Mutualistic Viruses and the Heteronomy of Life

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Final version published in

***Studies in History and Philosophy of Biological and Biomedical Sciences* (2016)**

(http://www.sciencedirect.com/science/article/pii/S1369848616300012)

Abstract

Though viruses have generally been characterized by their pathogenic and more generally harmful effects, many examples of mutualistic viruses exist. Here I explain how the idea of mutualistic viruses has been defended in recent virology, and I explore four important conceptual and practical consequences of this idea. I ask to what extent this research modifies the way scientists might search for new viruses, our notion of how the host immune system interacts with microbes, the development of new therapeutic approaches, and, finally, the role played by the criterion of autonomy in our understanding of living things. Overall, I suggest that the recognition of mutualistic viruses plays a major role in a wider ongoing revision of our conception of viruses.

Keywords

Autonomy, Co-infection, Immune system, Microbiota, Mutualism, Symbiosis, Virobiota, Virus.

Highlights

- Though viruses have generally been seen as harmful (e.g., pathogenic), many viruses are beneficial to their host.
- Mutualistic viruses are found across species, including in Bacteria, Archaea, plants, insects, and mammals.
- Beneficial viral effects on the host can be classified schematically into three categories: development, protection, or invasion capacities.
- The recognition of mutualistic viruses can be seen as an important ingredient of a more general reconceptualization of viruses in current virology.
- This recognition provides a useful bridge between medical and ecological-evolutionary approaches in virology.

1. Introduction

Viruses have generally been characterized by their detrimental effects, particularly their pathogenic ones. Examples abound of human, animal, and plant viruses that reduce host fitness, and Section 2 below recalls that, given the number of past and present human deaths due to viruses, it is by no means surprising that viruses are generally perceived as harmful.

In that context, the recent claim that many viruses can in fact be *mutualistic*, i.e., have beneficial effects on host fitness, was a bombshell to many (Ryan, 2009; Virgin, Wherry, & Ahmed, 2009; Roossinck, 2011). The aim of this paper is, via the analysis of several major examples of recently described mutualistic viruses, to assess the novelty of this claim as well as its conceptual and practical consequences. As explained below, the existence of mutualistic viruses has been known for some time, but the claim based on current data is different and much stronger that previous ones. Under its present form, the idea of mutualistic viruses

raises key questions about the way scientists might search for new viruses, microbe[1]-immune system interactions, the development of new therapeutic approaches, and, finally, the role the criterion of autonomy plays in our understanding of living things. I suggest that this idea can play an important role in a more general reconceptualization of viruses, at the interface between medical and ecological-evolutionary approaches.

The structure of this paper is as follows. Section 2 explains why viruses have generally been conceived as harmful. Section 3 describes in detail several examples of mutualistic viruses. Section 4 draws four conceptual and practical consequences from the existence of mutualistic viruses. Section 5 concludes.

2. Why viruses have generally been considered as harmful

Viruses have been identified at the end of the 19th century as *infectious* agents found in a solution filtered thanks to a Chamberland-Pasteur filter (a filter that retains bacteria) (Lustig & Levine, 1992); (Bos, 1999); (Cann, 2012)). In the footsteps of Ivanovksi, Beijerinck identified the tobacco mosaic virus as an infectious agent (a "contagious living fluid") exhibiting special features, in particular the capacity to pass through a filter that blocks bacterial agents ((van Helvoort, 1996); (Bos, 1999)). Similarly, it is the search for small-size infectious agents that led, in the first half of the 20th century, to the discovery of many viruses (including those of yellow fever, rabies, dengue fever, poliomyelitis, measles, rubella, etc.) (Hughes, 1977). Reflecting on these very important discoveries, Australian virologist and immunologist Frank Macfarlane Burnet (1899-1985) writes in his influential book *Viruses and Man*: "We can define a virus then as a microorganism responsible for disease which is capable of growth only within the living cells of a susceptible host – and which is normally considerably smaller than any bacterium." (Burnet, 1955). Viewing viruses as pathogenic is

[1] Throughout this text, the notion of "microbe" includes all microscopic biological entities, including viruses, regardless of any decision about their living vs. non-living status.

consistent with the etymology of the word (from the Latin *vira*, "poison"), and with the basic assumption of the germ theory of disease (defended, in particular, by Koch and Pasteur), which asserts that diseases are due to germs (though there were tensions between the germ theory of disease and the first conceptualizations of viruses, because several scientists maintained that only bacteria could provoke diseases).

The pathogenic view of viruses has been repeatedly expressed ever since, by both the lay public and many biologists. It is particularly true, of course, of medically oriented microbiologists, many of whom define viruses as "prototypic obligate intracellular pathogens" ((Nolan, Gaudieri, & Mallal, 2006); (Casadevall, 1998); (Kawamoto et al., 2003)). Significantly, similar definitions of viruses as pathogens are found in papers by molecular biologists (Anand, Schulte, Vogel-Bachmayr, Scheffzek, & Geyer, 2008), immunologists (Jirmo, Nagel, Bohnen, Sodeik, & Behrens, 2009), plant biologists (Wu, Lee, & Wang, 2011), and virologists (Cibulka, Fraiberk, & Forstova, 2012). Adding more weight to such definitions, a number of textbooks focus on viral pathogenesis (Nathanson, 2007), and Nobel Prizes awarded to the field of virology are often explicitly presented as rewarding the discovery of disease-causing viruses, such as HIV and human papilloma virus (which causes cervical cancer, and potentially other cancers as well) in 2008 (Weiss, 2008). Overall, as observed by William C. Summers, "The basic idea that viruses are the material basis for disease transmission has changed little in the past 150 years; what has changed is our understanding of the essential properties and biological capacities of viruses" (Summers, 2014).

Of course, pathogenicity (i.e., the capacity to cause disease) is not the only way viruses can be harmful. For example, some viruses reduce host fertility (Abbate, Kada, & Lion, 2015; Sait, Gage, & Cook, 1998), or manipulate host behavior (Hoover et al., 2011). It

seems more accurate, therefore, to say that viruses have generally been seen as fitness-reducing entities, most of the time through their pathogenic effects.

It is certainly not the aim of the present paper to deny that some viruses can cause significant harm. There have been dreadful viral infections in the past, including smallpox in 18th century Europe (estimated to have killed 400,000 people each year) and, following the First World War, the pandemic of influenza virus that killed over 40 million people worldwide (Loo & Gale, 2007) – many more than the war itself. Today, there are still many harmful viral infections; for example, it is estimated that by 2015, the human immunodeficiency virus (HIV) had infected more than 30 million people, with 1.8 million new infections and 1.7 million deaths in 2013 alone (Murray et al., 2014). Furthermore, many of the health alerts in the world in the last two decades were related to novel emerging viruses, including Severe Acute Respiratory Syndrome (SARS) coronavirus, and the 2009 pandemic influenza H1N1 (Chiu, 2013).

The immediate counterpart of the conception of viruses as infectious agents has been the exploration of how hosts are affected by those viruses, and the different antiviral defense mechanisms they can use. In particular, a key aspect of immunology has been devoted to understanding how hosts "fight" viruses. In textbook narratives about the historical sources of immunology, vaccination against different viral diseases is commonly the starting point (e.g., (Murphy, 2012)). This is related more generally to the interpretation of immune systems as defense systems, at war with pathogens, and especially viral pathogens ((Frank, 2002); (Clark, 2008)). As the rest of this paper will show, however, it is inadequate to see viruses exclusively as harmful and, relatedly, to conceive of the immune system only as a defense system, selected for its capacity to eliminate microbes.

3. Mutualistic Viruses

Though viruses have commonly been conceived as harmful, recent research has shown that many of them are *neutral* (not affecting host fitness) or even *mutualistic* (increasing host fitness) (Bao & Roossinck, 2013; Cadwell, 2015a, 2015b; Dennehy, 2014; Foxman & Iwasaki, 2011; MacDuff et al., 2015; Roossinck, 2008, 2011, 2015; Ryan, 2009; Shen, 2009; Stoltz & Whitfield, 2009; Virgin, 2014a). Here I focus on the case of mutualistic viruses. Mutualism is not a yes/no question, but rather a question of degree, and moreover it is often a contextual question (it depends on spatial and temporal conditions) (Méthot & Alizon, 2014; Van Baalen & Jansen, 2001). That said, the cases examined below are well-documented examples in which viruses clearly increase host fitness, or, in some cases, have even become indispensable to host development, survival, or reproduction.

In a mutualistic relation, the virus benefits most of the time from an appropriate niche, in which it can live and reproduce efficiently. The question is how the host can benefit. Viruses can increase host fitness in several ways. Three classes of positive effects on fitness can be schematically distinguished: they are *development*, *protection*, and *invasion*. **Table 1** offers an extensive list of mutualistic viruses, their hosts, and effects. In what follows I elaborate some particularly telling examples.

3.1. Viruses can have a mutualistic effect on host development

First, a virus can have a beneficial effect on host *development*. A first illustration concerns cases in which the realization of the life cycle of an organism is dependent on the presence of a virus within the host. Mutualistic polydnaviruses of parasitoid wasps constitute a very important and widespread example of this phenomenon. Many wasps (Hymenoptera, Ichneumonoidea, Braconidae) paralyze their hosts (which are arthropods, principally of the order Lepidoptera, Coleoptera, Hymenoptera and Hemiptera), and lay their eggs inside them, where the wasp larvae then develop. (In koinobiont species, the wasp larvae grow within the

still living host, and ruthlessly feed on it.[2]) Normally, the host immune system would wall off the wasp eggs (through a phenomenon called "encapsulation"), and eliminate them. Yet particles of viral (polydnavirus) origin suppress this immune response. In other words, the wasp eggs can survive and develop only because a virus integrated into the wasp's genome actively counters the immune defense of the host larva. Perhaps surprisingly, this phenomenon has been known for more than thirty years (Edson, Vinson, Stoltz, & Summers, 1981), but it is only recently that it has been understood in detail ((Espagne et al., 2004); (Bézier et al., 2009); (Herniou et al., 2013); (Drezen, Chevignon, Louis, & Huguet, 2014)).

It is also important to emphasize that the phenomenon described here is very frequent in nature. It is estimated that 30,000 species of endoparasitoid braconid and ichneumonid wasps have their own mutualistic viral species (Webb et al., 2006). The integration of polydnavirus into parasitoid wasps is also an evolutionarily ancient phenomenon, contrary to some other examples discussed below: it occurred several times, million years ago. In particular, polydnaviruses in the genus *Bracovirus* evolved approximately 100 million years ago from a nudivirus (Herniou et al., 2013).

Though there is no doubt that the genetic sequences that suppress the host immune response and make possible the life cycle of the parasitoid wasps are of viral origins, one could consider that the virus has been so tightly integrated into the host genome that it is no longer possible to see the virus and the wasp as separate entities (Roossinck, 2015). However, one of the reasons why polydnaviruses are so fascinating is that, even though they have evolved into vertically transmitted agents, they continue to function in many respects like more traditional viruses ((Herniou et al., 2013); (Drezen et al., 2014); (Strand & Burke, 2014); (Strand & Burke, 2015)). As noted by Herniou et al. (2013), bracoviruses in particular

[2] This phenomenon strongly impressed Darwin. It was a reason for him to doubt the existence of a potent and beneficent God: "I cannot persuade myself that a beneficent and omnipotent God would have designedly created the *Ichneumonidae* with the express intention of their feeding within the living bodies of caterpillars" (Letter to Gray, May 22, 1860).

are not fossil genomic remnants but active viruses, unlike most endogenous viral elements. Most classic viral functions have been conserved and bracoviruses undergo all the steps of a conventional virus cycle. Surprisingly, the main difference between polydnaviruses and classical viruses is the separation of the virus cycle between two cell types and hosts: the production of the infectious particles is done by calyx cells in the wasp ovaries, while the infection involves parasitized lepidopteran host cells.

A second example of a beneficial effect of a virus on host development concerns the role of endogenous retroviruses in the evolution of placenta in mammals. A *retrovirus* is an RNA virus that uses its own reverse transcriptase enzyme to produce DNA from its RNA genome, and then this DNA is integrated into the genome of the host cell. An *endogenous retrovirus* is a retrovirus that has been incorporated into the germline cells, and remains persistent in these cells. Recent research has shown that the evolution of placental mammals has been made possible thanks to an endogenous retrovirus. It was demonstrated first in sheep (Dunlap et al., 2006), and then in mice (Dupressoir et al., 2009), and it is certainly a phenomenon common to all mammals, including humans (Mallet et al., 2004). The fusion of cell membranes is required for the development of the placental syncytium, which is a major component of the immunological "barrier" of the fetus, and this syncytial fusion is possible only thanks to an endogenous retrovirus.

So here again, the realization of an organism's developmental cycle depends on the presence of an integrated virus. Interestingly, the event consisting in the incorporation of such a retrovirus occurred several times, independently, during mammalian evolution (Dupressoir et al., 2009). Thus placentation, a key event for all mammals including humans, has been made possible thanks to the incorporation of a virus into the mammal germline. This shows that mutualistic viruses can be involved in very significant evolutionary events.

In other cases, the beneficial effect of a virus on host development is due not to an old evolutionary legacy but to the present integration of a virus into the host. A striking recent paper has shown that a mouse enteric virus, the murine norovirus (MNV), can play a key role in the development and physiology of the host (Kernbauer, Ding, & Cadwell, 2014). It is well known that germ-free mice exhibit aberrant intestinal morphology and deficiencies in their lymphocytic compartments, which has been taken as a demonstration that some commensal bacteria are indispensable for a normal development, in particular for the development of the gut immune system (Round & Mazmanian, 2009)[3]. Kernbauer and colleagues show that MNV infection of germ-free mice can restore intestinal morphology and mucosal immunity, without triggering strong inflammation and disease, which demonstrates that a virus can exert mutualistic effects, exactly as some gut bacteria do.

An increasing number of biologists have started to study the "virobiota" (all the viruses that live in or on a host) and the "virome" (the set of all the genes of the virobiota), as a potentially crucial part of the "microbiota" and "microbiome"[4], respectively ((Reyes et al., 2010); (Moon & Stappenbeck, 2012); (Duerkop & Hooper, 2013); (Virgin, 2014b); (Cadwell, 2015b)). The human virobiota is made of both bacteriophages (i.e., viruses of bacteria)[5], and eukaryotic viruses (Virgin, 2014b). The study of the gut virobiota in health and disease seems to be particularly promising ((Reyes et al., 2010); (Reyes, Semenkovich, Whiteson, Rohwer, & Gordon, 2012); (Moon & Stappenbeck, 2012); (Norman et al., 2015); (Cadwell, 2015a)), and many authors are convinced that numerous mutualistic viruses will be discovered in the near future, in particular in humans and other mammals ((Lecuit & Eloit, 2013); (Kernbauer et al., 2014); (Cadwell, 2015b)). It is even possible that some beneficial effects on host

[3] For a philosophical analysis of symbiont-induced development, see (Pradeu, 2011, 2012)

[4] The "microbiota" refers to all the microbes that live in or on a host, and the "microbiome" to the set of all the genes of the microbiota.

[5] "Bacteriophages" or "phages" were discovered by Frederick Twort and Félix d'Hérelle in 1915-1917. They contributed significantly to the progress of molecular biology and bacterial genetics. On the many historical controversies around phages, see (van Helvoort, 1994).

development that have hitherto been attributed to mutualistic bacteria living in the host could in fact be due to mutualistic viruses. Overall, viruses can play a pivotal role in one of the most fundamental biological processes – that is, the very construction of an organism through development.

3.2. Viruses can have a mutualistic effect on host protection

Second, a virus can have a beneficial effect on host *protection*, that is, on the capacity of the host to protect itself from pathogens or diseases. A well-documented example concerns the protective role of pararetroviruses in plants (Roossinck, 2005). Pararetroviruses play a decisive role in protecting the plant host (e.g., tomatoes) against other viruses, which for their part are pathogenic, by generating small interfering RNAs (siRNAs) against them (Staginnus et al., 2007). In other cases (e.g., petunias), pararetroviruses prevent the entry of pathogenic viruses into the meristem of the plant.

In mammals as well, some viruses protect the host against diseases. This protection can concern viral diseases or non-viral diseases. An example of protection against viral diseases is the demonstration that, in humans, AIDS progresses much more slowly in patients infected by flavivirus GB virus C (GBV-C, also designated "hepatitis G virus", or HGV), which is a common, non-pathogenic, virus (Tillmann et al., 2001); (Bhattarai & Stapleton, 2012). Examples of protection against non-viral diseases are the several forms of latent herpesvirus that protect against some bacterial infections (*Listeria monocytogenes; Yersinia pestis*) (Barton et al., 2007), and the lymphotrophic viruses that protect against type 1 diabetes (Oldstone, 1988).

According to a recent study by Forest Rohwer's group (Barr et al., 2013), bacteriophages adhering to the gut mucus layer provide their animal hosts with immunity against pathogenic bacteria (the bacteriophages induce a reduction of bacterial attachment to

the mucus, lyse incoming bacteria, and cause a significant diminution of epithelial cell death). Thus, these bacteriophages have a mutualistic relationship with their host, by conferring a previously unrecognized virus-based immunity. Host-microbial relationships in the gut are extremely rich and complex, in particular because different microbes can have antagonistic or stimulating effects one on the other. Much work remains to be done on the overall effects on the host of these intricate microbial interactions.

Some cases are even more fascinating and entangled. As we have seen, many parasitoid wasps realize their life cycle by laying their eggs in arthropod hosts. But some hosts have evolved ways to protect themselves against the parasitoid wasps and their polydnaviruses. For example, an aphid (*Acyrthosiphon pisum*) host can kill the developing wasp (*Aphidius ervi*) larvae, and it has long been known that this protection is mediated by a symbiotic bacterium, *Hamiltonella defensa*. In 2009, however, it was demonstrated that the protective effect of this symbiotic bacterium is in fact due to the presence of a symbiotic bacteriophage within the bacterium (Oliver, Degnan, Hunter, & Moran, 2009) (see also (Moran, Degnan, Santos, Dunbar, & Ochman, 2005)). In that case, it is the virus, located within a bacterium, that is responsible for the protective effect on the host. In the words of the authors, the evolutionary "interests" of the three partners are "aligned," which means that by destroying the wasp larvae the bacteriophage increases not just its own fitness, but also the bacterium and the aphid fitness.

Such "Russian dolls" of mutualistic entities are probably common in nature and constitute a powerful evolutionary and ecological force, though they can be difficult to study (Ferrari & Vavre, 2011). To put this simply, the interaction between the aphid *Acyrthosiphon pisum* and the parasitoid wasp *Aphidius ervi* could be described as ultimately an arms race between two viruses, with the bacteriophage on one side, and the polydnavirus on the other (more exactly, there is a fitness alignment between three actors on the one hand – the aphid,

the bacterium, and the bacteriophage – and between two actors on the other hand – the parasitoid wasp and the polydnavirus). The conclusion is that in many cases, viruses, though they are generally seen as paradigmatic pathogenic entities, can actually be pivotal for host protection.

3.3. Viruses can have a mutualistic effect on host invasion capacity

Third, a virus can have a beneficial effect on host *invasion* capacity, that is, its capacity to colonize new territories at the expense of conspecifics, or to invade its own host. Mutualistic viruses can particularly help their hosts colonize new territories. For example, some bacteria harboring lysogenic bacteriophages[6] have an evolutionary advantage over bacteria that are not lysogenic for this bacteriophage, and this advantage can be important for both the invasion of new territories and the domination of a given territory (Bossi, Fuentes, Mora, & Figueroa-Bossi, 2003).

Plants and animals also possess persistent viruses, with no acute phase, which can destroy other related populations of plants and animals. This phenomenon may have been an important driving force in human history too. It is estimated that, ten years after the European invasion, 90% of the native people in Americas were dead; it is likely that viruses (including the viruses of smallpox and influenza) played a significant role in the rapid elimination of entire populations (Bianchine & Russo, 1992).

Another important example of a virus having a beneficial effect on the host's invasion capacity concerns virulence factors in bacteria. Bacteria can better colonize their hosts thanks to some virulence factors (including toxins). It is now known that many of these virulence factors, including diphtheria toxin, Shiga toxins, and cholera toxin (Boyd & Brüssow, 2002)

[6] "Lysogeny" describes the condition of bacteriophages that have existed for many generations integrated into their bacterial host genomes.

are expressed by the genome of a bacteriophage and not by the genome of the bacterium itself.

Finally, mutualistic viruses can be at the center of much more complex ecological relationships. The panic grass, *Dichanthelium lanuginosum*, grows in soils that are extremely hot (>50°C). This thermal tolerance requires a mutualistic fungus, *Curvularia protuberata*. Yet, the thermal tolerance is in fact mediated by a symbiotic virus within the symbiotic fungus (Márquez, Redman, Rodriguez, & Roossinck, 2007).

3.4. Conclusions about the different examples of mutualistic viruses presented in this section

Some important differences exist among the examples of mutualistic viruses examined in this section. For example, some viruses (e.g., wasp polydnaviruses and mammal retroviruses involved in placentation) are beneficial (and even indispensable) to the whole species (and, therefore, are evolutionarily ancient), while others (e.g., herpesviruses protecting the host against pathogens) are beneficial to the very organism that harbors them and, in some cases, are environmentally acquired at each generation.

That said, we can conclude from all the examples examined here that it is no longer adequate to see viruses as generally harmful. Many viruses – found in bacteria, plants, and animals (including humans) – increase the fitness of their hosts, and in particular their capacities to develop, defend themselves, or compete with other organisms in the invasion of new niches (Table 1).

What is the proportion of mutualistic viruses among all viruses? It is, unfortunately, impossible to answer this question. First, many viruses cannot be classified as entirely "mutualistic" or "pathogenic" since their interactions with the host can switch from one state to the other. Second, only a very limited number of viruses have been identified and studied

so far (Rohwer & Barott, 2013). Exploring viral biodiversity is one of the most exciting challenges of future biology, and this exploration in turn will shed light on the prevalence of mutualistic viruses.

4. Conceptual and practical consequences of the existence of mutualistic viruses

Why should biologists and philosophers or historians of biology should pay attention to the existence of mutualistic viruses? To what extent is it a novel and groundbreaking idea? Can it have an important impact on virology, from both a conceptual and practical point of view? In this section, I show that, even though the idea that viruses can be mutualistic is not entirely new, the data that support it are recent, and have made existing claims much more solid and exact.

The idea that viruses can be mutualistic has already been expressed in the past. In particular, Edgar Altenburg (1888-1967) suggested that all organisms contained numerous "viroids," that he saw as useful, or even indispensable to the host (Altenburg, 1946). For Altenburg, viroids could also give rise to pathogenic viruses, and to most cancers. Altenburg's hypotheses, though highly speculative, were extremely stimulating, and they had an impact on biologists who tried, in the second half of the twentieth century, to emphasize the importance of symbioses in nature (e.g., (Margulis & Sagan, 2002); see also (Sapp, 1994)).

Moreover, in many ecological and evolutionary approaches to viruses, the idea that viruses could enhance host fitness is well-accepted. As with any biological entity, a virus can be detrimental or beneficial to the fitness of another biological entity, depending on the circumstances (in particular, it can be beneficial at one period of time, and detrimental at another). For example, (Fellous & Salvaudon, 2009) analyze different cases of parasites being beneficial to the host in specific conditions, and they see viruses as one possible example of this (see also (Michalakis, Olivieri, Renaud, & Raymond, 1992)).

It would, therefore, be misleading to claim that the idea that viruses can be mutualistic is entirely new. And yet the present claim is clearly different from older ones. While the idea of mutualistic viruses in the past was highly speculative and based on a very limited number of examples, current research offers copious data about a wide diversity of examples across many species, as well as key molecular details about how the virus and host interact (Virgin et al., 2009; Roossinck, 2011, 2015; Cadwell, 2015b). For most scientists, there is a significant difference between a speculative idea and its empirical demonstration, and it is precisely this gap that recent data have filled in. Indeed, the current claim that viruses can be mutualistic may be seen as an important contribution to a more general revision of our conception of viruses. What is particularly significant is that the notion of mutualistic viruses, by undermining the still dominant view that viruses are generally harmful, can have key practical consequences on research in virology. Four of these conceptual and practical impacts can be explored here: the impact on how scientists search for new viruses, on the conception of how the host immune system interacts with microbes, on the potential use of viruses as therapeutic agents, and on the criterion of autonomy in the definition of living things.

4.1. A change in the way scientists might search for new viruses

One of the main reasons why mutualistic viruses have long been overlooked is simply that people did not think that viruses could be beneficial to their hosts, and therefore did not search for them (Virgin et al., 2009). Switching from the classic view to a conception according to which viruses can have all sorts of ecological interactions with other microbes and with their hosts opens up a whole new world for virus research.

This is in particular what has strikingly happened in recent marine virology, where, with the help of new technological advances including viral metagenomics, viruses have been proved to be much more diverse than expected (more than 5,000 viral genotypes or species in

100 liters of sea water, and up to 1 million species in 1 kg of marine sediment), and they are able to "manipulate" their environments (including their virus, protist, and metazoan environments) in many different ways (Rohwer & Thurber, 2009). As Rohwer and Thurber say, "So far, the study of marine viruses has been dominated by the search for pathogens, but this will need to change if we are to appreciate the diverse ways that viruses affect life on Earth." (On virus ecology, see O'Malley, this special issue).

The view that viruses can be mutualistic also has major potential consequences on medicine: until recently, looking for a virus meant in most cases looking for a virus associated with a specific pathogenic state, whereas now doctors and medically-oriented microbiologists may expect to find viruses associated with all sorts of neutral or fitness-enhancing effects (for more on the medical consequences of mutualistic viruses, see 4.3).

4.2. A modification of our conception of how the host immune system interacts with microbes

A crucial consequence of what has been said above about resident and mutualistic viruses is that one should reject the dominant view according to which the normal healthy situation for a given organism, and in particular for a human being, would be the *absence of infection*. All living things harbor numerous viruses as part of their normal constitution and functioning. A healthy human is infected by more than ten permanent chronic systemic viruses (including herpesviruses, polyomaviruses, anelloviruses, adenoviruses, papillomaviruses), and this number may in fact be far higher (Virgin, 2014b). The immune system cannot be defined anymore as a self-nonself discrimination system, given the huge number of commensal bacteria and viruses that live in a human being (Mokili, Rohwer, & Dutilh, 2012), and the fact that these genetically foreign components are not rejected, but rather immunologically regulated (Pradeu, 2012).

Most resident viruses do not cause harm to the host, and, as we saw, some of them are even useful, while others seem to do very little damage. The fact that a standard human being is infected by many viruses is largely underestimated in the use of control individuals in experimental settings and in the study of medical cohorts (Virgin et al., 2009). Moreover, lab organisms that are considered as identical simply because they possess the same genome are in fact, in most cases, not identical from the point of view of their virome, and this mere fact could potentially undermine (or at least alter) many experimental results.

Relatedly, in everyday life, a healthy immune system, far from being turned off, is actually in a constant state of basal activation, in particular through continuous stimulations by resident viruses. This "immunological imprint" (Virgin et al., 2009) is central to understanding both the normal functioning of the immune system and the way it will respond to novel encounters with other microbes. When it is infected by chronic viruses (or other chronic microbes), the immune system reaches a new equilibrium. These continuously exposed viral antigens subsequently constitute the new normal situation for the immune system. In other words, they form the new reference point with regard to which the immune system will detect discontinuities (Pradeu, Jaeger, & Vivier, 2013).

Because all animals and plants harbor many viruses, immunologists and microbiologists are increasingly taking stock of the complexity of host-microbe interactions, or rather host-microbe-microbe interactions. Indeed, when a host is infected by a microbe, it is crucial to understanding not only how the host will react, but also how other (resident and external) microbes will react. The field of co-infection[7] studies (e.g., (Virgin, 2007); (Lijek & Weiser, 2012); (Alizon, de Roode, & Michalakis, 2013); (Osborne et al., 2014); (Reese et al., 2014)) focuses on that question. When a host is immunocompromised after being infected by a microbe, other microbes (opportunistic ones) might invade it (as happens with HIV, for

[7] In that context, what "infects" the host is not necessarily harmful; it can be dangerous, benign, or mutualistic.

instance). Sometimes, two pathogens are much more dangerous if they attack their host together, leading to a combined assault (Jamieson et al., 2013). In many other cases, resident viruses protect the host against pathogenic microbes, as happens with latent herpesviruses that protect the host against *Listeria monocytogenes* and *Yersinia pestis* bacteria (Barton et al., 2007), as explained above. Overall, strong evidence supports the view that infection by chronic viruses constantly stimulates the immune system, making it able to respond better to different pathogens (Virgin, Wherry and Ahmed 2009). Although our understanding of viral infections and immune responses comes primarily from the study of acute infections, it seems crucial, given the ubiquity of chronic viruses, including many mutualistic viruses, to re-think radically host-virus interactions (Virgin et al., 2009).

Taking into account mutualistic viruses leads to a new, much more "ecological" view of the immune system and its interactions with microbes. According to the microbes it meets and the microbes it harbors (among which viruses seem to play a decisive role), each living thing reaches a complex ecological equilibrium, which can be relatively stable but can also be modified in some circumstances.

4.3. Virotherapy: The development of new therapeutic approaches

Attenuated or inactivated viruses have long been used for vaccination. But the existence of mutualistic viruses opens up new therapeutic avenues, particularly the use of bacteriophages to fight pathogens, the possibility of manipulating partners in co-infections, and the use of viruses to eliminate tumors.

First, it is in principle possible to eliminate or control pathogenic bacteria in a host through the use of bacteriophages (viruses of bacteria) that can kill those bacteria (thus creating an "alignment of fitness" of the host and the bacteriophage against the bacteria). "Phage therapy" was popular in several countries of the Soviet Union, especially Poland and

Georgia, but it was often neglected or considered too dangerous in Western countries ((Stone, 2002); (Chanishvili, 2012)). Yet recent years have witnessed a striking resurgence of interest in phage therapy, not least because of a growing concern about resistance to antibiotics among many bacteria ((Reardon, 2014); (Blaser, 2014); (Matsuzaki, Uchiyama, Takemura-Uchiyama, & Daibata, 2014); (Kingwell, 2015)). The aim is not to replace antibiotics, but to complement them, in particular for targets resistant to particular antibiotics. Phage therapies can be effective against multidrug resistant bacteria, and, because of the high specificity of phages for given bacterial strains, they are unlikely to modify the host normal flora (Matsuzaki et al., 2014). Different strategies can be used in phage therapy (Viertel, Ritter, & Horz, 2014), including direct use of bacteriophages to target bacteria, engineered phages ((Nobrega, Costa, Kluskens, & Azeredo, 2015); on the use of "phage cocktails", see (Chan, Abedon, & Loc-Carrillo, 2013)), combinations of phages with antimicrobial substances, the use of lytic enzymes (in particular endolysin, e.g., (Gupta & Prasad, 2011)), and the phage-mediated prevention of antibiotic resistance. Interesting recent results in phage therapy have been found for several bacterial infections, including infections of the urinary/genital tract (Międzybrodzki et al., 2012) and pulmonary infections (Abedon, 2015); they could also play an important role in the gut to improve gut disorders (Dalmasso, Hill, & Ross, 2014).

Importantly, there are currently no phage applications for humans that have either been approved or are in Phase III clinical trials in the European Union or the USA (Viertel et al., 2014). Nonetheless, things could change rapidly. Following the decision of the European Union in 2014 to prioritize the development of phage therapy[8], several research groups have accelerated their involvement in that domain, and two important phase II clinical trials have started in 2015 (Kingwell, 2015).

[8] In particular, the EU contributed €3.8 million to the "Phagoburn" project, which aims at exploring the use of phage therapy to treat burn wounds infected with bacteria (Reardon, 2014).

Some people might reject the idea that viruses that kill pathogens should be considered "mutualistic," but what is considered crucial here is the alignment of fitness between a host and a virus, whether that virus naturally possesses the capacity to eliminate the pathogen or has been engineered to do so. In addition, it seems likely that the recent resurgence of virotherapy research has been in part made possible by the growing recognition that viruses are not necessarily harmful to the host.

Second, as our knowledge of the ecological interactions occurring within a given host progresses, we can imagine being able to manipulate co-infections. For example, it seems possible to stimulate (or maintain) a given virus to fight or control a pathogen (a bacterium, fungus, helminth, or virus).

Finally, an extremely stimulating avenue consists in the use of viruses that can kill tumor cells, called "oncolytic viruses" ((Russell, Peng, & Bell, 2012); (Burke, Nieva, Borad, & Breitbach, 2015)), whether naturally (Roberts, Lorence, Groene, & Bamat, 2006) or because they have been engineered. The field already has a rich history (Kelly & Russell, 2007), but very promising results have been obtained recently, with no less than nine different virus families current being tested, including some in phase III clinical trials (Miest & Cattaneo, 2014). Because oncolytic vaccines can be engineered to kill tumor cells directly, modulate the kinetics of the antitumor immune response and reverse the immunosuppressive actions of the tumor, many specialists predict that they will play a decisive role in future anticancer therapies (Elsedawy & Russell, 2013), most likely in synergy with other components of immunotherapies (that is, the stimulation of the immune system through different ways, so that it eliminates tumors) ((Coffin, 2015); (Snyder, Zamarin, & Wolchok, 2015)).

4.4. A re-evaluation of the criterion of autonomy in our understanding of living things

Viruses are generally considered as dependent on a host to complete their life cycle, that is, as heteronomous (as opposed to autonomous) entities. This has served as one of the main arguments to exclude viruses from the category of living things (e.g., Moreira & López-García, 2009).

Yet what has been shown above, namely that viruses can be mutualistic, converges with arguments that the criterion of autonomy to define living beings seems very fragile (see (Dupré & O'Malley, 2009), and Dupré and Guttinger, this special issue). First, some viruses can realize host-independent morphogenetic processes (e.g., growing long filamentous tails) outside a host (Häring et al., 2005). Second, recent research on symbiosis and mutualism has shown that virtually every living thing is dependent on other living things, often of a microscopic nature, for its survival and its reproduction. Organisms as diverse as plants, marine animals, insects or mammals harbor huge quantities of microbes that play an indispensable role in their physiological activities, digestion, immune defense, development, etc. ((M. J. McFall-Ngai, 2002); (Pradeu & Carosella, 2006); (Pradeu, 2012); (M. McFall-Ngai et al., 2013); (Gilbert & Epel, 2015)). Each human, for example, hosts trillions of symbiotic bacteria, which are acquired from the environment, and are indispensable for digestion, normal development, and normal functioning of the immune system ((Bäckhed, Ley, Sonnenburg, Peterson, & Gordon, 2005); (Round & Mazmanian, 2009)).

Obligate mutualisms are widespread in nature. These obligate mutualisms can associate a host with bacteria, archaea, fungi or, as shown here, viruses. But if obligate mutualisms are so common, why should we consider viruses apart from the rest of the biological world under the pretext that they are "dependent" on a host? For instance, many bacteria cannot be cultured outside their host (this includes *Chlamydia trachomatis* (Byrne, 2003), but also, for instance, many members of the human gut microbiota ((Goodman et al., 2011); (Stewart, 2012)). Moreover, even if one focuses on parasitic interactions, there exist

many obligate intracellular parasitic bacteria (e.g., *Carsonella ruddii*) with less than 150 genes. Overall, "heteronomy" (that is, the fact for a biological entity to be dependent on other, persistent biological entities) (Pradeu, 2012) is ubiquitous in the biological world, so one of the main arguments to separate viruses from the rest of the biological world breaks down.

So could viruses be considered as "organisms"? (See also Forterre, this special issue). In previous work, I have suggested that any entity that engages in physiological interactions controlled by an immune system should be considered as an organism ((Pradeu, 2010); (Pradeu, 2012)). That view promotes the role of the immune system in the definition of organismality because the immune system constitutes a principle of inclusion and exclusion, in the sense that it determines what is part of a common entity, and what is excluded from it. Importantly, this criterion of inclusion-exclusion is not based on the origin of the entities under consideration (that is, on the opposition between the endogenous "self" and the exogenous "nonself"): resident bacteria and viruses in an animal, for example, are part of that animal (Pradeu, 2010).

Traditionally, viruses are not considered as organisms because they are said to lack metabolism (though this traditional view is questioned by some virologists, such as Claverie and Abergel, this special issue). But would viruses count as organisms with the "immunological view" presented here? For the moment, the answer is negative, because no formal immune system has been identified in viruses, contrary to the situation found in Bacteria and Archaea following the groundbreaking discovery of the CRISPR-Cas system (Barrangou et al., 2007). Nonetheless, future studies might well unravel an immune system of some kind in viruses, as some giant viruses can apparently be infected by virophages (viruses of viruses) ((La Scola et al., 2008); (Pearson, 2008); (Fischer & Suttle, 2011); (Yau et al., 2011)). It therefore seems extremely likely that they have evolved defense strategies against these potential invaders. From an immunological point of view, therefore, viruses could be

considered as “organisms”, if future research confirms that they possess immune-like mechanisms.

Be that as it may, it would still be very difficult to maintain that viruses are different from the rest of the biological world simply on the basis of the “lack of autonomy” argument. Autonomy clearly comes in degrees, and dependency of a biological entity on one or several other biological entities seems to be one of the most common features on Earth.

5. Conclusion

Viruses, which may well constitute the most abundant biological entities on Earth, remain largely unknown. In particular, mutualistic viruses, that is, viruses that increase host fitness, have accompanied us for a long time, but have remained largely “invisible”, and almost entirely neglected.

Two scientific and philosophical conclusions emerge from my discussion. The first concerns reconceptualizing biological individuality, autonomy, and competition. Viruses are everywhere in, on, and around living things, from prokaryotes to plants, fungi, and animals; they are sometimes detrimental, and at other times useful or even indispensable. By their ubiquity and diverse functional roles in every possible host, viruses extend the view that biological individuals are not autonomous but, on the contrary, heterogeneous (i.e., made of entities of different origins, often belonging to different kingdoms) and heteronomous (i.e., dependent, to some degree, on other biological entities to complete their life cycle). Moreover, ecological and evolutionary competition needs to be re-conceptualized, both spatially and temporally. Competition occurs not between selfish, autonomous, and homogeneous entities, but between temporarily constituted associations or aggregates (some of them long-lasting, others transient). In a given context, the three actors of the hierarchy made by a virus in a fungus in a plant (Márquez et al., 2007) help each other and constitute a

collaborative unit, in competition with other composite biological entities; however, in another context, this fitness alignment can break down. The study of biological competition and cooperation thus requires the careful investigation of how ecological interactions between and within hosts are established and broken down. Admittedly, many studies in ecology and evolution already pay attention to this highly dynamic nature of competitive/cooperative interactions, but my suggestion is that seeing the biological world through a viral lens strengthens this approach, and even makes it inevitable.

The second consequence of including viruses is more sociological in nature, but may also have more extended conceptual implications. It has often been observed that medically-oriented microbiology on the one hand, and evolutionarily and ecologically-oriented microbiology on the other, rarely interact either in research or in teaching (Smith, Rubinstein, Park, Kelly, & Klepac-Ceraj, 2015). Findings of mutualistic viruses now bring together these two branches of microbiology. Viruses are central to both human health and disease. They interact with us and regulate our relations with other resident entities, including bacteria, helminthes, and fungi. It is increasingly recognized that being infected by viruses is the norm rather than a deviation and resident viruses are a fundamental component of our basal immunity (Virgin, 2014a). They are, therefore, likely to play a key role in how we respond to any potentially pathological threat. These rich and reciprocal interactions call for the adoption by medical doctors of a "multiple infection" perspective, which draws on ecological and evolutionary concepts and models (Cadwell, 2015b; Choffnes, Olsen, & Mack, 2014). In turn ecologists and evolutionary biologists could avail themselves of the extensive molecular data accumulated by the medical sciences, especially about resident viruses (Woolhouse, Webster, Domingo, Charlesworth, & Levin, 2002). If this combination of medical and evolutionary-ecological approaches in microbiology is realized in teaching practice, we can expect from the

new generation of microbiologists an enriched vision of microbes and our interactions with them.

Acknowledgements

I thank Samuel Alizon, Eric Bapteste, Lynn Chiu, Jean-Michel Claverie, John Dupré, Jean Gayon, Stephan Guttinger, Philippe Huneman, Gladys Kostyrka, Maureen O'Malley, Karine Prévot, and Herbert 'Skip' Virgin for their comments on previous versions of this article.

Type of mutualism	Virus	Host	Effect	References
Development	Polydnavirus	Parasitoid wasps	Indispensable for the development of the wasp eggs in the host	; (Espagne et al., 2004); (Bézier et al., 2009); (Herniou et al., 2013)
	Endogenous retroviruses	Mammals	Made placentation possible	(Dunlap et al., 2006); (Dupressoir et al., 2009)
	Murine norovirus	Mice	Can replace the beneficial effect of commensal bacteria on intestinal development and homeostasis	(Kernbauer et al., 2014)
Protection against a pathogen or disease	Pararetroviruses	Plants	Protection against pathogenic viruses	(Roossinck, 2005); (Roossinck, 2008); (Roossinck, 2015)
	Flaviviridae viruses	Humans	Decrease in HIV infection	(Tillmann et al., 2001)
	Herpesviruses	Mice	Protection against bacterial infections	(Barton et al., 2007)
	Lymphotrophic viruses	Mice	Protection against diabetes	(Oldstone, 1988)
	Oncolytic viruses	Mice, humans	Elimination of tumors	(Parato, Senger, Forsyth, & Bell, 2005); (Miest & Cattaneo, 2014)
	Retrovirus, with ongoing endogenization	Koalas	(Probably) Immune protection	(Ryan, 2009); (Tarlinton, Meers, & Young, 2006)
	Bacteriophages	*Hamiltonella defensa* within aphid host	Elimination of parasitoid wasp	(Oliver et al., 2009)
		Bacteriophage within different animal hosts (e.g., Cnidarians, fish, humans)	Protection against pathogenic bacteria	(Barr et al., 2013)
Invasion of new hosts or	Lysogenic bacteriophages	Bacteria	Elimination of bacterial competitors	(Bossi et al., 2003)

niches	Bacteriophages	Bacteria	Invasion of host	(Boyd & Brüssow, 2002)
	Fungal virus	Fungus within a plant	Thermal tolerance	(Márquez et al., 2007)

Table 1. Examples of mutualistic viruses (based in particular on (Roossinck, 2011, 2015)).

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