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Are viruses alive? The replicator paradigm sheds decisive light on an old but misguided question

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ABSTRACT

The question whether or not "viruses are alive" has caused considerable debate over many years. Yet, the question is effectively without substance because the answer depends entirely on the definition of life or the state of "being alive" that is bound to be arbitrary. In contrast, the status of viruses among biological entities is readily defined within the replicator paradigm. All biological replicators form a continuum along the selfishness-cooperativity axis, from the completely selfish to fully cooperative forms. Within this range, typical, lytic viruses represent the selfish extreme whereas temperate viruses and various mobile elements occupy positions closer to the middle of the range. Selfish replicators not only belong to the biological realm but are intrinsic to any evolving system of replicators. No such system can evolve without the emergence of parasites, and moreover, parasites drive the evolution of biological complexity at multiple levels. The history of life is a story of parasite-host coevolution that includes both the incessant arms race and various forms of cooperation. All organisms are communities of interacting, coevolving replicators of different classes. A complete theory of replicator coevolution remains to be developed, but it appears likely that not only the differentiation between selfish and cooperative replicators but the emergence of the entire range of replication strategies, from selfish to cooperative, is intrinsic to biological evolution.

1. Introduction

"Alcohol-based hand sanitizers kill most types of bacteria, viruses and fungi in a few seconds" — claims a random ad in a family magazine. Regardless of the technical (in)accuracy of this statement, its anonymous author(s) has unwittingly answered, in the affirmative, a question that over several decades had been debated by many scientists: *Are viruses alive?* The logic here is simple and arguably undefeatable: you cannot kill something that is not alive. Much the same argument was made by a science writer in the top scientific journal *Nature*, on the occasion of the discovery of virophages, viruses that parasitize on other, giant viruses of amoeba. The same simple reasoning applies: if something can be sickened and eventually brought to death, it surely is alive to begin with (Pearson, 2008). In an influential conceptual paper stimulated by the discovery of giant viruses and virophages that parasitize on them, Raoult and Forterre classify viruses as one of the two fundamental categories of organisms (capsid-encoding organisms, in contrast to the ribosome-encoding organisms, i.e. cellular life forms), with the obvious implication that viruses are living beings (Raoult & Forterre, 2008). However, the opposite view has been forcefully propounded as well: viruses cannot be considered alive because of their inability to reproduce without a cellular host (Lopez-Garcia, 2012; Moreira & Lopez-Garcia, 2009). Each of these viewpoints certainly reflects distinct, important features of viruses: they combine "animate" (reproduction and the ensuing evolution) and "inanimate" features (lack of autonomy, existence of an inert state). This dichotomy fuels the perpetual "life vs non-life" debate among researchers, and even more so among scientific journalists and interested members of the public.

Certainly, the answer to the question "Are viruses alive?" depends on the definition of life or of the "state of being alive".

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Although this issue has been pondered at length for centuries, there is no generally accepted definition of life or "aliveness" (Trifonov, 2012, 2011), and it has been argued that such definitions are neither feasible nor needed (Bruylants, Bartik, & Reisse, 2010; Koonin, 2012; Szostak, 2012). Simple examples from different areas of biology show that a sharp boundary between the living and non-living (or animate vs inanimate) entities is but an illusion. Growing bacteria and archaea are certainly alive. However, many if not most of them enter a dormant (persistent) state under starvation and other forms of stress (Lewis, 2010; Wood, Knabel, & Kwan, 2013). The dormant cells have greatly reduced metabolic activity and are either able or unable to resume growth and division depending on the environmental conditions as well as random factors. Are dormant cells alive or not? Intuitively people are inclined to answer "yes": dormant cells are clearly not dead, because we can resume their growth under given conditions. But, from a biochemical standpoint, they dramatically differ from truly alive cells. Therefore, dormant cells exist in some third, "inert" state that is neither truly "alive" nor inanimate. Even more dramatically, Gram-positive bacteria, such as *Bacilli* and *Clostridia*, as well as cyanobacteria, sporulate under adverse conditions (Adams, 2000; Galperin et al., 2012; Paredes, Alsaker, & Papoutsakis, 2005). Spores are virtually inert biochemically and again, may or may not come back to active reproduction. Are they alive or dead? Or do they represent the third state as well? Thus, the "dead-alive" dichotomy in the classification of biological entities seems to present unsolvable conandra whereby the borders of life cannot be clearly defined.

Interestingly, the apparent paradoxes with respect to "aliveness" are not limited to prokaryotes. For example, micro-animals tardigrades can survive prolonged incubation in outer space where no biochemical reactions are possible (Jonsson, Rabbow, Schill, Harms-Ringdahl, & Rettberg, 2008). However, upon the return of the satellite to Earth, some of the tardigrades survived and even were able to produce offspring. Should they be admitted as "alive", in the regular sense, during this exposure? Many other situations in biology can be invoked, where a rational answer to the question "Is X alive or not?" is out of reach, but those mentioned above should suffice to make the point that this question generally does not allow a yes-or-no answer.

In the above discussion, we conflate the issue of the state of aliveness (whether or not a given object can be considered alive or not) with that of the category of animate (as opposed to inanimate) objects (whether or not a given object belongs to the category of living beings). In general, the two issues are distinct: a dead organism certainly still belongs within the living category. However, when it comes to viruses, these different aspects of aliveness are entangled and are typically discussed jointly. Indeed, viruses can be viewed as not belonging to the category of living beings because they are incapable of autonomous reproduction and extracellular virions are in a dormant (inert) state.

Given that the question on the "aliveness" of a particular class of entities is generally unanswerable (although for many objects the answer can be "intuitively obvious"), this appears to be a non-question. In contrast, in general, it is not difficult to delineate the range of biological phenomena. Although sometimes we cannot give a defendable answer to the question "is X alive?", we argue that it is always possible to tell whether a particular entity belongs to the realm of biology. Such an answer can be given within a fundamental concept that can be denoted the Replicator Paradigm, which we discuss in the following sections, with an emphasis on viruses.

2. The replicator paradigm

All life that is currently known centers around DNA or RNA molecules, replicating carriers of genetic information which all share fundamentally the same chemical structure. The regular structure of nucleic acids and the complementarity between purine and pyrimidine bases make nucleic acids uniquely suited for replication (and other processes that involve sequence copying, such as transcription). Replication with fidelity above the error catastrophe threshold (sometimes called the Eigen threshold) ensures inheritance of genetic information and automatically entails evolution via both selection and random drift (Eigen, 1971; Koonin, 2011; Szathmary & Demeter, 1987). Distinct, partly autonomous replicating units are known as replicators, a concept and a term that have been originally proposed by Richard Dawkins (Dawkins, 1982, 1976), and are widely used in theoretical modeling of evolution at different levels (Godfrey-Smith, 2000; Griesemer, 2000; Hull, Langman, & Glenn, 2001; Maynard Smith & Szathmary, 1995; Nanay, 2002). A key facet of the replicator concept as considered here is the (partial) autonomy with respect to genome replication.

Clearly, replicators are tightly linked to two other major biological concepts, the replicon and the genome. A replicon is literally a unit of replication (Jacob, 1993; Jacob & Brenner, 1963). The major difference from a replicator is that not all replicons possess any degree of autonomy, and conversely, a replicator does not have to be a single replicon. The concept of genome is effectively isomorphous with the replicator concept, but with a different emphasis: a genome is the entirety of nucleic acid sequences that are stably associated with a given replicator (we avoid speaking of "genetic information" here because parts of the genome often are not informative in the strict sense). Thus, each genome corresponds to a replicator that can encompass multiple replicons, e.g. in eukaryotes.

The (partial) replicative autonomy is the key feature that makes each replicator a distinct unit of evolution which employs a specific evolutionary strategy and evolves along a unique trajectory. Certainly, the autonomy of replicators is never complete, and no replicator can survive in isolation. The degree of a replicator's autonomy can be readily measured by the repertoire of the components of the replication machinery (enzymes and other proteins required for replication) that are encoded in the replicator genome, and by the presence of dedicated replication and/or transposition signals. Replicators form a continuum along the autonomy axis although with some degree of arbitrariness, distinct classes ranked by the level of autonomy can be envisaged (Fig. 1 and Table 1).

At the left end are "quasi-replicators", such as prokaryotic toxin-antitoxin (TA) and restriction-modification modules, ORF (Open Reading Frame)-less Group I self-splicing introns and mini-inteins, that have neither specific replication or transposition signals nor genes for any components of the replication machinery. Nevertheless, these entities possess properties that promote their survival and in some cases survival of other replicators on which they parasitize. A case in point are the TA modules that are "addictive" to prokaryotic cells because when the TA element is lost, the cell is killed by the toxin (Gerdes, Christensen, & Lobner-Olesen, 2005; Makarova, Wolf, & Koonin, 2009) (see Table 1). The Group I introns are ribozymes that catalyze their own excision and splicing of the flanking exons as well as reverse splicing which provides for limited spread to ectopic sites (Nielsen, 2012; Nielsen & Johansen, 2009). Mini-inteins are an extremely peculiar variety of parasitic or commensal quasi-replicators that autocatalytically excise from the target genes at the protein level while carrying no signals for replication or transposition (Mills, Johnson, & Perler, 2014; Starokadomskii, 2007).

Immediately to the right of the quasi-replicators are viroids, arguably, the simplest bona fide replicators. Viroids are small RNA molecules of only 400 nucleotides or so that encompass signals for replication initiation by the host DNA-dependent RNA polymerase or the RNA-dependent RNA polymerase of the "host" virus but

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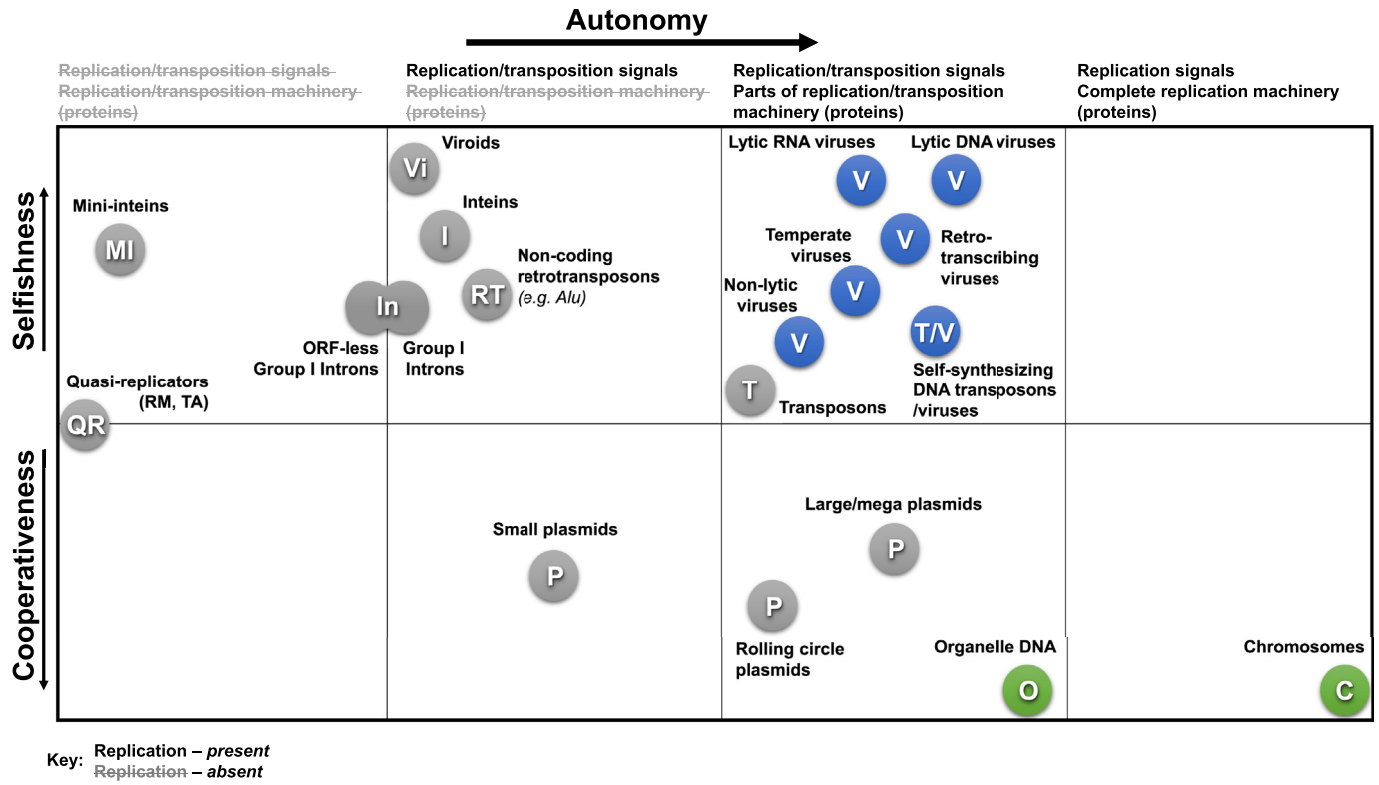

Fig. 1. The diversity of replicators: replicative autonomy vs selfishness-cooperativity. Although the distribution of different groups of replicators in this plane can be viewed as continuous, four classes are delineated by the criteria of presence or absence of signals for replication and/or transposition and the respective protein machinery. The specific positions of different replicators on the plane can be defined only qualitatively. The classes of replicators are denoted as follows: C, chromosomes (including organellar genomes); I, inteins; In, (self-splicing) introns; MI, mini-inteins; O, organellar genomes; P, plasmids; QR, quasi-replicators; RT, retrotransposons; T, (DNA) transposons; V, viruses; Vi, viroids. The three colors denote virion-less selfish elements, viruses and cellular life forms. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

encode no proteins (although some viroids possess ribozyme activity that is required for the processing of concatemers formed during replication) (Diener, 1989; Flores et al., 2015). Typical group I self-splicing introns that encode endonucleases involved in the intron transposition and inteins can be considered protein-coding replicators with the lowest degree of autonomy. Both types of elements, in addition to the catalytic moieties required for self-splicing (at the RNA and protein levels, respectively), encode homing endonucleases that enable transposition to homologous and less frequently to ectopic sites (Mills et al., 2014; Nielsen, 2012; Nielsen & Johansen, 2009; Starokadomskii, 2007).

Chromosomes, the cellular replicators, that encode all proteins involved in replication and a much greater number of accessory (with respect to replication) proteins and structural RNAs occupy the opposite end of the spectrum which corresponds to the maximum autonomy (Fig. 1). In between are all other diverse replicators including transposable elements, plasmids and viruses, as well as organelle genomes from mitochondria and chloroplasts (Fig. 1). The genomes of these non-cellular replicators span the range from about one kilobase (small transposons and satellite viruses) to over two megabase (giant viruses) and widely differ in terms of the complements of proteins they encode (Koonin & Dolja, 2013, 2014).

The only universal feature shared by all replicators is the presence of some signal that enables replicative autonomy. That signal can consist solely of nucleotide sequences that are recognized by the host replicative apparatus as is the case in many bacteriophages (e.g. lambda and its numerous relatives in the family *Siphoviridae*) that, however, encode a variety of accessory proteins (Kristensen et al., 2013; Liu, Glazko, & Mushegian, 2006). Many replicators with small genomes encode a single enzyme involved in replication such as the reverse transcriptase of retroelements, the replication initiator endonuclease-helicase of various rolling circle elements, and transposases (recombinases) of simple transposable elements (Koonin & Dolja, 2014; Koonin, Dolja, & Krupovic, 2015; Kristensen et al., 2013; Piegu, Bire, Arensburger, & Bigot, 2015). Replicators with larger genomes encode multiple proteins that comprise the replication machinery that can reach high complexity, e.g. in the giant viruses of the order "Megavirales" (Colson et al., 2013; Koonin & Yutin, 2010).

An orthogonal dimension of the replicator universe spans the range of reproduction strategies (or life styles), from complete selfishness (associated with parasitism) to full cooperativity (self reliance) (Fig. 1). Lytic viruses that replicate rapidly and kill the infected host in the process are the epitome of selfishness whereas cellular life forms can be considered ultimate cooperators that serve as self-reliant hosts to the selfish replicators (even though cellular life forms have evolved multiple layers of defense as discussed below). All other classes of replicators fall in between these two extremes. Temperate viruses either reproduce at a limited rate without killing the host cell or switch between integrated (lysogenic) and lytic reproduction strategies (Joh & Weitz, 2011; Oppenheim, Kobiler, Stavans, Court, & Adhya, 2005). Transposable elements propagate both within and together with the host genome at different paces that depend both on the intrinsic rates of replication and transposition, and by the interaction with the host defense mechanisms (Piegu et al., 2015; Wicker et al., 2007). Plasmids replicate under more or less tight control from the host, with some reaching high copy number and others represented by a single or a few copies per cell. Apart from the lytic viruses, all these

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Table 1
The diversity of replicators: genetic elements and their key features.

Genetic elements	Definition	Comment
Virus(es)	Obligate intracellular parasites that infect all cellular life forms. Viruses possess comparatively small genomes that consist ease, of single-stranded or double- stranded RNA or DNA. Most viruses encode at least one protein that forms the viral capsid that encases the genome.	Viruses are divided into 6 classes that differ in genome structure and replication-expression strategy: 1. Positive-strand RNA viruses 2. Negative-strand RNA viruses 3. Double-stranded RNA viruses 4. Retro-transcribing viruses 5. Single-stranded DNA viruses 6. Double-stranded DNA viruses.
Lytic virus	A virus that lyses (kills) the host cell after replication.	Lytic viruses typically cause death of the host or at least exert major deleterious effect.
Non-lytic virus	A virus that does not lyse the host cell.	These viruses are transmitted only vertically, via the host reproduction. Such is the lifestyle of most viruses infecting fungi.
Temperate (lysogenic) virus	A virus that does not immediately lyse the host cell after infection. The viral genome may remain dormant for many host generations, depending on external factors, and can deteriorate.	Temperate viruses, such as numerous tailed phages, typically do not substantially damage the host unless and until they are lysogenized.
Bacteriophage	A virus that infects bacteria bacteria and either lyses the bacterial cell or puts the cell into a lysogenic state, becoming a prophage.	
Prophage	A latent form of a bacteriophage whereby the viral genome is integrated into the host chromosome and its expression is regulated such that no virus is produced and the host cell is not lysed. Competent prophages can be induced to form infectious virus and lyse the cell whereas defective prohages cannot.	
Viroid	An infectious RNA molecule that encodes no proteins but recruits the host DNA-dependent RNA polymerase or viral RNA-dependent RNA polymerase for replication, and causes disease in plants.	
Virion	A complete viral particle that consists of RNA or DNA surrounded by a protein shell and often also a lipid membrane, and constitutes the infective form of a virus.	
Transposable element (transposon)	A segment of DNA that is capable of moving into a new position within the same or another chromosome or plasmid.	Most transposons encode enzymes, such as recombinases, transposases and integrases, that actively mediate transposition.
Plasmid	An autonomous genetic unit that does not encode virions but replicates within a cell (quasi)independent of the chromosomal DNA.	Most plasmids are double-stranded DNA molecules but some consist of single-straned DNA or RNA. Plasmids are vehicles of horizontal gene transfer.
Toxin-antitoxin (TA) system	A toxin-antitoxin system is a closely co-regulated system of two genes, one of which encodes a stable “poison” protein and another encodes an unstable protein or RNA “antidote”.	The TA systems possess addictive properties: due to the instability of the antitoxin, only the daughter cells that inherit the gene (chromosomal or on a plasmid) survive after cell division. The TA systems regulate the density of bacterial colonies and apparently mediates programmed cell death and dormancy induction under stress.
Restriction-modification (RM) module(s)	RM systems consist of a modification enzyme that methylates a specific DNA sequence in a genome and a restriction endonuclease that cleaves unmethylated DNA.	Conceptually, RM systems are a variety of TA modules.
Rolling circle plasmid	Plasmid that replicates through the rolling circle replication mechanism (a process of unidirectional nucleic acid replication that can rapidly synthesize multiple copies of circular molecules of DNA).	
Chromosome	A double-stranded DNA molecule that encompasses genes, regulatory elements and other non-coding nucleotide sequences, and encodes all or most of of the genetic information in cellular life forms.	
Organellar DNA	Double-stranded DNA molecules that represent the genome of enosymbiotic organelles of eukaryotic cells, namely mitochondria and chloroplasts (in plants, algae and some protists).	In principle, organellar DNA is similar to plasmids except for intracellular compartmentalization.
Group I intron(s)	Large self-splicing ribozymes, that catalyze their own excision from mRNA, tRNA or rRNA precursors.	Most Group I introns encode endonucleases that mediate the intron transposition and additionally facilitate splicing.
Group II intron(s)	Large self-splicing ribozymes, unrelated to Group I introns, that catalyze their own excision from mRNA precursors.	Most Group II introns encode large proteins containing a reverse transcriptase domain that mediates the intron transposition via reverse transcription and additionally facilitate splicing.
Intein	A part of a protein that is able to excise itself and join the remaining portions (the exteins) with a peptide bond in a process known as protein splicing.	Typically, inteins do not inactivate host proteins and are not substantially deleterious to the host. Inteins have been recruited as key regulators of animal development (the hedgehog family proteins).

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replicators depend on the long-term survival of their respective host cells, the ultimate cooperators, and thus combine selfishness with cooperativity in different proportions as captured in the recent classification of replicators into 5 classes along the selfishness-cooperativity axis (Jalasvuori, 2012; Jalasvuori & Koonin, 2015).

Arguably, an inverse relationship between the degree of autonomy and selfishness could be expected: ultimate, most aggressive parasites would shed all the genetic material other than that required for replication under selection to maximize the replication rate whereas parasites involved in complex interactions with the host would retain a diversified set of genes. Remarkably, however, there is at best a rough correspondence between autonomy, complexity and selfishness. For example, lytic viruses and virus-like agents that replicate rapidly and kill the host cell can have tiny genomes (as is the case for viroids, RNA bacteriophages or some ssDNA viruses) or large ones, exceeding in size those of many cellular life forms (as in giant viruses). Conversely, inteins and self-splicing introns, which are among the smallest replicators, as well as megaplasmids with genomes on par with those of the largest viruses, in most cases are harmless for the host. Thus, the choice of the strategy of interaction with the host (the degree of selfishness) appears to be largely uncoupled from the evolution of a replicator's genome, and similarly, the degree of autonomy (independence of the host with respect to replication) is uncoupled from the genetic complexity. Below, towards the end of the "Ubiquitous ecosystems of replicators" section, we touch upon the likely causes of this uncoupling.

2.1. Replicators, their vehicles and resource production

Obviously, replicators cannot reproduce in isolation. They require resources, such as nucleotides and amino acids, to build progeny genomes and devices for their reproduction that consist of proteins and RNA, as well as vehicles that facilitate the acquisition of the said resources and spread of the progeny (Dawkins, 1982, 1976; Jalasvuori, 2012; Jalasvuori & Koonin, 2015). As with autonomy and selfishness (see the preceding section), replicators span broad ranges of possibilities with respect to resource production and modification of the host metabolism, and the nature of the vehicles (Fig. 2). However, unlike replicative autonomy and selfishness, the capabilities of replicators with respect to resources and the nature and complexity of the vehicle are coupled. The replicators are sharply divided into two major categories with respect to resource production: i) producers that make all the resources required for replication or make some of the resources and actively import others in an energy-dependent manner, i.e. cellular life forms, and ii) non-producers that lack most of the biosynthetic and active transport capabilities, i.e. viruses and other parasitic replicators. The distinction between producers and non-producers reflects another type of autonomy that can be denoted "resource autonomy". Arguably, as far as the resource autonomy is concerned, there is a sharp distinction between autonomous cellular life forms and non-autonomous selfish replicators. Yet, as almost always is the case in biology, borderline situations exist.

While most of the producers (cellular life forms) make all of the energy they use and most if not all building blocks, some intracellular bacteria are energy parasites that obtain most if not all of their ATP from the host (Moran, 2002; Tamas, Klasson, Sandstrom, & Andersson, 2001). Conversely, some of the non-producers, such as large viruses, encode some metabolic enzymes, e.g. nucleotide kinases, that modify the production of building blocks in infected cells. The largest known viruses, in particular mimiviruses, encode many such enzymes including multiple components of the translation system such that multiple synthetic pathways in the infected cell are modified (Claverie, Abergel, & Ogata, 2009; Yutin, Raoult, Koonin, 2013). Among other processes, non-producers can contribute to energy conversion as is the case with cyanophages many of which encode cyanobacterial photosystems (Clokie & Mann, 2006; Thompson et al., 2011). Furthermore, even small non-producing replicators, such as RNA viruses, affect modifications of the host cell metabolism and the formation of structures, such as virus factories, that channel substrates into viral genome replication (Harak & Lohmann, 2015; Romero-Brey & Bartenschlager, 2014). All the prowess of viruses in the modulation of the host metabolism notwithstanding, producers and non-producers are clearly distinct: to the best of our current knowledge, non-producers never direct the formation of energizable membranes, extremely rarely encode complete metabolic pathways and never a complete translation system (Koonin & Dolja, 2013; Raoult & Forterre, 2008).

With regard to the vehicles, there are three distinct classes of replicators, those with: i) no vehicles (plasmids, transposons and other non-viral selfish elements), ii) virus-vehicles (virions), iii) cell-vehicles (Fig. 2). While the repertoire of genes and signals involved in replication defines the replicative autonomy of a replicator as discussed above, the type of vehicle determines a different dimension of autonomy that can be denoted biological or ecological. The resident replicators of cell-vehicles enjoy full or at least partial (in the case of parasites, symbionts and organelles) biological autonomy whereas the vehicle-less replicators and replicators with virus-vehicles depend on the cell-vehicles. Yet, the degree of autonomy is quite different between the two classes of parasitic replicators as the virus-vehicles provide for the long-term survival of extra-cellular virions and effective means for infecting new cells. Furthermore, the virions of many viruses, such as double-stranded RNA and negative-strand RNA viruses as well as retroviruses that package polymerases and other enzymes, are directly involved in the genome replication.

The fundamental distinction between cell-vehicles and virus-vehicles treads the same line as the distinction between producers and non-producers. The cell vehicles are dynamic, metabolically active entities bounded by energizable membranes and often capable of active movement whereas the virus vehicles are essentially inert although many contain enzymes that are activated within the host cell vehicle. Again, however, the boundary is not absolutely sharp, and the analogy between virions and bacterial spores (an inert version of the cell-vehicle) is hard to overlook.

A network of evolutionary relationships exists between replicators without vehicles (plasmids and various mobile elements) and bona fide viruses (Koonin & Dolja, 2014; Koonin et al., 2015). Transitions from one type of replicators to another have occurred on numerous occasions in the course of evolution. In a sharp contrast, there is no evidence of evolutionary transitions between cells and viruses. Claims to the contrary that have become rather popular in the wake of the discovery of giant viruses (Colson, de Lamballerie, Fournous, & Raoult, 2012; Claverie et al., 2006, 2009; Raoult et al., 2004) are readily refutable by phylogenomic analysis (Forterre, Krupovic, & Prangishvili, 2014; Yutin, Wolf, & Koonin, 2014). Indeed, evolutionary reconstructions strongly suggest that giant viruses have evolved, on multiple, independent occasions, from smaller, simpler viruses, rather than from a hypothetical "fourth domain of cellular life".

Neither is there any convincing evidence of origin of selfish elements from "escaped genes" (i.e., genes becoming autonomous, selfish replicators) of cellular life forms, notwithstanding the popularity of this scenario in the early days of virology. On the contrary, most of the essential viral genes (viral hallmark genes) have no close homologs among genes of cellular life forms (except for obvious cases of capture of viral genes by the hosts) and accordingly are likely to have originated in a primordial, pre-

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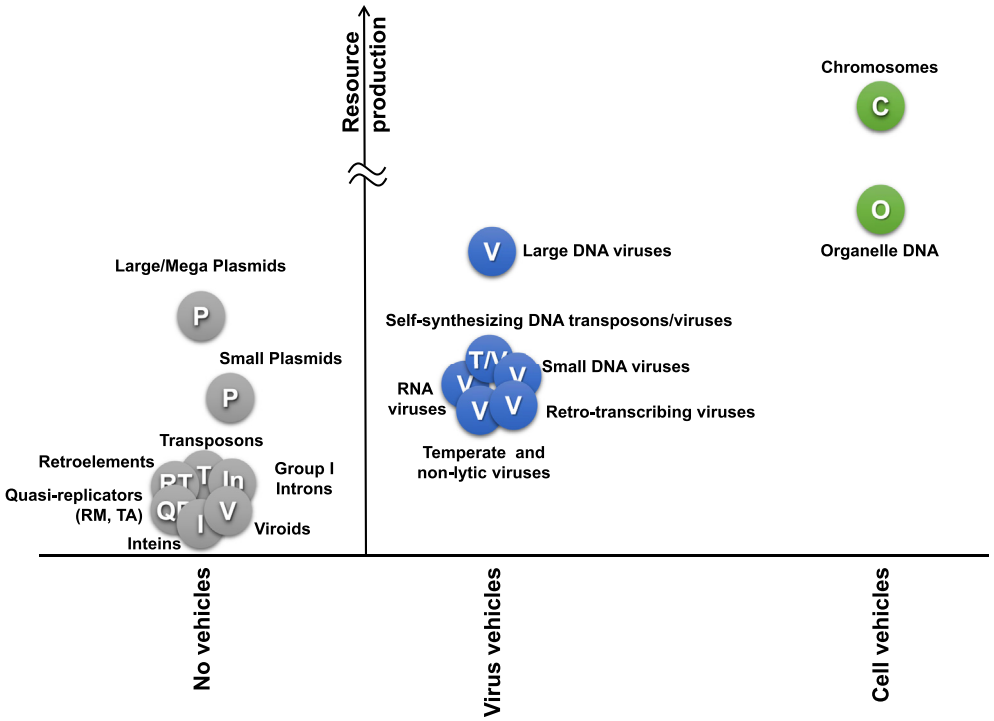

Fig. 2. Replicators, their vehicles and resource production. As in Fig. 1, the specific positions of different groups of replicators along the resource production axis is determined only qualitatively. However, on the vehicle axis, there are only three distinct positions for the two types of vehicles and the replicators without vehicles. The designations for the classes of replicators are as in Fig. 1.

cellular gene pool (E. V. Koonin, 2009; E. V. Koonin & Dolja, 2013; E. V. Koonin, Senkevich, & Dolja, 2006).

Thus, it is important to note that, whereas replicators form continua along the axes of selfishness-cooperativity and genome complexity, there is discontinuity when it comes to the vehicles and resource production (Fig. 2). The discontinuity extends also to the major differences in the gene content of cellular genomes and the genomes of selfish elements. All of the former encode the complete machineries for translation and for the maintenance of energizable membranes whereas none of the selfish replicators do even if genes for some components of these machineries are present. Arguably, this gulf between cells and selfish replicators that reflects fundamentally different survival strategies is the deepest divide between classes of biological entities (Koonin & Dolja, 2013).

2.2. Ubiquitous ecosystems of replicators

Every biological system, such as a unicellular or multicellular organism, is a complex, interwoven community of replicators of different types (Fig. 3). Indeed, all cells, with the possible exception of highly degraded intracellular parasites, carry multiple transposable elements; many cells also contain various plasmids; and all or nearly all cellular life forms are frequently attacked by viruses. The relationships between these diverse replicators span the range from mutualism to commensalism to antagonism. For example, plasmids often form a mutualistic link with the resident cellular replicators (chromosomes) by providing essential metabolic capacities (Petersen, Frank, Goker, & Pradella, 2013; Stasiak et al., 2014) or resistance to antibiotics (Andersson & Hughes, 2010). Prophages that are contained in most prokaryotic genomes can boost the host immunity to virus superinfection and apparently might provide other benefits such as stress resistance (Paul, 2008; Wang et al., 2010). Moreover, a distinct class of defective prophages known as Gene Transfer Agents serve as dedicated vehicles for gene transfer between prokaryotes (Lang, Zhaxybayeva, & Beatty, 2012).

Transposable elements generally should be considered commensals or even aggressive parasites of their cellular hosts. However, a large body of evidence indicates that sequences from these elements are routinely recruited as regulatory regions of host genes (Jordan, Rogozin, Glazko, & Koonin, 2003; Makalowski, 2000; Rebollo, Romanish, & Mager, 2012). Less frequently but also on many occasions, entire genes of mobile elements are captured to function in the host cells (Alzohairy, Gyulai, Jansen, & Bahieldin, 2013; Bowen & Jordan, 2007; Rebollo et al., 2012). The telomerase, a key enzyme in the replication of eukaryotic linear chromosomes, that was derived from retroelements is one striking example (Gladyshev & Arkhipova, 2011; Koonin, 2006), and the much more recent capture of syncytins, essential placental proteins, from retroviruses is another (Dupressoir, Lavialle, & Heidmann, 2012). Conversely, recruitment of genes from the

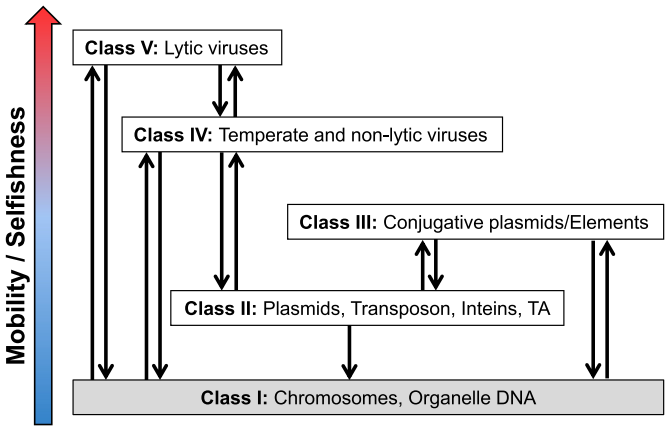

Fig. 3. Communities of interacting replicators. The 5 classes of replicators along the mobility/selfishness axis are denoted according to (Jalasvuori & Koonin, 2015). The arrows denote both physical fusion (integration) and parasitic, commensal or symbiotic relationships between different classes of replicators.

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cellular hosts, when viral genomes randomly captures host DNA that can be fixed in evolution if selected for a function beneficial to the virus, is a common route of evolution among viruses and other selfish replicators (Filee & Chandler, 2010; Filee, Pouget, & Chandler, 2008; Yutin & Koonin, 2012) (see more below on gene exchange between different classes of replicators).

Beyond gene exchange and recruitment, fusion of replicators (or more precisely replicons, for this occasion) is a ubiquitous phenomenon in all organisms (McGeoch & Bell, 2008). Obviously, this is an integral feature of the life cycles of transposable elements. Numerous integrated elements lose their autonomy and degrade, ultimately beyond recognition, to become parts of the host genomes. This process is ubiquitous across the spectrum of cellular life but was particularly massive in the evolution of animals and plants in which the content of transposon-derived sequences can exceed 90% of the genome (Diez, Meca, Tenaillon, & Gaut, 2014; Kidwell, 2002). Although not reaching such extravagant heights, amelioration of both transposable elements and proviruses is common also in bacteria and archaea. Moreover, analysis of archaeal genomes reveals multiple fossils of plasmids suggesting that plasmid accretion is a major path of genome evolution (Iyer, Makarova, Koonin, & Aravind, 2004; McGeoch & Bell, 2008). Even for lytic viruses, data are accumulating on frequent integration into the host genomes, even if this process is spurious with respect to virus reproduction (Chiba et al., 2011; Koonin, 2010; Liu et al., 2010). A striking example of fusion between distinct parasitic replicators are the IStrons which are hybrids between Group I self-splicing introns and insertion sequences, and combine properties of introns and DNA transposons (Tourasse, Stabell, & Kolsto, 2014). Similarly, large DNA viruses often harbor self-splicing introns and serve as vehicles for their dissemination (Edgell, Chalamcharla, & Belfort, 2011; Yoosuf et al., 2012). Apparently, genome fusion and integration along with interactions that do not involve physical joining connect all classes of replicators into a single network (Fig. 3).

The evolution of life is often described as an incessant arms race between hosts and parasites (Forterre & Prangishvili, 2009, 2013; Koonin & Dolja, 2013; Koonin & Krupovic, 2015b; Koonin & Wolf, 2012). Indeed, emergence of selfish, parasitic elements is inevitable in even the simplest replicator systems (Konnyu, Czaran, & Szathmary, 2008; Szathmary & Maynard Smith, 1997; Takeuchi & Hogeweg, 2007, 2012; Takeuchi, Hogeweg, & Koonin, 2011). Probably, a more accurate statement is that the entire history of life is a story of host-parasite coevolution. Arms race is a major aspect of this coevolution that, however, involves also multiple forms of cooperation, to different degrees for different classes of replicators (Dupre & O'Malley, 2009). This cooperation is manifested not only in gene exchange as outlined above but also in self-constraining strategies of numerous selfish replicators.

The arms race promotes evolution of multiple, intricate defense systems in all cellular life forms along with counter-defense systems in selfish replicators. Defense of cellular life forms typically consists of multiple layers including resistance mechanisms (such as rapid evolution of virus receptors), innate and often also adaptive immunity, and programmed cell death (Flajnik & Du Pasquier, 2004; Makarova, Anantharaman, Aravind, & Koonin, 2012, 2013; Rimer, Cohen, & Friedman, 2014). Strikingly, adaptive immunity systems in archaea and bacteria (the CRISPR-Cas systems) and in animals appear to have evolved through recruitment of different transposable elements (Koonin & Krupovic, 2015a), and a similar path of evolution led to the origin of an innate immunity system in ciliates (Swart & Nowacki, 2015). The finding that (at least) three distinct classes of transposons gave rise to three very different immunity systems implies a general principle whereby selfish replicators that are naturally evolved genome rearrangement devices are recruited for those defense mechanisms that involve such rearrangements (Koonin & Krupovic, 2015b). This principle extends even further in that many defense mechanisms, such as restriction-modification and toxins-antitoxins in prokaryotes, are "guns for hire" that are employed either for defense or for counter-defense by different classes of replicators (Koonin & Krupovic, 2015b). These "molecular weapons" are embodied in small, compact genetic units that possess a degree of selfishness and could be viewed as "quasi-replicators" because they lack replicative autonomy and hitchhike on replicators of different classes (Inglis, Bayramoglu, Gillor, & Ackermann, 2013; Kobayashi, 2001; Van Melderen & Saavedra De Bast, 2009). However, such "weapon units" possess addictive properties that allow them to promote their own survival and propagation, regardless of whether they provide any benefits to the host.

Counter-defense, i.e. adaptations of selfish replicators that enable them to escape and/or suppress the defense mechanisms of their hosts, varies dramatically among selfish replicators but virtually all of them encode some functions involved in inhibition of cell defense, such as inhibitors of programmed cell death or suppressors of the RNA interference response (Agol & Gmyl, 2010; Gewurz, Gaudet, Tortorella, Wang, & Ploegh, 2001; Koonin & Krupovic, 2015b; Wu, Wang, & Ding, 2010). Larger selfish replicators, such as viruses with large genomes, encompass numerous genes that encode multiple counter-defense mechanisms. Many if not most components of antidefense systems are recruited from the host defense although not all of them possess quasi-replicator properties (Gewurz et al., 2001; Ploegh, 1998; Vossen, Westerhout, Soderberg-Naucler, & Wiertz, 2002).

We have discussed several axes on which replicators occupy different positions. Yet another one is the axis of replication efficiency vs environmental adaptation. Any replicator faces the fundamental trade-off between maximizing the rate of replication as such and evolving adaptations to the respective environment that provide for maximization of the resource supply and genome protection. In the case of selfish replicators, the adaptations largely include counter-defense systems. Replicators are extremely widely spread along this axis. The trade-off effectively amounts to the well-known dichotomy, in ecology and evolution, between *r* and *K* strategy where the *r* strategy involves maximization of the reproduction rate whereas the *K* strategy entails elaborate adaptation (Hastings & Caswell, 1979; Molenaar, van Berlo, de Ridder, & Teusink, 2009). Generally, the *r* strategy wins in unstable, shifting environments that, however, provide plentiful resources for short time intervals, whereas the *K* strategy is advantageous in stable environment with limited resources (Ponge, 2013). Understanding in more specific terms how the fundamental choice between different evolutionary strategies is made, is key to the study of replicator coevolution and remains a major open research problem.

The coevolution of selfish and cooperative replicators appears to be a powerful driving force of evolution. Mathematical models of coevolution convincingly show that, in well-mixed populations of hosts, parasites cause collapse of the entire host-parasite system. Stable coevolution is possible only in structured populations (Takeuchi & Hogeweg, 2012, 2007; Takeuchi et al., 2011). Thus, selfish replicators promote evolution of complexity of the entire replicator ecosystem. More specifically, such major evolutionary transitions as the advent of DNA as a dedicated information storage device (Takeuchi et al., 2011) and the origin of multicellular life forms could have been promoted by the parasite-host arms race, in particular, through the evolution of programmed cell death as a defense (Iranzo, Lobkovsky, Wolf, & Koonin, 2014).

3. Concluding remarks

The question whether or not "viruses are alive" appears to be effectively meaningless because the positive or negative answer

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fully depends on the definition of life or the state of "being alive", and any such definition is bound to be arbitrary. Worse, any answer to this question does not seem to lead to any constructive developments. In contrast, the status of viruses in the realm of biology is naturally defined within the framework of the replicator paradigm. In the continuum of replicators along the selfishness-cooperativity axis, lytic viruses represent the selfish extreme whereas other parasitic replicators span a broad range. Selfish replicators are not only a part of the biological world but constitute an intrinsic, central part of that world. No replicator system can evolve without the emergence of parasites, and parasitic replicators drive the evolution of complexity at more than one level. The entire history of life is a story of parasite-host coevolution that includes both the inevitable arms race and various forms of cooperation. All evolving organisms are communities of interacting replicators of different classes, from the most selfish to the fully cooperative ones. Although a formal theory of replicator coevolution remains to be developed, an attractive hypothesis is that not only the split between selfish and cooperative replicators but the formation of the entire range of replication strategies is intrinsic to biological evolution.

Thus, unlike the discussions of "aliveness" of viruses, the replicator paradigm is constructive in that it provides the conceptual framework for theoretical and experimental study of the interactions within the replicator community that are among the key drivers of all evolution. One could plausibly argue that life cannot be reduced to replicators, and conversely, that replicators are not confined to the realm of biology. The first proposition is trivially true: indeed, production of energy and acquisition of resources is as intrinsic to life as replication (Dupre & O'Malley, 2009). Indeed, the complementarity of replication and metabolism (broadly defined to include energy production) is the biological manisfestation of the dualism of information (entropy) and energy, as beautifully explained by Schroedinger in his classic book (Schroedinger, 2003). We will not dwell here on the origin of life dilemma: replication or metabolism first? Different approaches are possible, the one that postulates joint origin of both groups of phenomena probably being the most coherent. The second proposition is more controversial. Typically, when speaking of non-biological replicators, one would mention memes (units of cultural inheritance introduced by Dawkins (Dawkins, 1976) and computer viruses along with various computational models of "artifical life". Whether or not memes can be legitimately considered true replicators, remains a matter of debate (Blackmore, 2000) whereas replication of computer viruses (and especially digital "genomes" specifically designed for that purpose) appears obvious. We will not discuss these alleged non-biological replicators in any detail but will make two salient remarks. First, these replicators are not biological entities per se but clearly are generated by biological systems. We are unaware of any plausible candidates for replicators of strictly non-biological origin. Second, and even more notable, these non-biological replicators clearly are virus-like elements endowed with different degrees of selfishness (much like the biological replicators described above; see Figs. 1 and 2) that exploit vehicles provided by other, more cooperative, biological replicators.

Replicators possesss the intrinsic property of evolvability, and it might be tempting to argue that evolvability rather than replication is the key feature of biological systems. We maintain, however, that this is not the case, and instead, evolution itself is an epiphenomenon of replication: selection, drift and parasite formation necessarily ensue as soon as replication with sufficient fidelity is established (Eigen, 1971; Koonin, 2011; Takeuchi & Hogeweg, 2012).

To conclude, we believe that the replicator paradigm is truly central to biology as both a conceptual framework and a research programme. Among other major issues, it settles the status of viruses in the biological world.

Author contributions

EVK and PS wrote the manuscript.

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