of population genetics and its explanatory power. This parallels the gradualist versus punctuated equilibrium debate over models of macroevolution. In this example, proponents of the gradualist school were often depicted as advocating a constant rate of evolutionary change when, in reality, no such stipulation about rates is made. Historically, population genetics and quasispecies represent two research traditions, and both have their own theoretical tools to explain evolutionary dynamics. For example, it is incorrect to think that population genetic models only allow for selection to act on individual variants, because models for which the unit of selection is the group or closely related kin have also been proposed. Consider, for instance, theories about the evolution of pathogen virulence. Although many are based on the trade-off between virulence and transmission rate acting at the individual level (thereby maximizing the overall reproductive rate of the pathogen), those based on group selection, where the level of virulence attained is that which maximizes group fit-

ness, have also been proposed (15). Indeed, there is a fundamental agreement between quasispecies theory and group selection models, wherein the former fulfils the three conditions that are required to make the population as a whole the target of selection. First, it considers replicative entities whose population structure promotes fast genetic divergence. Second, the members of the population are intimately related. Third, the whole distribution of mutants should be considered as an individual, rather than as a group. As such, population genetic models based on group selection could, if required, lead to quasispecies distributions. Of course, the critical issue is definitive evidence for selection acting at the level of the group, which is notable by its absence in most evolutionary studies.

To conclude, we have two pleas: first, that the term quasispecies only be employed when there is formal evidence for the particular cooperative population structure defined above, and second, that many more studies, both in vivo and in vitro, be undertaken to examine the basic mechanics of viral evolution, particularly the intricacies of natural selection and fitness landscapes. While we recognize and applaud the central role quasispecies theory has played in introducing evolutionary ideas into virology, we believe it is now time to look farther afield and to encompass the full range of ideas that exist in contemporary evolutionary biology. This may, in turn, lead to a new and more complete understanding of the processes that shape the evolution of RNA viruses.

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REFERENCES

1. **Burch, C. L., and L. Chao.** 1999. Evolution by small steps and rugged landscapes in the RNA virus ϕ6. Genetics **151:**921–927.
2. **Burch, C. L., and L. Chao.** 2000. Evolvability of an RNA virus is determined by its mutational neighbourhood. Nature **406:**625–628.
3. **De La Torre, J. C., and J. J. Holland.** 1990. RNA virus quasispecies populations can suppress vastly superior mutant progeny. J. Virol. **64:**6278–6281.
4. **Domingo, E., D. Sabo, T. Taniguchi, and C. Weissman.** 1978. Nucleotide sequence heterogeneity of an RNA phage population. Cell **13:**735–744.
5. **Domingo, E.** 1992. Genetic variation and quasispecies. Curr. Opin. Genet. Dev. **2:**61–63.
6. **Domingo, E., and J. J. Holland.** 1997. RNA virus mutations and fitness for survival. Annu. Rev. Microbiol. **51:**151–178.
7. **Eigen, M.** 1987. New concepts for dealing with the evolution of nucleic acids. Cold Spring Harbor Symp. Quant. Biol. **52:**307–319.
8. **Eigen, M.** 1996. On the nature of viral quasispecies. Trends Microbiol. **4:**216–218.
9. **Eigen, M.** 1996. Steps towards life. Oxford University Press Inc., New York, N.Y.
10. **Eigen, M., and P. Schuster.** 1977. A principle of natural self-organization. Naturwissenschaften **64:**541–565.
11. **Holland, J. J., J. C. De La Torre, and D. A. Steinhauer.** 1992. RNA virus populations as quasispecies. Curr. Top. Microbiol. Immunol. **176:**1–20.
12. **Huey, R. B., G. W. Gilchrist, M. L. Carlson, D. Berrigan, and L. Serra.** 2000. Rapid evolution of a geographic cline in size in an introduced fly. Science **287:**308–309.
13. **Jenkins, G. M., M. Worobey, C. H. Woelk, and E. C. Holmes.** Evidence for the non-quasispecies evolution of RNA viruses. Mol. Biol. Evol., in press.
14. **Miralles, R., P. J. Gerrish, A. Moya, and S. F. Elena.** 1999. Clonal interference and the evolution of RNA viruses. Science **285:**1745–1747.
15. **Miralles, R., A. Moya, and S. F. Elena.** 1997. Is group selection a factor modulating the virulence of RNA viruses? Genet. Res. **69:**165–172.
16. **Morse, S. S.** 1994. Toward an evolutionary biology of viruses, p. 1–28. *In* S. S. Morse (ed.), The evolutionary biology of viruses. Raven Press, New York, N.Y.
17. **Moya, A., S. F. Elena, R. Miralles, and E. Barrio.** 2000. The evolution of RNA viruses: a population genetics view. Proc. Natl. Acad. Sci. USA **97:** 6967–6973.
18. **Nowak, M. A.** 1992. What is a quasispecies? Trends Ecol. Evol. **7:**118–121.
19. **Ruiz-Jarabo, C. M., A. Arias, E. Baranowski, C. Escarmís, and E. Domingo.** 2000. Memory in viral quasispecies. J. Virol. **74:**3543–3547.
20. **Schuster, P., and P. F. Stadler.** 1999. Nature and evolution of early replicons, p. 1–24. *In* E. Domingo, R. Webster, and J. Holland (ed.), Origin and evolution of viruses. Academic Press, London, United Kingdom.
21. **Smith, D. B., J. McAllister, C. Casino, and P. Simmonds.** 1997. Virus "quasispecies": making a mountain out of a molehill? J. Gen. Virol. **78:**1511–1519.
22. **Steinhauer, D. A., and J. J. Holland.** 1987. Rapid evolution of RNA viruses. Annu. Rev. Microbiol. **41:**409–433.
23. **Steinhauer, D. A., J. C. De La Torre, E. Meier, and J. J. Holland.** 1989. Extreme heterogeneity in populations of vesicular stomatitis virus. J. Virol. **63:**2072–2080.

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