Move Over, Bacteria! Viruses Make Their Mark as Mutualistic Microbial Symbionts

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Viruses are being redefined as more than just pathogens. They are also critical symbiotic partners in the health of their hosts. In some cases, viruses have fused with their hosts in symbiogenetic relationships. Mutualistic interactions are found in plant, insect, and mammalian viruses, as well as with eukaryotic and prokaryotic microbes, and some interactions involve multiple players of the holobiont. With increased virus discovery, more mutualistic interactions are being described and more will undoubtedly be discovered.

While viruses have long had a very bad name as pathogens, and there are certainly many devastating human, animal, and plant diseases attributed to viruses, viruses are not all bad (1). Recent studies highlight the amazing intricacy of virus-host interactions that have evolved over long periods of time and involve interactions between the hosts and other entities, including other symbiotic microbes and vectors for transmission.

Bacteria are accepted as not just pathogens but also vital partners of eukaryotic life; it is clear that viruses are also essential to life (2). Over the past several years, more and more examples of beneficial viruses have been reported. In some cases, mutualistic symbioses have led to symbiogenesis, the fusion of entities to create a new entity. Mutualism in plant and insect viruses has been well documented, and more recently, mutualistic viruses have been described in mammalian health.

In this age of virus discovery, we are beginning to appreciate the enormous diversity of viruses, far beyond what we originally thought. Undoubtedly, many more will be understood as beneficial. This short review is not meant to be exhaustive but rather highlights some of the recent and dramatic examples of beneficial viruses, demonstrating why viruses need to be taken seriously not just as pathogens but as integrated members of the holobiont.

Symbiosis and symbiogenesis. Viruses have been recognized as symbiotic members of their hosts' microbial community (1). Symbiosis was first described in the late 19th century to explain lichen and was thought to be an oddity rather than the norm; now we recognize that all life is symbiotic. Symbiotic relationships can take many forms, from antagonistic to mutualistic, and viruses, like other symbionts, lie on a continuum that can shift with environmental changes (3, 4). Symbiotic relationships can lead to symbiogenesis, the fusion of two entities to create a new species, and the extent of virus-like sequences in the genomes of just about everything is evidence of the viral symbiogenesis that has shaped modern genomes (5). From an evolutionary perspective, symbiogenesis should follow a mutualistic symbiotic relationship, but this is not necessarily clear with viral symbiogenesis.

In some cases, the line between virus and host is blurred. For example, the polydnaviruses of the endoparasitoid braconid and ichneumonid wasps have integrated most of the virus genes into the wasp genome, leaving the virus particles to encapsidate wasp genes that suppress the immune system of the caterpillar hosts of the parasitoid wasps (6). It is not clear that the virus and wasp are

separate entities any longer, and this could be considered an example of mutualistic symbiosis in the process of becoming symbiogenetic.

In some cases, entire viruses genomes are integrated into the host genome, but these can exogenize and establish infections under some conditions. The badnavirus *Banana streak virus*, a pararetrovirus that has endogenized into the banana genome on multiple occasions (7), can exogenize and become active under various types of stress (8). Endogenization may provide a selective advantage to the host, acting as a method of immunization, as with endogenous pararetroviruses in tomato plants (9) and proposed for mammalian endogenous retroviruses (1), but this is not clear in banana plants. *Banana streak virus* may be an example of antagonistic symbiogenesis involving a selfish viral element that manages to hide in the host genome most of the time.

Other endogenous retroviruses have played a clear role in the evolution of their hosts. The mammalian genes for syncytin, essential in the establishment of the placenta, are retroviral *env* genes of viruses endogenized on several different occasions (10) and even function differently in ruminants versus other mammals (11). Many other symbiogenic viruses are integrated into the host genome. The role of these viruses, including many viruses beyond the retro- and pararetroviruses, is occasionally known. The endogenization events are often ancient, and these elements are considered viral fossils that can help us understand the deep evolution of viruses (12).

Mutualistic viruses and plants. In plants, viruses can ameliorate the effects of abiotic stress. Few plants can grow in the high soil temperatures found in the geothermal soils of Yellowstone National Park. However, one plant is commonly found in those hot soils, a tropical panic grass. The grass is colonized by a fungal endophyte that is, in turn, infected with a virus. All three, virus,
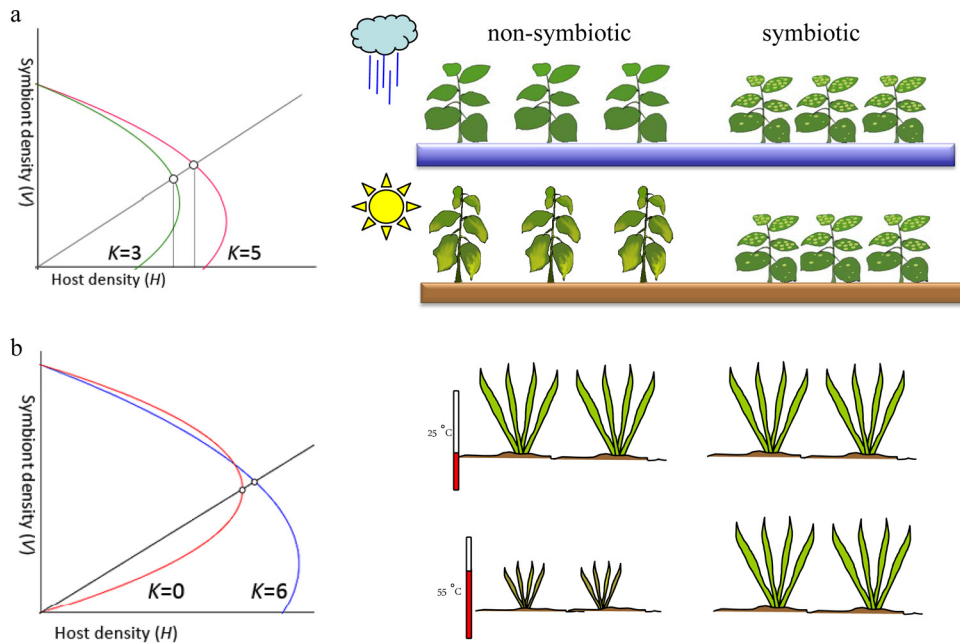
FIG 1 A simple model of biotic stress impacts on quality-selected mutualistic symbioses. The host isocline is a curve that intersects the *x* axis at the environmental carrying capacity (value *K*). The viral symbiont isocline is a line with a slope of the maximum titer, when the growth rate of the virus is much lower than the decay rate. The host and virus isoclines intersect at an equilibrium point that a pair of symbiotic partners can approach. (a) When an acute plant-pathogenic virus infects a plant (plant virus-plant host), the plant becomes tolerant to drought stress; without stress, the uninfected plant does better, but under stress, a decrease in *K* encourages a pathogenic virus to become mutualistic. (b) In a three-way mutualistic symbiosis (fungal virus-fungal endophyte-plant), the virus has no negative impact on the plant under normal conditions and is required for survival at high soil temperatures. Extremely low *K* and low viral virulence result in a stronger mutualistic symbiosis effect. Reproduced with permission from reference 3.

fungus, and plant, are required for survival in soils with temperatures of >50°C (13). Viruses can directly impact plants under abiotic stress as well. Several acute plant viruses conferred drought tolerance on a number of plants in greenhouse studies, and in at least one instance, virus infection also improves tolerance to cold (14) (Fig. 1).

Plant viruses also impact biotic stress factors. In white clover, infection with *White clover mosaic virus* makes the plant less attractive to fungus gnats (15). *Zucchini yellow mosaic virus* infects wild gourds and reduces the production of volatile compounds that attract beetles to the plants. The beetles are vectors of a bacterial wilt pathogen, so the virus reduces transmission of the wilt bacteria (16). These relationships are never black and white; there are costs and benefits, and the players must find a balance. Viruses can be on a continuum between mutualism and antagonism, and where they fall depends on the environment (3, 4).

Plants are very often infected with asymptomatic persistent viruses that differ somewhat from persistent viruses in other systems. These viruses are vertically transmitted at nearly 100% rates; no horizontal transmission has been demonstrated (17). They remain with their hosts for very long periods of time, perhaps thousands of years. *White clover cryptic virus*, a persistent virus ubiquitous in white clover, suppresses the formation of nitrogen-fixing nodules when adequate nitrogen is present in the soil (18), saving the plant from producing a costly organ when it is not needed. The biology of most other persistent plant viruses remains unknown, but such long associations and high levels of vertical transmission imply beneficial relationships.

Beneficial insect viruses. Besides the polydnaviruses discussed above, other beneficial insect viruses have recently been described.

For example, *Helicoverpa armigera densovirus 1* increases developmental rates in both the larva and pupa of its host, the cotton bollworm, and lengthens the life span and increases fecundity in female bollworms. As the bollworm is a serious crop pathogen, control measures are used in cotton fields, including the biopesticide *Bacillus thuringiensis* and biocontrol with a polyhedrosis virus. In field studies, the densovirus increased resistance to these agents (19), making it a bane for cotton farmers but a boon for the bollworm host. Another insect densovirus infects the rosy apple aphid. Virus infection results in the development of winged aphids that are smaller and have lower fecundity than their uninfected counterparts that do not develop wings. However, wings are an advantage to aphids when the plant becomes crowded. The virus is horizontally transmitted in aphid colonies using the plant as a vector. Since the virus does not replicate in the plant, it remains at a low level, but as the colony size increases, the odds that a nymph will acquire the virus increases, and eventually, an infected, winged aphid develops to move off and start a new colony (20).

A number of plant viruses can have dramatic impacts on their insect vectors (recently reviewed in reference 4). Some viruses infect both plants and insects, and while these are often deleterious to the plants, they can provide advantages to the insects. *Tomato spotted wilt virus* infects both plants and thrips, tiny insects that vector the virus between plants. The virus suppresses the antifeeding compounds produced by the plants in response to thrip damage, making virus-infected plants a better host for juvenile thrips than noninfected, thrip-damaged plants. The virus effect can extend to other insects; spider mites also do better on virus-infected

plants, even though they are not a host for the virus (4 and references therein).

Good viruses in mammalian health. Humans infected with *GB virus C* (GBV-C), also known as hepatitis G virus, do not show any clinical symptoms. However, HIV-positive patients who also have GBV-C show slower disease progression. Several effects of GBV-C on HIV have been shown in clinical studies and *in vitro*, including downregulation of cell receptors for HIV entry, reduced replication of HIV, effects on interferon synthesis, and interactions with interleukin pathways (21).

The importance of gut bacteria in digestion and gut architecture is well established. A recent study with mice showed that *Murine norovirus* establishes a latent infection in mice. In germ-free or antibiotic-treated mice, the norovirus can provide many beneficial functions that bacteria provide, including intestinal morphological characteristics and lymphocyte function (22).

Mammalian viruses can provide immunity to infection by bacterial pathogens, as shown in mice infected with a gammaherpesvirus that increases resistance to *Listeria monocytogenes* and *Yersinia pestis*. Latent herpesviruses also affect natural killer (NK) cells, an important line of defense against pathogens and cancer because they kill virus-infected cells and tumor cells, in addition to producing cytokines like interferon. While NK cells do not require specific sensitization (i.e., they can kill cells on first encounter), they do require arming before they can efficiently produce their cytotoxic effects. This arming function is provided by a latent herpesvirus in mice (23).

The guts of humans and other mammals are rich in viruses. These are largely still in the discovery phase. Many of the gut viruses are bacteriophage, although eukaryotic viruses are also part of the human virome, and these include both human viruses and viruses of eukaryotic symbionts (23). For the most part, little is known about how these viruses impact their hosts, but their ubiquitous presence implies functions. In some cases, phage may be regulating the populations of resident bacteria or they could affect the expression of bacterial genes that are involved in host digestion (24). In a recent study, bacteriophage were shown to adhere to mucous membranes in many different metazoan hosts. Mucous membranes are found at the point of entry of many bacterial pathogens. The phage are poised to provide the first line of defense against bacteria invading the metazoan host by infection and lysis. The metazoan host is benefitted by this early attack of potential pathogens, and viruses are benefitted by access to new hosts (25).

Viruses of microbes. In addition to providing benefits to the macrohosts of many microbes, viruses also directly benefit their microbial hosts. The killer viruses of yeasts and bacteria allow their hosts to invade new territories by killing off competitors while providing immunity to the virus-containing hosts (1). Phage also encode essential functions for bacteria, such as the production of toxins that allow them to invade their macrohosts, the horizontal gene transfer of essential elements, and in some cases, the ability to form biofilms (26). Viruses of other eukaryotic microbes have positive effects on the growth, fecundity, or persistence of their hosts (27).

Conclusions. Our new understanding of the role of viruses in mutualistic symbiotic relationships with their hosts is expanding as our knowledge of the virome, through new sequencing technologies and bioinformatic strategies, is rapidly increasing. Viewing viruses in the context of ecology (28) provides a framework for a deeper understanding of the intertwined relationships of all life, including viruses. It is inevitable that more examples of mutualistic viruses will be revealed as we continue this exciting phase in virology of virus discovery.

ACKNOWLEDGMENTS

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