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Quasispecies Theory in Virology

Holmes and Moya claim that quasispecies is an unnecessary and misleading description of RNA virus evolution, that virologists refer to quasispecies inappropriately, and that there is little evidence of quasispecies in RNA virus evolution. They wish to look for other ideas in evolutionary biology and to set down an agenda for future research. I argue here that real virus quasispecies often differ from the theoretical quasispecies as initially formulated and that this difference does not invalidate quasispecies as a suitable theoretical framework to understand viruses at the population level.

In the initial theoretical formulation to describe error-prone replication of simple RNA (or RNA-like) molecules, quasispecies were defined as stationary (equilibrium) mutant distributions of infinite size, centered around one or several master sequences (16, 20). However, as recently published by Eigen (17a), the nonlinear differential equations that define error-prone replication in finite populations have a linear approximation that can be solved. Thus, finite viral distributions can also be described as quasispecies (17a). The quasispecies theory established a link between Darwinian evolution and information theory and represented a deterministic approach to evolution. It was soon recognized that such an approach had limitations due to the nondeterministic nature of mutagenesis and to statistical fluctuations. Equilibrium in real viral populations is often perturbed by internal and external influences (environmental modifications, sampling events, etc.). The portion of sequence space explored by a mutant distribution depends on the population size, and it will be generally variable in space and time. The proportion of neutral sites which participate in such exploration is unknown and, given the evidence of phenotypic involvement of viral genomic RNA (in addition to its protein-coding function), such a proportion cannot be inferred from the frequency of synonymous mutations. No conclusions on quasispecies can be drawn from the analysis of consensus genomic sequences of virus isolates. The differences between steady-state, equilibrium mutant distributions and real viral populations have been recognized and extensively discussed (11, 13, 19, 28, 35). Disclosure of such differences, even if it were based on relevant observations, represents no novelty.

It must be stressed that treatments have been extended to finite populations (17a, 19, 27), and a number of phase transitions inherent to quasispecies theory have proven relevant to RNA virus evolution: time-evolution of fitness and virus entry into error catastrophe (1, 20, 35, 37). The scope of application of the quasispecies theory has broadened as have many concepts in science, including those of population genetics (see page 146 in reference 11 and page 221 in reference 38). Quasispecies has a physical, a chemical, and a biological definition (13, 18). Virologists use the chemical definition as rated distributions of nonidentical but closely related RNA genomes (10, 11, 17, 18, 27). Biologically, the quasispecies is the target of selection since ensembles of mutants rather than individual genomes rise to dominance and individual genomes may have only a fleeting existence (9, 11, 13, 18, 23). When virologists refer to unique features of RNA genetics, they mean the great adaptive potential of RNA viruses—the capacity to explore sequence space—resulting from high mutation rates and population complexity (10, 11, 13, 21, 23, 26). Virologists do not mean that mutations, competition, or selection is of a particular sort. Darwinian principles in connection with quasispecies have been explicitly invoked by theoreticians and experimentalists alike (11, 13, 16, 35).

What is the evidence of quasispecies dynamics in RNA virus populations, and why is quasispecies theory exerting an influence in virology? The initial experiment with phage Qβ which provided the first experimental support for a quasispecies dynamics in an RNA virus (14, 17) has now been carried out with biological and molecular clones of representatives of the major groups of human, animal, and plant RNA viruses, including immunodeficiency viruses and hepatitis C virus, both in cell culture and in vivo (11). Support for quasispecies has also come from studies on replication of RNA molecules in vitro (4). Upon replication, an infectious genome evolves into a mutant spectrum that may or may not maintain a stable consensus or average sequence (population equilibrium) over many generations, in cell culture or during natural infections (14, 17, 21, 23). The consensus sequence need not be identical to the dominant or master sequence. Direct fitness measurements of components of mutant spectra provide little evidence of strict neutrality (14, 15, 30). In the absence of evidence of a clonal origin, virologists sometimes infer a quasispecies structure, and this indeed is not a rigorous use of the concept (17). Yet, from our current knowledge of the composition of mutant spectra, polymorphism does not seem an accurate term either (11). A few authors have made considerable efforts to attribute the complexity of the mutant spectra to artifactual mutations introduced during the process of reverse transcription-PCR amplification of viral RNA. Yet it must be clear to any observant scientist that the quasispecies nature of several RNA viruses was documented before in vitro amplification procedures were available (reviewed in reference 12) and that with the available methodology, sequencing of biological clones (not subject to amplification uncertainties) and molecular clones provides a similar description of mutant spectrum complexity (2).

Measurements of high mutation rates together with the framework provided by quasispecies theory have been crucial to understanding that virus populations are made of extremely complex and dynamic mutant swarms and that the consensus sequence may not even exist physically in the populations under study (6, 11, 18, 22). Mutant spectra provide a rich repertoire of genetic and phenotypic variants for adaptability, a repertoire which is continuously replenished upon RNA replication. This has been the main reason for the influence of quasispecies in virology, as it bears not only on virus population structure and its evolution but also on viral pathogenesis and disease control strategies. Such influence had not been achieved by other theories of population biology for reasons previously discussed (11), the main one being that quasispecies put the emphasis on error-prone replication so that the wild type existed only as an average of different structures (10, 12, 16–20, 23, 27, 28, 35). Furthermore, quasispecies has represented the introduction of studies on complexity to virology, a field that is currently under development and that goes beyond specific models of population biology. I list a few relevant observations and developments that bear on the quasispecies nature of RNA virus populations: (i) the decrease in the frequency of the most abundant genome in a mutant spectrum (the "master" in quasispecies theory) as the populations come

closer to the error threshold (36); (ii) the existence of thresholds for genetic and phenotypic expression, not only with vesicular stomatitis virus, the pioneer observation (9), but with other phenotypes both in cell culture and in vivo (11); (iii) a predictable nonlinear behavior with the presence of critical (divergence) points during viral competitions (31) and the recently introduced concept of contingent neutrality (30); (iv) the presence of memory genomes in viral quasispecies (33), with its implications for viral diagnosis (memory consists of the persistence in the mutant spectrum of minority genomes which were dominant in the prior evolutionary history of the viral quasispecies, a point misunderstood by Holmes and Moya); (v) a possible connection between mutant spectrum complexity and host range size (34), pathogenic potential of virus populations, and outcome of antiviral therapies (11, 29); (vi) the recognition of combination therapies and multivalent vaccines as a means to counteract the adaptive potential of dynamic mutant spectra (6, 10, 11, 22); (vii) the identification in mutant spectra of mutations for fitness gain of the dominant genome in the quasispecies (3); and (viii) virus extinction through increased mutagenesis (7, 8, 24, 25, 36). This is a promising new development, directly predicted by quasispecies theory, which may result in a new antiviral strategy. It will never be possible to know how many of these developments would have taken place, and at what pace, in the absence of quasispecies theory, but it is highly questionable that quasispecies was a misleading influence. In these experiments, ensembles of mutants, rather than individual genomes, rise to dominance, there are modulating effects of the mutant spectra, there is memory as a property of the quasispecies as a whole, and there is a collective transition to higher complexity and loss of infectivity in response to increased mutagenesis. There is cross-talk among components of the mutant spectra. We could have interpreted some individual observations by alternative evolutionary models, but quasispecies theory provides a coherent picture for all of them. Conclusions and predictions from model studies in cell culture fit many observations made during natural viral infections (6, 11, 12, 21, 22). Obviously, as in any active field of research, new questions are continuously being posed, and many remain unanswered.

Nothing impedes Holmes and Moya from developing a new theory or using old ones. In fact, new theories that combine stochastic and deterministic features have been developed for application to virology (32). In the literature on quasispecies, use is frequently made of concepts of population genetics when needed (birth and death model, Muller's ratchet, competitive exclusion principle, Red Queen hypothesis, Wrightian adaptive landscapes, punctuated equilibrium, etc., as reported in references 5, 11, 22, and 27). The main objective has to be the understanding of the nature of viral populations and their adaptive capacities. Will other theories replace quasispecies in the future? Certainly. Progress in science is basically associated with new technological developments (instruments and experimental procedures) and new theories that replace old ones. However, a new theory does not become dominant as a result of the will of one or a few scientists. For it to replace quasispecies, a new theory must have broader explanatory and experiment-provoking powers than quasispecies. When it comes, virologists will not miss the opportunity to use it to confront the challenges of current virology.

Due to space limitations, only a few references on primary work are included. Additional ones can be found in the quoted review articles and books.

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