In their recent review, Más et al. propose that the existence of quasispecies has become a universal rule for RNA viruses and may be extended to other biological systems. Although the historical perspective on quasispecies theory provided by Más et al. serves as a valuable introduction to the subject, their article presents a polarized view of the applicability of quasispecies theory to RNA virus evolution and contains a number of important misunderstandings. Herein, I present a counterargument: that for all its power as a theory, the data needed to demonstrate the existence of quasispecies in natural populations of RNA viruses are still lacking. Although elements of quasispecies theory have been debated for a number of years, there is clearly still a major intellectual chasm between the proponents and opponents of quasispecies dynamics in RNA viruses.

Even the strongest supporters of quasispecies theory should acknowledge that the overwhelming majority of papers claiming to report evidence of RNA virus quasispecies do nothing of the sort. In common parlance, the term ‘quasispecies’ is used by virologists to refer to any type of intra-host genetic variation. For example, it is now de rigueur to refer to intra-host populations of hepatitis C virus as quasispecies. The reality, however, is that in every case the relevant authors have simply observed intra-host genetic variation, which is necessary, yet not sufficient, to demonstrate quasispecies behavior. While this terminological misuse does little harm in itself, it does provide false support for the applicability of quasispecies theory to RNA viruses. Although a variety of definitions of quasispecies exist, a common theme is that the target of natural selection is the viral population as a whole, rather than individual variants as is the case in most population genetic models. This particular form of group selection arises because mutation rates are so high in RNA systems that there is effectively a ‘mutational coupling’ between variants, so that they evolve as a unit. As noted by Más et al., this focus on the group rather than on the individual leads to the most interesting prediction of quasispecies theory: that populations with mutant distributions that do not differ greatly in fitness can sometimes outcompete those that contain mutants with a far wider range of fitness values, including some of very high individual fitness. Such a phenomenon has been dubbed ‘survival of the flattest’ to distinguish it from the Darwinian notion of ‘survival of the fittest’ in which mutations of individual highest fitness are favored. The survival of the flattest can also be thought of as a form of ‘mutational robustness’, that is, the
maintenance of phenotype in the face of deleterious mutation pressure.

There is no doubt that the mathematical theory behind the quasispecies is both elegant and meaningful if the key assumptions are upheld. It is also true that there is some experimental evidence that RNA viruses form quasispecies when certain conditions are upheld. But it is the careful examination of these conditions that tells us why, on current data, quasispecies theory is unlikely to be relevant for RNA viruses in nature. Specifically, those experimental studies that provide the best evidence for quasispecies dynamics all involve artificially elevated mutation rates, and even here, it can be argued that quasispecies behavior will be transient. In the case of two viroids infecting chrysanthemum plants, populations possessing ‘normal’ mutation rates did not behave like quasispecies: the target of selection was the fittest rather than the flattest.\(^5\) It was only when mutation rates were artificially elevated through treatment of the host plants with ultraviolet C that the survival of the flattest was observed. Essentially the same observation was made with vesicular stomatitis virus, in this case using the mutagens 5-fluorouracil and 5-azacytidine to elevate mutation rates.\(^6\) Such a lack of mutational power also seems to be true of studies using ‘digital organisms’. Although it is possible to evolve, \textit{in silico}, populations that exhibit survival of the flattest (high mutational robustness\(^6\)), the mutation rates required to do so are perhaps so high as to be unrealistic for natural systems.\(^7\) In sum, although RNA viruses are the exemplars of rapid mutation, it may be that their mutation rates in nature are not high enough to see the regular occurrence of quasispecies dynamics. Ironically, this means that the human immunodeficiency virus, long considered the poster child of quasispecies behavior, is unlikely to form a quasispecies as the background error rate of reverse transcriptase is perhaps 3- to 5-fold lower than that of RNA-dependent RNA polymerase.\(^8\)

Más et al. discuss two other pieces of evidence that RNA viruses form quasispecies: that quasispecies theory provides the theoretical bedrock to antiviral therapy through ‘lethal mutagenesis’ and that quasispecies may be central to viral pathology.\(^9\) Although there is no denying that quasispecies theory has been central to the development of treatment strategies based on the application of mutagens,\(^10,11\) it is another matter to claim that the quasispecies explains lethal mutagenesis. This rests on the subtle, but essential, difference between generating an ‘error catastrophe’, which proponents of quasispecies theory claim to explain lethal mutagenesis, and breaching an ‘extinction threshold’, which may be a more realistic explanation and which does not rely on quasispecies dynamics.\(^12,13\)

Of course, debating the true explanation for lethal mutagenesis should not detract from the importance of its action. The observation that neuropathology in poliovirus infection required rapidly mutating and diverse viral populations has been lauded as a powerful demonstration of the quasispecies in action.\(^14\) Although this work undoubtedly provides an exciting new perspective on the factors shaping the evolution of mutation rates in RNA viruses, it is also the case that there was no clear demonstration that the target of natural selection was the group rather than the individual.

One of the most intriguing suggestions made in the poliovirus study was that ‘cooperation’ between mutational variants allowed the brain infection to occur.\(^15\) The notion that RNA viruses cooperate was also a major theme running through the paper of Más et al.\(^1\) Although the RNA virus quasispecies is often said to involve cooperation, mechanistically it is difficult to imagine how such cooperation could ever occur. More pertinently, the quasispecies is demonstrably \textit{not} about cooperation, but rather is based on the joint effects of mutation and selective competition. If the quasispecies is to be taken seriously by evolutionary biologists, it is essential that it is described in scientifically rigorous terms and not in a manner that engenders it with almost mystical properties such as cooperation. In reality, the quasispecies is nothing more than an interesting form of ‘mutation–selection balance’ that occurs at very high mutation rates.

Más et al. suggest that quasispecies theory challenges the Darwinian dogma that natural selection favors individual variants.\(^1\) To me, the true dogma to be challenged is that all RNA viruses form quasispecies without considering the defining feature of this theory: that natural selection favors groups rather than individuals. The true accomplishment of quasispecies theory is that it has introduced useful and important evolutionary ideas into virology. The danger is that over-hyping the RNA virus quasispecies, and giving it properties that are poorly defined, will damage the credibility of virology as an evolutionary science. Indeed, I contend that there is currently no evidence for the defining feature of quasispecies dynamics in natural populations of RNA viruses; that natural selection acts on the group rather than the individual. In part, this could be a function of the fact that most studies of natural selection in RNA viruses have considered mutants with major fitness effects, such as those that confer immune escape or resistance to antiviral drugs. In these cases, natural selection uniformly seems to favor the fittest. The goal for the future should be to consider the nature of selection on mutants with smaller fitness effects. More generally, my challenge for students of viral evolution for the next decade is to provide unequivocal evidence that RNA viruses in nature indeed form quasispecies. At present, the quasispecies is a theoretically elegant, but perhaps unnecessary, evolutionary theory for RNA viruses. At worst, it may be positively misleading.

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