CHAPTER 1

DEFINING THE ECOLOGY OF VIRUSES*

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CONTENTS

1.1 INTRODUCTION

The goal of virology is to understand the viruses and their behavior. Virology is an interesting subject and even has contributed to the concepts of what we consider to represent dieties and art. Sekhmet, an ancient Egyptian goddess, was for a time considered to be the source of both causation and cure for many of the diseases that we now know to be caused by

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* This chapter represents a revision of "Defining the ecology of viruses", which appeared as chapter 1 of the book *Viral Ecology*, edited by Christon J. Hurst, published in 2000 by Academic Press. All of the artwork contained in this chapter appears courtesy of Christon J. Hurst.

Studies in Viral Ecology, Second Edition. Edited by Christon J. Hurst.

FIGURE 1.1 Image of Sekhmet, “Bust Fragment from a colossal statue of Sekhmet,” Cincinnati Art Museum, John J. Emery Fund, Accession #1945.65 Cincinnati, Ohio. Originally the warrior goddess of Upper Egypt, Sekhmet was for a time believed to be the bringer of disease. She would inflict pestilence if not properly appeased, and if appeased could cure such illness.

viruses (Figure 1.1). Influenza, a viral-induced disease of vertebrates, was once assumed to be caused by the influence of the stars, and that is represented by the origin of it’s name which is derived from Italian. The following was a rhyme which children in the United Sates sang while skipping rope during the influenza pandemic of 1918–1919:

I had a little bird
And its name was Enza
I opened the door
And in-flew-Enza.

(Source: The flu of 1918, by Eileen A Lynch, The Pennsylvania Gazette November/December 1998 (http://www.upenn.edu/gazette/1198/lynch.html).

And a bit more recently an interesting poem was written about viruses (Source: Michael Newman, 1984):

“The Virus”

Observe this virus: think how small
Its arsenal, and yet how loud its call;
It took my cell, now takes your cell,
And when it leaves will take our genes as well.
Genes that are master keys to growth
That turn it on, or turn it off, or both;
Should it return to me or you
It will own the skeleton keys to do
A number on our tumblers; stage a coup.

But would you kill the us in it,
The sequence that it carries, bit by bit?
The virus was the first to live,
Or lean in that direction; now we give
Attention to its way with locks,
And how its tickings influence our clocks;
Its gears fit in our clockworking,
Its habits of expression have a ring
That makes our carburetors start to ping.

This happens when cells start to choke
As red cells must in monoxidic smoke,
Where membranes get the guest-list wrong
And single-file becomes a teeming throng,
And growth exists for its own sake;
Then soon enough the healthy genes must break;
If we permit this with our cells,
With molecules abet the clanging bells;
Lend our particular tone to our death knells.

The purpose of this book is to define the ecology of viruses and, in so doing, try to approach the question of what life is like from a "virocentric" (as opposed to our normal anthropocentric) point of view. Ecology is defined as the branch of science which addresses the relationships between an organism of interest and the other organisms with which it interacts, the interactions between the organism of interest and its environment, and the geographic distribution of the organism of interest. The objective of this chapter is to introduce the main concepts of viral ecology. The remaining chapters of this book will then address those concepts in greater detail and illustrate the way in which those concepts apply to various host systems.

1.1.1 What is a Virus?

Viruses are biological entities which possess a genome composed of either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). Some virus groups produce single stranded genomes, and other virus groups produce either fully or partially double stranded genomes. Viruses are infectious agents which do not possess a cellular structure of their own, and hence they are "acellular infectious agents." In 2000, (Hurst, 2000) I proposed a biological domain that would represent the acellular infectious agents which possess nucleic acid genomes (termed "genomic acellular infectious agents"), and its constituent members would be the infectious agents commonly termed to be either viruses, satellite viruses, virusoids or viroids. The proposed domain title was Akamara (ακαμαρα), whose derivation from Greek (α + καμαρα) would translate as meaning "without chamber" or "without vault," and I suggested that name as describing the fact that these agents lack a cellular structure of their own. I feel honored by the recognition that my suggestion has received https://es.wikipedia.org/wiki/Akamara; https://prezi.com/accqbr5jjusj/christon-j-hurst/; https://www.timetoast.com/timelines/historia-de-la-clasificacion-de-los-seres-vivos-2b8474a8-2d53-45ba-8516-5338c25fd224.

Furthermore, the viruses are obligate intracellular parasites, meaning that they live (if that can be said of viruses) and replicate within living host cells at the expense of those host cells. Viruses accomplish their replication by usurping control of the host cell's biomolecular machinery. Those which are termed "classical viruses" will form a physical structure termed a "virion" or viral particle that consists of their RNA or DNA genome surrounded by a layer of proteins (termed "capsid proteins") which form a shell or "capsid" that protects the genomic material. Together, this capsid structure and its enclosed genomic material are often referred to as being a "nucleocapsid". If the question becomes one of "Which came first, the virus or the capisd proteins?," then Jalasvuori and coauthors (2015) have suggested that capsid proteins came first as a means of facilitating horizontal gene transfer.

Two of the most basic categories of capsid structures are those described as being helical versus icosahedral. The genetic coding for the capsid proteins generally is carried by the viral genome. Most of the presently known virus types code for their own capsid proteins. However, there are some viruses which are termed as being "satellite viruses." The satellite viruses encapsidate with proteins that are coded for by the genome of another virus which coinfects (simultaneously infects) that same host cell. That virus which loans its help by giving its capsid proteins to the satellite virus is termed as being a "helper virus." The capsid or nucleocapsid is, in the case of some groups of viruses, surrounded in turn by one or more concentric lipid bilayer membranes which are obtained from the host cell. Viruses are grouped taxonomically from the levels of species and genus, on upward through to higher taxonomic levels. The basis for those taxonomic designations includes viral morphology, host range, and replication strategy. Viral taxonomic designations historically incorporated distinctions that were based upon viral antigenic cross reactivity. The usage of antigenic cross reactivity has largely been replaced by taxonomic designations that reflect viral genomic analysis.

Figure 1.2 is a drawing of a helical nucleocapsid structure showing how the capsid

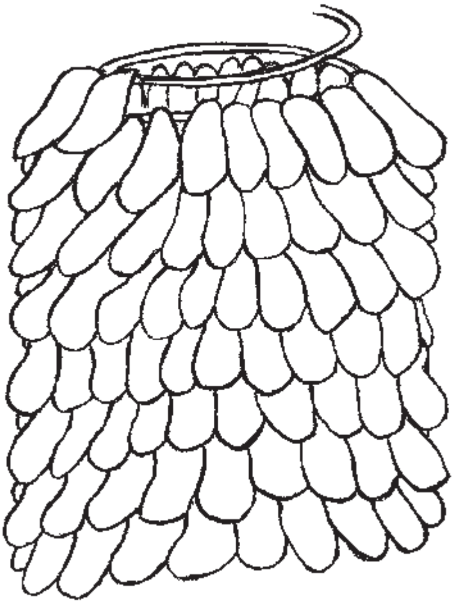

FIGURE 1.2 Drawing of a helical capsid structure showing how the capsid proteins attach to the helical coil of the viral nucleic acid genome. Presumably, all of the capsid proteins are identical to one another in a helical structure.

proteins attach to the helical coil of the viral nucleic acid genome. Presumably, all of the capsid proteins are identical to one another in a helical structure. Those viruses that possess helical capsid structures generally have single stranded RNA genomes. Some of the virus families which possess single stranded RNA genomes have a genome that is positive sense, which means that their genome has the coding of a messenger RNA molecule and can be translated. There also are virus families that have negative sense single stranded RNA genomes, which means that their genome must be copied to produce complimentary strands, and those complimentary strands can be translated.

Figure 1.3 is a photograph of the assembled model published by Hurst et al. (1987) showing the protein arrangement in an icosahedral capsid structure. As mentioned above, viruses have genus and species names, plus higher viral taxonomy levels also exist. This particular structure is a representation of the viral

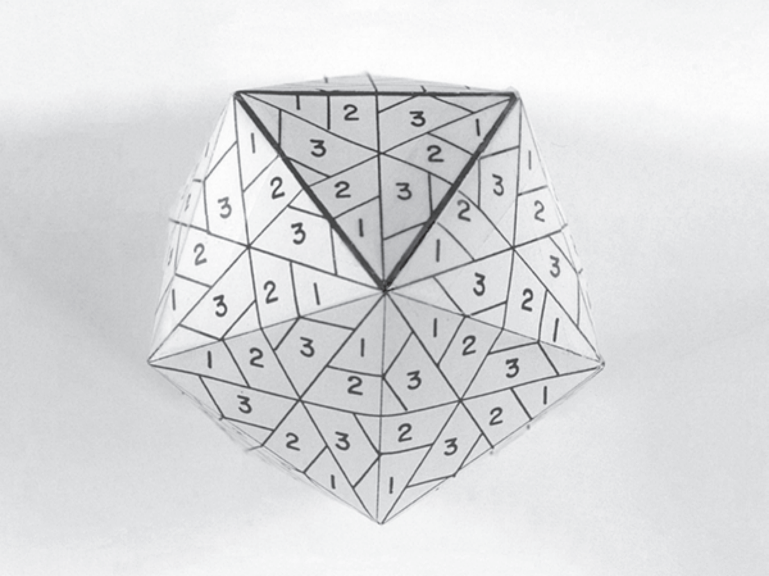

FIGURE 1.3 Photograph of the assembled model published by Hurst et al. (1987) showing the protein arrangement in an icosahedral capsid structure. This particular structure is a representation of the viral family Picornaviridae. The members of this family produce capsids that contain multiple copies of three major (larger-sized, numbered 1, 2, and 3) capsid proteins and one minor (smaller-sized, numbered 4) capsid protein. The relative positions of the three major capsid proteins are shown in this illustration as trapezoids numbered 1, 2, and 3. The trapezoidal shape is used for illustrative purposes, as the true shapes of these proteins is more complex and not truly trapezoidal. The darkly outlined triangle represents one of the twenty sides of the viral capsid. Although these sides are often referred to as "faces," the term icosahedron literally interprets from the Greek as meaning that this structure has twenty surfaces upon which it could rest.

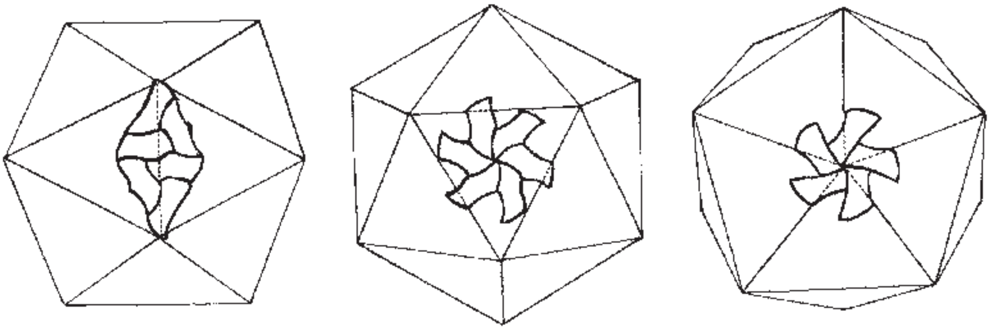

FIGURE 1.4 Drawing of an icosahedral capsid structure showing what would be a mirror image of the shape of the capsid proteins for the viral family Bromoviridae. Unlike the picornaviral model, the bromoviral capsid seems to contain multiple copies of only one type of capsid protein. Presumably, those copies of the same protein would be rotated into different relative positions such that they can arrange into an icosahedron. This drawing shows how those capsid proteins combine to produce the twofold (left), threefold (center), and fivefold (right) axes of symmetry that define an icosahedral structure.

family Picornaviridae. The members of this family have a single stranded RNA genome that is positive sense. Picornaviruses produce capsids that contain multiple copies of three major (larger-sized, numbered 1, 2, and 3) capsid proteins and one minor (smaller-sized) capsid protein. The relative positions of the three major capsid proteins are shown in Figure 1.3 as trapezoids numbered 1, 2, and 3. The trapezoidal shape is used for illustrative purposes, as the true shapes of these proteins is more complex and not truly trapezoidal.

Figure 1.4 is a drawing of an icosahedral capsid structure showing what would be a mirror image of the shape of the capsid proteins for the viral family Bromoviridae. The bromoviruses have a single stranded RNA genome that is positive sense. Unlike the picornaviral model, the bromoviral capsid seems to contain multiple copies of only one type of capsid protein. Presumably, those copies of the same protein would be rotated into different relative positions such that they can arrange into an icosahedron. An icosahedral structure is defined by having twofold (left), threefold (center), and fivefold (right) axes of symmetry. Figure 1.3 shows a fivefold axis of symmetry.

Figure 1.5 is an electron micrograph of coronaviruses and these particular virions belong to the species *Gammacoronavirus avian coronavirus*. The coronaviruses are members of the viral family Coronaviridae, and they have a single stranded RNA genome that is positive sense. The coronavirus has a helical nucleocapsid, and its mature virions possess a outer lipid membrane with characteristic club-shaped spikes that protrude from the membrane. The Latin origin of the name,

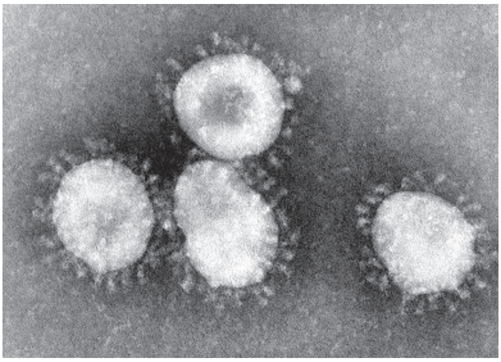

FIGURE 1.5 Transmission electron micrograph of coronaviruses. Viruses have genus and species names, plus higher taxonomy levels also exist. The virus shown in this image is designated *Gammacoronavirus avian coronavirus*, and it belongs to the family Coronaviridae. Viruses are designated taxonomically on the basis of their morphology and host range. Viral taxonomic designations also used to include distinctions that were based upon viral antigenic cross reactivity. The usage of antigenic cross reactivity has largely been replaced by taxonomic designations that reflect viral genomic analysis. The title of this image is “Coronaviruses 004 lores.jpg,” it is a Public Domain image from the Centers for Disease Control and Prevention’s Public Health Image Library (PHIL), with identification number #4814.

corona, given to this virus group means crown or wreath and refers to an item worn on the head as adornment. Those spikes enable the coronavirus virions to bind onto the host cell molecule which the virus uses as a receptor. Virus groups differ with respect to which host cell component they have evolved to use as their receptor, but the commonality is that a receptor will be part of a molecule that is exposed on the surface of their targeted host cells. Binding to its receptor is a necessary initial step, after which the viral nucleic acid genome enters the host cell and the virus can then begin its replicative process. For many virus groups, that replicative process may result in the immediate production of progeny virions. Other virus groups incorporate reproductive strategies in which progeny virions are produced less often, with the virus instead using such maintenance strategies as latency, endogeny and lysogeny, as will be explained later in this chapter.

Figure 1.6 is a transmission electron micrograph of multiple bacteriophage attached to the cell wall of a bacterial host. The term phage refers to viruses that infect microorganisms. There are many viral families that include members which are infectious for bacteria, and those members are known as bacteriophage. I would guess that these particular virions belong to the viral family Siphoviridae. Siphoviruses have double-stranded DNA genomes packaged within an icosahedral structure. The siphoviral icosahedron has a flexible noncontractile tail and short fibers that extend outward from the bottom, distal end, of the tail.

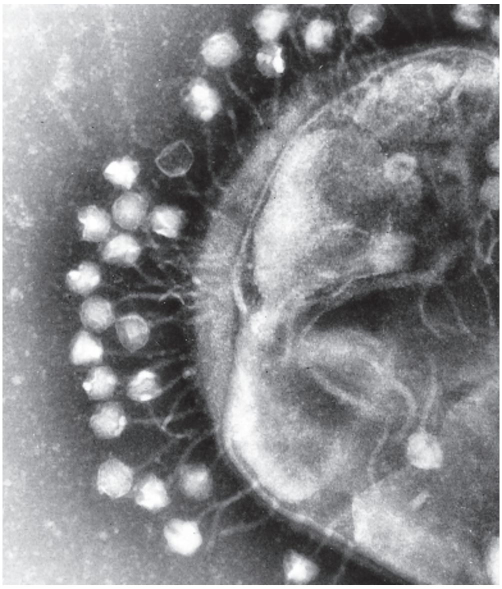

FIGURE 1.6 Transmission electron micrograph of bacterial viruses, termed bacteriophage, attached to a bacterial cell wall. My guess, based upon their taxonomic structure, would be that the viruses shown in this image belong to the family Siphoviridae. This image is titled "Phage.jpg" by author Dr. Graham Beards, and it is being used under a Creative Commons Attribution-Share Alike 3.0 Unported license. The magnification is approximately 200,000. https://en.wikipedia.org/wiki/File:Phage.jpg.

There also exist many other types of acellular infectious agents which have commonalities with the classical viruses in terms of their ecology. Two of these other types of acellular infectious agents, the viroids and prions, are included in this book and are addressed within their own respective chapters (chapter 7 by Flores, Di Serio, Navarro, Duran-Vila and Owens, chapter 17 on viruses of humans by Hurst). Viroids are biological entities akin to the classical viruses and likewise can replicate only within host cells. The viroids possess RNA genomes but lack capsid proteins. The agents which we refer to as prions were once considered to be nonclassical viruses. However, we now know that the prions appear to be aberrant cellular protein products which, at least in the case of those afflicting mammals, have acquired the potential to be environmentally transmitted. The natural environmental acquisition of a prion infection occurs when a susceptible host mammal ingests the bodily material of an infected host mammal. The reproduction of prions is not a replication, but rather seems to result from a conversion of a normal host protein into an abnormal form (Hurst chapter 17 on viruses of humans). A prion would be a 'non-genomic acellular infectious agent'.

All members of the known virus families complete their reproduction when they are internal to a host cell, with one exception.

That unique exception is the species Acidianus two-tailed virus (genus *Bicaudavirus*, family Bicaudaviridae) which undergoes a morphological maturation following its release from a host cell (Hochstein et al. 2018). This uniqueness of the Acidianus two-tailed virus suggests that it may represent the initial discovery of an entirely new category of biological entities.

1.1.2 What is Viral Ecology?

Ecology is the study of the relationships between organisms and their surroundings. Viral ecology is, therefore, the relationship between viruses, other organisms, and the environments which a virus must face as it attempts to comply with the basic biological imperatives of genetic survival and replication. As shown in Figure 1.7, interactions between

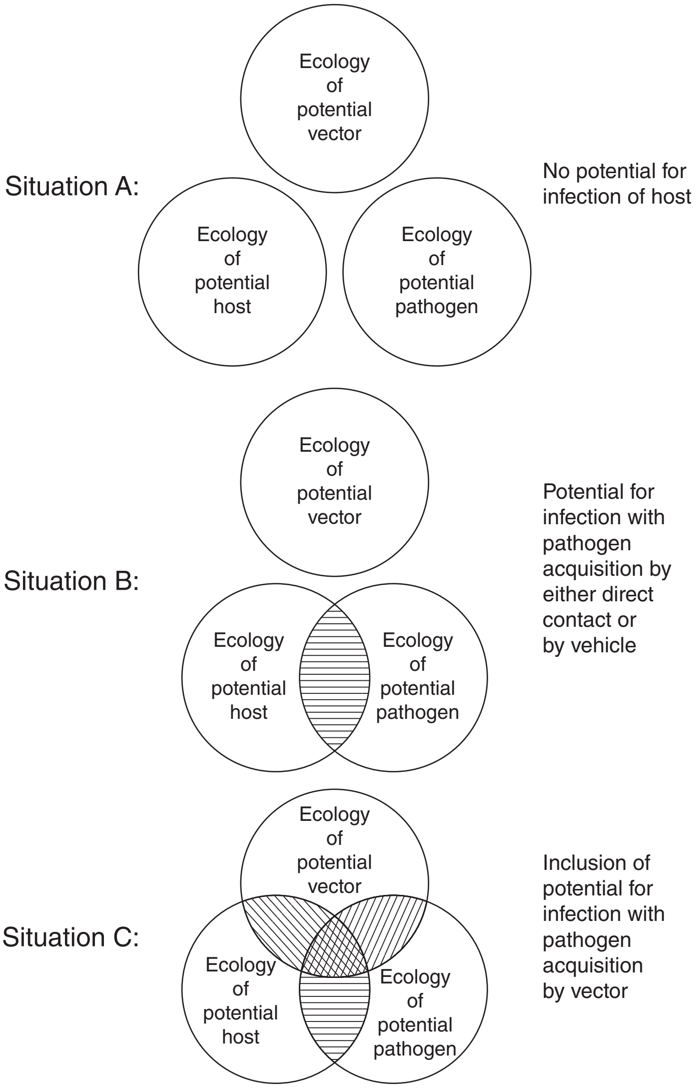

FIGURE 1.7 Interactions between organisms (biological entities) occur in the areas where the physical and chemical ecologies of the involved organisms overlap. Infectious disease is a type of interaction in which a microorganism acts as a parasitic predator. The microorganism is referred to as a pathogen in these instances.

species and their constituent individual organisms (biological entities) occur in the areas where there exist overlaps in the temporal, physical, and biomolecular (or biochemical) aspects of the ecological zones of those different species. Many types of interactions can develop between species as they share an environment. One of the possible types of interactions is predation. When a microorganism is the predator, that predator is referred to as being a pathogen and the prey is referred to as being a host. Viruses are predators, and later in this chapter I will return to explaining that concept.

When we study viral ecology we can view the two genetic imperatives that every biological entity must face, namely, that it survive and that it reproduce, in the perspective of a biological life cycle. A generalized biological life cycle is presented in Figure 1.8. This type of cycle exists, in its most basic form, at the level of the individual virus or individual cellular being. However, it must be understood that in the case of a multicellular being this biological life cycle exists not only at the level of each individual cell, but also at the tissue or tissue system level, and at the organ level. This biological life cycle likewise exists on even larger scales, where it operates at levels which describe the existence of each species as a whole, at the biological genus level, and also seems to operate further upward to at least the biological family level. Ecologically, the life cycles of those different individuals and respective species which affect one another will become interconnected both temporarily, geographically, and biologically. Thus, there will occur an evolution of the entire biological assemblage and, in turn, this process of biotic evolution will be obliged to adapt to any abiotic changes that occur in the environment which those organisms share. While a species physiologic capacities establish the potential limits of the niche which it could occupy within this shared environment, the actual operational boundaries of it's niche are more restricted and defined by it's interspecies connections and biological competitions. The concepts of evolution, habitat and niche, including the difference between a potential niche versus an operational niche, recently have been discussed by Hurst (2016, 2021).

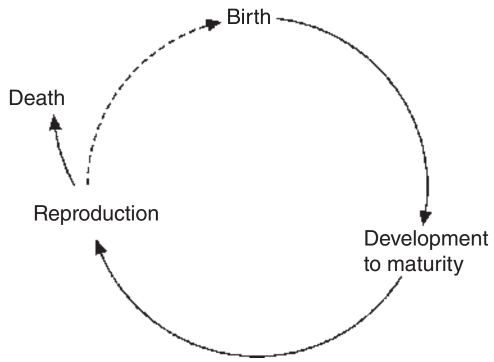

FIGURE 1.8 Generalized biological life cycle. Ecologically, the life cycles of different organisms which affect one another are temporally interconnected.

1.1.3 Why Study Viral Ecology?

The interplay which occurs between a virus and the living organisms which surround it, while all simultaneously pursue their own biological drive to achieve genetic survival and replication, creates an interest for studying the ecology of viruses (Larson, 1998). While examining this topic, we improve our understanding of the behavioral nature of viruses as predatory biological entities. It is important to realize that in nature both the viruses of macroorganisms and the viruses of microorganisms normally exist in a cycle with their respective hosts. Under normal conditions, the impact of viruses upon their natural hosts may be barely apparent due to factors such as evolutionary coadaptation between the virus and its host (evolutionary coadaptation is the process by which species try to achieve a mutually acceptable coexistence by evolving in ways which enable them to adapt to one another). However, when viruses find access to new types of hosts and alternate transmission cycles, or when they encounter a concentrated population of susceptible genetically similar hosts such as occurs in densely populated human communities, communities of cultivated plants or animals, or algal blooms, then the impact of the virus upon its host population can appear catastrophic. The term "biological invasion" is used to describe some of these catastrophic encounters. Biological invasions will be discussed in Section 1.2 of this chapter.

As we study viral ecology we come to understand not only those interconnections which exist between the entities of virus and host, but also the interconnections between these two entities and any vectors or vehicles which the virus may utilize. As shown in Figure 1.9, this interplay can be represented by the four vertices of a tetrahedron. The possible routes by which a virus may move from one host organism to another host organism can be illustrated as the interconnecting lines between those vertices which represent two hosts (present and proximate) plus one vertice apiece representing the concepts of vector and vehicle. Figure 1.10, which represents a flattened form of the tetrahedron shown in the previous figure (Figure 1.9) can be considered our point of reference as we move forward in examining viral ecology. The virus must survive when in association with the present host and then successfully move from that (infected) host organism (center of Figure 1.10) to another host organism. This movement, or transmission, may occur via direct contact between the two host organisms or via routes which involve vectors and vehicles (Hurst and Murphy 1996). Vectors are, by definition, animate (living) objects. Vehicles are, by definition, inanimate (non-living) objects. Any virus which utilizes either vectors or vehicles must possess the means to survive when in association with those vectors and vehicles in order to sustain its cycle of transmission within a population of host organisms. If a virus replicates enough to increase its population while in association with a vector, then that vector is termed to be "biological" in nature. If the virus population does not increase while in association with a vector, then that vector is termed to be "mechanical" in nature. Because viruses are obligate intracellular parasites, and vehicles are by definition non-living, then we must assume that the virus cannot increase its population while in association with a vehicle.

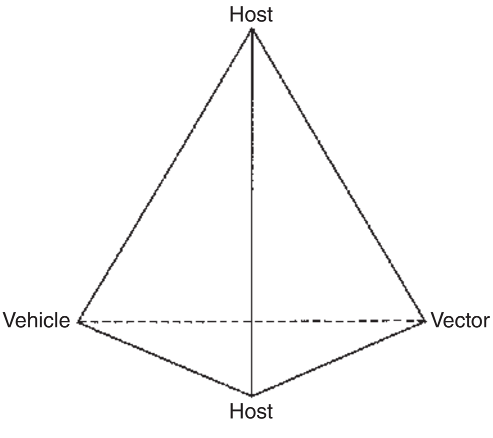

FIGURE 1.9 The lines connecting the four vertices of this tetrahedron represent the possible routes by which a virus can move from one host organism to another host organism.

Environmentally, there are several organizational levels at which a virus must function. The first and most basic of those levels is the individual host cell. That one cell may comprise the entire host organism. Elsewise, that host cell may be part of a tissue. If within a tissue, then the tissue will be contained within a larger structure termed either a tissue system (plant terminology) or an organ (plant and animal terminology). That tissue system or organ will be contained within an organism. The host organism is exposed to the open (ambient) environment, where it is but one part of a population of other organisms belonging to its same species. The members of that host species will be surrounded by populations of other types of organisms. Those populations of other types of organisms will be serving as hosts and vectors for either the same or other viruses. Each one of these organizational levels represents a different environment which the virus must successfully confront. A virus' affects upon it's hosts and vectors will draw responses against which the virus must defend itself if the virus is to survive. Also, the virus must always be ready to do battle with it's potential

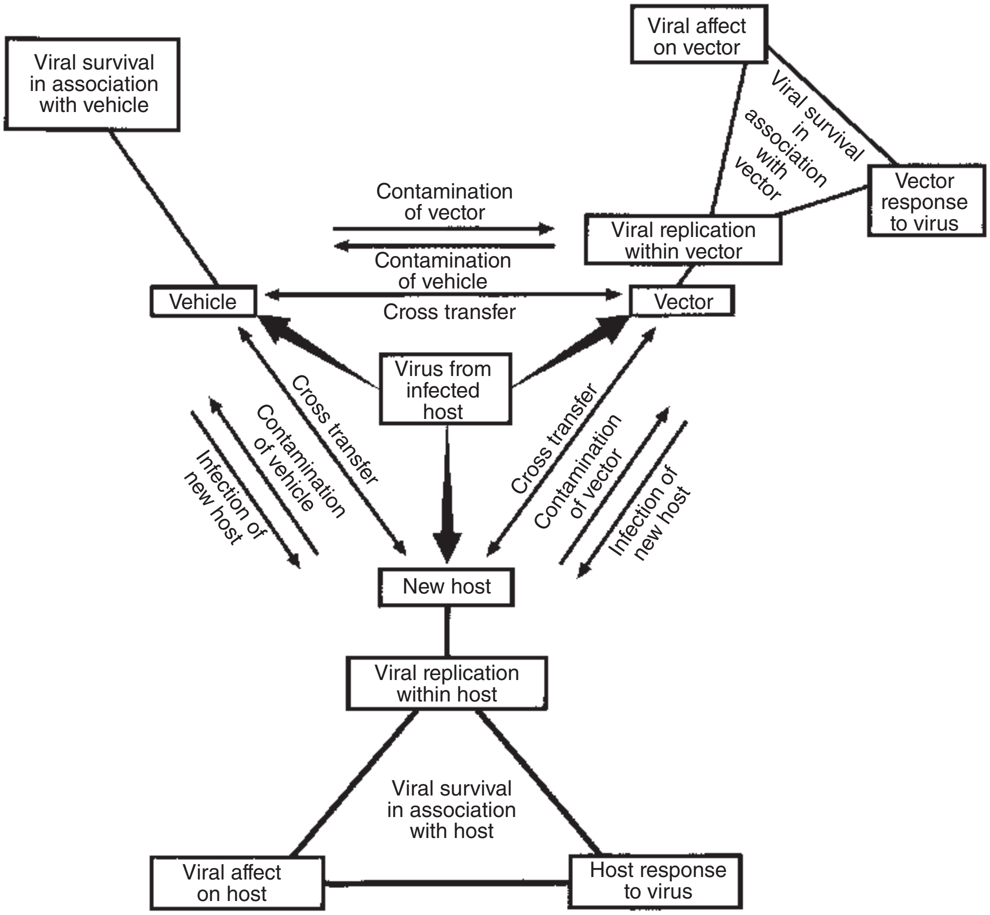

FIGURE 1.10 Viral ecology can be represented by this diagram, which represents a flattened form of the tetrahedron shown in the previous figure (Figure 1.9). The virus must successfully move from an infected host organism (center of figure) to another host organism. This movement, or transmission, may occur via direct transfer or via routes which involve vehicles and vectors. In order to sustain this cycle of transmission within a population of host organisms, the virus must survive when in association with the subsequently encountered hosts, vehicles and vectors.

biological competitors. Contrariwise, the virus must be open to considering newly encountered (or reencountered) species as possible hosts or vectors. Because of their acellular nature, when viruses are viewed in the ambiental environments (air, soil and water) they appear to exist in a form that essentially is biologically inert. However, they have a very actively involved behavior when viewed in these many other organismal environments.

Considering the fact that viruses are obligate intracellular parasites, their ecology must be presented in terms which also include aspects of the ecology of their hosts and any vectors which they may utilize. Those factors or aspects of viral ecology which we study, and thus which will be considered in this book, include the following:

Host Related Issues

1. what are the principal and alternate hosts for the viruses;
2. what types of replication strategies do the viruses employ on a host cellular level, host tissue or tissue system level,

host organ level, the level of the host as a whole being, and the host population level;

3. what types of survival strategies have the viruses evolved that protect them as they confront and biologically interact with the environments internal to their host (many of those internal environments are actively hostile, as the hosts have developed powerful defensive mechanisms);
4. what direct effects does a virus in question have upon its hosts, i.e. do the hosts get sick and, if the hosts get sick, then how severe is the disease and does that disease directly threaten the life of the host;
5. what indirect effects does the virus have upon its hosts, i.e., if the virus does not directly cause the death of the hosts or if viral-induced death occurs in a temporarily delayed manner as is the case with slow or inapparent viral infections, then how might that virus affect the fitness of the host to compete for food resources or to avoid the host's predators;

General Transmission-Related Issue

6. what types of transmission strategies do the viruses employ as they move between hosts, including their principal and alternate transmission routes which may include vehicles and vectors; and

Vector-Related Issues

7. in reference to biological vectors (during association with a biological vector the virus will replicate and usually is carried within the body of the vector), what types of replication strategies do the viruses employ on a vector cellular level, vector tissue or tissue system level, vector organ level, the level of the vector as a whole being, and also on a vector population level;
8. in reference to biological vectors, what types of survival strategies have the viruses evolved that protect them as they confront and biologically interact with the environments internal to their vectors (those internal environments may be actively hostile, as vectors have developed many powerful defensive mechanisms);
9. in reference to biological vectors, what direct effects does a virus in question have upon its vectors, i.e. do the vectors get sick and, if the vectors get sick, then how severe is the disease and does that disease directly threaten the lives of the vectors;
10. in reference to biological vectors, what indirect effects does the virus have upon its vectors, i.e., if the virus does not directly cause the death of the vectors or if viral-induced death occurs in a temporarily delayed manner as is the case with slow or inapparent viral infections, then how might that virus affect the fitness of the vectors to compete for food resources or to avoid the vector's predators;
11. in reference to mechanical vectors, what types of survival strategies have been evolved by those viruses which are transmitted by (and during that event usually carried on the external surfaces of) mechanical vectors, since while in association with a mechanical vector the virus must successfully confront any compounds naturally present on the body surface of the vector plus confront the passively hostile ambiental environments of either air, water or soil through which the vector will be moving; and

Vehicle-Related Issue

12. what types of survival strategies have been evolved by those viruses which are transmitted by way of vehicles and which thereby must successfully confront the passively hostile ambiental environments of either air, water or soil as the virus itself is transferred through those environments.

If biological curiosity alone were not a sufficient reason for studying viral ecology, then perhaps we would study the viruses out of a desire to both understand them as predators and to contemplate the ways in which we might enlist their aid as ecological tools. One of the ways in which we enlist the viruses is to use them as viral vectors in gene therapy (Lundstrom 2018).

1.2 SURVIVING THE GAME: THE VIRUS AND IT'S HOST

Remember that: *so long as the virus finds a new host, whether or not the current host survives is unimportant*. Although it may be beneficial to not kill a current host until that host has reproduced to help provide a new generation of potential host organisms, if the host to virus ratio is large enough, then even this latter point may be unimportant. This section presents in general terms the relationship between a virus and host. The generalities of relationships between viruses, vectors, and vehicles will be discussed in Section 1.3 of this chapter. The specific subject of the practical limits to viral virulence in association with hosts and vectors will be addressed in Section 1.4 of this chapter.

Biological invasions are events that occur when a host population encounters a virus which in some way seems new. Impact from the virus can make the invasion appear catastrophic when viewed from the hosts perspective. These invasions likely also would appear catastrophic when viewed from the virus perspective.

The categories of biological invasion are:

Three categories of events can lead to biological invasion of a virus into a host population. These categories are: first, that this virus species and host species (or sub-population of the host species) may never have previously encountered one another; second, if there have been previous encounters, the virus may have since changed to the point that antigenically it appears new to the host population; and third, even if the two species may have had previous encounters, this subpopulation of the host species subsequently may have been geographically isolated for such a lengthy period of time that most of the current host population represents a completely new generation of susceptible individuals.

"No previous encounter between virus species and host" can be represented by the introduction of measles into Pacific Islands as described by Shanks (Shanks 2016) and the introduction of HIV. Humans as a species have coevolved with the successfully invading virus which causes measles (species *Measles morbillivirus*, genus *Morbillivirus*, family Paramyxoviridae). Sadly, the biological invasion of HIV viruses into human populations also seems to have been successful (Caldwell and Caldwell 1996) and the extreme host death rate associated with this invasion can be assumed to indicate that the two species have not had time to coevolve with one another. The human immunodeficiency viruses cause HIV and they belong to the genus *Lentivirus* (family Retroviridae).

"Previously encountered virus species has changed" can be represented by the H1N1 influenza pandemic of 1918–1919 caused by a virus which developed novelty by reassortment of its genomic segments, and the SARS-CoV-2 coronavirus pandemic of 2019–2021 caused by a virus unofficially designated as strain 2 of the species *Severe acute respiratory syndrome-related coronavirus*, (genus *Betacoronavirus*, family Coronaviridae) which is a genetic variant of the species *Severe acute respiratory syndrome-related coronavirus*.

"New generation of the host species, possibly susceptible because of geographic isolation" can be represented by outbreaks of viral disease in remotely

> isolated host communities that are located on small islands, and in mountainous or jungle areas, with those outbreaks related to the occasional arrival of ill visitors. For human populations, this is only of historical importance because the development of tourism facilitated by steamship travel and air travel have increased the frequency of interaction with human communities that once were seldom encountered due to geographic isolation. This type of outbreak pattern certainly does still seem relevant for some animal species which live in geographical isolation. This category of biological invasion may also be a contributing factor in some of the "bloom and bust" cycles noticed to occur in photosynthetic aquatic microorganisms, during which the population of a host species may rapidly increase only to suffer a dramatic decline caused by a virus.

Influenza invasions generally always prove successful, and influenza refers to infections by members of the genera *Alphainfluenzavirus*, *Betainfluenzavirus*, and *Gammainfluenzavirus*, all belonging to the family Orthomyxoviridae. Disease outbreaks cause by the Ebola (species *Zaire ebolavirus*, genus *Ebolavirus*, family Filoviridae) and Lassa (species *Lassa mammarenavirus*, genus *Mammarenavirus*, family Arenaviridae) viruses generally have represented examples of unsuccessful biological invasions. The zoonotic introductions of these viruses into human populations cause sporadic, but often limited, outbreaks of hemmorrhagic fever and notably produce fear. The observation of extremely virulent and fulminate symptomatology in humans that has been associated with infections by some strains of Ebola and Lassa can generally be assumed to indicate either that we humans are not the natural host for these viruses or, at the very least, that these two viral species have not had time to coevolve with humans. Fortunately, the relatively limited chain of transmission for most viral strains associated with these two illnesses (for Ebola see Coltart et al. 2017, for Lassa see Fuller 1974), with their serial transfers often being limited to only two or three hosts in succession, represents what will occur when a virus species appears genetically unable to establish a stable relationship with a host species. The Western African Ebola virus epidemic of 2013–2016 was a very unfortunate exception and notable for the extensive chain of human transmissivity associated with that viral strain.

An overblown immune response by the host (Spear 1998) seems to cause the extreme symptomatology and mortality which result in humans that have Ebola or Lassa infection. This type of host response has been described as a cytokine storm (Younan et al. 2017) and similarly can occur in some cases when humans have coronavirus infection (Gao et al. 2020). The severe cytokine reaction which develops in cases of Ebola and Lassa disease seems to occur in nearly all instances of human infection and it is noted for producing massive hemorrhage, most obviously observed by blood leaking from eyes, nose, mouth, and pores of the skin. The cytokine reaction which develops in a minority of coronavirus disease infections is most noted for producing pneumonia. While having self-induced death of a host individual occur as the product of an encounter with a pathogen may seem like a dire outcome, this outcome represents a mechanism of defense operating at the species level benefiting the host population. If a particular infectious agent is something against which members of the host population could not easily defend themselves, then it may be better to have that infected host individual die (and die very quickly!) to reduce possible spread of the contagion to other members of the host population.

While in association with a host, the virus has only one principle goal. This goal is for the virus to replicate itself to a sufficient level that it can achieve transmission to another host. Attaining the goal can be done by one of two basic strategies. The first of these strategies would be a productive infection, for which five basic patterns can be defined. The second strategy would be a non-productive infection.

The patterns of productive infection are:

"Short term – initial" in which viral production has only a short term initial course, after which the viral infection ends and there no longer is a presence of that virus within the body of the host individual, although subsequent reinfection can occur; the outcome from this pattern of infection depends upon the virus type and historical exposure to that type within the host population, the situation being that in otherwise healthy members of a multicellular host population with which the virus has coevolved, these infections are usually mild and by themselves normally associated with a fairly low incidence of mortality;

"Recurrent" in which repeated episodes of viral production occur, this pattern often has a very pronounced initial period of viral production, after which the virus persists in a latent state within the body of the host with periodic reinitiations of viral production that usually are not life threatening;

"Increasing to end-stage" in which viral infection is normally associated with a slow, almost innocuous start followed by a gradual progression associated with an increasing level of viral production and eventual death of the host, in these instances death of the host may relate to destruction of the host's immunological defense systems which then results in death by secondary infections;

"Persistent-episodic" is a pattern that represents a prolonged nonfatal infection which may persist for the remainder of the hosts natural lifetime associated with a continuous production of virions within the host, but interestingly the infection only episodically results in symptoms, the viral genome does not become quiescent, the host remains infectious throughout the course of this associative interaction, and very notably some members of the family Picobirnaviridae often produce this pattern of productive infection;

"Persistent but inapparent" is a pattern that represents a prolonged nonfatal infection which seemingly never results in overt symptoms of illness attributable to that particular virus, the viral genome never becomes quiescent and viral infections that follow this pattern are persistently productive with the host often remaining infectious for the remainder of their natural lifetime, with notable examples of viruses which produce this pattern being members of the family Anelloviridae, and it also occurs in certain rare instances of infection by Human immunodeficiency virus 2 which is a member of the genus *Lentivirus* of the family Retroviridae.

The goal of a productive infection is for the virus to actively produce infectious viral particles (those capable of infecting cells) which are termed "virions", during the period of virus' association with the current host. Subsequent spread of the infection to the next host occurs by transfer of these produced virions. A virus often interferes with the natural generation of host cell proteins (Gao et al. 2016) while the virus is instead redirecting the cell to produce viral proteins. Productive viral infections involve an energetic cost to the host cell, and that cost mostly is associated with the translation of viral proteins (Mahmoudabadi et al. 2017). This cost which a productive viral infection imposes upon its host cell is directly related to the burst size, which is the number of progeny viral particles released from each infected cell. The cost per infected cell will be much greater with a large burst size. Mahmoudabadi et al. (2017) have estimated these costs of viral infections relative to the energy budget of their host, suggesting that an *Escherichia* virus T4 infection (genus *Tequatrovirus*, family Myoviridae) with a typical burst size of two hundred virus particles may consume approximately a third of its unicellular host’s total energy budget. By contrast, an influenza viral (family Orthomyxoviridae) infection with a typical burst size of six thousand progeny virus particles may consume only about one percent of a cells energy budget for the multicellular host. The difference is due in large part to the much larger size of the cells that host influenza virus infections.

Some bacteriophage can facilitate their replication by phenotypic reconfiguration of a host cell to redirect energy and substrates towards viral synthesis (Warwick-Dugdale et al. 2019). The process of phenotypic reconfiguration uses auxiliary metabolic genes that represent genetic information which the virus progenitors originally acquired from host genomes and then subsequently adapted within their viral genomes. An infecting virus also may improve host fitness, which is termed phenotypic augmentation (Warwick-Dugdale et al. 2019).

There are two options to the “Short term – initial” pattern. The first option is a very rapid, highly virulent approach which is termed “fulminate” (seemingly explosive) and usually results in rapid death of the host organism. This first option usually represents the product of an encounter between a virus and a host with which that virus has not coevolved. The second option is for the virus to gradually become less virulent as it sequentially passes from host to host, with the virus species and its host species eventually coevolving to a point that the virus causes infections which often progress more slowly and appear more benign to the host.

The “Recurrent” pattern as defined here represents virus families that incorporate latency into their replicative scheme. Latency is a relationship in which the viral genome never is eradicated and remains within cells of the infected host for the entire lifetime of the host. The initial infection of a host by one of these viruses may produce very obvious symptoms, after which latent viruses generally show only sporadic replication. Reactivation of latent virus genomes results in a resumed production of progeny viruses and recurrence of disease symptoms. The latent viral genome is considered to be quiescent during those periods of time when progeny virions are not being produced by the infected cells.

Presumably it is patrolling by the host immune system which enforces the need for a virus to maintain latency. The immune system seems not to recognize and attack quiescently infected cells that contain the latent viral genomes. Immune suppression due to stress, coinfections (instances of simultaneous infection by more than a single species of pathogenic organism), and medications, can however facilitate the reactivation of latent viruses. Traumatic damage to cells which contain the latent viral genomes also can result in viral reactivation. Thusly, latent infections may become either episodically symptomatic or symptomatically inapparent depending upon the virus type and immune status of the infected host. Latency reduces the impact which the virus infection imposes upon the hosts energy budget, and latency also increases at least the short term survival prospects for the infected host. Latent infections may indeed never be life threatening to the host. Herpes viruses (family Herpesviridae) represent an example of the viral groups which utilize latency. The Herpes viruses most typically are associated with recurring blisters of the skin and mucosal epithelial cells. Some herpes viruses maintain their latency within nerve cells, while other herpes viruses maintain their latency within immune cells. Itzhaki (2018) has suggested that latent Herpes simplex virus (species *Human alphaherpesvirus 1*, genus *Simplexvirus*, family Herpesviridae) infections in humans may have a role in the development of Alzheimer’s disease. Latency is not a replicative strategy whose usage is limited to viruses affecting animals. Indeed, many viral groups that are infective of plants use latency (Takahashi et al. 2019).

The pattern of "Increasing to end-stage" represents permanently active infections. The Human immunodeficiency viruses (genus *Lentivirus*, family Retroviridae) represent an example of viral infections that typically increase in severity until death of the host.

The pattern of "Persistent but inapparent" can be represented by members of the viral family Baculoviridae, which often cause covert rather than overt infections of their insect hosts. The prevalence of covert Baculoviridae infection sometimes exceeds fifty percent. Covert infection may have the form of either a non-productive latency, or else a sublethal infection during which there is only a low level production of viral progeny (Williams et al. 2017).

The product of interspecies encounters between a virus and it's natural coevolved host will usually lead to a relatively benign (mild, or not directly fatal), statistically predictable, outcome that results from adaptive coevolution between the two species. Still, these normal relationships do not represent a static coexistence between the virus and the natural host, but rather a tenuous equilibrium. Both the virus species and it's evolved host species will be struggling to get the upper hand during each of their encounters. The result will normally be some morbidity and even some mortality among the host population as a result of infection by that virus. Yet, because the virus as a species may not be able to survive without this particular natural host species, excessive viral-related mortality in a host population may not be in the long term best interest of the virus. Non-productive viral infection strategies offer a survival option.

A non-productive infection pattern may either seldom or never produce actual virions. Thus, the usual goal of a non-productive infection strategy is to pass the infection to the next host by directly transferring only viral genomic sequences. If those viral sequences are genetically inherited, then they are considered to be endogenous viral elements (Dennis et al. 2019; Edwards et al. 2018; Feschotte 2010; Katzourakis and Gifford 2010; van der Kuyl et al. 1995). There also has been a suggestion that genes from the viral family Mimiviridae can be laterally transferred into eukaryotic host genomes (Filée 2014) but I will not further discuss that concept in this chapter.

The patterns of non-productive infection include:

Endogeny which describes intracellular viral genomic information genetically inherited by progeny of multicellular organisms, the endogenous DNA generally is contained within the genome of a multicellular host; and

Lysogeny which generally describes intracellular viral genomic information genetically inherited by progeny of unicellular organisms, it is a term approximately equivalent to endogeny, and the viral genomic information of the lysogenic virus may be contained either within the genome of its unicellular host or exist as an independent genetic entity within the host cell. That viral genetic material may be either newly gained as a consequence of recent infection or have been inherited from earlier generations of the host. The term lysogeny has been used to describe some additional types of viral infections, such as the development of a genomically integrated provirus which can result when adeno-associated viruses (AAV, genus *Dependoparvovirus*) become intracellularly established within animal cells in the absence of a helper virus. Establishment of the AAV provirus is considered to be a type of latency, and the AAV provirus can become activated if the cell subsequently is superinfected by a helper virus. The result of that proviral activation will be production of progeny AAV.

Endogeny is one type of viral strategy for achieving a non-productive, or virtually non-productive, pattern of infection. Achieving an endogenous state implies that the genome of the virus is passed through the host's germ cells to all offspring of the infected host (van der Kuyl et al. 1995; Villarreal 2016). Both endogeny and lysogeny represent a high degree of coevolution between the virus and host species. Endogeny and lysogeny serve to maintain viability of a host and allow host propagation during conditions that otherwise might limit the possibility of an infecting virus achieving transmission. Endogenous and lysogenous infections may indeed never be life threatening to the host. Were an endogenous or lysogenous virus instead to enter a replicative cycle, then it might be that neither the individual virus nor its host would survive. Mutations occurring in the endogenous or lysogenous viral genomic information can result in that viral information becoming incapable of producing an infectious virus particle, in which case that viral genomic information is said to have become "grounded."

Endogeny and lysogeny, as with latency, also serve to reduce the impact which the virus infection imposes upon the hosts energy budget. Inheritance of endogenous and lysogenous infections, and successfully maintaining the status of endogeny and lysogeny in the subsequent offspring, also increases not only the short term survival of each infected host individual but also the long term survival of the host species.

The ability of a virus to enter latency similarly results from coevolution between a virus species and its host species, but the benefit which a host derives by surviving the latent infections must be established anew consequent to each generation of the host encountering and being infected by the virus.

Any non-viral DNA that is introduced to cells via transfection or viral transduction is termed to be an exogenous factor. Incorporation of exogenous DNA into the genetic information of a cell and subsequent inheritance of that DNA by progeny cells means that the newly added DNA has become endogenous.

When considering the presence of endogenous viral elements in animal genomes, we typically may think of retroviruses (family Retroviridae). Retroviruses have an RNA genome, they replicate their nucleic acid in the cell nucleus, and are unique among the currently identified viral families in that retroviruses must incorporate a DNA copy of their viral genome into the host cell genome as an obligatory early step for their viral replication. Retroviruses code for their own integrase enzyme. Pistello and Antonelli (2016) have suggested that integration of retroviral genomes into the host cellular DNA offers the virus an initial advantage by helping to ensure that host cells are persistently infected, but the advantage might shift over time in favour of the infected cell if mutational genetic changes in the integrated viral genome neutralize ability of the virus for generating progeny particles. It may be that the remnants of retroviral genomes which are contained in host genomes offer a benefit to the host by representing an acquirement of resistance to either the same or related retroviruses.

Placental mammals, including humans, permanently have incorporated species of endogenous retroviruses into the chromosomes of their genomes. Genes from those retroviruses can be useful to the host. An example of that usefulness are syncytins, they are proteins derived from endogenous retroviral elements and the syncytins are crucial in placental development (Soygur and Sati 2016). It additionally has been hypothesized that endogenous retroviral elements facilitated evolution of the placental mammals by suppressing maternal immunity during pregnancy (Villarreal 2016).

The presence of endogenous viral elements in animal host cells is not limited to only retroviruses. There are endogenous viral elements representing RNA viral families which replicate their genomes in the cellular nucleus, among those being Bornaviridae elements found in mammals and reptiles, and Orthomyxoviridae elements found in insects. Endogenous viral elements representing RNA viral families which replicate their genomes in the cytoplasm include Filoviridae elements found in mammals, Flaviviridae elements found in insects, Peribunyaviridae elements found in insects, Reoviridae elements found in insects, and Rhabdoviridae elements found in insects. Endogenous viral elements that represent DNA virus families which replicate their nucleic acid in the cell nucleus include the following: Circoviridae sequences having being found in amphibians, birds, fish, reptiles and mammals; Hepadnaviridae sequences in birds and reptiles; and Parvoviridae sequences both in mammals as well as in a reptile.

Gilbert et al. (2014) reported on the discovery of endogenous bornaviruses (family Bornaviridae), circoviruses (family Circoviridae), and hepadnaviruses (family Hepadnaviridae) in snakes. Pénzes et al. (2018) reported finding Parvoviridae sequences both in a vole (*Ellobius lutescens*) and a pit-viper snake (*Protobothrops mucrosquamatus*). In nature, a pit viper eagerly would eat a vole, and viruses will be acquired by ingesting virally infected animals, but it is uncertain as to whether a predation of voles might be related to the presence of endogenous Parvoviridae sequences in snakes.

A summary of endogenous viral elements in animals has been published by Katzourakis and Gifford (2010). A recent summary of Circoviridae sequences in vertebrates has been presented by Dennis et al. (2019).

There additionally has been a suggestion that Mimiviridae genes can be laterally transferred into eukaryotic host genomes (Filée 2014). Mimiviridae replication occurs both in the nucleus and the cytoplasm. Mimiviridae are part of the phylum Nucleocytoviricota, which are large nucleo-cytoplasmic DNA viruses, and many eukaryotes contain endogenous sequences from Nucleocytoviricota even in the absence of evidence that those eukaryotes are infected by members of this viral group (Gallot-Lavallée and Blanc 2017).

Some of the viruses that are endogenous have evolved to offer a survival-related benefit to their natural host, and this can give an added measure of stability to their mutual relationship. Two examples of this type of relationship are the hypovirulence elements associated with some strains of Chestnut blight fungi, and *Bracovirus* in wasps. The hypovirulence (reduced virulence) which the virus-derived genetic elements afford to the fungi that cause Chestnut blight disease reduce the virulence of those fungi (chapter 5 by Hillman and Milgroom). This reduced virulence allows the host tree, and in turn the fungus, to survive. An example of the benefit from endogenous bracoviruses is that members of the genus *Bracovirus* seem to play a crucial role in the *Cotesia congregata* wasp life cycle. There are enndogenous *Bracovirus* within the wasp genome, and those endogenous sequences produce viral particles within the wasp body. The progeny *Bracovirus* particles are injected into parasitized lepidopteran hosts during wasp oviposition. The *Bracovirus* particles encapsidate multiple dsDNA circles that encode virulence genes, and expression of those genes within the parasitized caterpillars suppresses the caterpillars immune system which is essential for wasp success (Louis et al. 2013).

There also are viruses which have left behind traces of their genomes as endogenous elements of plants (Takahashi et al. 2019), including the endogenous plant pararetroviruses which are considered to be genomic counterparts of the family Caulimoviridae. Caulimoviruses have DNA genomes that replicate in the nucleus, and caulimoviruses utilize reverse transcription (Geering 2020), but caulimoviruses do not code for an integrase enzyme.

Indeed, many of the endogenous viral elements found in animals, and endogenous viral elements found in plants, represent viral families that do not code for an integrase enzyme. That absence of an integrase leaves open for consideration the possibility that either a host cell integrase, or an integrase from another virus genome, installed those viral genomic materials into the host cell genome.

Viruses actually may have contributed to the evolution of immune systems (Broecker and Moelling 2019). Fragments of the virus genomes belonging to some RNA viral groups may be reverse-transcribed early during infection, with this process directed by endogenous retrotransposons. The produced viral complimentary DNA (cDNA) may become integrated into the host genome as endogenous viral elements and resultingly produce antisense Piwi-RNAs (piRNAs; Tassetto et al. 2019). The piRNA molecules represent part of the innate immune system as will be mentioned later in this chapter.

Viral insertions and endogenized viral genetic information are not necessarily harmless to the host! The endogenous retrovirus envelope syncytin genes, which are crucial to the initiation of a placenta, also seem to induce the spread of HIV-1 establishment of HIV reservoirs in the placenta (Tang et al. 2020). Human endogenous retrovirus envelope genes may play a role in the development of multiple sclerosis and also amyotrophic lateral sclerosis (Küry et al. 2018). Endogenized retroviral sequences have been associated with human tumors. Honda (2017) has suggested that endogenized viral elements may contribute to human cancer, and specifically, endogenous bornavirus (family Bornaviridae) sequences present in humans may be involved in predisposition to lung adenocarcinoma among nonsmokers (Honda 2017). Edwards et al. (2018) found that Filoviridae homologs in bats suppress the innate immune system, suggesting that genomically integrated sequences from non-retrovirus RNA viruses had been coopted and in that case possibly serve as regulators of innate immune signaling. Non-endogenized genomic viral insertions of Hepatitis B virus (species Hepatitis B virus, genus *Orthohepadnavirus*, family Hepadnaviridae) in humans are involved in the development of hepatocellular carcinoma.

Lysogeny historically has been represented by bacteriophage which insert their genomic DNA into that of the host genome, a task often done using viral encoded integrase genes. The inserted viral genome is termed to be a prophage, and the bacteria which contain those prophage are termed "lysogens." Lysogeny also is represented by other bacterial viruses which create a prophage that exists as a circular replicon within the bacterial cell and their bacterial hosts similarly are termed lysogens. Lysogens are immune to infection by viruses related to the prophage. If the prophage excises itself and begins a lytic replication, then the host bacteria will die from the resulting lysis which releases progeny bacterial viruses. There are at least some lysogenic viruses that must remain transcriptionally active, and their regulator proteins be expressed and functional, in order for the lysogenic state to be maintained (Schubert et al. 2007). That requirement for transcriptional activity represents a major difference between endogeny and lysogeny, as most endogenous viruses seem to remain stably endogenous without a need for the virus to be transcriptionally active.

As described above, having viruses exist as prophage in the bacterial genome thus is protective for the host cell provided that those prophage do not become lytic. Mutations occurring in the prophage can result in the prophage becoming "grounded," meaning that the prophage are unable to excise themselves (Ramisetty and Sudhakari 2019) and thus a grounded lysogen cannot become lytic.

1.2.1 Cell Sweet Cell, and Struggles at Home

As diagramed in Figure 1.11, viruses can arrive at their new host (solid arrows) either directly from the previously infected host, via an intermediate vehicle, or via an intermediate vector. Viral survival in association with the new host will first depend upon the virus finding it's appropriate receptor molecules on the host cell's surface (Spear 1998). After this initial location, the virus must be capable of entering (Cohen 2016) and modifying the host cell so that the virus can reproduce within that cell. If the host is multicellular, then the virus may first have to successfully navigate within the body of the host until it finds the particular host tissue which contains it's correct host cells. Throughout this process the host is not defenseless!

Molecular antiviral defenses begin at the most basic level which would be non-specific mechanisms. These conceptually include DNA

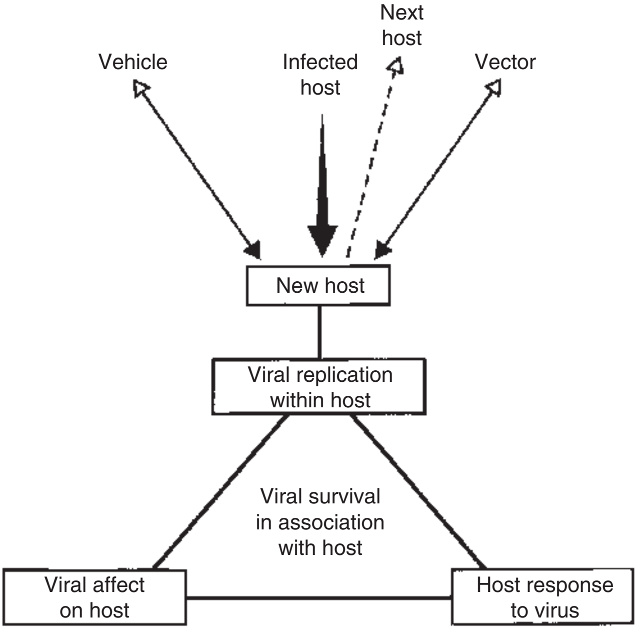

FIGURE 1.11 Viruses can arrive at their new host (filled arrows) either directly from the previously infected host, via an intermediate vehicle, or via an intermediate vector. Viral survival in association with that new host depends upon: viral replication within that new host, the effects which the virus has upon that host, and the response of that host to the virus. Successful viral survival in association with this new host will allow a possible subsequent transfer of the virus (open arrows) to its next host either directly, via a vehicle, or via a vector. This represents a segment from Figure 1.10.

restriction and modification systems. The viruses can counter these defenses in many ways, including the use of such techniques as virally-encoded restriction-like systems which chop-up the DNA genome of their host cells to provide a ready source of nucleic acids for the production of progeny viral genomes. The range of molecular defenses against viruses progresses upward with greater complexity to the use of post transcriptional processing (Russev 2007). Plants in fact heavily rely upon molecular defenses such as post-transcriptional control (chapter 6 by Patil and Fauquet). There also are viruses which try to shut down post-transcriptional defenses, and these are most clearly noticed among some viruses infective of plants.

Within a multicellular host, the virus may face anatomically associated barriers including membranous tissues in animals. The virus also may face non-specific, non-immune biological defenses (Moffat 1994), including such chemical factors as the enzymes found in both tears and saliva, and the acid found in gastric secretions. The types of anatomical and non-specific, non-immune defenses encountered can vary depending upon the viral transmission route and the portal by which the virus gains entry into the host's body. After a virus finds it's initial host cell and succeeds in beginning it's replication, the effects which the virus has upon the host can then draw a defensive biological response. The category of non-specific non-immune responses which a virus may encounter at this stage include even such things as changes in host body temperature for mammals. As if in a game of spy versus spy, the virus most importantly must survive the host's specific immune defenses.

The listing and adequate explanation of antiviral defense techniques would by itself be enough to nearly fill a library. But, I will attempt to summarize some of them here and help the reader to track those through this book.

Plants often try to wall off an infection, hoping essentially to live their lives despite presence of the infectious agent and hoping not to pass the infection along to their offspring through viral contamination of their germ cells. With vertebrates, the end goal can be perceived as ridding the body of the pathogen even if that end goal is not always achieved. Vertebrates have developed active mechanisms for hunting down and destroying pathogens and pathogen-infected cells within their bodies, and yet there are pathogens against which those active mechanisms do not work. Thus, the technique of "walling-off" a pathogen still occurs in vertebrates with an example being the development of tubercules in some mycobacterial and fungal infections.

Antimicrobial peptides are an additional defensive mechanism found in all classes of life, and represent a main part of the insect defensive system (Chapter 10 by Schroeder and Kevill).

Apoptosis, the targeting of individual cells within the body of the host for selective destruction by the host, commonly exists across the animal kingdom. This mechanism is used by many invertebrates (chapters 8 by Renault, 9 by Bateman, 10 by Schroeder and Kevill), as well as by vertebrates, to destroy any virally infected cells which may be present within their bodies. However, apoptosis is a weapon that can be used by both of the combatants. Using apoptosis to destroy virally-infected cells before the virus contained within those cells can assemble progeny virions is an effective approach when used carefully by the host. As might be expected, some viruses therefore defensively try either to shut-down the process of apoptosis, or at least to shut-down that process until the virus is ready to use apoptosis as a mechanism for assisting in the liberation of assembled virions from the infected host cell.

Vertebrates, and some of the invertebrates, have more complex body plans and can use them with good effectiveness in combating infections. With the evolutionary development of more complex body plans, comes the possibility of dedicating cells and even organs to the task of fighting pathogenic invaders. Those invertebrates with more complex body plans are represented in the anti-viral fight by their use of lymphoid organs to actively collect and either sequester or specifically assault and destroy the microbial offenders. Some of the aquatic crustaceans (Bateman chapter) tend to rely upon sequestering an infection and must hope to breed a new generation of their own progeny before they, themselves, are killed by the infection which they have sequestered within their body. At the same time, the infected parents must hope not to pass along the sequestered infection to their offspring through contamination of their eggs and sperm. Such viral collection and sequestration techniques are found upward through the evolutionary line and likewise used by the vertebrates.

Higher on the scale of defensive responses are things which we term to be immunological in nature. Some of these we term to be innate, others we call adaptive. A good starting point for this discussion of immunological responses is the capacity for distinguishing self versus non-self, accompanied by the capability for biochemically destroying cells that are determined to be non-self. This approach exists from at least the level of fungi (chapter 5 by Hillman and Milgroom) upwards for the non-animals, and among the animals this approach begins with at least the corals. Determining and acting upon the distinction of self versus non-self likely may have developed as a system that helps to support successful competition for growth in a crowded habitat, but it serves well against pathogenic organisms. As a health issue, this process sadly plays a role in autoimmune diseases and we try to suppress it when hoping to use organ and tissue transplantation to save human lives.

Immune systems exist as a collection of tools which enables the capacity to recognize and engage infecting viruses as part of an evolutionary struggle (Maarouf et al. 2018). These mechanisms of host response include intracellular components of the innate immune system, which has been described as the dominant immune system response for plants, fungi, insects, and primitive multicellular organisms. The innate immune system includes Piwi-interacting RNA (piRNA) molecules (Tassetto et al. 2019) and while their activities typically include a participation in the silencing of transposons, these RNA molecules seem also to represent a form of antiviral defense. Post-transcriptional activity of the RNA induced gene silencing (RNAi) machinery, previously also known by other names including "post-transcriptional gene silencing," acts as an additional antiviral defense mechanism (Andino Lab 2017). MicroRNA (miRNA) functions in RNA silencing and post-transcriptional regulation of gene expression and is involved with the regulation of innate immune responses. The innate immune system also includes pattern recognition receptors among which are toll-like receptors, the RIG-I and RIG-I like receptors (retinoic acid-inducible gene-I and gene-I-like receptors), NOD-like receptors (nucleotide-binding oligomerization domain-like receptors), and interferons. Interferons and their homologues are protein systems which vertebrates have developed and use effectively against some viruses, and correspondingly many viral groups contain mechanisms for suppressing interferon production (Muñoz-Jordán and Fredericksen 2010). Coronaviridae provide an example of viruses having learned to counteract the Type I interferon (IFN-I) response by using Inositol-requiring enzyme 1α (IRE1α) downmodulation of the host microRNA (miR) miR-30a-5p abundance (Ma et al. 2018). Coronaviruses also increase expression of the Suppressor of Cytokine Signaling genes SOCS1 and SOCS3 which additionally dampens the IFN-I antiviral response (Ma et al. 2018). The jawed vertebrates additionally possess immune systems which are termed adaptive and to some extent the innate immune system controls the adaptive immune system (Jain and Pasare 2017). Vertebrate adaptive immune systems respond in ways that involve B and T cells, and include the production of protein antibodies that can be highly specific. Further information on the role of immune systems in jawed vertebrates can be found in others chapters of this book (chapters 11 by Nerland, Øvergård and Patel, 12 by Chinchar, Duffus and Brunner, 13 by Marschang, Meddings and Ariel, 14 by Verhagen, 15 by Kramer and Tavakoli, 16 by Fereidouni, and 17 human viruses chapter by Hurst).

One of the more curious suggestions I have seen is that phagocytosis of bacteriophage present within mammals potentially might modulate aspects of both innate and adaptive immunity (Van Belleghem et al. 2018). By definition, bacteriophage are infectious of bacteria and are not infective for mammals. However, the presence of bacteria certainly will involve the presence of bacteriophage. Those bacteriophage likely would get engulfed by the phagocytic immune cells that are part of the innate immune system and those phagocytic cells have responsibility for helping to initiate the adaptive immune response.

One of the landmark milestones in virology was honored when the 1954 Nobel Prize in Physiology or Medicine was awarded jointly to John F. Enders, Frederick C. Robbins, and Thomas H. Weller for demonstrating the successful cultivation of animal tissue cells, which enabled use of those cells for cultivation of polioviruses (Enders et al. 1964). Cultivation of polioviruses was a key step that eventually led to the development of vaccines which prime the adaptive immune system against polioviruses (species Enterovirus C, genus *Enterovirus*, family Picornaviridae). Cohen-Dvashi et al. (2020) have discussed the design of immunotherapy against viruses.

Options for surviving the immune defenses of the host can include such techniques as:

"You don't know me" a virus may infect an accidental host, in which case a very rapid viral proliferation may occur, with an example being Lassa fever in humans;

"Being very, very quiet" some viruses form a pattern of latency in association with the virus' persistence within that host, an example being herpesviruses;

"Virus of a thousand faces" the practice of antigen shifting occurs, with an example being the lentiviruses;

"Keep to his left, that's his blind spot" some pathogenic agents maintain low antigenicity, an approach used by viroids and prions;

"Committing the perfect crime" there are viruses that infect the immune system, with this approach being taken by many retroviruses and herpesviruses; and

"Finding a permanent home" a high level of coevolution can result in a virus taking up permanent genetic residency within the host and thereby the virus automatically becomes transmitted to the host's progeny, this is an approach taken by viroids, endogenous retroviruses, and LTR retrotransposons.

Each virus must successfully confront it's host's defenses while at the same time the virus is trying to replicate sufficiently for accomplishing transmission to another candidate host. Some viruses, such as the retroviruses that cause HIV, directly confront the enemy by infecting and then replicating within the immune cells! Failure to successfully confront the host's defensive responses will result in genetic termination of the virus and, on a broader scale, such failure may eventually result in extinction for that viral species.

1.2.2 I Want a Niche, Just Like the Niche, That Nurtured Dear Old Mom and Dad

The initial tissue type in which a virus replicates may be linked inextricably with the initial transmission mode and portal (or site) of entry into the body of the host. For example, those viruses of mammals which are acquired by fecal – oral transmission tend to initiate their replication either in the nasopharyngeal tissues or else in the gastrointestinal tissues. There then are subsequent host tissue and organ types that will be affected, some of which may be related to the virus' efforts at trying to reach it's proper portal of exit. Others of the host tissues affected by the virus may be unrelated to interhost viral transmission, although the viral affect upon those other tissues may play a strong role in the severity of illness which is associated with that viral infection. An example of the latter would be the encephalitic infection of brain neurons in association with echoviral conjunctivitis, an infection which initially would be acquired from fomites as part of a fecal–oral transmission pattern. In this case, the encephalitis causes nearly all of the associated morbidity but does not seem to benefit transmission of the virus (personal observation by author C. J. Hurst).

1.2.3 Being Societal

Successful viral survival in association with a new host will allow a possible subsequent transfer of the virus (Figure 1.11, open arrows) to its next host either directly, via a vehicle, or via a vector. The movement of a viral infection through a population of host organisms can be examined and mathematically modeled. An epidemic transmission pattern, characterized by a short term, higher than normal rate of infection within a host population is represented by the compartmental model shown in Figure 1.12 (Hurst and Murphy 1996). An endemic transmission pattern, characterized by a long term, relatively constant incidence rate of infection within a host population is represented by the compartmental model shown in Figure 1.13 (Hurst and Murphy 1996).

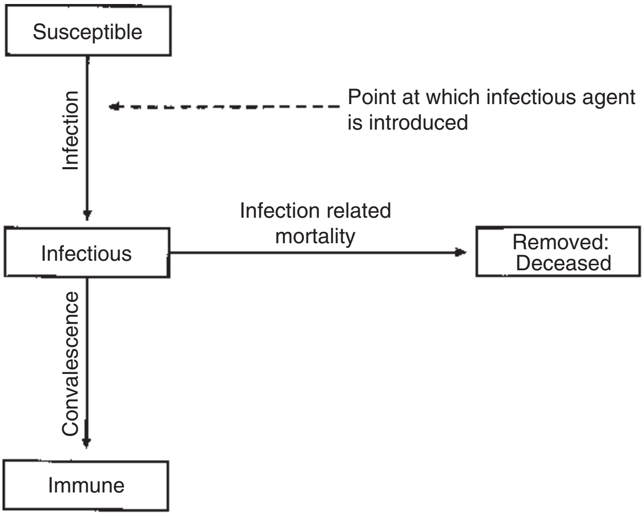

FIGURE 1.12 Epidemic transmission of a virus within a host population is represented by this type of compartment model (Hurst and Murphy 1996). Each of the boxes, referred to as compartments, represents a decimal fraction of the host population with the sum of those decimal fractions equaling 1.0. The compartments which represent actively included members of the host population are those labeled susceptible, infectious, and immune. This model incorporates only a single category of removed individuals, representing those whose demise was due to infection related mortality. The solid arrows represent the rates at which individual members of the host species move between the different compartments during the course of an epidemic. Those rates of movement are often expressed in terms of individuals per day as described by Hurst and Murphy (1996). Used with permission of the author and Cambridge University Press.

1.3 STEPPIN' OUT AND TAKING THE A TRAIN: REACHING OUT AND TOUCHING SOMEONE BY VECTOR OR VEHICLE

Remember that: *host-vector choices, cycles and vehicle utilizations as they exist today may (and probably do!) reflect evolutionary progression from prior species interactions and ecological relationships.*

After a virus has successfully replicated within the body of it's current (present) host, it must seek successful transmission to it's next (proximate) host. The resulting chain of transmission usually is the ultimate goal of viral reproduction. There are three basic approaches by which this can be attained: transmission by direct contact between the present and proximate hosts, transmission mediated by a vector, and transmission mediated by a vehicle (Hurst and Murphy 1996). While considering these approaches, it is important to keep in mind that the chains of transmission originate by random chance followed by evolutionary adaptation. Viruses generally will have one primary route of transmission and that route will have been optimized by their evolution. Alternate, or secondary, transmission routes can happen but will be of relatively minor importance.

1.3.1 "Down and Dirty" (Just Between Us Hosts)

This section heading is one which can be used to describe host to host transmission (transmission by host to host contact) and that transmission generally is by passing of virions. While this is one of the most notorious transmission routes, it is not the most common route of viral transmission between animals. This route may serve to a perhaps even more limited extent in microbes. This route likely functions still

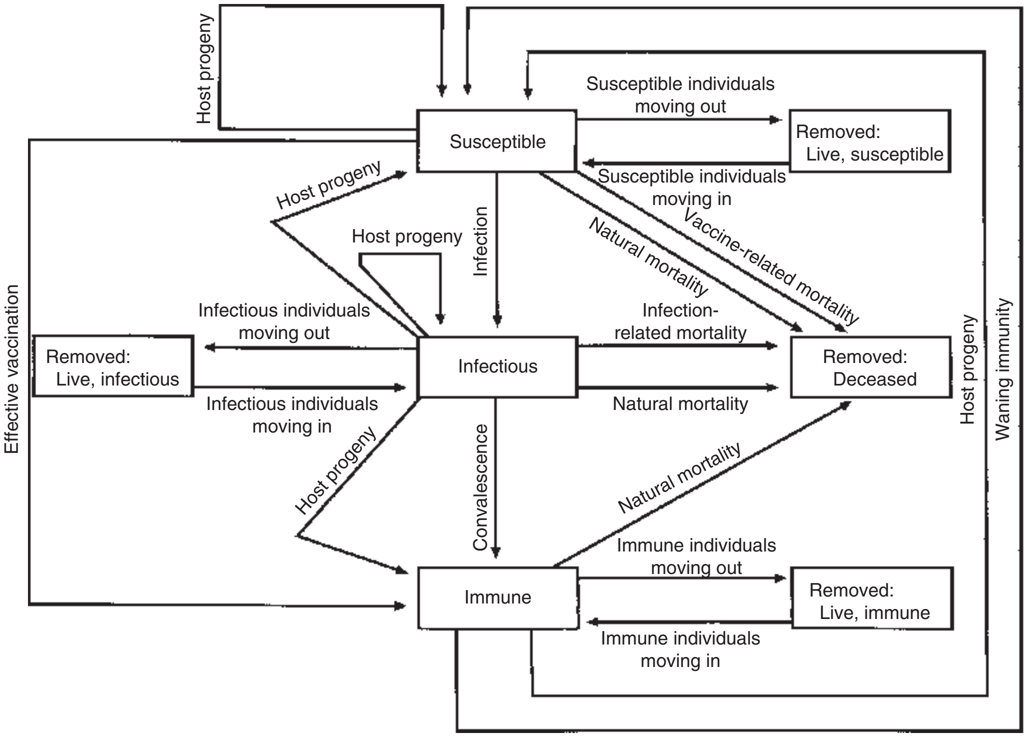

FIGURE 1.13 Endemic transmission of a virus within a host population is represented by this type of compartment model (Hurst and Murphy 1996). This model is essentially an extension of the model presented in Figure 1.12. This model contains the same three compartments (susceptible, infectious, and immune) representing actively included individuals and the category of individuals removed by infection related mortality as were described for Figure 1.12. This model differs in that it must also consider the various possible categories of live removed individuals which can move into and out from the compartments of actively included individuals. Their removal represents the fact that they do not interact with the actively included individuals in such a way that the virus can reach them, often due to spatial isolation. This model also includes the fact that the immune status of individuals can naturally wane or diminish with time such that immune individuals return to the compartment labeled susceptible; production of host progeny, representing reproductive success of the members of the host species; natural mortality, as a means of removing members of the population; and the possible use of vaccination to circumvent the infectious process plus the associated vaccine – related mortality. Please notice that the progeny of infectious individuals may be susceptible, infectious, or immune at the time of their birth depending upon the type of virus which is involved and whether or not that viral infection is passed to the progeny. Used with permission of the author and Cambridge University Press.

worse in vascular plants perhaps due in part to the relative immobility of those hosts.

It is important to remember that many of the viral infections present in animals can be transferred as virions directly from parent to offspring either prior to birth or during the birth process. These include viruses which move transovarially (Lequime et al. 2016; Nanfack-Minkeu et al. 2019) and viruses which move transplacentally. Notable examples of transplacentally acquired viral infections are the species Human immunodeficiency virus 1 and Human immunodeficiency virus 2 (both belonging to the genus *Lentivirus*, family Retroviridae), Rubella virus (genus *Rubivirus*, family Matonaviridae), and Zika virus (genus *Flavivirus*, family Flaviviridae).

Viral infections which utilize latency presumably are not inherited in a latent form as viral genomic DNA, but instead are transmitted to the hosts offspring in the form of virions. Examples of this would be members of the viral families Herpesviridae and Papillomaviridae which utilize latency and can be transmitted as virions between viviparous mammals as congenital infections, acquired during the process of vaginal birth.

Both endogenous viruses as well as retrotransposons that insert and maintain themselves in the genome of their host are transmitted directly through the germ cell line as DNA rather than as virions.

1.3.2 "The Hitchhiker" (Finding a Vector)

Transmission by vectors may be the most prevalent route by which the viruses of plants are spread among their hosts. This route clearly also exists for some viruses of animals. However, this route has not yet been defined in terms of viruses which infect microbes. Vectors are, by definition, animate objects, and more specifically they are live organisms. The considerations of natural fitness for a virus species must include the constraints which evolution has imposed upon its vectors. Being a vector implies, although by definition does not require, that the entity serving as vector has self-mobility. Thus, plants could serve by definition as vectors, although when we consider the topic of viral vectors we usually tend to think in terms of the vectors as being invertebrate animals. Vertebrate animals can also serve as vectors, as likewise can some cellular microbes.

There are two categories of vectors: biological and mechanical. As was stated earlier, if the virus increases it's numbers while in association with a vector, then that vector is termed as being biological. Conversely, the vector is termed to be mechanical if the virus does not increase it's numbers while in association with that vector. Beyond this there lie some deeper differences between mechanical and biological vectors. These differences include the fact that the acquisition of a virus by a biological vector usually involves a feeding process. Phagic habits of the biological vector result in the virus being acquired from an infected host when the vector ingests virally contaminated host body materials, acquired through biting. Subsequent transfer of the infection from the contaminated biological vector to the virus' next host occurs when the biological vector wounds and feeds upon the next host. Actual transference of the virus to that next host occurs incidentally when the vector contaminates the wound by discharging viruses contained either in the vector's saliva, regurgitated stomach or intestinal contents, or else discharged feces and urine. Essentially any animal is capable of serving as a potential biological vector provided that the wound which it inflicts while feeding upon a host plant or animal will not result in the death of that new host until the virus would have had the chance to replicate within and subsequently be transmitted onward from that new host. There are many issues surrounding the question of what makes a good biological vector. These issues include: physical contact between the virus' host and the potential vector during a feeding event, viral reproduction within that potential vector, and that the infected vector be able to survive long enough to transmit the virus to a new host. It also helps if there is some factor driving the vector to pass along the infection, such as the virus finding it's way into the vector's saliva, or the virus increasing the physical aggressiveness of the vector.

The fact that biological vectors usually acquire the viral contaminant while wounding and ingesting tissues from an infected host brings us to another distinguishing difference between biological and mechanical vectors: viral contamination of a biological vector usually is associated with the virus being carried internal to the body of the vector. Replication of the virus then occurs within the body of the biological vector. Contrastingly, viral contamination of a mechanical vector usually occurs on the external surface of the vector and the virus subsequently tends to remain on the external surface of the mechanical vector. One possible example of mechanical vectoring would be the acquisition of plant viruses by pollinating animals such as bees and bats during their feeding process. These pollinators can serve as mechanical vectors if subsequently they are able to passively transfer the virus from their body surface to the next plant from which they will feed. In the case of these pollinators, the acquired virus presumably is carried external to the pollinator's body. Conversely, it is possible that a plant being visited by a pollinator might become contaminated by viruses afflicting that pollinator, and the plant could then passively serve as a mechanical vector if subsequent pollinators should become infected when they visit that plant. Biting flies can serve as biological vectors if, during feeding, they ingest a pathogen which can replicate in association with that fly and then be passed onward when the fly bites it's next victim (Hurst and Murphy 1996). Non-biting flies can passively serve as mechanical vectors if they feed upon contaminated material and then subsequently transmit those microbial contaminants to the food of a new host without that pathogen having been able to replicate while in association with the non-biting fly (Hurst and Murphy 1996). Arthropods such as wasps, which repeatedly can sting multiple animals, could serve as mechanical vectors by transporting viruses on the surfaces of their stingers. Also, passive surface contamination of pets that occurs unrelated to a feeding event can result in the pets serving as mechanical vectors (Hurst and Murphy 1996).

When a virus is transported inside the body of the vector, then that transportation is referred to as being an "internal carriage." Contrastingly, transportation of a virus on the external body surfaces of a vector is referred to as being an "external carriage." As will be described in chapter 6 by Patil and Fauquet, there are some plant viruses which are transported through internal carriage by invertebrates that represent mechanical vectors (because the virus does not increase its population level when in association with those invertebrates). Thus, although the biological vectoring of a virus usually involves internal carriage, the fact of internal carriage does not alone always indicate that the vectoring is biological. Humans, interestingly, can serve as mechanical vectors via internal carriage for plant viruses that would be consumed with food and later excreted in feces (Zhang et al. 2006).

Because a virus must (by definition!) replicate in association with the biological vector; we can view the viral – vector association (Figure 1.14) in the same manner as was done for that of a virus and it's host (Figure 1.11). Indeed, it often is difficult to know which species is actually the viral host and which is actually the viral vector; to distinguish which is the victim and which serves as the messenger. Traditionally, we have often taken the view that humans are a high form of life and presumed there is a decreasing hierarchy down to the microbes. From this traditional, and sadly very anthropocentric, viewpoint we might assume that any living thing that transmits a virus between humans must be the vector as humans surely must be in the respectable position of serving as the host. Another version of this philosophy would consider a vertebrate to be the host and any invertebrate to be the vector. Still a third version has been based upon relative size, with the largest creature considered as the host and the smaller considered as the vector. Since we stated earlier that this chapter is intended to consider life from a virocentric perspective, we could easily accept the virocentric view which finds that there may be no clear distinction between host and vector. Rather, any biological vector can likewise be viewed as a host. The argument as to which one, the traditional host or traditional vector, really serves as the host would then become moot.

Because many types of viruses are capable of infecting more than a single species of host, we are also left to ponder about determining which is the principle host versus those which serve as alternate hosts. Settlement of the distinction asked by this latter question is usually done by examining the comparative virulence of the virus in the different types of hosting species. That species for which the virus seems less virulent is assumed to be the more natural, most coevolved, host. It then is assumed that the species for which the virus seems to have

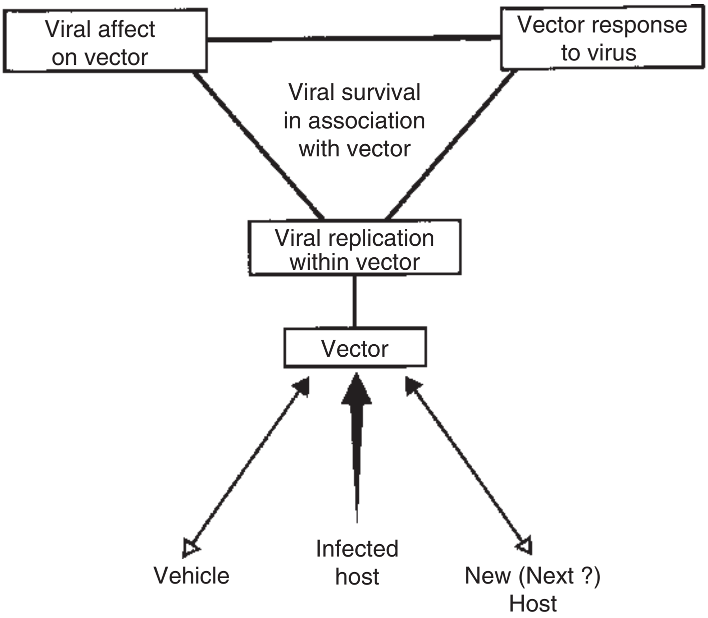

FIGURE 1.14 This figure addresses viral association with a biological vector and represents a segment from Figure 1.10. Vectors are, by definition, animate objects and are categorized either as "biological," meaning that the virus increases in number during association with that vector, or "mechanical," meaning that the virus does not increase in number during association with that vector. Biological vectors seem to have far greater importance than do mechanical vectors in terms of the spread of viral infections. Viruses can arrive at the biological vector (filled arrows) either directly from an infected host or via an intermediate vehicle. Transmission of the virus, via this vector, to a new host (or perhaps more accurately the "next" host since, in the case of viruses, biological vectors may be considered as alternate hosts) requires that the virus both survive and replicate while in association with that biological vector. Thus, examining viral survival in association with a biological vector also involves considering the effects which viral replication has upon that vector and the response of that vector to the virus. Successful viral survival in association with the vector will allow a possible subsequent transfer of the virus to its next host either directly or via a vehicle (open arrows).

greater virulence are alternate hosts. While trying to appreciate this conundrum, it must be understood that from a virocentric perspective both the principle and alternate hosts, as well as any biological vectors utilized by a virus, will all represent hosting species, and thus we may never be able to sort out the answers. Any further discussion of this particular issue is best left to only the most insistent of philosophers! Perhaps the only things left to be said of this issue are that examples of the transmission of a virus by a biological vector are represented in Figure 1.15, and that ecological interactions between a virus and it's principle hosts, alternate hosts, and biological vectors can be represented by the example shown in Figure 1.16.

1.3.3 "In a Dirty Glass" (Going There by Vehicle)

Viruses also can be transmitted by vehicles. Vehicles are, by definition, inanimate objects. More specifically, the term vehicle applies to all objects other than living organisms. There are four general categories of vehicles and these are: foods, water, fomites (pronounced fo mi tez, defined as contaminated environmental surfaces which can serve in the transmission of pathogens), and aerosols. Viruses that are transported by either water or wind may be associated with pieces of particulate matter which serve as carrier particles. The virus might naturally be imbedded in particulate

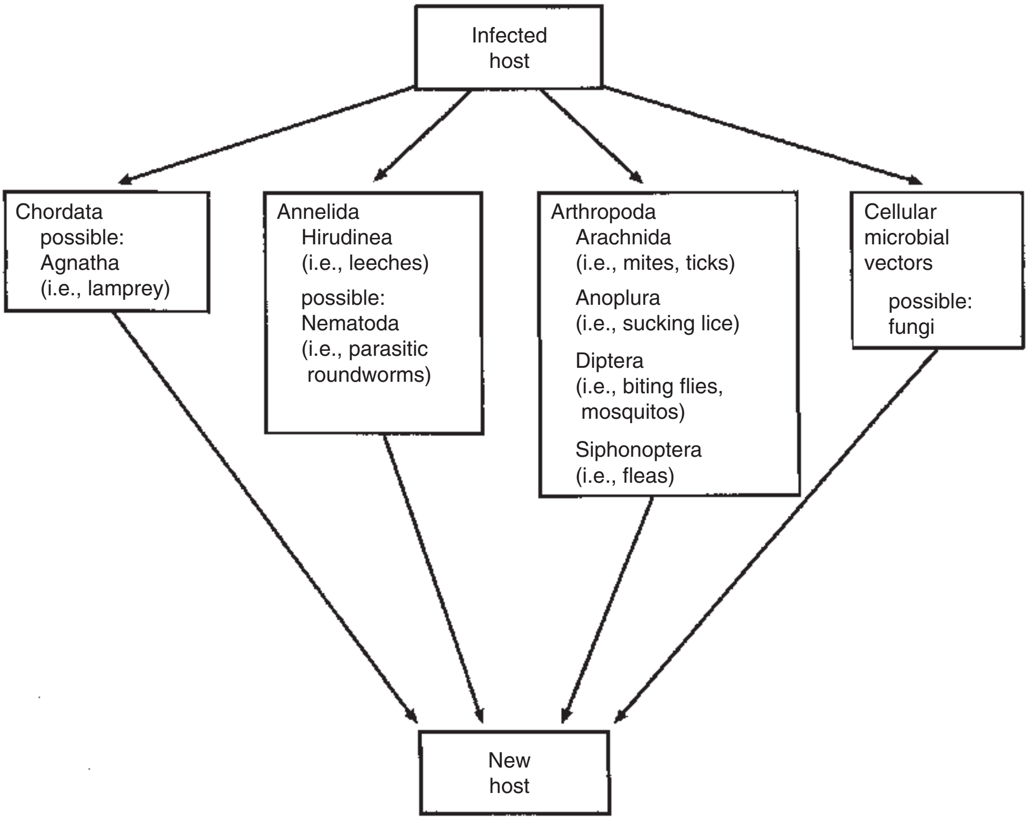

FIGURE 1.15 The transmission of a virus via a biological vector can be represented by this diagram. The virus is acquired as the biological vector feeds upon natural bodily fluids or else enzymatically liquefied bodily components of the infected host. Subsequent transmission of the virus to a new host results when the vector releases contaminated excretions or secretions while feeding upon that new host.

organic matter that has been shed from its last host. Alternatively, the virus may have become adsorbed to the surface of either an organic or inorganic particle encountered in the environment, and this commonly happens in water. Transport of the virus by either water or wind then is tied to transport of the carrier particle. Adsorption of viruses to carrier particles in water can increase viral environmental stability.

Figure 1.17 represents viral association with a vehicle. Transmission of the virus, via a vehicle, to a new host first requires contamination of that vehicle (shown by the filled arrows in Figure 1.17). The virus must then survive while in association with the vehicle. Because viruses are by definition obligate intracellular parasites, and by definition vehicles are non-living, then a virus neither can replicate on nor within a vehicle. Likewise, because vehicles are by definition non-living, we do not expect that any specific antiviral response will be produced by the vehicle. Transference of the virus to its next host can occur either directly or via a vector (shown as the open arrows in Figure 1.17). One possible indication as to the difference between a vector and a vehicle is that, while a live mosquito can serve as a biological vector, after it's death that same mosquito instead represents a vehicle. The transmission of a virus via a vehicle can be represented by the diagram shown in Figure 1.18. Acquisition of the virus by either the new host, or the next vector, from that contaminated vehicle results from either

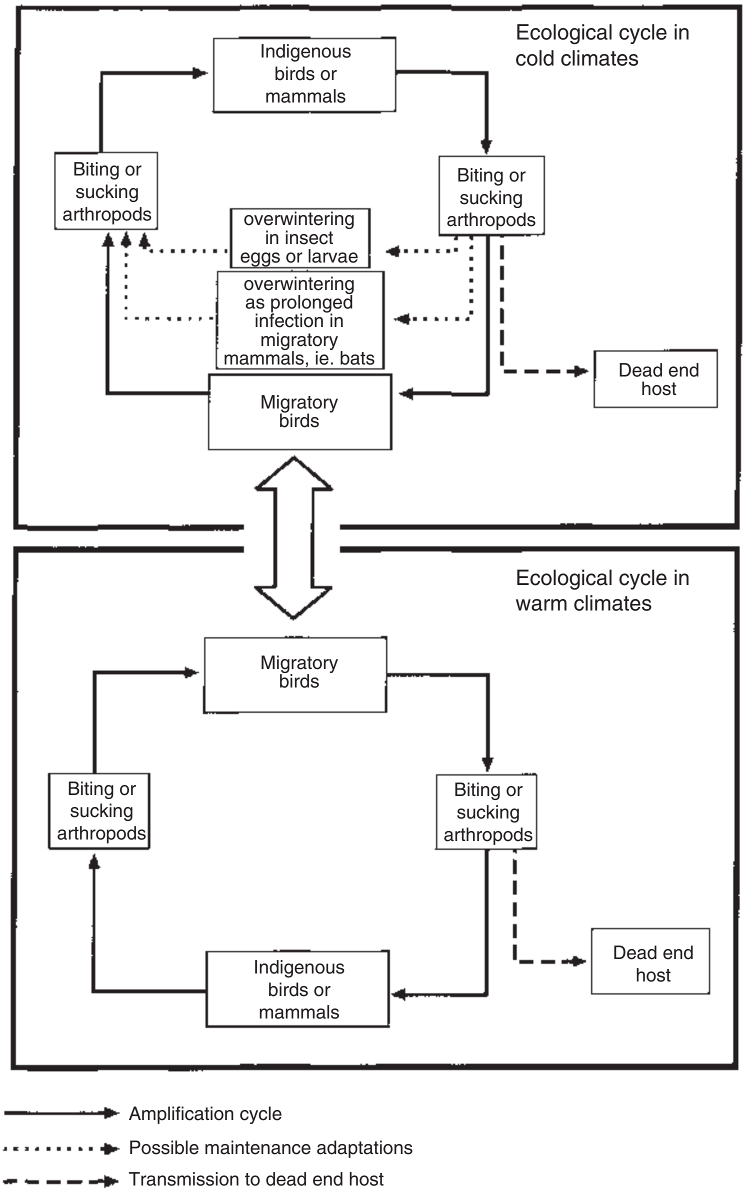

FIGURE 1.16 This figure represents a generalization of the ecological interactions which lead to insect-transmitted viral encephalitids. These infections generally are either enzootic or epizootic, meaning that their natural hosts are animals. Humans normally represent dead – end hosts for these viruses, meaning that the virus is not efficiently transmitted from infected humans to other hosts. The example shown in this figure is of a virus which has evolved ecological cycles both in warm, tropical climates and in cold, temperate climates. The cycle that has evolved in the warm climates can utilize arthropod vectors which do not have to go through the process of overwintering, thus allowing for an active year-round transmission cycle. Migratory birds, which may travel thousands of miles during their seasonal migrations, can shuttle the virus infection to the temperate zones. In the temperate zones, the virus' ecological cycle may need to include strategies for overwintering in insect eggs or larva and the possibility of survival as a prolonged infection in animals which may migrate lesser distances, such as bats.

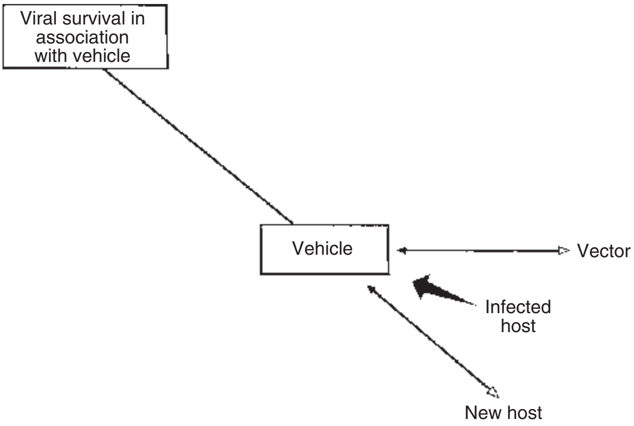

FIGURE 1.17 This figure addresses viral association with a vehicle and represents a segment from Figure 1.10. Viral transmission between hosts can occur by means of a vehicle. Vehicles are by definition inanimate objects. Viral contaminants can reach the vehicle (filled arrows) either directly from an infected host or via an intermediate vector. Transmission of the virus, via this vehicle, to a new host requires that the virus survive in association with the vehicle. Transference of the virus to its next host can occur either directly or via a vector (open arrows).

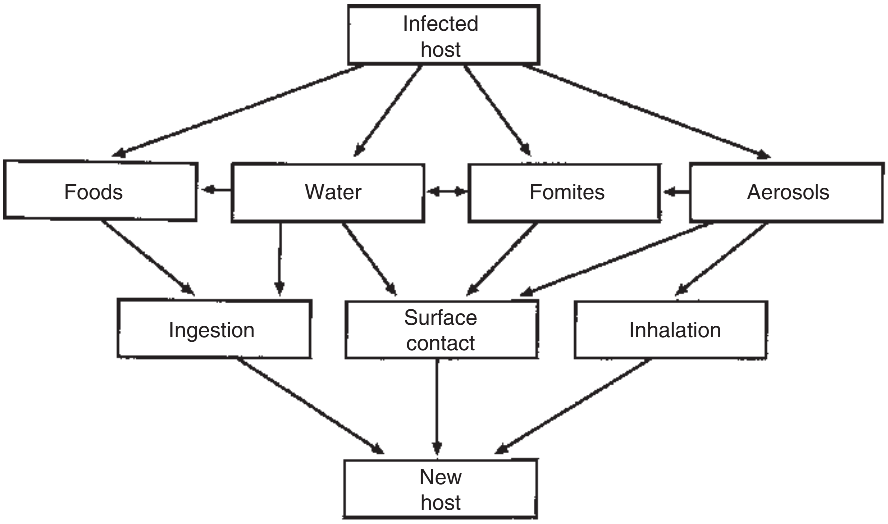

FIGURE 1.18 The transmission of virus via vehicles can be represented by this diagram. Food items can be contaminated by the action of an infected host. Alternatively, the food in question may actually be the body of an infected host that subsequently is consumed by a susceptible, predatory new host. Viral contaminants present in water can be acquired by a new host either directly, as the result of external or internal exposure to the contaminated water including ingestion of the water; or indirectly, following contact between the new host and an environmental surface (serving as a secondary, intermediate vehicle) that has been contaminated by that water. Fomites are solid environmental (non-food) objects whose surfaces may be involved in the transfer of infectious agents. Viral aerosols may result in the infection of a new host either directly through inhalation of the aerosol, or indirectly following contact between the new host and some other vehicle (either food, water, or a fomite) contaminated by that aerosol. Cross- contamination of vehicles is a serious problem and occurs when contaminants are transferred from one material to another. The horizontal arrows in this figure represent one aspect of cross-contamination. Arrows pointing to the box "Surface Contact" also represent cross-contamination.

ingestion of the vehicle (associated with foods and water), surface contact with either contaminated water or a contaminated solid object (a fomite), or inhalation (aerosols). The horizontal arrows shown in Figure 1.18 indicate cross contamination of vehicles, as do those arrows which point to the box "Surface Contact." Examples of cross contamination include: aerosols can land onto fomites and also contaminate either food or water; contamination of fomites such as containers used for food and beverage will result in contamination of any food or beverage which is placed into those containers; and similarly any contaminated food preparation surfaces, implements, and utensils will contaminate food.

Although, from a human perspective, we might tend to associate waterborne transmission with animals and in particular human diseases (chapter 17 on viruses of humans by Hurst), the waterborne approach will play a major role in viral transmission for viruses that infect cyanobacteria (chapter 2 by Chopyk, Nasko and Sakowski), eukaryotic microalgae (chapter 3 by Jackson, Allen and Monier), seaweeds (chapter 4 by Schroeder and McKeown), marine molluscs (chapter 8 by Renault), crustaceans (chapter 9 by Bateman), fish (chapter 11 by Nerland, Øvergård and Patel), marine mammals (chapter 16 by Fereidouni), and amphibians (chapter 12 on viruses of amphibians by Chinchar, Duffus and Brunner). There are even viruses of terrestrial plants, including some carmoviruses of the viral family Tombusviridae, which seem as though they might be transmitted by water. The list of vehicles associated with viral transmission even includes agricultural tools and other work implements.

The topic of vehicle-associated transmission of pathogens was discussed at length in the reference by Hurst and Murphy (1996). The transmission of microbial pathogens by food and water has been reviewed by Hurst and McDonnell (2020). The risk probabilities of infection, illness, and death associated with human ingestion of waterborne viruses relative to the risks associated with ingested waterborne bacteria and protozoa has been reviewed by Hurst (2018). Toilet aerosols as a route of water-associated microbial transmission has been researched by Gerba et al. (1975), who determined that aerosols produced by toiled flushing contained seeded viruses and bacteria which had been placed into the toilet water prior to the flushing. Gerba et al. (1975) concluded that in addition to inhalation risks, those toilet aerosols contaminate environmental surfaces in the bathroom area. Knowlton et al. (2018) detected bacteria in toilet aerosols at a hospital.

1.3.4 Bringing Concepts Together

Biological entities exist over a spectrum of complexities, ranging from the viruses, viroids and prions (yes, even the prions are biological entities!) to multicellular organisms. The process of maintaining the viability of even the largest of organisms is, and perhaps must, be organized at small levels. Biologically, this has been achieved by a highly evolved process of internal compartmentalization of functions with a systemic coordination. If we consider for a moment one of the most enormous of the currently living multicelled organisms, the blue whale (*Balaenoptera musculus*), we notice that this kind of compartmentalization and coordination begins all of the way down at the level of the subcellular structures and organelles within each individual cell. The compartmentalization and coordination then continue upward through a number of levels including the various individual types of cells, the tissues into which those cells are organized, the organs which the tissues comprise, and finally the total internal coordination of all of these through nerve signaling and hormonal regulation. At every one of these biological levels there is a "taking from" and a "leaving behind" exchange of material with respect to the immediate surrounding environment. This results in the existence of dramatic environmental differences at all levels, even down to the many microenvironments which exist within the organizational regions of a single cell.

Every virus must try to comply with the basic biological imperatives of genetic survival and replication. While complying with these imperatives the viruses must, as obligate intracellular parasites, not only face but also survive within and successfully be transported through the various environments which are internal to the host. Those viruses which are transmitted by biological vectors must also have evolved the capability to survive and be transported through internal environments faced within the vector. Viruses which are transmitted by mechanical vectors generally must possess an additional evolved ability to survive on the surface of that vector. Likewise, both those viruses transmitted by mechanical vectors and viruses transmitted by vehicles must possess the ability to survive exposure to natural ambiental environments encountered either in the atmosphere, hydrosphere or lithosphere. These numerous environments are summarized in Figure 1.19. Conditions confronted at the interface zones, as indicated by the dashed lines in Figure 1.19, represent areas of still additional environmental complexity. While viruses appear biologically inert when viewed in the ambiental environments, they display their biology and interact with their surroundings when they reach the environments internal to their hosts and biological vectors.

The adaptability of a species in terms of its biological cycle and biological needs will determine that species' potential distribution range. This potential distribution range is limited in actuality to a smaller range based upon interspecies relationships and competitions. Ourselves being large multicellular creatures, we humans normally think of a distribution range as being geographical in nature and identifiable on a map. As microbiologists, many of us have come to understand the concept of distribution range in finer detail; an example being the depth within a body of water where a particular species of microorganism normally will be found. At the level of viral ecology, the concept of species distribution range encompasses everything from tissue and organ tropisms (those tissues and organs which a virus seems to attack preferentially) upwards to the geographical availability of host species, vector species, and the prevailing directional flow of appropriate vehicles such as air and water. The larger, geographical end of this scale is represented in Figure 1.20.

While considering the factors addressed in Figure 1.20, it is important to keep in mind that albeit the virus' election of hosts, vectors, and routes of transmission would all originate by random chance, the attainment of reliable continued viral success would require that such random selection events be followed and strengthened by evolution. This explains the reason why viruses do not appear suddenly to develop the ability to use a different vehicle. Indeed, it is perhaps likely that in order to use a vehicle such as air or water, the virus must have preadapted itself to the conditions which it will encounter in association with that vehicle. Nearly each individual species of virus which achieves transmission by vehicles seems invariably to use only one type of vehicle. This trait likewise seems to hold true for all species belonging to any given viral genus. Furthermore, this identification seems to nearly always hold true at the level of viral family. In fact, this is one of the defining characteristics of the ecology of a viral group. The only virus which seems to have evolved the ability to utilize more than a single vehicle is the Hepatitis A virus (Hurst and Murphy 1996), which has evolved a most remarkable ability to be effectively transmitted both by water and on fomites. It is for these reasons, that fears expressed in the public press suggesting viruses such as Ebola may suddenly take flight and be transmitted over large distances via aerosols amount to nothing more than frightening speculation. Why is it just speculation? Because that route of transmission is not a part of the virus' ecology. Invasive medical devices such as syringes, endoscopes and other surgical implements, plus transplanted animal tissues including transfused blood and blood products, and grafted plant material, represent exceptions to this rule. These devices and transplanted tissues represent unnatural vehicles which, by

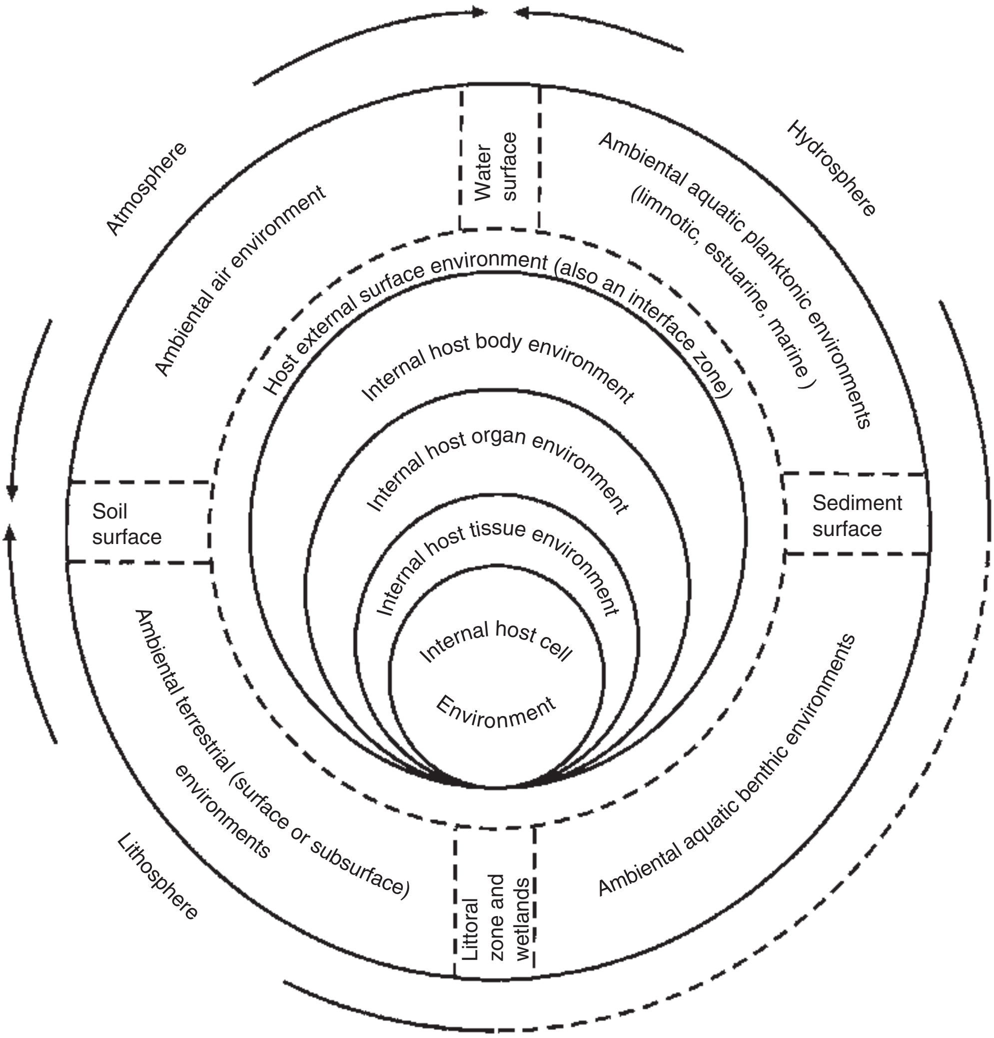

FIGURE 1.19 This figure integrates the concepts of host, vehicle and biological vector by representing the environments potentially faced by a virus. As obligate intracellular parasites, the viruses must face, survive within, and successfully be transported through environments which are internal to the host. Those viruses which are transmitted by biological vectors must also have evolved the capability to survive and be transported through internal environments faced within the vector. Viruses which are transmitted by vehicles and mechanical vectors must additionally possess an evolved ability to survive in natural ambiental environments (atmosphere, hydrosphere and lithosphere). Conditions confronted at the interface zones, as indicated by dashed lines, represent areas of additional environmental complexity.

their nature, allow the virus an abnormal access to the interior of a new host (Hurst and Murphy 1996). Any virus which would naturally be transmissible by direct contact with either an infected host or any type of vector can also be transmitted by one of these unnatural vehicular routes.

Viruses occasionally will appear in association with “apparently new” (unexpected) hosts and biological vectors. These latter occurrences with unexpected hosts or vectors represent the identification of sporadic events which occur when geographical boundaries are breached by the movement of those potential

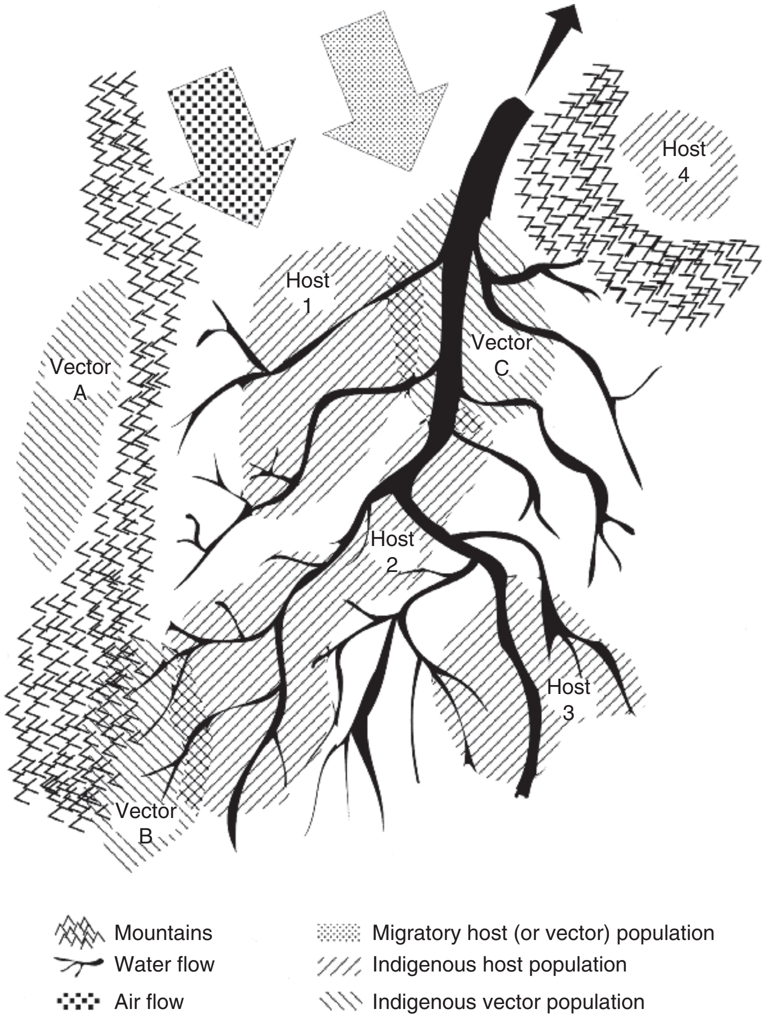

FIGURE 1.20 This figure presents a hypothetical example of the way in which the ecology of a virus is delineated by the spatial relationships between its potential hosts, vectors, and vehicles. The figure represents a viral infection existing in a watershed basin whose area covers tens of millions of hectares. An assumption is made that the four potential indigenous host populations and three potential indigenous vector populations are terrestrial organisms whose ecological areas are delineated and that these organisms do not migrate outside of their own respective ecological areas. Indigenous host populations 1, 2, and 3 reside in riverine ecological areas within the basin. Indigenous vector population B has a highland ecology, while vector population C has a lowland ecology, and both of these vector populations reside within the basin. Indigenous vector population A and indigenous host population 4 are excluded from participation in the viral infection cycle due to their geographical isolation and, because of their geographical exclusion from the basin, we do not need to be concerned with the nature of their ecological zones. Vector population B is capable of interacting in a cycle of transmission involving host population 2. Vector population C is capable of interacting in a cycle of transmission involving host populations 1 and 2. None of the indigenous vector populations is capable of interacting in a cycle of transmission involving host population 3. A virus capable of being transmitted by surface waters could move from host population 3 to host population 2, since host population 2 is located downstream of host population 3. That same surface waterborne route could not spread the virus to host population 1, because host population 1 is not situated downstream of either host populations 2 or 3. Likewise, neither could the surface waterborne route spread the virus in a upstream direction from host population 1 to host population 2, nor from host population 2 to host population 3. Alternatively, a migratory host or vector population could carry the virus from host population 1 to host populations 2 and 3, as likewise could air flow if the virus is capable of being transmitted as an aerosol.

hosts and vectors for which the virus in question already has a preevolved disposition. These preevolved dispositions may represent, at some basic level, the renewal of old acquaintances between a virus, vector, and host. Alternatively, if these particular viral, host, and vector species truly never have met before, then an important aspect which can factor into these encounters is the biological relatedness between these "apparently new" hosts or vectors and those other hosts or vectors which the virus more normally would use.

1.3.5 Is There No Hope?

Many host-related factors do play a role in the transmission of viral -induced illnesses. These include:

"Finding the wrong host" the "oops" or accidental occurrence factor wherein viruses occasionally will encounter and successfully infect living beings other than their natural hosting species, an event which represents a mistake not only for the host (which often will be fated to die for want of having inherited an evolved capability to mount an effective defense against that virus) but also is a mistake for the virus (which often will not be able to subsequently find one of its natural hosts and hence also loses it's existence);

"Only the good die young" culling the herd for communal protection can have some advantage for the host population as a whole if those individuals that demonstrate a lesser ability to resist the virus are weakened enough by the infection that they then are more easily killed by predators (this is an act that both reduces the likelihood that other members of the host population will become infected by that virus strain and also may improve the gene pool of the host species by selectively eliminating it's most susceptible members);

"Being your own worst enemy" behavioral opportunities for disease transmission do exist, and ethnic or social customs often play a role in disease transmission including the probable reality that a lack of male circumcision has spelled disaster for the human population of Africa by facilitating the heterosexual transmission of HIV (Caldwell and Caldwell 1996), and in fact most of those vector borne diseases that afflict humans can be avoided by changes in host behavior.

If we view this situation from the human perspective, there does exist a basis for hope in terms of the health of hosts. Our most important advantage lies in the use of barriers, which represent a very effective means by which we can reduce the transmission of all types of infectious agents. Barriers can be classified by their nature as physical (Table 1.1), chemical (Table 1.2), and biological (Table 1.3). In many cases, these barriers already exist in nature. Natural examples of barriers include both high and low temperatures (thermal, a physical barrier), sunlight (radiation, a physical barrier), the natural salinity

TABLE 1.1 Categories of Physical Barriers

Thermal
Acoustic (usually ultrasonic)
Pressure
  barometric
  hydrostatic
  osmotic
Radiation
  electronic
  neutronic
  photonic
  protonic
Impaction (includes gravitational)
Adhesion (adsorption)
  electrostatic
  van der Waals
Filtration (size exclusion)
Geographic features
Atmospheric factors (includes such meteorological aspects as humidity, precipitation, and prevailing winds)

TABLE 1.2 Categories of Chemical Barriers

- Ionic (includes pH and salinity)
- Surfactant
- Oxidant
- Alkylant
- Desiccant
- Denaturant

TABLE 1.3 Categories of Biological Barriers

- Immunological (includes specific as well as nonspecific)
  - naturally induced (intrinsic response)
  - naturally transferred (lacteal, transovarian, transplacental, etc.)
  - artificially transferred (includes injection with antiserum and tissue transfers such as transfusion and grafting)
- Biomolecular resistance (not immune-related)
  - lack of receptor molecules
  - molecular attack mechanisms (includes nucleotide-based restrictions)
  - antibiotic compounds (metabolic inhibitors, either intrinsic or artificially supplied)
- Competitive (other species in ecological competition with either the virus, its vectors, or its hosts)

of water (both osmotic, a physical barrier and also desiccant, a chemical barrier), and ecological competition (competitive, a biological barrier). The intentional use of barriers can involve both individual and combined applications. One example of a combined barrier application is the retorting of canned products, a process which employs a combination of elevated temperature and hydrostatic pressure to achieve either disinfection or sterilization (this process is similar to autoclaving). Many of these barrier concepts, such as filtration acting as a physical barrier, can be applied at different levels. For example: some particle exclusion filtration devices have pore sizes small enough that they can act as a filtration barrier against virus particles themselves; natural latex condoms and disposable gloves act as filtration barriers against a liquid vehicle (they contain pores which are larger than the virus particles yet smaller than the droplets of liquid in which the virus is contained); window screens and mosquito netting act as filtration barriers against flying vectors; and walls, fences, doors and gates can act as filtration barriers against infected hosts. The ingestion of food and water is associated with natural digestive treatments such as pH changes and secreted enzymes, both of which represent chemical barriers. When viewed from the virocentric perspective, the use of barrier techniques for preventing viral transmission would represent cause for despair instead of hope. There is, however, a notable exception represented by the idea of some viruses such as the polyhedrin-forming members of the viral families Baculoviridae and Reoviridae seeming to require digestive treatment as an aid to their infectivity for their insect hosts.

1.4 WHY THINGS ARE THE WAY THEY ARE

The ability of a virus to pass on it's genetic content is the key consideration of the virus. We now understand how this gets done on a molecular level. What still remains to be understood are how this thing gets done and has come about at the species level.

1.4.1 To Kill or Not to Kill – A Question of Virulence

One of the nagging questions which a virus must face is what should be the extent of it's virulence, i.e., whether or not it should kill it's hosts and biological vectors as a consequence of their encounters. When considered in purely evolutionary terms, virulence is the ability of the disease agent to reduce reproductive fitness of that host. The relative virulence of a virus with respect to one of it's hosts or biological vectors is generally presumed to be a marker of co-evolution. More specifically stated, it seems that the less virulent is the virus for one of it's hosts or vectors, the more greatly coevolved is the relationship. Why should this be so? It should clearly be the case that, were a virus to infect an individual member of a host or biological vector population prior to that individual having reached reproductive age, it would be in the virus' best interest to not kill that host or vector. Contrariwise, in a very strict sense, death of that host or biological vector should not matter to the virus if that individual host or biological vector has passed the end of the normal reproductive lifespan. The reason for this latter philosophy is that, even if this particular host were to survive, it would not produce more susceptible offspring. Additionally, within each species of potential host or biological vector, there would be a strong genetic drive to enable their infants to mount sufficient immunological defense so as to reach the age of reproductive maturity. That same genetic drive does not, by definition, act upon the preservation of individuals who have passed their reproductive years. One example of the result from interaction of these forces is the fact that infections caused by the Hepatitis A virus can go nearly unnoticed in human infants, yet Hepatitis A virus infections can be disastrous in human adults.

Figure 1.21 represents the question of how the success of a virus relates to its' virulence. The virus will not be successful if the result of

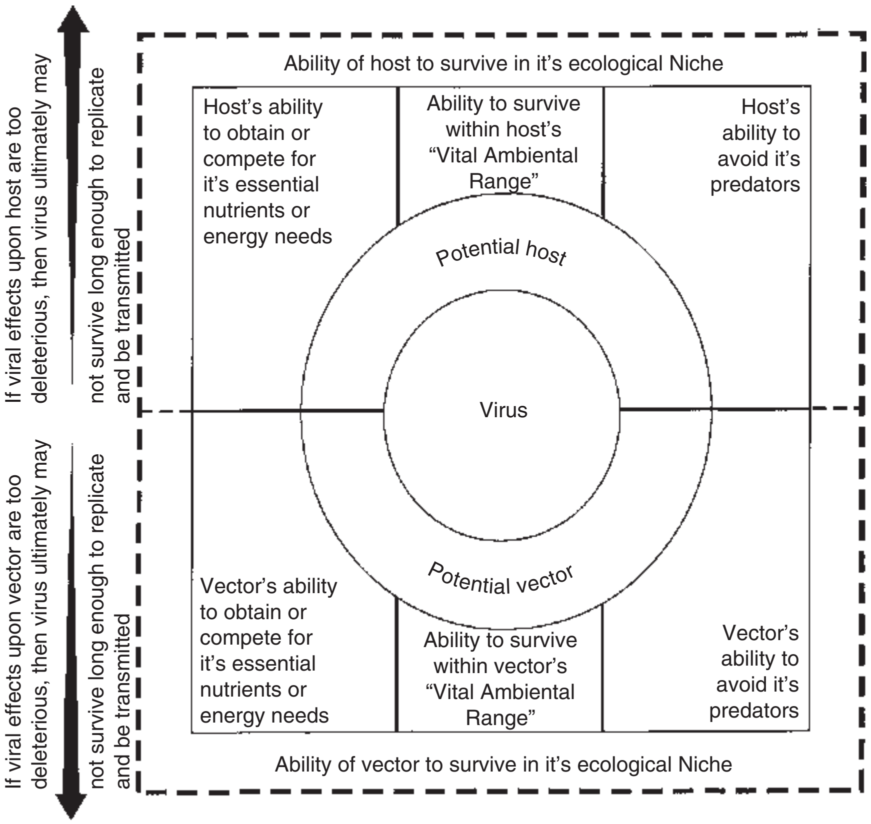

FIGURE 1.21 This figure represents the question of how the success of a virus relates to its' virulence. Success requires that the virus replicate within the bodies of its hosts and any biological vectors to concentrations which are high enough that the virus has a reasonable chance of being passed onward to infect either its next host or its next biological vector. The virus will not be successful if, within this period of replication, the result of viral infection is too deleterious in terms of affecting the ability of the present host or biological vector to survive within its own respective ecological niche. The survival requirements of those potential hosts and biological vectors include: the respective ability of those hosts and biological vectors to compete for their essential needs; their ability to survive within their own vital ambiental range as defined by factors such as temperature, plus either humidity and altitude (if terrestrial) or depth and salinity (if aquatic); and their ability to avoid being consumed by predatory individuals.

viral infection is too deleterious in terms of affecting the ability of the present host or biological vector to survive before that virus has been able to achieve transmission to it's next host or biological vector.

1.4.2 Genetic Equilibrium (versus Disequilibrium)

One of the hallmarks of relationships between virus species and their host species is their apparent goal of reaching a mutually acceptable genetically-based equilibrium (Dennehy et al. 2006). Some viruses also seem to have interchanged genetic material with their hosts while striving to evolutionarily reach a level of mutual coexistence.

There are many considerations associated with an apparent genetic equilibrium. In most instances of endemic viral infection in populations of a coevolved host or biological vector, the infections appear relatively unnoticed or relatively innocuous. This may change when the virus encounters a concentrated population of genetically similar susceptible hosts or biological vectors concentrated within a small radius, perhaps resulting in an epidemic. It also may change when the virus invades a population of novel hosts or vectors (hosts or vectors to which that virus appears to be new); this is termed a "biological invasion." Excessive virulence may represent reduced genetic fitness with respect to the virus, host, or biological vector. Limited virulence on the part of the virus seems to represent a state of coevolution but with some remaining flux in the virus-host interaction. This state may have a beneficial effect by acting as a genetic screening upon both the host species and the viral species. In contrast, avirulence may represent a far more evolved steady state, although evolutionarily it may not be the final state, between the viral and host populations. Avirulence is normally acquired by repeated successive passage of the virus through members of a host or biological vector population.

What are the considerations associated with an apparent genetic disequilibrium? If the virus seems to make all of the members of a species extremely sick, then presumably it normally may not be hosted or vectored by that species. If a virus causes a reduction in the genetic fitness of the host (ability of the host to pass on it's genetic heritage) then the virus is viewed as being in disequilibrium with the host. Incompatible genetic differences may both fuel the fires of virulence and allow a constant state of genetic disequilibrium to exist. Genetic equilibria need time to establish. Constant disequilibrium may be viewed as a competitive strategy effected via "Evolutionary Cheating" (included in loving memory of Dr. Alex Frasier who taught me to understand evolution). Evolutionary cheating involves finding ways to change the rules of fair competition and thereby tilt the playing field in favor of your species. One good example of evolutionary cheating would be to eat your competing species. Viruses tend to steal genes from their hosts (Balter 1998), and this would represent another example of evolutionary cheating.

1.4.3 Evolution

Understanding evolutionary history involves study and speculation. We also try to guess at a purpose for the events which we suggest have occurred. Posing evolutionary questions as to "how and why" does hopefully lead us to logically answering those questions.

How and when did viruses originate?

As we look at the relationships between viruses and their hosts and vectors, we might ask ourselves that age-old question of "Which came first, the virus or the cell?" (Koonin et al. 2006). It is perhaps more likely that the viruses and their host cells arose simultaneously, and possibly with interdependence between host and virus (Durzyńska and Goździcka-Józefiak 2015). The range of virus groups that we currently know may represent many separate origination events. Bioinformatic analyses have suggested that retroviruses possibly evolved early in the Palaeozoic Era, between 460 and 550 million years ago, providing the oldest inferred estimate for age of any of the presently known virus groups (Hayward 2017).

Presumably the viruses and living cells have thus been struggling to find mutually agreeable terms with one another for quite a long time. Viruses and hosts genetically steal from one another, and some of the viral capsid proteins may have begun as cellular proteins (Krupovic and Koonin 2017).

The question of coevolution between viruses and their hosts has been addressed by many people (Kaján et al. 2020; Koskella and Brockhurst 2014; Laanto et al. 2017; Leeks et al. 2018; Lythgoe et al. 2017; Petrie et al. 2018). Interestingly, Enard et al. (2016) have indeed suggested that viruses are among the most dominant drivers of evolutionary change for mammalian as well as human proteomes.

Gibbs and Weiller (1999) interestingly offered the suggestion that plant viruses may be able to switch hosts to infect vertebrates, that members of different virus families can recombine during the natural course of evolution, and that DNA viruses can incorporate genes from RNA viruses presumably during coinfection even when neither of those viruses possesses reverse transcriptase. Their observations and discussion concerned genetic viral elements of vertebrates, with those sequences of interest seeming to contain both nanovirus information including a nanovirus origin of replication, and also calicivirus information. Nanoviruses (genus *Nanovirus*, family Nanoviridae) have DNA genomes and are known to infect plants. Caliciviruses (family Caliciviridae), which have RNA genomes, typically infect animals and mostly have been found in vertebrates. The suggestion of Gibbs and Weiller (1999) was that evolutionary conservation of a nanovirus origin of replication indicated the transferred nanoviral viral genetic material had maintained an ability to replicate. Their speculation was that this host-switch had occurred when a vertebrate was exposed to sap from an infected plant.

Why are the viruses still around?

Viruses seemingly modulate the way in which microorganisms drive most ecosystems (Gregory et al. 2019). One very notable example which represents that aspect of viral ecology is the extent to which bacteriophage infections play a role in the control and structure of marine cyanobacterial communities (Puxty et al. 2018, Zhong et al. 2018). Genomic analyses suggest that there is an evolutionary pressure upon those viruses which infect marine microbes to accumulate genes which relate to viral propagation strategies (Nishimura et al. 2017), and so perhaps there never are too many tools in the viral toolbox.

The viruses might serve as an evolutionary benefit to the cellular organisms by gradually transferring genetic information between different sources and serving as a source for genomic development. Perhaps this is the reason why their hosting species continue allowing the viruses to exist. Perhaps the pure beauty of a virus, when viewed as an evolutionary element, is that it can break free from one host to enter another host. Gradually, that virus could coevolve until at last it might settle upon a permanent home as some endogenous genetic element within a single hosting species. Alternatively, the virus may play the role of eternally being a rebel in search of a cause. Oh, to be so free as a species!

What will the viruses become with time?

As stated above, in a strictly evolutionary sense it is not necessary for the viruses to be leading to anywhere. However, if we can draw parallels and make an assumption that the relationship between virus and host moves with time towards both avirulence and eventual genetic equilibrium, then we can make hypotheses. Perhaps some of the viruses will indeed continue the way of being predatory outsiders. Others, however, seem destined for symbiosis and thus to become a part of us. We see at least two clues pointing to the latter type of destiny. One of these lies in Villareal's hypothesis (Villarreal 2016) that by evolving to have the same biological agenda as their placental mammalian hosts, the endogenous retroviruses have symbiotically joined with their hosts to create a single species. The hypovirulence elements of the fungi which cause Chestnut blight disease are another clue (chapter 5 by Hillman and Milgroom), these elements apparently evolved from a virus and seemingly have achieved symbiosis. The hypovirulence elements sustain their existence by reducing the virulence of their host fungi, so that in turn the host fungus does not kill the tree upon which the fungus feeds, enabling all to survive.

We do not know either to what, or to where, the viruses are leading. We also must understand that in a true biological sense it is not necessary for the viruses to "lead" anywhere. From a virocentric view, a perfectly organized virus reproducing from host to host (perhaps with a few vectors included for spice) and transmitting its genetic information over time is a sufficient trend. In considering the evolution of viruses, we must remember the wisdom of Niles Eldredge (1991), that no existing biological entity can be said to represent an end product of evolution. Rather, it is only the extinct biological life forms that clearly can be said to have represented end products of evolution. Likewise, we do not and perhaps never may know if viruses arose only once or else have arisen at many times, with their evolutionary arisal bounded only by the practical limits of some definable adaptive zone. Understanding this comes from the realization that thus far, sabre-toothed cats and sabre-toothed cat-like animals have evolved at several different times during history and that each evolution came from a different biological lineage (Eldredge 1991; Hurst 2016). The evolution of sabre-toothed carnivores at each time event would have corresponded to the opening of the appropriate niche, and each of their extinctions would have corresponded to the closing of that niche. For just as it is true that the availability of a niche can drive evolution, so too can the closure of a niche drive extinction. One of our goals must be developing a better understanding regarding the natural philosophy of viral ecology. That will require us to successfully view the habitats and niches of viruses from a perspective which recognizes the conceptional framework of competitive exclusions. Those exclusions limit the locations and activities of biological families, genera and species from their broadly defined potential habitats and niches to more narrowly defined operational habitats and operational niches (Hurst 2016, 2021).

Although the lack of viral fossils restricts our efforts at following the evolution of viruses, we can draw hypotheses by looking at parallels between a few of the virus groups and their hosts. To begin this process, we have seen that some of the presently existing viral families (we know nothing about those viral families that may be extinct) seem restricted to different host groups. It is likely that as time has gone by, these viruses and their hosts have coevolved and perhaps even undergone phylogenation (the evolution of phylogenetic groupings) in parallel. For example, those viruses which we know as the viral family Myoviridae seem restricted to infecting prokaryotic cells. This could suggest either that the ancestors of the Myoviridae are relatively new or else relatively ancient. Members of the viral family Siphoviridae, which also infect prokaryotes, have developed a relatively stable mechanism of endogeny (in their case referred to as lysogeny), which may be suggestive of these viruses having had a long period of coevolutionary adaptation with their host cells. We can see that the viroids of plants, which genetically bear a link to the viruses (chapter 7 by Flores, Di Serio, Navarro, Duran-Vila and Owens) and prions (chapter 17 on viruses of humans by Hurst) seemingly have developed such a highly evolved endogenous state that they never produce anything resembling a virion and indeed may not use or even need a natural route of transmission because they remain internal to their host. Additional examination of the existing viral groups, and the establishment of parallels between these and the known evolution of animal phyla, reveals that virus groups such as the family Iridoviridae, which do produce virions, seem restricted to invertebrates and poikilothermic vertebrates. This latter examination could lead to the suggestion that ancestors of the iridoviruses followed the animal phylogenation pathway upward to a point just short of the evolution of euthermia. The family Retroviridae have gone onward to infect euthermic animals, and it has been hypothesized that at least some retroviruses have coevolved with their hosts to the extent that the viruses enabled the development of placental mammals (Villarreal 2016).

Alas, it might also be true that the evolution of viruses represents a question which we cannot yet even try to answer.

1.5 SUMMARY (CAN THERE BE CONCLUSIONS?)

The ecology of a virus primarily consists of it's interactions with the organisms that serve as it's hosting species (principle hosts, alternate hosts, and vectors). The routes by which viruses achieve transmission between these other organisms represent a second aspect of the ecology of viruses. Furthermore, an examination of the interactions between a virus and it's hosts and biological vectors brings up many questions. Principle among these questions is the reason why the outcome of viral infections sometimes appears to be so disastrous, and yet at other times appears unnoticeable.

One of the founding principles in biology is that natural selection serves as the basis for the population dynamics which produce those many different outcomes which we observe as scientists. When we use this principle as the lense through which to examine interactions between viruses and their host and vector species, we notice that many possible strategies exist, more than can be explained. The strategies which we do find in evidence began at random and exist because selection has not done away with them. While we do not know how the viruses have arisen, or what will be their destiny, we can assume that there may be viruses for as long as there are cells.

ACKNOWLEDGEMENT

I am thankful to my friend and former colleague Dr. H. D. Alan Lindquist for his assistance with the preparation of a much older version of this chapter. My hopes and best wishes go out to him.

A REMEMBRANCE OF RICARDO FLORES

I wish to share my remembrance of a dear colleague, Ricardo Flores Pedauyé. It was in 2008 that Ricardo enthusiastically and expertly took the reins of our chapter on the subject of viroids as we worked together for this project which presents a collective understanding of viral ecology. Collaborating with Ricardo always seemed to be an honor and my appreciation for him constantly grew stronger through the 12 years that I knew him. Ricardo passed from this life on the 20th of December in 2020. Fortunately, I am able to share with you this photograph of Doctor Ricardo Flores giving a presentation at the Eighth Australasian Plant Virology Workshop, held 19–22 November 2008, in Rotorua, New Zealand. This image appears courtesy of the Australasian Plant Pathology Society. I have a bottle of cava wine from Spain which I will open in honor of Ricardo. Now, if only I could wish for a slice of tortilla, some paella, and of course Ricardo with whom to share the celebration of his scientific career and of our friendship.

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