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CONCEPTS & SYNTHESIS

Quasispecies productivity

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Abstract
The quasispecies theory is a helpful concept in the explanation of RNA virus evolution and behaviour, with a relevant impact on methods used to fight viral diseases. It has undergone some adaptations to integrate new empirical data, especially the non-deterministic nature of mutagenesis, and the variety of behavioural motifs in cooperation, competition, communication, innovation, integration, and exaptation. Also, the consortial structure of quasispecies with complementary roles of memory genomes of minority populations better fits the empirical data than did the original concept of a master sequence and its mutant spectra. The high productivity of quasispecies variants generates unique sequences that never existed before and will never exist again. In the present essay, we underline that such sequences represent really new ontological entities, not just error copies of previous ones. Their primary unique property, the incredible variant production, is suggested here as quasispecies productivity, which replaces the error-replication narrative to better fit into a new relationship between mankind and living nature in the twenty-first century.

Keywords Virus · Mutation · Genetic innovation · Consortial properties · RNA networks · Early evolution

Introduction

Since the advent of deep sequencing methodologies and their application to metagenomics surveys in the last decades, the generally accepted view is that viruses are the most abundant biological entities on this planet (Koonin 2009; Forterre 2013). They outnumber cellular organisms by ten times and affect all cellular entities during their whole lifetime from the beginning of evolution to date (Forterre and Prangishvili 2013; Rohwer et al. 2014). Most viruses do not cause diseases and epidemics but invade host genomes persistently without harming the host (Villarreal 2005). As defective viral parts, they remain exapted or co-opted as effective regulatory RNAs for cellular needs (Vignuzzi and López 2019). Without the abundance of viral host interactions, no cellular life could exist, and no evolutionary progress in the emergence of new species would have occurred (Koonin et al. 2022). The virosphere represents the evolutionary and regulatory key player within the roots and stem of the tree of life (Villarreal and Witzany 2010). One reason for this success story of viruses is their high mutation rate, which guarantees nearly incomprehensible variability in the virus populations as well as in the virus-host relationship. The reason for this high variability is explained in the quasispecies concept of Manfred Eigen and Peter Schuster (Eigen and Schuster 1977).

Additionally, our knowledge about RNA networks and their roles in gene regulation dramatically increased. Short RNA sequences may snap back and build stem-loop structures with a stem of double-stranded RNAs and loops that are single-stranded but highly binding prone to foreign RNAs (Higgs and Lehman 2015). Through various evolutionary ligation procedures, they conserved the tRNA structures and ribosomal subunits (Briones et al. 2009). Such RNA stem-loops spontaneously generate bulges and loops and change their interaction potential (Smit et al. 2006). In contrast to the leading opinion in the twentieth century on gene expression, more recently it has been found that most genes in complex organisms express regulatory RNAs (Mattick and Amaral 2022).

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This paper summarises the original quasispecies concept and its necessary adaptations to more recent empirical data on behavioural motifs of viruses with its evolutionary impact on cellular life.

The origin of the quasispecies theory

In the early 70s of the twentieth century, Manfred Eigen and Peter Schuster formulated a fundamental physico-chemical concept that integrated Darwinian evolution, mathematical information theory, and statistical mechanics as a purely deterministic approach. The concept assumed a steady-state mutant distribution of virus isolates in equilibrium far from environmental impacts. Additionally, this concept provided an explanatory model for the origin of life driven by the inherent processuality of RNA replicators (Eigen and Schuster 1977). At the core of this concept, the replication of RNA molecules with a high error rate was described. Quasispecies represent RNA collectives that are error products of replication with prevalent production of mutant genomes. They called this 'mutant distribution' of infinite size around one or more master sequences. The application of quasispecies theory represented the first attempt to coherently explain the planetary unique variability of RNA viruses.

Additionally, a second important consequence of these quasispecies populations is that there exists a limitation to the information that can be inherited. If this limitation is overstepped by too high a mutation rate, a so-called error threshold leads to a population collapse of quasispecies until extinction (Eigen 1971). This seminal concept has developed into lethal mutagenesis as a strategy currently used in antiviral pharmacology (Domingo 2005; Perales et al. 2019).

The context-dependent nature of mutagenesis

Despite the initial quasispecies theory having been formulated deterministically for mathematical tractability, it was soon realised that extensions to non-deterministic models would best fit the reality of viral quasispecies, and generalizations of the initial quasispecies theory were developed (Saakian and Hu 2006; Domingo and Schuster 2016). The non-deterministic nature of mutant swarm formation and their evolutionary outcomes has several underlying influences. A very initial one is the unpredictable generation of mutations due to quantum mechanical indeterminations in the electronic distributions in nucleobases that influence the occurrence and frequency of the different tautomeric forms of a base. Such tautomeric variations are one of the drivers of base-to-base mispairings and, thus, of introduction of mutations during RNA genome replication. This initial unpredictable mutation occurrence is followed by random events, due to bottlenecks at various stages of the infectious cycle of viruses, notably during cell-to-cell or host-to-host transmission. In practical terms, the master sequence that presided the original, deterministic quasispecies formulation is blurred in favor of broad mutant spectra organised in functional ensembles that have been referred to as cooperative consortia (Villarreal and Witzany 2013).

The extremely high adaptive capability of RNA viruses was explained by their error-prone replication and the production of closely related variant genomes in interaction with a more static DNA-based biosphere (Holland et al. 1982). Interestingly, Holland and colleagues mentioned that error-prone replication of a quasispecies genome swarm in infected individuals is not only an error in copying the master sequence. It is a really new and unique entity, which 'has never existed before and will ever exist again' (Holland et al. 1992).

This newly adapted quasispecies concept is also valid in the explanation of real-life world environments; the changing conditions and non-deterministic contextuality of quasispecies now are far away from observations limited to virus isolates. It better explains the various forms of genetic variation, competition, and selection and gives better insights into viral fitness, such as fitness gain through genome segmentation (Garcia-Arriaza et al. 2004). It pertains also to the multiple recombinant (and recombination intermediate) forms often inherent to replication in some RNA viruses that compete to provide molecularly viable (functional) solutions (Bentley et al. 2021). The medical benefit of this concept is that with technically constructed error thresholds, the epidemic infection waves may be stopped because infecting viral clouds crash down by information overload (Lázaro et al. 2003).

Too much information: error threshold

Crossing the limit into overproduction with increased mutational loads leads to RNA virus extinction because the critical limit for heritable information is overstepped. The error threshold introduced by Eigen and Schuster means the maximum at which the dominant sequence can stabilise the whole mutant consortium.

Quasispecies populations of RNA viruses explore new sequence space, generating new and non-deterministically identified variants, which are subject to biological selection processes. Such exploration continues being active even upon extensive replication in the absence of externally applied perturbations, as shown with experimental evolution studies with the hepatitis C virus in which the occupation of sequence space was monitored (Moreno et al. 2017). Therefore, the continuous input of new variants is an important driver of genetic novelty and of impact on host evolution

(Domingo et al. 2012). The various forms of error threshold that limit virus adaptability by information damage are neutralised by the virus/virus competitions that may lead to dominating variant clouds out of nearly outcompeted parts of populations in producing better variations than previously existed (Cuesta et al. 2011). Error threshold in real is exploring the limits of variant possibilities until the end. However, surprisingly, the end does not exist because, unless the mutational input becomes unbearably high due to externally introduced mutagenic agents, quasispecies cooperation and competition always succeed (Domingo-Calap et al. 2019). This is important for virus-host co-evolution because, principally, it opens new ways of adaptation to whatever will happen in the real-life world.

History-dependent memory genomes

Additionally, another feature has been noticed to play important roles in the quasispecies mutant spectrum. Minority genomes that cannot be identified by analyses of the consensus sequence play crucial roles in resistance to antiviral inhibitors, or neutralizing antibodies. Thus, minority genomes play complementary roles to the viral clouds of viral quasispecies populations and add important features to viral identities according to environmental (and contextual) circumstances such as the immune system of the host (Briones and Domingo 2008). These memory genomes are endowed with history-dependent consortial properties, which enrich the adaptability of the whole quasispecies population (Ruiz-Jarabo et al. 2000). Minority genomes may store information of previous selection processes in a minority of populations. These may play complementary roles in quasispecies fitness (Arias et al. 2004; Garcia-Arriaza et al. 2004). Not to forget the various motifs of recombination with other virus types, which lead to assemblies of RNA (+/-) DNA and retroviruses, with high extension of variables and increasing tendency to cross host barriers (Stedman 2015). The adaptive potential of this quasispecies cooperation cannot be simply error-based. The common use of 'error copy' generally designates a deficient or damaged variant of an original entity, not an optimised and better variant.

How clouds of genetic parasites persistently invade host genomes

With the hypercycle model, Eigen and Schuster could explain the evolutionary step from self-replicating quasispecies to DNA storage medium of cellular life, not to forget the essential roles of reverse transcriptases that translate RNA information into DNA (Eigen and Schuster 1978). However, the origin of cellular life did not stop and is constantly affected by viral infection events and integration of genetic material into host cell DNA. To coherently explain the most common lifeform of viruses, the host persistence, Villarreal suggested the model of 'addiction modules' (Villarreal 2012). Shortly summarised, competing viral clouds together with host immune systems reach equilibrium status that is inheritable and dramatically changes host genetic identity such that the host now uses defective viral sequence parts to better adapt to environmental circumstances. The resulting addiction module of counterbalancing parts of former competing genetic parasites now serves as an immune function against related genetic parasites (Villarreal 2009a). The parts are addicted to one another as a stable toxin and an unstable antitoxin. It protects the host, compared to members of the same population that do not possess this module. They can be found as various RNA networks such as mobile genetic elements and all the non-coding RNAs being relevant in every step of cell development and regulation. Prominent examples of addiction modules that can be found in bacteria include toxin/antitoxin and restriction/modification modules, but any insertion deletion module, and even other counter-regulated pathways, may derive from such competitive viral clouds with host equilibrium status (Mruk and Kobayashi 2014). The addiction module can become unstable again through different circumstances, e.g. stress situations, which may inactivate the counter balance and become virulent again for some time. This is demonstrated by various forms of symptoms of human herpes virus (Villarreal 2009b).

Insufficiently complex

The deterministic approach of the original concept of Eigen and Schuster is insufficiently complex to integrate more recently found empirical data about the social life of viruses (Díaz-Muñoz et al. 2017). Although Eigen mentioned that quasispecies represent self-instructing species that 'resemble, in many ways, social behaviour', a deterministic approach cannot integrate the variety of cooperative interactions on intra- and inter-cellular levels. Extensions of the initial deterministic theory to embrace non-equilibrium conditions have been developed (Saakian and Hu 2006; Domingo and Schuster 2016). In addition, cooperative and communicative competencies opened new insights to a rich social life of viruses, which affects the whole range of cell-based evolution and diversity of the tree of life from its roots unto its leaves (Sanjuán 2021). The social behaviour of biotic agents cannot be completely formalised because the communication needs signals, commonly shared rules for their use, and a real-life world context, which determine the final meaning of the signals. Especially, the context-dependent meaning of genetic information is out of the current scope of mathematical equations (Witzany 2014).

Quasispecies are competent in innovation, integration, regulation, and exaptation

It is important to notice that RNA viruses within RNA networks and their infective capacity enrich genomic habitats and sequence space as open systems. Therefore, any given host sequence may be affected in its integrity by invading genetic parasites. The newly added sequence parts provided by the genetic parasites change the genetic identity of hosts with serious consequences for the host populations, influencing self/non-self recognition. The core competencies of such genetic parasites are innovation, integration, regulation, and exaptation (Villarreal 2008). This means the infected host may have some benefits from viral infection such as innovation of the genetic identity, the integration of new features, newly derived tools for regulation, and the newly adapted regulatory tools of former viral parts into the host regulatory system; these features have been documented in the vast majority of RNA networks being crucial players in host cell regulation (Feschotte 2008). In addition to this, another case of innovation lies in the reactivation of ancient states, such as interactions or activities, that remained latent or silenced within a host cell after a viral infection. This ability to rescue and rekindle pre-existing processes would significantly contribute to cellular adaptation and plasticity (Schwartz and Stern-Ginossar 2023).

In this perspective, the evolution of species depends on virus features for innovation and transfer of genetic information as can be seen, for example, in the evolution of the adaptive immune system (Villarreal 2009b). The regulatory complexity differentiates humans from *C. elegans* both with a nearly equal number of genes (Mattick and Amaral 2022). The regulatory tools in all steps and substeps of transcription, translation, immunity, and repair in cellular organisms are outlined by RNA networks derived from former infections of genetic parasites (Witzany 2011). Interestingly, the crucial driving motif of RNA group interactions is not competition but cooperation (Mizuuchi and Ichihashi 2018). It was shown that cooperative RNA stem loop groups outcompete selfish ones (Vaidya et al. 2012). At the dawn of biotic behaviour and biological selection in an early RNA world cooperation is the dominant interacting motif (Briones et al. 2009).

The genetic code and its editors

Manfred Eigen assumed the genetic code as a real language, not just a metaphor; however, his model of natural languages and communication was determined by mathematical theories of language such as the information theory, which describes language as a quantifiable set of signs, or the Noam Chomsky's concept of a 'universal syntax' hidden in material reality, which determines the meaning of informational contents. The self-organisation of matter into life was constructed by Eigen in the light of Alan Turing's and John v. Neumann's self-reproducing automaton (Eigen and Winkler 1993). However, these theoretical preconditions are falsified by the modern communication theory (Witzany 2014).

Natural languages do not function as algorithm-based decision machines, and therefore, since the first proposals of Turing and v. Neumann, not even one self-reproducing machine has been constructed or seen until today. Also, Chomsky's universal syntax does not meet reality because information meaning depends on context, not on syntax. The genetic code is a real natural code, but as no natural language speaks itself, no natural code codes itself; all available empirical data indicate that every natural language or code requires social interacting groups of living agents that use these languages or codes in real-life world context to coordinate and organise common behaviour (Witzany and Baluška 2012). These processes are called communication, and natural communication basically is a social event of interacting agents (Witzany 2019). The meaning of information depends on the context in which it is used by these agents, not on a 'universal syntax'. As convincingly demonstrated by epigenetics, the context of use represents the pragmatic and highly adaptive nature of the genetic information (Jablonka 2013). This requires the participation of populations of competent code-using agents. Social interacting groups of living agents not only generate meaningful sequences in messages or information storage, but also generate new unexpected sequences depending on the communication context that cannot be computed or formalised by algorithm-based mechanisms (Villarreal and Witzany 2023). In light of these clarifications, the following can be better understood: the communication of viruses, their rich social lives, the innovative generation of really new sequences, the integration of viral sequences or their defectives into host genetic sequences and the various consequences, changing host genetic identity, host gene regulation, and exaptation. Virus populations provide this editorial competence of genetic sequences according to the real-life context (Witzany 2011). The evolutionary outcomes then are innovations in geno- and phenotypes caused by genetic parasites, e.g. as demonstrated in the evolution of the placenta or the evolution of neuronal communication, which are clearly not results of accumulation of error replication events (Pérot et al. 2012; Pastuzyn et al. 2018).

The error narrative revisited

Regarding cell biology, mutations are generally defined as error replications of DNA content. The mutation rate of an organism can be viewed as the probability that a change in the genetic information can be inherited by the next generation. If DNA in cellular genomes is damaged through environmental influences such as radiation or by biotic influences such as genetic parasites or an error in cellular replication, empirical data confirms two directions of consequences: first, the damaged or destroyed DNA sequence in the cellular genome is repaired by a variety of highly coordinated pathways that restructure the original sequence. Up to 100 proteins encoded in the human genome are involved in maintaining cellular DNA integrity. The second consequence may be deleterious, i.e. the genetic content remains deficient, which may lead to disease or even death of the organism. Overwhelmingly, in most observations, DNA mutations in cells cause no better variant and do not lead to better replicons than the parent string. Deleterious mutations occur far more frequently than beneficial mutations. Error replication in most cases yields a worse variant than the original copy. Evolutionary biology has developed a narrative to coherently integrate these facts into evolutionary processes. Some few error replications on cellular DNA are neutral or better variants. Over the long evolutionary time scales, the few neutral or better variants may accumulate and lead to a new or better adapted phenotype of the organism.

When the high mutation rates of RNA viruses, as outlined in the quasispecies concept, are considered, it must be noticed that there are some relevant phenomena that do not fit into the error-replication narrative. These phenomena include low evolutionary rate in cases of virus/host persistence, immune escape strategies by variant productions, complementary interaction with history-dependent memory genomes of minority populations, the variety of cooperation mechanisms, and often fast-changing roles from cooperative agents to competitive agents and vice versa (Ciota et al. 2012; Shirogane et al. 2012). They include also the non-formalisable mutant spectra of viral clouds and non-predictable outcomes and, last but not least, the generation of new sequences, the innovative sequence content, and the integration into host genomes without damaging protein coding sequences. This is at odds with a mere error replication formulation that results in an increase of quasispecies fitness (Villarreal 2008).

Therefore, as an updated narrative, we suggest the term ‘productivity’ as more adequate than error-prone replication. The high mutation rate of quasispecies represents an inherent high productivity of variants, which are not mathematically predictable in a real-life world context. Quasispecies productivity integrates all features of mutant spectra, the various interaction strategies in cooperation, competition, and complementary functions. The high productivity guarantees maximum of survival and adaptation probability of these populations that are essential agents within the roots and the stem of the tree of life. If productivity is too high, information overload will destroy heritable information in a productivity threshold.

What is the benefit of revising the error replication narrative? The time during which the quasispecies concept was originally developed was a time with a very dominant mechanistic paradigm in molecular biology. Most researchers were convinced that life is physics and chemistry that functions mechanistically in a machine-like fashion and that it could be computed by formalisable procedures and mathematical equations (Eigen and Winkler 1993). Yet, the complexity of viral quasispecies can give rise to emerging properties based on interactions that are exerted among components of the same mutant spectrum. Evidence that mutant spectra cannot be considered as mere aggregates of mutant genomes with their expression products includes the following: (i) fitness (overall replicative capacity) of individual biological clones of a virus is lower than the fitness of the parental population from which the clones were isolated (Domingo et al. 1978; Duarte et al. 1994). (ii) Cooperation among components of a mutant spectrum can give rise to emergent phenotypes (recent examples in Shirogane et al. 2023; Yousaf et al. 2023). These observations underline the consortium nature (Villarreal and Witzany 2013) in the expression of some phenotypic traits of RNA viruses and accentuate the productivity feature of viral quasispecies. RNA viruses are suitable experimental systems to understand the molecular mechanisms of the emergence of new biological features. The evidence of biological complexity that was initially portrayed by viral quasispecies was extended in subsequent decades to the cellular world. Such extension consisted also in the recognition of cellular heterogeneities, in this case due to genetic and epigenetic factors, as well as communication processes among cells, tissues, and organs. These events conform to the complexity of biological systems, with their potential for emerging properties, in which viruses play a role as interacting entities (Solé and Goodwin 2000; Domingo 2020). It is in the sense of recognition of the potential of emergent behaviours in which we propose to modify previous narratives that dominated molecular biology and that were overt simplifications (one gene-one protein, junk DNA in complex genomes, viruses as mere disease agents, etc.). The new narrative that we emphasize with the term ‘productivity’ clearly surpasses the mere ‘error-prone replication’ picture often used to frame quasispecies behaviour. Interestingly, the narrative centered in biological complexity and its power for emergent behaviour fits what has been termed ‘stochastic thinking’, or

how apparently random events may help culminating biological functions, including key processes of cell biology (Domingo et al. 2023). Quasispecies productivity cannot be disconnected from events that exert great influence on our biosphere. For example, it fuels virus occupation of new biological niches, promoted by environmental modifications, which are severely accentuated by climate change.

For interactions among components of a mutant spectrum to maintain genomes by complementation or to generate new phenotypes by cooperation, the presence of multiple virus particles in an infected cell is a key mediator. Multiple particles are achieved by a high multiplicity of infection (expressed as the number of infectious particles per cell). A favorable event for a cell to capture multiple viral particles with non-identical genomes for intra-mutant spectrum interactions is provided by their en-bloc transmission through extracellular vesicles, an occurrence that has been documented with several viral-host systems (Chen et al. 2015; Cattaneo et al. 2019; Kerviel et al. 2021). Thus, complementation or cooperation events may take place through interactions among mutants that have arisen de novo within an infected cell or among more genetically divergent genomes that have reached the same cell from distant origins, either by individual viral particle or by groups of particles enveloped into different classes of extracellular vesicles.

Therefore, we propose replacing the error-replication narrative by a productivity narrative that emphasizes the emergence of phenotypic traits that can follow from mutant spectra acting as productive consortia. Quasispecies productivity is the molecular origin of a wealth of new biological potentialities. To some extent, this brings viruses closer to Charles Darwin's view, expressed in the Origin of Species of '…..endless forms most beautiful and most wonderful have been, and are being evolved….'.

Conclusions

Although the quasispecies theory is more than 50 years old, it is an appropriate concept to explain RNA virus evolution at the dawn of biological selection processes and therefore at the origin of life. Some assumptions, such as mutagenesis, cannot be coherently explained within a deterministic approach and had to be updated to better fit into more recent empirical data. The same holds true for various behavioural motifs of quasispecies consortia, especially the complementarity of minority genomes, various cooperation and communication skills of viral populations, and the information on theoretical pre-assumptions about the genetic code, which does not meet the relevance of context of use and meaning (function) of nucleotide sequences, in contrast to pure analyses of the molecular syntax. Finally, the error-replication paradigm of mutations remains a historical curiosity if we want to explain the incredible diversity in the evolution of organisms with all its regulatory pathways in developmental processes by selective accumulations of replication errors. If we look at the current knowledge about the interactional dynamics of quasispecies and RNA networks, the more appropriate term for this never-ending potential to generate new, unexpected, and non-formalisable abundance of variants, we therefore suggest 'quasispecies productivity', which in our opinion better fits into an integrated picture of life processes in the twenty-first century.

Declarations

Conflict of interest The authors declare no competing interests.

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