Chapter 7: Viruses

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7.1 INTRODUCTION

The most commonly studied invertebrate viruses are those that frequently cause overt disease in their hosts. As such, many of the examples presented in this chapter involve insect pests of crops or forests that have been studied in the search for effective biological control agents. The majority of these approaches have focused on the use of viruses, mostly baculoviruses (*Baculoviridae*), as the active ingredient in biological insecticides. These types of products are usually applied in an inundative strategy of biological control in order to infect and kill a high proportion of pest insects in a short period of time. An alternative approach involves an inoculative strategy in which small amounts of pathogen are released into the pest population. The pathogen multiplies over several transmission cycles until the pathogen population is sufficiently large to effectively control the pest population through the development of epizootics of disease.

The interest generated in invertebrate viruses largely depends on whether the host is considered to be of benefit or not, to humans. Viruses that kill pests and vectors are generally viewed favorably and considerable information has been obtained on the ecology of these diseases. In contrast, viruses of beneficial or commercially valuable invertebrates such as insect pollinators or shellfish are studied primarily when disease has a tangible economic impact on their populations. The same applies to insect mass rearing facilities that produce massive numbers of insects for use in pest or vector control programs involving the sterile insect technique (Kariithi et al., 2013). The foremost example of viruses infecting beneficials, however, is that of pathogenic viruses of honeybees that have attracted a great deal of attention over the past decade in the search for the causative agent(s) of colony collapse disorder which has been decimating bee populations in many parts of North America and Europe (Cox-Foster et al., 2007; Martin et al., 2012). Pathogens of beneficial invertebrates in terrestrial and aquatic ecosystems are considered elsewhere in this book (see Chapters 14 and 15), and are only considered briefly here.

The use of the terms pathogenicity and virulence often vary across the literature on invertebrate viruses. This is because ecologists, evolutionary biologists and invertebrate pathologists have applied different definitions depending on the focus of their studies, or have used different combinations of metrics to define each concept (see a discussion of these issues by Thomas and Elkinton, 2004; Shapiro-Ilan et al., 2005). To avoid confusion, and because I have

drawn examples from across all of the disciplines involving host-virus interactions, I have opted mostly to avoid these terms in favor of the metrics that were employed, such as infectivity (the capacity to infect), dose or concentration-mortality relationships, and speed of kill.

In addition to baculoviruses, many other invertebrate viruses are known to infect invertebrates in terrestrial or aquatic habitats (Williams et al., 2016; Rybov 2016). However, this chapter has restricted its focus to the better known virus families for which most information is available. That said, a world of opportunities remains available for any researcher wishing to study the ecology of the better or lesser-known viruses (tetraviruses, nodaviruses, birnaviruses, idnoreoviruses, herpesviruses, nidoviruses, etc.) that infect insects and other invertebrates.

7.2 DIVERSITY OF INVERTEBRATE PATHOGENIC VIRUSES

The virus pathogens of invertebrates are classified in orders, families, genera and species based on multiple criteria related to the physical characteristics of the virus particle, the genome properties such as type of viral nucleic acid, genome organization and gene content, deduced phylogenetic relationships, the replication cycle within the host cell, and ecological characteristics such as types of host infected and the nature of virus disease (pathology), among others. Although virus species is a recognized concept and has an established definition, isolates of viruses are given names that are not italicized, even if they include the name of the host species (Kuhn and Jahrling, 2010). I have adopted this practice here. Virus family names, in contrast, are italicized.

One key characteristic that determines the ecology of these pathogens is the presence or absence of an occlusion body (OB) (Table 1). This is a crystalline matrix of protein that surrounds the virus particle (virion) and protects it during periods outside the host. This structure is particularly important in the transmission of viruses that infect invertebrates in terrestrial habitats, as the OB allows the virus to persist on plant surfaces, where plant secondary chemicals and solar ultraviolet (UV) radiation can inactivate the virus, or in the soil where enzymes released by microorganisms may otherwise degrade viral proteins and nucleic acids.

The baculoviruses, entomopoxviruses and cypoviruses are all characterized by forming large OBs, typically 0.5 - 4 μm in diameter that can be visualized using a phase contrast microscope. The non-occluded viruses, such as the densoviruses, nudiviruses, iflaviruses, hytrosaviruses and iridescent viruses, tend to exploit routes of transmission that do not involve extended periods in the environment. The ascoviruses and polydnaviruses have intimate relationships with parasitoid wasps. These viruses are carried between hosts by the parasitoids and are never exposed, or only very briefly, to environmental conditions outside the host insect.

As most of our understanding of invertebrate virus ecology comes from baculoviruses, it is worth briefly mentioning the baculovirus transmission cycle here. When a susceptible lepidopteran larva consumes OBs, these break down in the alkaline insect midgut and release occlusion derived virions (ODVs). These virions have to cross the peritrophic membrane, which is a tube of chitin and glycoproteins that lines the midgut and protects it from abrasion and pathogens. The ODVs then infect midgut epithelial cells, where they undergo replication to

produce virions that bud through of the basal membrane of the cell into the hemolymph. The budded virions disperse in the hemolymph to infect other cells during the systemic phase of infection (Rohrmann, 2013). Following multiple rounds of systemic infection the infected cells accumulate large numbers of OBs that are then released into the environment, often following the death of the insect, for transmission to other susceptible larvae (see Chapter 3).

Of the virus families listed in Table 1, only the cypoviruses, dicistroviruses and iflaviruses have an RNA genome, whereas all the others are DNA viruses. The type, organization and quantity of nucleic acid in the viral genome have implications for the infection strategy, replication scheme and the ability to carry supplementary, non-essential genes that improve aspects of virus fitness. Thus, with the exception of the densoviruses, the genomes of DNA viruses tend to be far larger than the genomes of RNA viruses. This reflects the diversity of "survival strategies" that viruses can adopt, ranging from structurally simple particles with a small compact genome to large complex particles with an extensive array of genes with structural, replication and auxiliary functions. The ecological consequences of this diversity will be explored in the course of this chapter.

7.3 DISTRIBUTION OF INVERTEBRATE PATHOGENIC VIRUSES

Invertebrate pathogenic viruses are present on all continents of the world, in terrestrial, freshwater and marine habitats. A recent metagenomic study even reported the presence of dicistroviruses, iflaviruses and iridoviruses in a remote Antarctic lake that was frozen for most of the year, although the host species were not identified (López-Bueno et al., 2015). As obligate intracellular microparasites, the primary factor that determines the presence of pathogenic viruses in a particular locality is, of course, the presence of the invertebrate host. The principal factors that determine the presence of the host are suitable climatic conditions and the availability of a suitable food supply, be it a plant in the case of phytophagous insects, a vertebrate host in the case of hematophagous arthropods, or plankton, algae or organic particles in the case of marine crustaceans or mollusks (see Chapters 4 and 6). Even for occluded viruses than can persist in the environment for extended periods, the periodic presence of the host population is required to maintain a viable pathogen population.

Our current understanding of the diversity and distribution of invertebrate pathogens has less to do with the geographical distribution of pathogens and much more to do with the geographical distribution of invertebrate pathologists and the availability of scientific infrastructure for the study of diseased insects and other invertebrates. This was clearly reflected in a qualitative analysis of the development of virus-based biological insecticides in different geographical regions, in which North America and Europe were developing more viruses for pest control than the countries of Africa, Central and South America, Oceania or the Indian subcontinent (Entwistle, 1998). That said, the rapid growth in the study of insect pathogenic viruses in China over the past two decades has resulted in significant advances in the use of these pathogens in pest control (Sun, 2015). As a result of the use of viruses in biological control,

particularly baculoviruses, many of the following examples are from viruses of insect pests in forest and agricultural ecosystems.

7.4 KEY ASPECTS OF PATHOGEN ECOLOGY

The survival of a pathogen in a particular host population depends on a complex set of interactions that modulate transmission. Transmission itself is the process by which a pathogen or parasite is passed from an infected host to a susceptible host of the same or subsequent generations (see Chapter 1). Because of this, the mechanistic aspects of virus transmission, i.e., the route by which the virus leaves one host and gains entry to a new host to achieve infection, are highly influential in the ecology of virus diseases.

The majority of invertebrate viruses employ direct transmission (Tanada and Kaya, 1993). This means that the virus passes directly from one host to another through reproduction or sexual contact. Alternatively, some common viruses have an intermediate step in which they leave the infected host and wait in the environment until encountered by a new susceptible host. In contrast, indirectly transmitted pathogens require an intermediate (secondary) host or a vector organism in order to pass from one primary host to another primary host.

In the following sections it will become clear that pathogen survival involves the interplay of transmission with persistence in the environment (in the case of occluded viruses) and dispersal across a range of spatial scales, from local movement between plants and soil to regional dispersal, usually via host-mediated migration. Virus transmission, persistence and dispersal are modulated by host-related factors such as foraging behavior, and by biotic factors, often involving the host plant in the case of phytophagous insects, and abiotic factors that reflect specific characteristics of the environment. It is therefore important to bear in mind in the following sections that transmission, persistence and dispersal should not be viewed in isolation but rather as a set of interacting and interdependent processes.

With the development of molecular tools over the past two decades we are slowly becoming aware that many invertebrate species harbor covert (inapparent) infections by viral pathogens that can affect different aspects of their development or reproductive capacity in the absence of clear signs of disease. However, our understanding of the ecology of non-lethal viruses lags many years behind that of lethal virus pathogens. There are several reasons for this – obvious diseases tend to attract the attention of researchers that study these organisms, studies are more easily targeted at individuals showing specific signs of morbidity or mortality in a population, the massive proliferation of the virus in lethally-infected individuals and the associated pathological changes in tissues and organs simplify the correct identification of the causative agent, and standard laboratory techniques have usually been developed and verified for the detection and identification of the most serious invertebrate diseases. That said, advances in molecular detection techniques now allow the screening of large numbers of organisms in the search for particular pathogens, including viruses from insects (Zwart et al., 2008; Virto et al., 2014; Zhou et al., 2015), or other invertebrates (Ren et al., 2010; Panichareon et al., 2011). Alternatively, transcriptome studies and metagenomics approaches are proving highly

informative in the discovery of non-lethal viruses in ants (Valles et al., 2012), bees (Cox-Foster et al., 2007), mosquitoes (Cook et al., 2013), dragonflies (Rosario et al., 2011), moths (Pascual et al., 2012; Jakubowska et al., 2014, 2015), among others.

Serendipity has also played an important role in the discovery of non-lethal viruses. The detection of non-lethal viruses has frequently been accidental during the study of apparently healthy individuals (Lacey and Brooks, 1997), when working with apparently healthy cell lines (Carrillo-Tripp et al., 2014), or during the study of lethal viruses in which non-lethal viruses can appear as contaminants (Wagner et al., 1974; Jakubowska et al., 2016). As a result, most of the examples in the following sections focus on lethal viruses of insects, particularly baculoviruses, which are by far the best understood insect-virus pathosystems (Cory, 2010). However, when working on virus ecology it is important to bear in mind that just because an experimental individual or population appears to be healthy, this is not evidence that it is not infected by one or more pathogenic viruses.

7.5 TRANSMISSION

Transmission is described as *horizontal* when the pathogen leaves an infected host and passes to a susceptible host (other than the host's offspring). This involves a spatial component in transmission, even for viruses that adopt a sit-and-wait strategy during the environmental phase of transmission. Virus particles that remain infectious outside the host can infect individuals from the same generation or subsequent generations. Alternatively, *vertical* transmission occurs when infected parents reproduce and pass the pathogen to their offspring. As such, vertical transmission is a mechanism for trans-generational transmission in pathogens that do not kill their host prior to reproduction. In fact many pathogenic invertebrate viruses adopt a mixed strategy involving both horizontal and vertical transmission, depending on the conditions within the infected host and the relative probability of successful transmission by either route.

7.5.1 Horizontal Transmission

For lethal viruses, the death of the host is usually followed by the release of massive numbers of virus particles. This is characteristic of baculoviruses, such as nucleopolyhedroviruses and granuloviruses that infect lepidopteran larvae. Many baculoviruses have genes for cathepsin and chitinase enzymes that rapidly break down the host tissues and liquefy the virus-killed insect (Ishimwe et al., 2015). This facilitates the release of virus OBs that are then spread over the surfaces of the leaves and stems of the host plant through gravity or the action of wind and rain. A single infected late-instar larva can release enormous numbers (~10^6-10^9) of OBs (Shapiro, 1986). As susceptible larvae may become infected following the consumption of a single or a few OBs, depending on species and the growth stage of the larva, the death of a single infected insect can have the potential to transmit the infection to many other larvae that consume OB-contaminated foliage.

In agricultural settings, in which baculoviruses are used as insecticides, OBs are usually applied to the whole crop, resulting in a near-uniform distribution of OBs. Similarly, viruses applied as insecticides are present at high densities on the crop so that pest insects rapidly

acquire a lethal infection during periods of feeding in the hours following the application of OBs (Lasa et al., 2007). In natural settings, insects acquire infections from OBs in the environment that likely have a random or clumped distribution (Dwyer, 1991). A clumped distribution of OBs reflects the local distribution of recent deaths of infected insects from which viral OBs have been released (Vasconcelos et al., 1996b; D'Amico et al., 2005). As such, we would expect the patterns of transmission in areas where natural populations of viruses exist to be quite different to those in crops treated with virus-based insecticides, although the basic principles related to transmission remain the same.

An insect's susceptibility to virus infection usually decreases markedly as it grows. For example, in baculoviruses, the 50% lethal dose of OBs increases by 10^3 - 10^5 fold between the first and final instars in many species of Lepidoptera (Briese, 1986). The mechanism of this developmental resistance likely involves three main factors: (i) a decreasing surface area:volume ratio in the gut of growing larvae, which means that in order to reach midgut cells, virus particles must pass through an increasing volume of food bolus as the larvae age (Hochberg, 1991a), (ii) a stage-related increase in the thickness and reduction in the porosity of the peritrophic membrane through which virions must pass to reach and infect midgut epithelial cells (Wang and Granados, 2000; Levy et al., 2012), and (iii) an increase in the rate of sloughing of infected midgut cells in later, compared to earlier, instars (Kirkpatrick et al., 1998). Cell sloughing reduces the period available for the virus to replicate in infected midgut cells and produce the budded virions that establish a systemic infection (McNeil et al., 2010). As the peritrophic membrane represents a major barrier to pathogens infecting through the gut, several granuloviruses and nucleopolyhedroviruses produce mucin glycoprotein-degrading enzymes that they carry in the OBs or the virions, to degrade the membrane and facilitate access to midgut cells (Peng et al., 1999b; Slavicek and Popham, 2005; Hoover et al., 2010). Similarly, a chitinase domain in the fusolin protein of entomopoxviruses is activated in the insect midgut to degrade the chitin component of the peritrophic membrane and facilitate access of the large entomopoxvirus virions to midgut cells (Mitsuhashi and Miyamoto, 2003; Chiu et al., 2015).

As a result, the probability of horizontal transmission depends on complex interactions among the density of inoculum OBs in the environment, the spatial distribution of inoculum (uniform, random or clumped), the host density, feeding behavior and the susceptibility of insects to infection (Dwyer, 1991; Goulson et al., 1995; D'Amico et al., 1996; Reeson et al., 2000; Parker et al., 2010). As many of these variables differ markedly between distinct species of insects and their viruses, it is clear that quantitative estimates of transmission require an understanding of the behavior of healthy and infected insects, the rate of decay of OBs in the environment, and variation generated through heterogeneity in host susceptibility and host plant effects (for herbivorous hosts), in addition to the usual estimates of host and pathogen densities in each pathosystem.

A clear example of how density affects transmission comes from comparative studies on lepidopteran larvae that live alone or in groups. Solitary species usually exist at relatively low

densities, as dispersal behavior or cannibalism reduce the numbers of individuals in a particular locality. In contrast, gregarious species experience high local densities of conspecifics within each group of individuals. In the case of solitary species resistance to infection depends mainly on larval weight, whereas in gregarious larvae, resistance to infection increases faster than body weight gain as the risks of virus transmission for each individual in a group-living species increase with age (Hochberg, 1991a).

Two additional routes of horizontal transmission are frequently found in invertebrate viruses; cannibalism of infected individuals and transmission during sexual contact. As both of these routes involve specific behaviors they are considered in sections 7.9.2 and 7.9.3.

7.5.1.1 Estimating Horizontal Transmission

Initial attempts to quantify the transmission process in baculoviruses adopted the principle of mass action, in which transmission is directly proportional to the density of susceptible and infected individuals (or infectious OBs in the case of baculoviruses) in the local population (Anderson and May, 1981). The mass action principle assumes that the efficiency of transmission is a constant (the transmission coefficient), reflecting a fixed probability of infection following contact between a susceptible individual and a pathogen particle in the environment (McCullum et al., 2001). For the host population this can be written as: $dS/dt = -vSP$, where S is the density of susceptible hosts, P is the density of virus particles in the environment and v is a constant describing the probability of transmission.

However, a series of field studies with nucleopolyhedroviruses have demonstrated that the efficiency of transmission is not constant but varies with the density of susceptible insects and the pathogen (D'Amico et al., 1996), insect growth stage and the area of foliage consumed (Goulson et al., 1995), pathogen clumping (Dwyer 1991; D'Amico et al., 2005), heterogeneity in host susceptibility (Dwyer et al., 1997; Reeson et al., 2000; Hudson et al., 2016), duration of exposure to the pathogen, and density-dependent variation in insect behavior (Reeson et al., 2000). Similar findings were reported in laboratory studies on a granulovirus of the meal moth, *Plodia interpunctella*, that was transmitted via cannibalism of infected individuals. In this case the transmission coefficient increased with the density of susceptible hosts and decreased with the density of infected cadavers (Knell et al., 1998). The transmission efficiency also declined over time as infected cadavers were rapidly consumed by cannibalistic larvae in the first few hours of the experiments, resulting in a reduction in the overall pathogen density.

Behavioral, physiological and environmental factors may affect both the probability of contact between pathogen particles and susceptible insects, and the probability of successful infection once contact has occurred. Consequently, an alternative approach has been developed in which a proportion of the host population is considered to occupy a pathogen-free refuge, the size of which can vary according to the size of the pathogen or susceptible insect populations (Hails et al., 2002). This approach proved useful for comparison of transmission risks in lepidopteran populations exposed to wild-type and recombinant baculoviruses (Hails et al., 2002), and mosquito larvae exposed to an iridescent virus (Marina et al., 2005), highlighting the

versatility of the procedure. The value of other formal approaches to the study of virus transmission in insect populations is discussed in detail in Chapter 12 of this book.

7.5.2 Vertical Transmission

Vertical transmission of invertebrate viruses, from parents to offspring, is a route that is only available to pathogens that do not invariably kill their hosts prior to reproduction. The presence of vertically transmitted infections in insects has been suspected since early observations that the offspring of seemingly healthy insects could spontaneously succumb to virus diseases, even under clean laboratory conditions (Kukan, 1999). Vertical transmission is also an issue of concern in laboratory colonies of insects that are required to be pathogen free for use in a diversity of experimental settings (Fuxa et al., 1999; Helms and Raun 1971), or in insect mass-production facilities where the impact of vertically-transmitted pathogens can be devastating (Greenberg, 1970; Boucias et al., 2013; Morales-Ramos et al., 2014).

Molecular studies have demonstrated that vertical transmission is a common feature of viruses in natural populations of insects (Carpenter et al., 2007; Virto et al., 2014; Cory, 2015), including beneficial insects such as honeybees (de Miranda and Fries, 2008), and other invertebrates (Cowley et al., 2002; Barbosa-Solomieu et al., 2005). Similarly, electron microscopy studies have provided evidence for the presence of virus particles in the ovarian tissues or developing eggs in adult female Lepidoptera infected with nucleopolyhedrovirus (Smith-Johannsen et al., 1986), densovirus (Garzon and Kurstak, 1968), or nudiviruses (Raina et al., 2000; Rallis and Burand, 2002), as well as a hytrosavirus of the tsetse fly, *Glossina pallidipes* (Jura et al., 1989), and an ascovirus and iflavirus in parasitoid wasps (Bigot et al., 1997; Reineke and Asgari, 2005), among many others. In the case of rhabdoviruses or entomopoxviruses of parasitoid wasps, the viruses may replicate in the poison or accessory glands of the infected female wasp before being injected into host insects together with the parasitoid egg(s), which are subsequently infected by the virus (Lawrence and Akin, 1990; Lawrence and Matos, 2005).

Studies focusing on male involvement in vertical transmission are less common than studies on females. Nevertheless, evidence in favor of male involvement includes observations on the presence of virus in the testes for nucleopolyhedroviruses, granuloviruses and nudiviruses in Lepidoptera (Lewis et al., 1977; Burden et al., 2002; Pereira et al., 2008), reovirus in Coleoptera (Kitajima et al., 1985) and rhabdoviruses in Diptera (Longdon et al., 2011), among others. Indeed, male involvement in transmission during mating has been reported in densoviruses (Barik et al., 2016), sigmaviruses (Longdon and Jiggins, 2012), nucleopolyhedroviruses (Knell and Webberley, 2004), iflaviruses (Yue et al., 2007), nudiviruses (Zelazny, 1976; Burand, 2009), iridescent viruses (Marina et al., 1999; Adamo et al., 2014), among others.

To determine whether the virus particles responsible for vertical transmission are present inside, or on the surfaces of eggs, experimental egg masses are often subjected to surface decontamination using formalin or hypochlorite solutions. If no difference is observed in the

incidence of virus infection in the progeny from surface decontaminated versus untreated eggs it is usually concluded that the virus is likely to have been transmitted in the developing embryos within eggs, which is known as transovarial transmission (see Chapters 1 and 3). In contrast, if surface decontamination of eggs markedly reduces the incidence of infection in the offspring, it is likely that most infections are acquired by ingestion of virus particles on the exterior egg surface that the hatching larvae consume as they chew their way out of the egg. If the contaminating virus particles were deposited by the female during oviposition, this is known as transovum transmission. The parental origin of the contaminating virus defines vertical transmission and differs in that respect from eggs that become contaminated from environmental sources of inoculum (Murray and Elkinton, 1989). In the case of baculoviruses, scanning electron microscopy has been used to confirm the presence of viral OBs on the exterior egg chorion (Hamm and Young, 1974; Nordin et al., 1990).

In a few cases, viruses have developed a symbiotic, mutualistic relationship with their hosts that depends on vertical transmission. One example is seen in the ascoviruses that infect lepidopteran larvae (Bideshi et al., 2010). Horizontal transmission in ascoviruses is normally achieved when a female parasitoid wasp carries the virus on her ovipositor from an infected to a susceptible caterpillar. The Diadromus pulchellus ascovirus 4a (DpAV-4a) differs from other ascoviruses in that it replicates in the parasitoid ovary (Bigot et al., 1997). During oviposition the wasp injects the virus into pupae of the leek moth *Acrolepiopsis assectella*. The virus suppresses the leek moth immune response, allowing development of the parasitoid progeny that themselves acquire the virus for future cycles of vertical transmission (Renault et al., 2002).

The symbiotic relationship with ichneumonid or braconid parasitoid wasps has evolved further in another family of viruses, the polydnaviruses (Strand, 2010). The polydnaviruses are transmitted to offspring as so-called proviruses that replicate in the wasp's reproductive tract, producing encapsidated particles that are injected into the wasp's host (usually a caterpillar) during oviposition. Once injected, the polydnavirus particles enter the cells of different caterpillar tissues but do not replicate. Instead they carry and express a series of genes from the wasp's genome that favor the survival of the wasp's offspring. The most important effect of polydnaviruses is to protect the developing wasp egg or larva by suppressing the caterpillar immune response that would otherwise encapsulate the developing parasitoid within hemocytes, followed by melanization and parasitoid death (Gundersen-Rindal et al., 2013). Therefore the virus is essential for the successful development of the parasitoid and the virus, which is in reality an extension of the genome of the wasp, achieves continuous cycles of vertical transmission (Strand, 2012).

7.6 PERSISTENCE

Viruses can persist in two quite different ways: (i) as a covert infection within the host in an attempt to achieve vertical transmission through host reproduction, or (ii) in the environment as long-lived infective stages. Whether a virus adopts a lethal or non-lethal strategy of host exploitation depends largely on the relative opportunities for horizontal and vertical

transmission, which is the clearest indicator of virus fitness (Cory and Franklin, 2012). So-called mixed-mode transmission strategies are observed in many of the invertebrate viruses.

7.6.1 Persistence Within the Host

Inapparent infections are common in a wide range of invertebrates but have not been quantified because of a lack of interest in infections that do not cause immediate patent disease, because their detection usually requires considerable knowledge of invertebrate pathology and molecular techniques and an appreciation of the complexity of host-virus relationships, and because of difficulties in identifying novel viruses below the level of virus family (Okamura, 2016).

Publications on this topic variously refer to covert, inapparent, silent, occult, persistent or sublethal infection, in which the virus replicates at a low level without killing the host. In this sense covert infection differs from latent infection, in which the virus genome is integrated into the host genome, or persists in an inactive state in host cells with minimal replication (Lin et al., 1999; Fang et al., 2016). The latent state is poorly understood in invertebrate viruses, although it may involve the suppression of cell epigenetic silencing and the production of viral miRNAs that inhibit the expression of lytic viral genes (Wu et al., 2011; Hussain and Asgari, 2014).

For studies in which the presence of the pathogen has been confirmed, sublethal disease is characterized by decreased body weight and adult eclosion, decreased reproduction and longevity (Marina et al., 2003; Sood et al., 2010; Cabodevilla et al., 2011b), as well as adverse effects on sperm production (Sait et al., 1998). Due to the association between covert infections and reduced reproduction, sublethal disease has been implicated as a potentially important factor modulating the population dynamics of insect populations (Boots et al., 2003; Bonsall et al., 2005; Myers and Cory, 2016), although empirical evidence for this is sparse.

Sublethal effects have their origins in three possible explanations: (i) a direct result of the pathological effects of the virus within the host, (ii) the metabolic costs incurred from mounting an immune response to suppress the pathogen or, (iii) the result of host traits that are corrected with disease resistant phenotypes (Myers and Kuken, 1995; Rothman and Myers, 1996; Bouwer et al., 2009). Fortunately, molecular techniques now allow covertly infected individuals to be identified with a high degree of confidence. These individuals can now be differentiated from those individuals that were exposed to viral inoculum but did not become infected, or those that became infected but managed to rid themselves of the infection.

Covert infections were initially detected using DNA hybridization techniques (Christian, 1992; Kukan and Myers, 1995) that were superseded by PCR or multiplex PCR to detect viral genomic DNA in host tissues (Williams, 1993; Hughes et al., 1997; Lupiani et al., 1999; Arzul et al., 2002; Abd-Alla et al., 2007; Kemp et al., 2011), or the use of reverse transcriptase PCR (RT-PCR) to detect gene transcription as an indicator of virus replication (Burden et al., 2002; Martínez et al., 2005; Vilaplana et al., 2010). The use of expressed sequence tag (EST) libraries based on mRNA sequences purified from the host have also proved useful, although only viruses with high titers are likely to be detected using this method (Liu et al., 2011). Quantitative PCR

(qPCR) now allows researchers to detect very low numbers of gene copies in experimental samples (Yue et al., 2007; Murillo et al., 2011; Blanchard et al., 2014), as do recently-developed amplification techniques (Xia et al., 2014, 2015), as well as transcriptomics and next generation sequencing (Liu et al., 2011; Ma et al., 2011; Kolliopoulou et al., 2015; Webster et al., 2015).

Interestingly, the amounts of nucleopolyhedrovirus present in covertly infected adult Lepidoptera differed markedly in different parts of the adult body, with particularly high virus loads in the head, legs and wings, whereas previously researchers have tended to focus on tissues and organs within the insect abdomen, such as the fat body. The whole-body virus load also differed with life stage, being highest in eggs and neonate larvae and lowest in final instar and adult insects (Graham et al., 2015).

7.6.2 Persistence Outside the Host

Viruses vary markedly in their ability to persist outside the host (Ignoffo, 1992). These differences reflect the importance of environmental persistence in their transmission cycle. As mentioned in section 7.2, the virions of occluded viruses, namely the baculoviruses, entomopoxviruses and cypoviruses, are protected by the protein matrix that comprises the OB. This structure allows virions to persist for months or years in protected environments (Jaques, 1985). Some non-occluded viruses, such as densoviruses (*Parvoviridae*) are also capable of extended periods of survival outside the host (Kawase and Kurstak, 1991), whereas the Oryctes nudivirus is inactivated within a few days in the environment (Zelazny, 1972). That said, with the exception of a number of baculoviruses, the persistence of invertebrate pathogenic viruses has not been the subject of systematic study or quantification, so that information on environmental persistence in many virus families is limited.

One important factor to take into account in studies on virus persistence is that research performed prior to the development of PCR-based detection almost invariably employed bioassay techniques or serological reactions to estimate the quantities of infectious virus present in a particular sample. In contrast, molecular techniques are used to detect or quantify viral DNA or RNA in environmental samples (Hewson et al., 2011; Krokene et al., 2012), which is not necessarily equivalent to a measure of the quantity of virus that retains infectivity for the target host. Where possible, the results of molecular analyses should, therefore, be verified using biological assays.

7.6.2.1 Persistence on Plants

The sit-and-wait strategy of transmission of baculovirus, cypovirus and entomopoxvirus pathogens of phytophagous insects necessitates that these viruses persist in an infective state on the food plant until consumed by a suitable host. However, virus persistence on plants involves a series of complex interactions of virus particles with plant architecture, leaf epidermal structure, leaf surface chemistry and plant phenology that usually have to be studied as a set of variables rather than as individual factors. Environmental factors also interact with plant-related variables to influence virus persistence. For example, following the release of baculovirus OBs from an infected insect, OBs may be washed by rainfall on to the upperside or underside of leaves or

plant stems. Each of these locations will differ in the presence, density and physical characteristics of surface hairs (trichomes), surface wrinkles and pits, stomata, glandular structures and epicuticular waxes that are all likely to affect OB adhesion and retention. Laboratory studies on the forces involved in OB attachment to hydrocarbons, such as those present in leaf waxes, have indicated that strong hydrophobic interactions are probably important in maintaining OB adhesion (Small et al., 1986). The upper and lower leaf surfaces and stems will also differ in their exposure to solar UV radiation; a major factor in the inactivation of virus pathogens in the environment (see section 7.11.1). The presence of plant exudates can also result in different chemical environments present at these sites. For example, in the case of plants of the family Malvaceae, leaf surface pH values are high (pH 8-11) and can differ markedly between the upper and lower phylloplanes, depending on plant species. Phylloplane pH also tends to increase as the leaf ages (Harr et al., 1984). The alkalinity of the leaf surfaces is due to the presence of glandular trichomes that secrete carbonates and bicarbonates of magnesium, calcium and potassium (Elleman and Entwistle, 1982). This contrasts with the phylloplane of many other plants that tends to be slightly acid, e.g., pH 5 - 6 in the case of maize (Derridj, 1996).

When applied to cotton leaves nucleopolyhedrovirus OBs were inactivated within 24 h, even when plants were not exposed to solar radiation (Young and Yearian, 1974; Elleman and Entwistle, 1985a). The presence of dew droplets on the leaves appears to solubilize the exudates and likely speeds the inactivation of OBs (Young et al., 1977), Although OBs exposed to cotton exudates retain their polyhedral structure, exposure to metal cations in leaf exudate may have reduced the solubility of these OBs in the insect midgut (Elleman and Entwistle, 1985a, 1985b).

OBs on bark or plant stems represent an important pathogen reservoir in populations of the gypsy moth (*Lymantria dispar*). OBs on bark can infect neonate larvae as they search for suitable foliage (Woods et al., 1989), or can be washed by rainfall and contaminate egg masses prior to hatching (Murray and Elkinton, 1989).

Plant phenology will often influence OB persistence both in terms of the types of plant structures available (leaves, flowers, fruits, etc.), and changes in plant architecture during growth and development. For example, the leaf whorl of maize plants is the preferred feeding site of larvae of the fall armyworm (*Spodoptera frugiperda*). The leaf whorl also provides a natural cup-like structure that protects OBs from solar radiation as developing leaves expand and grow out of the whorl (Castillejos et al., 2002). Similarly, the ability of OBs to persist on the surfaces of fruit can be quite different from that of leaves, which can have important implications for the effectiveness of OBs applied as biological insecticides, such as the nucleopolyhedrovirus used to control *Heliothis virescens* on cotton (Fuxa, 2008), the granuloviruses used to control larvae of the codling moth, *Cydia pomonella*, and lepidopteran pests of citrus (Ballard et al., 2000; Moore et al., 2015). In contrast, although root feeding invertebrates are infected by a number of occluded and non-occluded viruses, the role of root structure, root exudates and root microbiota on virus persistence on and around root systems remains unknown.

Adopting a formal approach, Fuller et al. (2012) have argued that OB persistence on foliage cannot be estimated accurately unless virus decay is measured independently of infectiousness. To achieve this they varied the density of infected *L. dispar* cadavers and exposure time. Following different periods of decay, cadavers on oak branches were enclosed in gauze bags with healthy larvae to estimate transmission. Importantly, OB-contaminated foliage within gauze bags was protected from additional decay during the period in which transmission (infectiousness) was determined. The best estimates of average virus persistence in this system varied from 2.5 days in 2008 to 14.3 days in 2007, although the 2007 estimates were judged unreliable due to predation of experimental larvae. In contrast, data taken from studies by other authors using purified OB suspensions indicated average persistence times of 0.9 – 1.5 days for L. dispar nucleopolyhedrovirus on oak (Webb et al., 1999, 2001), 0.5 – 1.3 days for Trichoplusia ni nucleopolyhedrovirus on cabbage (e.g., Jaques, 1972), or 0.4 – 0.9 days (half-life) for Helicoverpa armigera nucleopolyhedrovirus on cotton (Sun et al., 2004). These findings provide support for previous observations that OBs released from infected cadavers persist on trees and field crops significantly longer than purified OBs applied as biological insecticides (Magnoler, 1968; Evans and Entwistle, 1982; Young and Yearian, 1989; Pessoa et al., 2014). This is probably because the debris and substances from the insect cadaver provide improved adhesion to plant surfaces and/or protection from UV radiation (see section 7.11.1).

A special case of virus persistence on plants is that of viruses of insect pollinators on flowers. Several recent studies have implicated flowers as sites that can become contaminated with parasites or viruses such as deformed wing virus (*Iflaviridae*), when infected insects visit flowers. These parasites and pathogens can infect other susceptible pollinators that subsequently visit contaminated flowers, such as honeybees, bumblebees, or wasps (Singh et al., 2010; Evison et al., 2012; Fürst et al., 2014; McMahon et al., 2015). Consequently, the role of pollen, nectar or other flower traits in the persistence and transmission of pollinator viruses has begun to generate interest among researchers concerned about recent global pollinator declines (McArt et al., 2014). The high visitation rates to flowers by pollinators and the ability of non-host pollinators to disperse pathogens and parasites from contaminated to non-contaminated flowers (Graystock et al., 2015), suggests that the persistence of pollinator viruses on flowers may play a significant, but poorly understood, aspect of the ecology of these pathogens.

7.6.2.2 Persistence in Soil

The soil is the most important environmental reservoir for occluded viruses. Rain splash, surface water and wind-blown dust can move OB-contaminated soil particles from the soil on to plants where they can be consumed by susceptible insects (Hochberg, 1989; Fuxa et al., 2007). When infected plant-feeding insects die they fall onto the soil surface or remain on the plant and release large numbers of OBs that are subsequently washed from leaf surfaces onto the soil. Alternatively, OB-contaminated leaves and stems senesce, fall to the soil and are subsequently incorporated into the soil by agricultural practices such as tillage (Fuxa and Richter, 1996), or by the soil fauna. In forests, the leaf litter is also an abundant virus reservoir in which OBs can

persist for extended periods with little loss of infectivity (Podgwaite et al., 1979; Thompson and Scott, 1979).

In a systematic study on tillage and precipitation, viable nucleopolyhedrovirus OBs were detected in the soil of soybean fields at depths of 0-25 cm, but were far less abundant at depths of 25 - 50 cm (Fuxa and Richter, 1996). Soil microcosm experiments indicated that nucleopolyhedrovirus OBs released from virus-killed larvae or applied to the soil in water underwent a 3-logarithm reduction in viable OBs over a 17 month period (Fuxa et al., 2001). This may seem like a large reduction, but the enormous quantity of OBs produced in each infected insect means that even after extended periods in the soil, many OBs remain viable and have the potential to infect and replicate if consumed by susceptible insects. Indeed, the abundance of OBs in soil closely reflects the prevalence of infection in the host insect population in forests (Thompson et al., 1981), field crops (Fuxa and Richter, 2001), greenhouse crops (Murillo et al., 2007), and in pastures attacked by soil-dwelling pests (Kalmakoff and Crawford, 1982). Such is the stability of the OB structure that viable OBs of a forest pest, *Orgyia pseudotsugata*, have been detected in soils several decades after the forest was cleared (Thompson et al., 1981).

Recognizing that soil represents a major environmental reservoir of OBs also means that it represents a unique resource for the discovery of novel virus isolates. Studies on open field agricultural soils and greenhouse substrates have proved that soils contain a high diversity of nucleopolyhedroviruses and granuloviruses (Murillo et al., 2007; Rios-Velasco et al., 2011; Gómez-Bonilla et al., 2012). These can be isolated using a simple bioassay technique in which soil samples are mixed with artificial diet and fed to early instar larvae that succumb to virus disease if sufficient OBs are present (Richards and Christian, 1999). Viable isolates of Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) were obtained from 29 – 38% of the greenhouse soil substrate samples tested using this technique (Murillo et al., 2007).

There is an intimate association between OBs and soil particles. However, as soil is one of the most heterogeneous habitats on Earth, the findings on OB populations in one type of soil may not be readily extrapolated to other types of soils. The clay component of soil is particularly important and OB retention in soil depends on the relative abundance and type of clay component. Indeed, once bound to clay, baculovirus OBs can be very difficult to recover. In a study on seven different clays, Helicoverpa armigera nucleopolyhedrovirus OBs bound strongly to all the clays tested, whereas two non-occluded viruses, cricket paralysis virus (*Dicistroviridae*) and an iridescent virus (*Iridoviridae*), preferentially bound to certain clay types but not others (Christian et al., 2006). OB binding to soil components is likely to be affected by the cation exchange capacity of the soil, which is determined largely by soil pH, the presence of clay minerals, and organic matter (Hunter-Fujita et al., 1998). The presence of iron-based minerals is likely to be a good indicator of soils that are suitable for baculovirus OB populations (Christian et al., 2006).

Baculoviruses are not the only invertebrate viruses that persist in soils. The occluded cypoviruses (Tanada et al., 1974) and entomopoxviruses (Hurpin and Robert, 1976), and the non-occluded densoviruses (Watanabe and Shimizu, 1980), iridescent viruses (Reyes et al., 2004), and nodaviruses (Felix et al., 2011), have all been found to persist in soil. That said, the relationship between the soil virus populations and prevalence of infection in host invertebrates, such as that observed in soil-dwelling lepidopteran pests, remains poorly understood in general (Kalmakoff and Crawford, 1982; Bourner et al., 1992; Prater et al., 2006).

7.6.2.3 Persistence in Water

Viruses are often stored in water for periods of months or years in laboratory refrigerators. However there are no systematic studies of the persistence of occluded viruses in natural water bodies, probably because of a lack of interest in the use of these pathogens for the control of aquatic insects. The viruses that naturally infect hosts in aquatic habitats might be expected to be stable in water, but this is not always the case. The infectious titer of abalone herpesvirus fell markedly following 1 - 5 days of incubation in seawater at 15 °C (Corbeil et al., 2012). Similarly, qPCR-based studies indicated a >99.9% reduction in the number of genomes of the oyster herpesvirus (OsHV-1) in a 24 hour period in seawater, whereas the virus appeared to persist at high titers in the tissues of dead, infected oysters over a 7 day period (Hick et al., 2016).

Mosquito larvae are susceptible to nucleopolyhedroviruses and cypoviruses, the infectivity of which is modulated by calcium and magnesium ions present in solution (Becnel, 2006). The ability of these viruses to persist in the aquatic environment has not been studied in detail, although it is likely that they have retained the occlusion body structure in order to persist in the soil habitat of pools that undergo periods of drying when rainfall is scarce. Storage of invertebrate iridescent virus 3 in water at 27 °C resulted in a near exponential reduction in the infectious titer as determined by bioassay in larvae of *Aedes taeniorhynchus*. The virus persisted approximately twice as long in brackish water than in freshwater, possibly reflecting an adaptation to the brackish water habitat of the mosquito host (Linley and Nielsen, 1968).

The persistence of nucleopolyhedrovirus OBs of the spruce budworm (*Choristoneura fumiferana*), a terrestrial lepidopteran, was monitored over three years in aquatic microcosms that had been inoculated with a large quantity of OBs (>10^{10} OBs) in a forested area of Ontario, Canada. Viral DNA was detected in 8 - 9 out of 12 microcosms after one year, but only samples taken close to the bottom sediment proved positive by PCR after 3 years (Holmes et al., 2008). Quantitative PCR analysis of environmental samples from an island off the coast of Maine, USA, indicated the presence of nucleopolyhedrovirus in soil under chokecherry trees (*Prunus virginiana*) infested by webworms (*Hyphantria* spp.), and also in the sediment of freshwater pools, sea foam and marine plankton samples. The widespread presence of OBs was attributed to the runoff from infected webworms and webworm feces during a period of frequent rainfall on the island (Hewson et al., 2011).

7.7. DISPERSAL

7.7.1. Host-Mediated Dispersal

Probably one of the most important yet least understood mechanisms of virus dispersal involves the movement of infected hosts. For insects, this usually occurs on two broad scales: (i) local movement on or among food plants by infected larvae that die and release OBs at a site different from the site where they acquired the infection and, (ii) flight of adult insects carrying covert infections that are transmitted vertically to their offspring at an oviposition site distant from the original site of infection of the parent.

The first issue of local movement by infected larvae has mainly been examined in relation to behavioral manipulation of the host insect by the virus (section 7.9.4). This results in increased vertical and horizontal dispersal by infected insects, which improves the dispersal and transmission of the pathogen. Infected *Mamestra brassicae* larvae moved twice as far as healthy larvae in cabbage plots; an effect that was particularly evident during the 2-3 day period prior to death. The virus was also effectively dispersed by infected insects that crawled over a distance up to 45 cm from the initial point of release (Vasconcelos et al., 1996b).

An excellent example of host-mediated dispersal comes from the Oryctes nudivirus that infects the gut of both larval and adult rhinoceros beetles and has been successfully used for biological control of this pest (Hochberg and Waage, 1991). Adult beetles are good fliers and spend alternating periods feeding in the apices of coconut palms and reproducing beneath decomposing palm trunks. Infected adults live for ~4 weeks and during this period they excrete large quantities of virus as they move between feeding and breeding sites, thereby contaminating both types of habitat and transmitting the virus to developing larvae or other adult conspecifics (Jackson, 2009). The rate of spread of this virus through the dispersal of infected adult beetles was estimated at between ~1 and 3 km/month on different islands in the Pacific and at 4 km/month in the Seychelles (Bedford, 1980; Lomer, 1986).

The dispersal of covertly infected adults of the African armyworm, *Spodoptera exempta*, is likely to represent the principal means by which its nucleopolyhedrovirus (SpexNPV) travels along migration routes over distances of hundreds of kilometers during periodic outbreaks of this pest (Vilaplana et al., 2010). The prevalence of covert infection in adult moths collected during an outbreak in Tanzania ranged between 60 and 97%, depending on the PCR detection technique used. Infections were naturally efficiently transmitted to the progeny of infected parents. Outbreaks of this pest in Kenya, Tanzania and other parts of East Africa tend to terminate in epizootics of virus disease. That said, the influence of covert infection on the dispersal of infected *S. exempta* adults and the factors that trigger the activation of lethal disease in their offspring have yet to be determined.

In a study on the invasion of forests in Wisconsin by *L. dispar*, the rate of dispersal of the pest was estimated at ~12 km/year but, having arrived in a new section of the forest, the insect population then required several generations to reach densities at which transmission of its nucleopolyhedrovirus (LdMNPV) was likely to occur (Hajek and Tobin, 2011). This meant that

the virus began to regulate the pest population some 4 years after the pest had established in a particular location, compared to a 3-year delay in the case of a fungal pathogen.

The dispersal of non-occluded viruses that infect highly mobile insects, such as the viruses of crickets, drosophilids and other dipterans, remains largely unstudied from an ecological perspective. An example of host-mediated dispersal of a non-occluded virus comes from terrestrial isopods (woodlice, pillbugs) infected by an iridescent virus, which was influenced by the distance between suitable patches of habitat (Grosholz, 1993). The probability of dispersal decreased as interpatch distances increased. Habitat patchiness was also influential in the prevalence of virus disease – low levels of patchiness during the wet spring months were associated with a high prevalence of infection, which decreased as the dispersal of infected isopods became more restricted during the dry summer and fall months.

7.7.2. Environmental Factors Involved in Dispersal

There are many anecdotal accounts of virus dispersal through the action of rainfall and wind-blown dust. For example, the contamination of egg masses on foliage by OBs of the Douglas-fir tussock moth (*O. pseudotsugata*) increased from 12 to 100% as the remains of infected cadavers were washed over foliage by a day of light rain (Brookes et al., 1978). Virus-decontaminated branches became contaminated by a sawfly nucleopolyhedrovirus washed down by rain from infected cadavers on the upper branches of spruce trees (Evans and Entwistle, 1982). Indeed, the presence of OBs in raindrops hanging from pine needles beneath diseased sawfly (*Neodiprion sertifer*) colonies was quantified at 10^8 OBs/ml by direct counting under a microscope (Olofsson, 1989). This effect was confirmed in experiments using simulated and natural rainfall applied to infected cadavers of *L. dispar* on oak trees, in which branches below cadavers became contaminated by OBs washed down from higher branches (D'Amico and Elkinton, 1995). Simulated rainfall also strongly influenced the vertical distribution of nucleopolyhedroviruses OBs on cabbage plants and in the soil of field plots (Goulson, 1997). Irrigation water may also be an effective means of virus dispersal in crops that are routinely irrigated (Young, 1990). Virus-contaminated dust was implicated in the dispersal of OBs from the soil to colonies of sawfly larvae feeding on pine at varying distances from a forest dirt track (Olofsson, 1988a).

Quantitative studies of local virus dispersal are rare. Simulated rainfall transported between 56 and 226 OBs of Helicoverpa zea single nucleopolyhedrovirus (HzSNPV) from different types of soil onto cotton plants in a greenhouse experiment. OB transport increased with increasing speed of air currents and more OBs were transported from dry compared to wet soils. Of the three soils tested, OB retention was lowest in sandy soil and highest in clay soil. No OB transport was detected in the absence of simulated rainfall (Fuxa and Richter, 2001). In subsequent experiments, simulated rainfall was capable of transporting soil OBs distances of 30 – 75 cm onand to cotton plants, whereas air currents transported OBs 60 – 80 cm, irrespective of soil type. Transport from soil was detected for OBs at depths of up to 2 cm. In all cases the lower

portions of cotton plants were more heavily contaminated than the upper portions by wind- and rain-transported OBs (Fuxa et al., 2007).

7.7.3. Biotic Factors that Assist the Dispersal of Viruses

7.7.3.1. Predators

Numerous species of insect predators have been demonstrated to act as potential agents for the dispersal of baculovirus OBs. This occurs as most predators have an acidic gut and the OBs in infected larvae pass through the gut without dissolution and are excreted in the predator's feces, sometimes for several days following the consumption of an infected prey item.

Birds appear to be particularly effective agents of dispersal of baculoviruses, not only because they are important predators of insect larvae, but also because of the large distances they can fly and disperse OBs in their feces between feeding sites. In a study on bird species trapped in and around pine forests treated with nucleopolyhedroviruses to control larvae of the Pine beauty moth (*Panolis flammea*), a total of nine bird species, representing 11-77% of birds captured, were found to produce viable OBs in their feces. Each bird dropping contained between $5x10^4$and $5x10^7$ OBs, which represented a great many lethal doses of the viruses for early instar larvae of *P. flammea* (Entwistle et al., 1993). Studies in field crop and pasture systems have reported similar quantities of OBs in bird droppings (Crawford and Kalmakoff, 1978; Hostetter and Bell, 1985). In a separate study on sawfly control using nucleopolyhedrovirus applied to spruce trees, 90% of bird droppings collected from spruce trees contained viable OBs. Virus-contaminated bird droppings were collected at distances up to 6 km from sawfly infestations (Entwistle et al., 1977). Similarly, the feces of birds foraging for earthworms in OB-contaminated soil also tested positive for sawfly nucleopolyhedrovirus OBs (Olofsson, 1989). Birds can additionally spread virus by processing infected larvae prior to consumption (Reilly and Hajek, 2012). In an aviary study, chickadees (*Poecile atricapilla*) consumed most infected larvae and excreted most OBs, but larvae were usually swallowed whole. In contrast, vireos (*Vireo olivaceus*) beat the urticating hairs off *L. dispar* larvae before eating them; an act that sprayed droplets of liquefied larval tissues onto nearby foliage. As a result virus transmission resulting from rigorous prey processing by vireos exceeded transmission through the passage of OBs in feces (Reilly and Hajek, 2012).

Small mammals have been reported to be common dispersal agents for baculoviruses in forest ecosystems and up to 75% of fecal samples may contain important quantities of OBs (Hostetter and Bell, 1985). However, most studies on agricultural pests have focused on predatory arthropods that consume moribund and virus-killed lepidopteran larvae. These studies have implicated carabids (Vasconcelos et al., 1996a), predatory hemipterans (Young and Yearian, 1987), earwigs (Dermaptera) (Castillejos et al., 2001), neuropterans (Boughton et al., 2003), spiders (Fuxa and Richter, 1994), crickets and scavenging flies (Lee and Fuxa, 2000a), in the dissemination of OBs in their feces over periods of several days. The nests of paper wasps of the genus *Polistes* were found to contain OBs of several different nucleopolyhedroviruses,

cypoviruses and entomopoxviruses, reflecting the diseases of their lepidopteran prey (Morel and Fouillaud, 1994).

In soybean plots, virus dispersal was estimated at 80 – 120 cm/day and occurred in all directions from plots treated with AgMNPV. Virus dispersal was significantly correlated with the presence of predatory arthropods that tested positive for OBs in their feces. In greenhouse microcosms of collard plants, dispersal by larvae of the cabbage looper, *Trichoplusia ni*, infected with a wild-type nucleopolyhedrovirus (AcMNPV) averaged 22 - 45 cm/day, whereas the virus dispersal rate increased to 38 – 71 cm/day in the presence of predators and a scavenging fly (Lee and Fuxa, 2000b). The susceptibility of diseased larvae to predation may be greater (Young and Kring, 1991), similar (Vasconcelos et al., 1996a), or less (Castillejos et al., 2001), than that of healthy conspecifics, depending on the predator-prey system and the severity of the disease.

7.7.3.2. Parasitoids

The abundance and high mobility of insect parasitoids means that they can also be highly effective agents for the dispersal of invertebrate viruses. Indeed one family of viruses, the ascoviruses, depend almost entirely on endoparasitoid wasps as vectors to transmit infections to healthy noctuid hosts (Stasiak et al., 2005). Virus dispersal and transmission via endoparasitoid wasps is often highly efficient because the ovipositor becomes contaminated with virus during oviposition into an infected insect and virions are injected directly into the host hemolymph during subsequent acts of oviposition in healthy insects (Brooks, 1993). There are numerous examples of studies on endoparasitoid wasps that demonstrate that wasps can vector viruses between hosts infected by baculoviruses (Cossentine, 2009), an entomopoxvirus (Lawrence, 2002), iridescent virus (López et al., 2002), and densovirus (Kurstak and Vago, 1967). Limited evidence from field experiments supports the idea that female endoparasitoids effectively disperse viruses under natural conditions (Hochberg, 1991b; Fuxa and Richter, 1994; López et al., 2002). However, due to the difficulties in tracking individual wasps, the rate of parasitoid-mediated dispersal of invertebrate viruses in the field has not been quantified. In some cases ectoparasitoids may also be efficient vectors of viruses (Stoianova et al., 2012).

7.7.3.3. Other Organisms

Earthworms are normally abundant in agricultural and forest soils and laboratory studies indicate that they are capable of moving nucleopolyhedrovirus OBs from the soil surface to lower depths where they are protected from exposure to UV radiation or high temperatures. Earthworms are capable of transporting OBs because their guts are slightly acidic and OBs can pass through without loss of infectivity (Infante-Rodríguez et al., 2016).

Finally, livestock were implicated in the dispersal of nucleopolyhedrovirus, granulovirus and entomopoxvirus of the soil-dwelling pest complex, *Wiseana* spp., in New Zealand. The transport of these viruses on the hooves of the animals resulted in the spread and increased prevalence of virus diseases in pastures (Kalmakoff and Crawford, 1982). Similarly, by moving virus from the soil onto pasture grasses, the presence of cattle increased the prevalence of nucleopolyhedrovirus disease in *S. frugiperda*, in the United States (Fuxa, 1991).

7.7.4. Agricultural Practices that Affect Dispersal

Agronomic practices are likely to have a major influence on virus populations in the environment. The avoidance of tillage in the production of soybean over a two year period was shown to increase soil populations of nucleopolyhedrovirus OBs to the point where natural epizootics were initiated in velvetbean caterpillar, *Anticarsia gemmatalis* populations in Brazil (Moscardi, 1989). In contrast, following the application of the same virus in the United States, tillage moved virus from the soil onto plants and resulted in an elevated prevalence of disease in the pest population (Young and Yearian, 1986). In another study in soybean, most agricultural operations did not influence the vertical distribution of established soil OB populations although a decline in soil OBs was observed following the removal of crop refuse on the soil surface by disking (Fuxa and Richter, 1996). Other types of practices, such as the use of herbicidal or hormonal defoliants that cause near total loss of foliage immediately prior to the harvest of cotton crops, are also likely to contribute a large influx of occluded viruses of cotton pests into the soil reservoir, although studies are lacking.

The movement of honeybee hives by commercial apiaries across large areas of the United States in response to demands for pollination services, in combination with an increase in the average size of apiaries, intensification of honey production and the international trade in queens and bee semen, all provide opportunities for the transmission and dispersal of honeybee viruses and parasitic vectors of bee diseases (Smith et al., 2013; Mutinelli, 2011). The intensive movement of hives also affords opportunities for the exchange of viruses and other pathogens between wild and commercial bees (Fürst et al., 2014). These are issues of major concern given the recent declines in natural and managed bee populations in the United States and Europe.

7.7.5. Spatial Patterns of Dispersal

Patterns of disease dispersal from the initial epicenter of an epizootic are often modeled as a reaction-diffusion model borrowed from chemical reaction kinetics with a diffusion component to describe spatial movement (White et al., 2000). This appears as a moving wave-front of infection, which is effectively a spatial transition zone, on one side of which the prevalence of disease is high (closer to the epicenter) and on the other side the prevalence of disease is low. The speed of the wave is determined by a combination of individual-level processes involving transmission, production of progeny virus particles, virus decay in the environment and movement of host insects. Evidence from studies on a spruce-feeding sawfly, *Gilpinia hercyniae*, and its nucleopolyhedrovirus in Wales revealed that after travelling approximately 1000 m through the forest, the wave of infection began to breakdown as other minor waves of disease travel outwards from the periphery of the major wave. These secondary epicenters were likely initiated by biotic vectors of the disease such as birds (Entwistle et al., 1983), although this interpretation has been challenged in favor of seasonal effects on wave behavior (White et al., 1999).

The travelling wave model performed well in describing small scale dispersal of Orgyia pseudotsugata nucleopolyhedrovirus infection in an experimental system of fir seedlings

(Dwyer, 1992). On a larger scale, ballooning of *L. dispar* larvae on silk threads was found to contribute to the initial wave-front of disease during a period of several weeks over a distance of ~100 m, but subsequently the model showed poor match to the observed spatial distribution of infections, possible due to parasitoid vectoring of the virus (Dwyer and Elkinton, 1995). A detailed description of the use of models to understand the spatial spread of these pathogens is given in Chapter 12.

7.8. GENETIC DIVERSITY IN VIRUSES

7.8.1. Genetic Diversity is Pervasive in Virus Populations

Genetic diversity is present in all invertebrate virus populations but has been particularly studied in baculoviruses. The fact that genetic diversity is maintained and transmitted between host generations indicates that this variation is selectively advantageous to each virus. Estimates of genetic diversity in baculoviruses depend largely on the techniques employed. Studies using restriction endonuclease enzymes, beginning in the 1980's, started to characterize diversity within and between baculovirus isolates from the same and different host species. It also became apparent that many natural isolates comprised mixtures of genotypes that could be separated by cloning using *in vitro* (cell culture) or *in vivo* techniques. *In vivo* cloning involves serial inoculation of larvae with very low doses of OBs or by injecting larvae with low concentrations of budded virions from the hemolymph of an infected insect. These techniques can give quite different results in terms of the diversity and characteristics of the genotypes isolated, due to the divergent conditions required for replication and transmission in insects compared to *in vitro* systems (Erlandson, 2009). An alternative approach now involves deep sequencing and metagenomic analyses to determine the diversity of genotypic variants present in natural virus populations that have not been subjected to prior cloning steps (Baillie and Bouwer, 2012b; Chateigner et al., 2015).

Nucleopolyhedroviruses and granuloviruses tend to differ in their genetic diversity characteristics. Nucleopolyhedroviruses tend to be genetically heterogeneous with many variants occluded within a single OB. Therefore, most nucleopolyhedrovirus infections involve mixtures of genotypes. In contrast, each granulovirus OB contains a single virion with a single genome and infections tend to involve very little diversity (Erlandson, 2009; Eberle et al., 2009), although mixed infections in granuloviruses may be adaptive under certain circumstances, such as when the host population is resistant to one genotype (Graillot et al., 2016).

The diversity present in baculovirus genomes consists of single nucleotide polymorphisms (SNPs) and indels (insertions and deletions), which are often located around putative origins of replication (*hrs*) and baculovirus repeated open reading frames (*bro*) that have multiple functions in baculoviruses (Erlandson, 2009). Large deletions, sometimes representing over 10% of the genome, are present in a quarter or a third of all genotypes in some nucleopolyhedrovirus populations (Simón et al., 2004a; Redman et al., 2010). These deletion variants can only persist in the presence of complete genotypes that provide the missing gene products in cells that are simultaneously infected by complete and deletion genotypes, a process

known as complementation. This is possible because during the final phase of systemic nucleopolyhedrovirus infection, each cell is infected by approximately four budded virions, each of which contains a single genome that may be a deletion variant or a complete genotype (Bull et al., 2001). Coinfection of cells by multiple genotypes also provides numerous opportunities to generate diversity through recombination among genotypic variants, a mechanism known to be important for generating novel genotypes in baculoviruses (Kondo and Maeda, 1991; Kamita et al., 2003).

Ultradeep sequencing has revealed that the diversity present in an isolate of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is astonishing, with every possible combination of variants present, albeit at different frequencies. Each genotypic variant was found to comprise an average of 94 single nucleotide polymorphisms scattered across the genome and 25% of variants had large deletions (Chateigner et al., 2015). Other studies have identified important variation in genes encoding proteins that are located in the envelope of ODVs. These proteins include *per os* infection factors (PIFs) and ODV-E66 that are critical for primary infection of midgut cells (Simón et al., 2011; Craveiro et al., 2013; Thézé et al., 2014). Variation has also been identified in core genes involved in replication (Baillie and Bouwer 2012a; Chateigner et al., 2015), and auxiliary genes such as *chitinase*, *egt* and *enhancin* that improve transmission (D'Amico et al., 2013b; Harrison 2013; Martemyanov et al., 2015a). Additional diversity may also arise from the presence of mobile genetic elements such as transposons, indicating that invertebrate viruses can act as vectors for these elements (Gilbert et al., 2014). As might be expected, this notable diversity at the nucleic acid, gene and genomic levels is reflected in numerous phenotypic traits that modulate virus fitness within and between host insects and their populations.

7.8.2. Genetic Diversity Favors Virus Survival

The genetic diversity in nucleopolyhedrovirus populations is selectively advantageous and has clear ecological and evolutionary benefits to these viruses. When individual genotypic variants are examined, each variant usually exhibits a particular combination of phenotypic characteristics that are often presented in terms of OB dose-mortality metrics, speed of kill, OB production in each insect and OB production per milligram of insect tissue. These traits are clearly important for virus transmission because they determine the likelihood of acquiring an infection, the time taken between initial infection and the release of progeny OBs that can infect other susceptible larvae, the total number of OBs released from each insect, and the efficiency with which the virus converts host resources into virus progeny. However, it is not possible to maximize all these traits simultaneously as many involve correlations and tradeoffs imposed by biological constraints.

One of the best-characterized tradeoffs is that of speed of kill and total OB production. Slow-killing variants allow infected insects to continue feeding and growing during virus replication, thereby providing additional resources for the production of virus progeny. Fast-killing variants kill the host shortly following infection so that each host represents a near-fixed

resource to be exploited for progeny production. Additional evidence for the ecological role of the tradeoff between speed of kill and OB production in baculoviruses comes from a study of the *L. dispar* - LdMNPV system. Field isolates of LdMNPV varied in their tendency to kill larvae rapidly without producing progeny OBs, and also varied in the period during which infected larvae could grow prior to death, as indicated by post mortem body size (Fleming-Davies and Dwyer, 2015). Cadaver size was positively correlated with the prevalence of infection in neonate larvae exposed to bark pieces that had overwintered under natural conditions, indicating that rapid speed of kill was costly to virus environmental persistence and transmission to the following generation in this pathosystem.

As most nucleopolyhedrovirus infections involve mixtures of genotypes, the analysis of individual clonal genotypes is unlikely to provide ecologically useful information. Instead, the role of mixed genotype infections and the interactions among genotypes is likely to approximate natural virus populations to a far greater degree. For example, mixtures of genotypes present in natural (wild-type) isolates can increase the virus' ability to establish lethal infections (López-Ferber et al., 2003; Bernal et al., 2013; Redman et al., 2016), and increase the total production of OBs in infected insects (Barrera et al., 2013; Bernal et al., 2013). In these cases the infectivity and OB production values of the wild-type population exceed that of the component variants, indicating a degree of cooperation among genotypes, which, in the case of one nucleopolyhedrovirus, is known to be mediated through the expression of a *pif* gene (Simón et al., 2013).

Genotypic heterogeneity in virus populations may also provide preadaptation that allows the pathogen to exploit novel hosts, new host genotypes, or food plants with novel chemical defenses. In such cases, rare genotypes are likely to be favored over common genotypes in a given population through a process of negative frequency dependent selection.

Finally diversity provides opportunities for risk spreading by the virus in response to environmental stochasticity. Examples include the presence of genotypes with divergent tendencies for vertical or horizontal transmission that may be differentially favored as host densities fluctuate (Cabodevilla et al., 2011a). Similarly, viral *chitinase* and *cathepsin* genes are responsible for post mortem melting of infected cadavers, which increases the rate of transmission (Goulson et al., 1995), but also exposes OBs to UV inactivation. Therefore, for a given speed of kill phenotype, variation in the frequency of genotypes lacking the viral *chitinase* gene (Vieira et al., 2012; D'Amico et al., 2013b), could determine the probabilities of transmission within and between host generations as a bet-hedging strategy in response to variation in opportunities for horizontal transmission over time.

7.8.3. What Generates So Much Genetic Diversity?

Genetic diversity is present within individual hosts, between different host insects, and between populations that are segregated by geographical, behavioral, or ecological factors. Genetic variation within and between virus populations will arise from host-related and other

ecological processes, of which I consider three here: heterogeneity in host susceptibility to infection, the roles of food plants, and host species-mediated selection.

Heterogeneity in susceptibility to infection in *L. dispar* promotes polymorphism in the infectivity phenotype of LdMNPV (Fleming-Davies et al., 2015). This arises from a tradeoff between transmission rate and variation in susceptibility in this insect. High variation in susceptibility results in a fraction of the host population that acquire an infection at very low pathogen densities and a fraction that is resistant to infection even at high pathogen densities. In contrast, when variation in susceptibility is low, the probably of infection gradually increases with increasing pathogen density in the environment (Fleming-Davies et al., 2015). Similarly, heterogeneity in host susceptibility promotes mixed genotype infections when the inoculum comprises mixtures of genotypes, as the probability of each viral variant establishing infection varies with host genotype (van der Werf et al., 2011). Additional support for this concept comes from a study demonstrating differential susceptibility of sibling groups of *L. dispar* larvae to infection by different strains of LdMNPV. From this it was apparent that virus variants differed in their capacity to produce lethal infection of family groups and that susceptibility to infection also varied across families, indicating that the outcome of exposure to LdMNPV inoculum depends on viral genotype x host genotype interactions (Hudson et al., 2016).

Food plants can have major effects on the transmission of baculoviruses (section 7.12.1). Individual genotypic variants and mixed genotype populations can differ markedly in dose-mortality responses, speed of kill and total OB yields when host larvae feed on different species of food plant, so that the transmission of one variant is favored over that of other variants on a given plant (Hodgson et al., 2002; Raymond et al., 2002). In such situations the genotypic composition of the virus population is expected to vary according to the local species composition of available food plants. In another plant-insect-virus system, different species of crop and pasture grasses had no significant role in modulating the genetic composition of the virus population (Shapiro et al., 1991).

In the case of nucleopolyhedroviruses with an extended host range, laboratory studies indicate that infection of other host species with differing susceptibility can result in selection for certain genotypes or mixtures of genotypes within particular hosts, which represents an additional mechanism for maintaining genetic diversity in sympatric species that share a common virus pathogen (Hitchman et al., 2007; Zwart et al., 2009).

7.8.4. How is Genetic Diversity Transmitted?

As many as 20 or more genotypic variants can be present within individual nucleopolyhedrovirus-infected insects (Cory et al., 2005; Baillie and Bouwer, 2012b). So how is this diversity transmitted? For vertically transmitted infections, virus variants have to be transmitted from the parental reproductive organs to the egg surface, or within the developing embryo of the offspring. This is likely to represent a bottleneck in the transmission of many variants, although studies have yet to address this issue.

For horizontally transmitted infections, the key to variant transmission is the OB. Nucleopolyhedrovirus OBs usually occlude groups of 30 – 80 ODVs, and each ODV typically contains between 1 and 10 nucleocapsids enveloped within the virion. Moreover, nucleocapsids each containing a different genotype can be enveloped within an ODV (Clavijo et al., 2010). Therefore, when a larva consumes a single OB, numerous ODVs are released into the midgut lumen, and each ODV that infects a midgut cell can transmit between 1 and 10 genomes of potentially different genotypes. ODVs act independently in highly susceptible hosts, each with a certain, albeit low, probability of establishing a productive infection. This situation changes in less susceptible hosts in which high doses of OBs are required to establish infection. In this case, ODVs appear to require a critical threshold before a productive infection can be established, possibly due to intrinsic host defense mechanisms (Zwart et al., 2009).

If deletion genotypes and complete genotypes are present in the same virion (i.e., both genotypes had replicated together in a coinfected cell prior to being occluded in an OB), then these genotypes will have shared the common pool of proteins necessary for peroral transmission and both variants will be transmitted together (Clavijo et al., 2009, 2010). When the dose of OBs consumed is very low, the opportunities for coinfection and complementation with complete genotypes are reduced. In such cases primary infection in the insect midgut represents an important bottleneck to diversity (Zwart and Elena, 2015).

In contrast to the situation when larvae consume low doses of OBs, the density of OBs in the environment increases greatly during the development of an epizootic. In consequence, the average number of inoculum OBs consumed by the insect and the probability of the transmission of genotypic diversity increase accordingly, so that *a priori* we may expect diversity in virus infections to increase during epizootics (Hodgson et al., 2003). However, this idea has been contradicted by observations on a nudivirus that infects shrimp, in which mixed genotype infections were less prevalent during disease outbreaks (Hoa et al., 2011).

Finally the possibility that nucleopolyhedroviruses are capable of generating genetic diversity *de novo* has come from a study on Helicoverpa armigera nucleopolyhedrovirus in which larvae inoculated with a high dose of OBs (95% mortality) were found to produce progeny OBs with a similar diversity of genetic variants as that present in the inoculum. In contrast, larvae inoculated with a single OB (5% mortality) produced OBs with a significantly higher number of variants, and variants that were less similar, compared to those present in the virus sample from which the inoculum OB was obtained (Baillie and Bouwer, 2013). The processes behind these intriguing observations have yet to be elucidated.

7.9. ROLE OF HOST BEHAVIOR IN VIRUS ECOLOGY

Despite the critical importance of host behavior in the transmission of most invertebrate virus pathogens, this aspect is often neglected in studies of pathogen ecology or during field testing of virus-based insecticides. For invertebrates that acquire infections by ingestion of contaminated food, the choice of what and where to eat will clearly influence the survival and

reproduction of the host, but will also determine the probability of infection and the host's ability to resist or overcome the pathogen.

7.9.1. Foraging Decisions: What and Where to Eat

Decisions made by ovipositing females of polyphagous species as to the food plant species or the position of eggs laid on the plant can influence the prevalence of disease in their offspring. For example, in *S. frugiperda* the prevalence of nucleopolyhedrovirus disease at field sites was positively correlated with the presence of signalgrass (*Brachiaria platyphylla*), and negatively correlated with two other grasses (Fuxa and Geaghan, 1983), although this did not reflect the quantities of virus OBs required for acquisition of lethal disease on each type of plant (Richter et al., 1987). Similarly, the food plant species was clearly demonstrated to affect virus fitness in the winter moth, *Operophtera brumata* (Raymond et al., 2002), and immune system function and disease resistance in *T. ni* larvae (Shikano et al., 2010).

The choice of host plant can also determine the virulence of the virus strain that an insect is at risk of acquiring. In the case of the Western tent caterpillar, *Malacosoma californicum pluviale*, virus isolates present on particular plant species killed their hosts faster when larvae consumed inoculum OBs on foliage of the same plant (Cory and Myers, 2004). However, whereas phytopathogenic viruses can increase oviposition by insect vectors on infected plants (Chen et al., 2013), it is not clear whether any species of adult female invertebrates are capable of detecting the presence of invertebrate pathogenic viruses, or the remains of virus-killed individuals on plants, and can modify their ovipositional decisions accordingly. The only exception to this comes from observations that females of *C. pomonella* reduced oviposition on apple cultivars that had been treated with a granulovirus-based insecticide, probably due to components in the product formulation that altered leaf surface metabolites rather than a response to the presence of granulovirus OBs (Lombarkia et al., 2013).

For phytophagous insects, decisions on where to feed on the plant can also have implications for the transmission of their pathogens. For example, in *L. dispar*, late instars avoid feeding on leaves contaminated by the remains of diseased cadavers, but do not avoid foliage contaminated by purified nucleopolyhedrovirus OBs (Capinera et al., 1976). In a later study, *L. dispar* larvae appeared able to detect and avoid virus-infected cadavers from a distance of at least 5 mm and the behavior varied between family groups, indicating a significant degree of heritability in this trait (Parker et al., 2010). A model developed from these observations suggested that the ability to detect infected cadavers from a close distance (<1 mm) would result in a decrease in the prevalence of infection by 4 – 7%, which appears modest in a single round of transmission, but may have a significant impact on the risk of infection during the multiple cycles of transmission that occur during the development of an epizootic (Eakin et al., 2015). In contrast, *Spodoptera exigua* larvae showed no preference to avoid contact or consumption of leaf disks contaminated by infected cadavers (Rebolledo et al., 2015).

7.9.2. The Risks of Cannibalism

Insects from several orders show cannibalistic behavior, particularly during the final larval stages, or in situations of low food availability, or high population density. The ecological and evolutionary consequences of this behavior have been reviewed elsewhere (Richardson et al., 2010). Cannibalism is also an efficient route for the transmission for certain pathogens, including baculoviruses, when healthy larvae consume moribund infected conspecifics prior to death (Dhandapani et al., 1993; Boots, 1998; Chapman et al., 1999). Cannibalism has also been shown to result in transmission of densovirus and entomopoxviruses in Orthoptera (Streett and McGuire, 1990; Weissman et al., 2012), and iridescent viruses in Lepidoptera, Orthoptera, Diptera and terrestrial isopods (Crustacea) (Williams, 2008). This is because diseased individuals often become lethargic in the final stages of infection and are unable to defend themselves from aggressive conspecifics. When this behavior occurs infected cadavers are eaten, it should more correctly be described as conspecific necrophagy.

7.9.3. Sexually-Transmitted Virus Diseases

For sexually transmitted viruses the choice of sexual partner will often affect reproductive success and may also directly affect survival of the individual or their offspring. Indeed, in a review Knell and Webberley (2004) identified 17 pathosystems in which sexual transmission of a virus resulted in reduced insect reproduction and/or reduced offspring survival. Recent examples include that of healthy *S. exigua* females that mated with nucleopolyhedrovirus-infected male moths. A quarter of the offspring from these matings were covertly infected by a nucleopolyhedrovirus (Virto et al., 2013). Similarly, healthy females that mated with iflavirus-infected males efficiently transmitted the infection to their offspring (Virto et al., 2014). The semen of infected male honeybees was found to contain deformed wing virus (*Iflaviridae*) and transmission to the offspring of healthy queens was 100% efficient (Yue et al., 2007). Similarly, densovirus-infected male *Anopheles* mosquitoes inseminated healthy females with semen containing over 10^6 genomes of the virus (Barik et al., 2016). Sexual transmission of nudiviruses is a particularly well characterized example of the efficiency of transmission during mating (see section 7.5.1). The application of molecular tools to the study of these pathosystems is greatly improving our understanding of the role of mating systems on the transmission of invertebrate pathogens in general.

7.9.4. Ecological Consequences of Host Manipulation by Viruses

There are many reports of changes in the behavior of virus-infected invertebrates. These may be related to pathological effects or may be adaptive. In the latter case, behavioral changes can be classified into three broad groups:

(i.) The extended phenotype. In this group of examples the virus manipulates the host to improve its own fitness through the expression of viral genes; the so-called extended phenotype of the virus. Indeed, several viruses have the capacity to manipulate invertebrate hosts to improve their transmission (Han et al., 2015b). Baculovirus-infected insects often show enhanced locomotory activity, or hyperactivity, mid-way through the course of the infection, which increases their rate of dispersal (Kamita et al., 2005). A closely related, but apparently

distinct behavior, is baculovirus-induced climbing behavior that occurs shortly before death in many species of Lepidoptera. This behavior was first described in nucleopolyhedrovirus-infected larvae of the nun moth, *Lymantria monacha*, over a century ago, and was named tree-top disease due to the tendency of larvae to die and hang suspended from the branches and foliage in the highest parts of coniferous trees. Since then, the behavior has been reported in *Mythimna* (*Pseudaletia*) *separata* (Ohbayashi and Iwabuchi, 1991), *L. dispar* (Murray and Elkinton, 1992), *M. brassicae* (Goulson, 1997), *Orgyia antigua* (Richards et al., 1999), *S. exigua* (van Houte et al., 2014b), and *T. ni* (Ros et al., 2015), among other lepidopteran species, whereas downward movement of infected larvae has been reported in *O. brumata* (Raymond et al., 2005). Although common among nucleopolyhedrovirus-infected insects, climbing behavior has occasionally been reported in granulovirusinfected individuals (Moore et al., 2011), but apparently not in other occluded insect viruses.

This behavior appears to be adaptive for the virus as infected larvae that die on the uppermost parts of plants are likely to improve the transmission and dispersal of the pathogen by: (i) releasing large quantities of OBs that subsequently contaminate foliage lower in the plant canopy by liquefaction or the action of rainfall, and (ii) being more susceptible to predation by birds that are efficient agents for virus dispersal. The climbing behavior clearly depends on a positive phototactic response, although the molecular basis for this remains unknown (van Houte et al., 2014b). In the case of *S. exigua* larvae, virus-induced climbing behavior increased the probability of encounters with healthy conspecific larvae that became infected following necrophagy of virus-killed cadavers (Rebolledo et al., 2015).

Invertebrate viruses are also capable of manipulating the sexual activity of their hosts. Infection of cotton bollworm, *Helicoverpa zea*, adults by the nudivirus Hz-2v often results in malformation of the reproductive system and sterility, although sexual activity was enhanced. Infected females called for mates more frequently, produced more mating pheromone, and attracted more mates than healthy females. During copulation the genitals of males become contaminated by virions in a waxy vaginal plug. Contaminated males then transmitted the virus to other females during subsequent matings. Moreover, a portion of the female population is fertile and asymptomatic and produces infected sterile offspring through which the virus is transmitted. The molecular basis for these complex behavioral changes has yet to be elucidated (Burand, 2009). Similarly, iridescent virus infection of the cricket, *Gryllus texensis*, causes sterility in both sexes. However, infected individuals continue to engage in mating behavior and infected males are quicker than uninfected males to court females. The virus is transmitted to healthy crickets through sexual activity, leading to its description as a "viral aphrodisiac" (Adamo et al., 2014).

(ii) The host responds to infection by adopting behaviors that reduce the costs of infection, such as self-medication, involving a specific therapeutic and adaptive change in behavior in response to disease. For example, larvae of *S. exempta* infected with a nucleopolyhedrovirus immediately adjusted their diet to reduce carbohydrate intake while

protein consumption gradually increased over time, resulting in markedly improved survival compared to insects that could not adjust their diet. The high protein - low carbohydrate diet was associated with higher levels of antimicrobial activity in the hemolymph and improved immune system function. This provides clear evidence of an adaptive self-medication response in this species (Lee et al., 2006; Povey et al., 2014).

(iii) In the third case the behavioral changes represent a shared phenotype arising from the expression of both virus and host genes. As such, a virus may elicit a particular behavior in one host species but not in another. The outcome and magnitude of shared phenotype behavioral changes are therefore likely to depend on combinations of host, virus and environment interactions (van Houte et al., 2013). For example, in contrast to the self-medication response of *S. exempta* mentioned above, modified feeding responses in infected *T. ni* larvae were dependent on both temperature and the identity of the virus (Shikano and Cory, 2015, 2016).

7.9.4.1. Molecular Basis for Host Manipulation

Behavioral changes observed in infected individuals are likely to be due to pathological effects on the nervous system (Wang et al., 2015), or changes in metabolism or physiology (Thompson and Sikorowski, 1980; Chen et al., 2014). In a small number of cases the molecular basis for viral manipulation of host behavior has been elucidated. The first to be identified was that of the ecdysteroid UDP-glucosyltransferase (*egt*) gene in baculoviruses (O'Reilly and Miller, 1991). The EGT enzyme inactivates ecdysteroid hormones by conjugation with sugars, which delays molting to the following instar. This results in continued feeding and growth, a higher production of OBs and improved probability of transmission compared to gene deletion viruses (Cory et al., 2004). The fact that *egt*-deletion variants are present in natural populations of nucleopolyhedroviruses suggests that variation in speed of kill and OB production may be selectively advantageous under certain circumstances (Harrison et al., 2008; Simón et al., 2012). The *egt* gene has also attracted attention in the development of baculovirus insecticides as its deletion results in an improved speed of kill compared to wild-type virus (Popham et al., 2016).

The *egt* gene has also been implicated in the climbing behavior of infected of *L. dispar* larvae prior to death (Hoover et al., 2011). However, *egt* is not responsible for this behavior in all insect-nucleopolyhedrovirus pathosystems (Ros et al., 2015). In *S. exigua* larvae infected by AcMNPV, climbing behavior was dependent on molting during the infection period, and larvae that died without molting tended to move downwards rather than upwards. In contrast, in *S. exigua* larvae infected by the homologous virus (SeMNPV), the *egt* gene was shown to extend the lifespan of the infected host and facilitate climbing behavior (Han et al., 2015a).

Enhanced locomotory activity was linked to the expression of a baculoviral protein tyrosine phosphatase (*ptp*) gene in the silkworm, *Bombyx mori*, in a process that was light-activated (Kamita et al., 2005). In BmNPV-infected silkworms, the PTP protein does not require enzymatic activity to elicit enhanced locomotory activity but appears to be a viral structural protein present in the envelope of budded virions that interacts with a protein that modulates the actin cytoskeleton of the infected cell and manipulates host behavior via infection of larval brain

tissues (Katsuma, 2015a). In the case of AcMNPV, phosphatase activity is required for enhanced locomotory activity, although the target substrate is presently unknown, and appears to exert its effect independently of the processes that govern climbing behavior (van Houte et al., 2014a; Katsuma, 2015b). Understanding the molecular basis for host manipulation by pathogens is an issue that is currently generating excitement among invertebrate pathologists and evolutionary biologists.

7.10. DYNAMICS OF VIRUSES IN HOST POPULATIONS

7.10.1. Pathogenic Viruses Can Regulate Populations

Viruses can be major mortality factors in populations of some invertebrates, particularly forest-feeding Lepidoptera (Erebiidae and Lasiocampidae) and sawflies (Hymenoptera, Diprionidae), as well as beneficial insects such as silkworms and honeybees. Outbreaks of baculovirus diseases have been associated with the cyclic dynamics of some forest pests, with a periodicity of several generations (typically 5 – 15 years), particularly in temperate regions. In these systems the density of the host population increases until it exceeds a threshold value at which point the pathogen can spread rapidly through the population. This is followed by dramatic declines in the host population that then falls below the threshold density. The virus persists as a vertically-transmitted covert infection or in environment reservoirs until the threshold density is exceeded once more and sustained transmission is possible again (Briggs et al., 1995). Following the seminal population model (model G) of Anderson and May (1981), significant advances have been made in developing models that accurately describe population dynamics of insect-virus pathosystems (Elderd, 2013). The *L. dispar* – LdMNPV system has been particularly well characterized in this respect (see Chapter 12 of this book for a detailed review).

Another excellent example of insect-virus dynamics is that of the Western tent caterpillar, *M. c. pluviale*, and its nucleopolyhedrovirus in British Colombia, Canada (Myers and Cory, 2016). This univoltine gregarious insect feeds on a variety of host trees and has cyclic populations with a periodicity of 8 – 11 years. The prevalence of virus infection over a 24 year period closely tracked host population density, indicated by the density of silk tents that families of larvae inhabit (Fig. 7.2a). During epizootics, 80 – 100% of larval families within tents were diseased and host population densities fell rapidly. A significant negative correlation between the rate of population change between one year and the next and the percentage of virus-diseased families was detected (Fig. 7.2b), which is indicative of population regulation by the pathogen. A lag in the recovery of the host population is required for cyclic dynamics and reduced fecundity following an epizootic of disease was identified as the most probable cause for delayed recovery in the host population (Cory and Myers, 2009). Potential causes of the reduced fecundity were considered, including reduced food quality or availability following defoliation of trees, costs incurred from resistance to infection or from maintaining an immune response to control a covert infection, or the pathological effects of sublethal disease in covertly-infected insects. Sublethal infection is often associated with reduced fecundity or fertility in other species of Lepidoptera

(Rothman and Myers, 1996). In the case of *M. c. pluviale* a significant negative correlation was detected between the prevalence of infection and the numbers of eggs laid in egg masses (Fig. 7.2c). The low fecundity observed during years with disease epizootics was followed by reduced population density the following years, a prerequisite for cyclic dynamics. Myers and Cory (2016) therefore concluded that this delayed density dependent effect on fecundity was most likely due to sublethal disease in *M. c. pluviale* populations, although testing for this using molecular methods was problematic during periods between outbreaks due the paucity of insects present in low density populations.

7.10.2. Ecosystem Characteristics that Favor Virus Transmission

Epizootics of infection in high density pest populations have been reported in a number of agricultural systems and have led researchers to consider developing baculoviruses as biological insecticides, sometimes with great success (Moscardi, 1999). Fuxa (2004) proposed that agroecosystems could be classified as permissive or non-permissive based on their propensity to sustain a high prevalence of baculovirus infection in agricultural pests. Examples of such systems include the Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV) which is capable of sustained annual epizootics in a permissive system involving pasture grasses, but not in crops such as maize or sorghum (non-permissive systems). This was attributed to the behavior of larvae that are solitary and do not disperse between maize and sorghum plants but move readily between low-growing grasses. The pasture grasses are also very likely to become contaminated from the soil OB reservoir (Fuxa, 1982).

Plant-pest systems involving *A. gemmatalis* larvae on soybean, and *T. ni* larvae on collards, were also considered permissive to epizootics due to plant contamination by soil OB populations and a high prevalence of dispersal of their respective viruses by predatory arthropods. In contrast, the cotton – *Helicoverpa* spp. system was considered as non-permissive, as introductions of the virus in one season were never reflected in lethal disease the following season, possibly due to the poor persistence of OBs on cotton foliage (Fuxa, 2004). Forest ecosystems, in contrast, have a combination of factors that favor virus persistence including an undisturbed soil reservoir, physical protection for OBs in tree bark crevices on twigs and branches, the dispersal of OBs by biotic and abiotic factors, and the opportunities for horizontal and vertical transmission in resident insect populations that do not exist, or exist to a limited degree, in ephemeral crop habitats.

Of the host-related factors that favor epizootics, in addition to heterogeneity in resistance to infection (see section 7.8.3), it has become increasingly recognized that the risk of disease is likely to increase with population density as transmission is density dependent (section 7.5). As such, it would be advantageous for species that experience large fluctuations in density to be able to increase their investment in immune defenses to match the threat posed by pathogens in high density populations; a response known as density dependent prophylaxis (Cotter et al., 2004). This risk is particularly relevant to gregarious feeding species in which the death from virosis of a single individual can rapidly spread to other group members (Hochberg, 1991a).

An additional physiological response to the presence of high densities of conspecifics is that of phase polyphenism. Numerous species of Lepidoptera and some other taxa exhibit a switch to a dark larval phase when reared at high densities, which is due to the melanization of the cuticle. Dark phase individuals often differ from their pale conspecifics in metabolic rate, growth rate, and other variables that affect fitness, including their susceptibility to nucleopolyhedrovirus disease (Goulson and Cory, 1995).

Interestingly, species that show phase polyphenism also have the capacity for density dependent prophylaxis. In *S. exempta*, both rearing density and phase polyphenism were positively associated with increased phenoloxidase levels in the hemolymph and reduced susceptibility to nucleopolyhedrovirus disease. Under field conditions transmission and mortality were also lower in larvae that had been reared under crowded conditions (reviewed in Wilson and Cotter, 2009). Similarly, the armyworm *M. separata* became highly resistant to nucleopolyhedrovirus and granulovirus infection when reared at high densities. When virions were injected directly into the hemolymph however, no difference was observed in the susceptibility of larvae that had been reared singly or in groups, suggesting that resistance to infection may be related to primary infection in the insect midgut (Kunimi and Yamada, 1990).

In *L. dispar*, which is not a phase polyphenic species, rearing at high densities did not result in density dependent prophylaxis. In fact the ability to resist lethal infection and survival times of infected insects were reduced at high rearing densities (Reilly and Hajek, 2008). In contrast, *A. gemmatalis* appears to represent an intermediate species, in which larvae are phase polyphenic and can experience high density populations but are not gregarious. In *A. gemmatalis* the switch to the melanic form is an all-or-nothing response to the presence of one or more conspecifics. Larvae reared at high densities had a stronger encapsulation response, higher numbers of hemocytes and survived longer when infected, but no significant differences were detected among phase phenotypes for disease resistance (Silva et al., 2013). Nonetheless, the dark phenotype was observed to have a thicker and more robust peritrophic membrane than that of pale conspecifics (Silva et al., 2016), providing support for the idea that the midgut barrier may provide an important contribution to disease resistance in melanic phase insects. Rearing temperature was highly influential in determining the prevalence of each phase phenotype, immune response and the survival of infected insects (Silva and Elliot, 2016). In light of climate change, these findings have potential implications for insect-virus population dynamics in phase polyphenic species, as insects may become less susceptible to pathogens in a warmer climate.

7.10.3. Climate Change and Insect-Virus Population Dynamics

Our understanding of the likely impact of global warming on invertebrate-virus population dynamics is extremely limited, mainly due to a paucity of empirical testing of theoretical models. It is clear however that rising temperatures are expected to influence disease dynamics in agricultural, forest and aquatic ecosystems. Two studies have indicated that warmer temperatures may increase the impact of virus pathogens on insect populations. The prevalence of lethal infection by SfMNPV in *S. frugiperda* larvae increased at higher temperatures, possibly

due to increased feeding rates. The transmission rate itself was not temperature sensitive, but heterogeneity in the risk of disease decreased with increasing temperature, resulting in higher mortality than observed at lower temperatures (Elderd and Reilly, 2014). As climate warming and increased CO_2 is associated with decreased protein:carbohydate ratio in plants, Shikano and Cory (2015) compared the interaction of temperature and nutrition on the growth and survival of *T. ni* larvae that were infected by nucleopolyhedroviruses. The survival of virus-challenged insects was positively correlated with dietary protein:carbohydate ratio, suggesting that these pathogens could exacerbate the negative effects of reduced protein (nitrogen) availability in plants in a global warming scenario.

Conversely, increasing rearing temperatures did not influence the susceptibility of *M. c. pluviale* larvae to nucleopolyhedrovirus infection or the estimated yield of progeny OBs, although survival times of infected larvae declined rapidly with increasing temperature (Frid and Myers, 2002). One particular feature of this species is that it appears capable of temperature regulation through sun basking behavior, possibly rendering it less susceptible to climatic variation than non-regulating insects.

7.11. INFLUENCE OF ABIOTIC FACTORS ON VIRUSES

Viruses in the environment are subjected to a series of abiotic challenges that they do not face within the host. Their ability to retain infectivity during periods in the environment varies widely depending on the ecosystem and the presence of the OB structure. In the present section I consider the effects of the most influential abiotic factors: UV radiation, seasonality, temperature, precipitation and pH.

7.11.1. Effect of Ultraviolet Light on Viruses

The environmental factor that has attracted most attention from researchers is solar UV radiation. This is because UV rapidly inactivates viruses applied to plants as biological insecticides, thereby limiting their effectiveness for pest control. As such, the half-life of occluded viruses exposed to direct sunlight can often be measured in terms of hours (Ignoffo et al., 1997; Sajap et al., 2007), such that only a small fraction of the original inoculum remains viable a few days after applying OBs as a biological insecticide (Sun et al., 2004). As mentioned previously (section 7.6.2.1), the remains of infected cadavers appear to provide protection against UV degradation so that OBs released from cadavers persist approximately twice as long as purified OBs on foliage (Fuller et al., 2012). In contrast, a large fraction (~50%) of the OBs applied to plants grown under the UV-protective structure of a plastic greenhouse can still be viable a week after the initial application (Bianchi et al., 1999; Lasa et al., 2007).

Exposure to solar UV is highest in tropical regions and at high altitudes and decreases with increasing latitude. Temperate regions also experience marked seasonal changes in UV irradiation and climatic conditions, particularly due to cloud cover and precipitation. UV-B (280 - 315 nm wavelength) is the most biologically harmful part of the solar radiation spectrum that arrives at the Earth's surface. UV-B has the ability to cause breaks in strands of nucleic acids or, more frequently, fuse adjacent thymine bases in DNA strands, forming a cyclobutane thymine

dimer which blocks normal DNA synthesis and often results in mutations. In viruses with a dsRNA genome, uracil dimers may also accumulate as a result of UV radiation.

Of the occluded viruses, entomopoxviruses were classified as the most resistant to UV-B in laboratory conditions and granuloviruses were the most sensitive to UV-B inactivation, whereas nucleopolyhedroviruses and cypoviruses were intermediate in their susceptibility (Ignoffo et al., 1977). Light with a longer wavelength and a lower energy, such as UV-A (315–400 nm) and visible light, can also inactivate viruses given extended periods of exposure (Shapiro and Domek, 2002).

To counter UV-induced damage, several insect pathogenic viruses encode class II photolyase enzymes that use the energy of visible light to repair pyrimidine dimers and return them to their original state. To date, photolyase genes have been identified in several entomopoxviruses, a granulovirus and a growing number of nucleopolyhedroviruses (van Oers et al., 2008; Rabalski et al., 2016). Phylogenetic evidence indicates that baculoviruses probably obtained their photolyase genes by horizontal gene transfer from an ancestral lepidopteran host (Biernat et al., 2011). Other families of invertebrate DNA viruses have a selection of genes to repair different types of damage to their genomes, including strand breaks and base or nucleotide excision repair (Blanc-Mathieu and Otata, 2016).

For OBs on plant foliage, on stems, or bark, the degree of shading provided by the upper layers of foliage or adjacent plants will reduce exposure to solar UV (Jaques, 1985). Consequently, OBs on foliage at the middle or lower parts of the plant canopy will receive a lower dose of UV and tend to persist longer than OBs at the top of the plant. For example, compared to the upper or middle sections of plants, the density of OBs was markedly higher on the lower parts of soybean plants and pine trees (*Pinus cortata*) that were shaded from direct sunlight by upper canopy foliage (Young and Yearian, 1989; Richards et al., 1999). Viral OBs were most abundant on heather (*Calluna vulgaris*) growing under pine trees which represented the most shaded habitat in a pine plantation and comprised a major environmental reservoir of a lymantriine nucleopolyhedrovirus (Richards et al., 1999).

OBs persisted longer on the undersides of cotton, cabbage and soybean leaves compared to those on the upper surfaces (Young and Yearian, 1974; Biever and Hostetter, 1985; Peng et al., 1999a). Similarly, because of the angle of incidence of solar radiation, nucleopolyhedrovirus OBs on south-facing foliage of pine trees received an approximately five-fold higher dose of UV than north-facing foliage for trees growing in the northern hemisphere (Killick and Warden, 1991), whereas the opposite effect was observed for granulovirus OBs applied to citrus trees growing in the southern hemisphere (Moore et al., 2015). OBs in the crevices at the bases of pine needles, or on the bark of twigs, branches and trunks of trees may also be protected from UV radiation, so that they can remain viable during the winter period when host larvae are absent (Kaupp, 1983; Olofsson, 1988b). As such, surfaces of trees can constitute a reservoir of OBs in the environment that retain their infectivity compared to OBs on plants in unshaded locations. For example, OBs of the winter moth (*O. brumata*) NPV retained infectivity on oak (*Quercus*

robur) and sitka spruce (*Picea sitchensis*) in forested areas whereas OBs on heather in unshaded habitats were rapidly inactivated (Raymond et al., 2005).

The use of UV-lamps has been evaluated for the inactivation of pathogens such as white spot syndrome virus (WSSV), a whispovirus that can persist in water used for shrimp farming (Chang et al., 1998). However, the influence of natural sunlight on virus persistence in water has been little studied. One exception is the study on particles of an iridescent virus in trays of fresh water which lost 97% of their infectious titer over a 60 hour period under shaded tropical conditions, but lost 99.99% of their infectious titer in a 36 h period, when exposed to direct sunlight. The persistence of the virus was negatively correlated with the accumulated dose of solar UV radiation (Hernández et al., 2005). Of course, viruses in tropical habitats are likely to experience a more severe combination of insolation and elevated temperatures compared to those in temperate zones.

7.11.2. Seasonal Effects on Viruses

Seasonality affects virus persistence in tropical and temperate regions by way of seasonal fluctuations in biotic and abiotic factors such as UV radiation, precipitation, and temperature and the presence of the host plant foliage and plant phenology. That said, early studies on baculovirus ecology recognized that the seasonality of virus dynamics in soil and on the foliage of plants were driven by the seasonal characteristics of the host life cycle. Following an initial application of nucleopolyhedrovirus OBs to soil of cabbage plots, the density of soil OBs reached a peak during the fall (500-1000 OBs/mg soil), remained high or fell very slightly during the winter, then fell due to tillage of plots in the spring (5 – 30 OBs/mg soil), before returning to peak levels during the summer and fall. This cyclic pattern was repeated during the 5 years of the study (Jaques, 1974). In each of these years the density of OBs on cabbage leaves increased rapidly from 1-10 OBs/cm^2 following planting, as plants became contaminated with OBs from the soil reservoir, to 100-1000 OBs/cm^2 in samples taken in the summer and fall when *Trichoplusia ni* larvae had become infected and OBs were subsequently washed by rainfall over cabbage leaves and onto the soil (Jaques, 1985). Similar patterns were seen in studies of soybean fields following the introduction of a nucleopolyhedrovirus to control *A. gemmatalis*, in which OB densities in soil and on plants showed synchronous seasonal fluctuations over a three-year period, with the main loss of OBs in the soil occurring over the winter months (Fuxa and Richter, 1994, 1996).

Plants die or lose leaves during the fall (temperate regions) or dry season (tropics) and this is a mechanism by which OB contaminated foliage can become incorporated into the soil reservoir. For example, the horizontal distribution of the virus population in the soil reflected the spatial pattern of fallen leaves at distances of up to 15 m from large poplar and plane trees contaminated with a nucleopolyhedrovirus of the fall webworm, *Hyphantria cunea* (Hukuhara, 1973).

7.11.3. Effect of Temperature on Viruses

The influence of temperature on the stability of occluded viruses has been summarized by others (Jaques, 1985; Benz, 1987; Ouellette et al., 2010). At normal environmental temperatures (10 – 40°C) these occluded viruses can usually retain infectivity for weeks, months or even years (David and Gardiner, 1967). However, these studies were often complicated by environmental factors including different levels of moisture and the presence of contaminants such as enzymes and other microorganisms. Exposure to elevated temperatures (≥60 °C) results in loss of infectivity within a few minutes (Ribeiro and Pavan, 1994).

Non-occluded viruses differ in their sensitivity to heat. The infectivity of nucleopolyhedrovirus budded virions was significantly reduced following exposure to temperatures exceeding 45 °C (Michealsky et al., 2008). Purified particles of iridescent virus were rapidly inactivated above 50 °C, but gradually lost between 0.5 and 1 logarithm of infectivity over a 50 day period at temperatures of 4 or 25 °C, or the ambient temperature of a tropical pond (Marina et al., 2000; Martínez et al., 2003). The nodavirus, Flock house virus, lost 1-2 logarithms of infectivity following 10 minutes of exposure to 53-58 °C (Scotti et al., 1983). In contrast, densoviruses and iflaviruses are stable at high temperatures (50 – 60 °C), but are inactivated above 70 °C (Seki et al., 1986; Jakubowska et al., 2016). In general, all the invertebrate viruses are stable for periods of years when frozen.

7.11.4. Humidity, Moisture and Precipitation

Several viruses that infect aquatic or soil-dwelling invertebrates are sensitive to desiccation. An iridescent virus lost two logarithms of infectivity in 24 h in dry soil (6.4% moisture, -1000 kPa matric potential) whereas its half-life was 4.9 days in a natural soil and 6.3 days in sterilized soil. The moisture content of the natural and sterilized soils was 17-37% (−114 to -9.0 kPa), but this moisture range did not significantly influence virus stability (Reyes et al., 2004). Similarly, the shrimp whispovirus (WSSV) was totally inactivated following 48 h of dry conditions (LeBlanc and Overstreet, 1991).

Occluded viruses are not generally affected by humidity (David et al., 1971; Ignoffo, 1992). However, wetted deposits of nucleopolyhedrovirus or granulovirus OBs, such as would occur following rainfall or early morning dew, were more rapidly inactivated by UV radiation than dry deposits (Ignoffo and Garcia, 1992). Periods of damp weather have been reported to increase the prevalence of nucleopolyhedrovirus infection in *L. monacha* and the armyworm, *S. exempta* (Persson, 1981; Benz, 1987), but not in the gypsy moth, *L. dispar* (Hajek and Tobin, 2011). However, as high humidity is a stressor, it is possible that the reports reflect the activation of covert infections, triggering lethal disease in natural populations of these insects (Fuxa et al., 1999).

The persistence of OBs of Anticarsia gemmatalis nucleopolyhedrovirus (AgMNPV) over 28 months in an agricultural soil was affected by moisture, with the highest persistence in saturated soil (45% moisture, 0 kPa matric potential), lowest in damp soil (30% moisture, -30 kPa), and intermediate in the driest soil treatment (15% moisture, -500 kPa). A very different

pattern was observed in soil taken from a marsh, possibly due to differences in physicochemical properties and the presence of microorganisms (Peng et al., 1999a).

The role of precipitation in the persistence of viruses has little to do with inactivation and more to do with transport and dispersal in the environment. Early studies recognized that it was difficult to wash baculovirus or cypovirus OBs from plant foliage exposed to natural or simulated rainfall (Burgerjon and Grison, 1965; David and Gardiner, 1966; Bullock, 1967). In a controlled field study, natural or simulated rainfall applied to LdMNPV-infected *L. dispar* cadavers on oak trees resulted in a reduction in the prevalence of infection of conspecific larvae from approximately 42-60% prior to rainfall, to 20-35% of infection following rainfall, indicating a significant loss of inoculum from oak foliage through the action of rain (values estimated from figures in D'Amico and Elkinton, 1995). Clearly humidity and precipitation are factors that tend to interact with other environmental variables in each particular habitat and across different types of ecosystem.

7. 11.5. Effect of pH on Viruses

The pH of the environment can be highly influential in the stability and persistence of invertebrate viruses. As mentioned in section 7.6.2.1., the alkaline pH of cotton leaf surfaces can rapidly inactivate nucleopolyhedrovirus OBs (Young and Yearian 1974; Elleman and Entwistle, 1985a). In contrast, simulated acid rain (pH 3) reduced the mortality of infected sawfly larvae feeding on pine foliage, not due to a direct effect on the virus but likely due to a pH-mediated change in the quality of plant foliage that improved larval survival (Neuvonen et al., 1990).

Most studies on the effect of pH in the environment have focused on the pH of the soil. As OBs break down in the presence of alkaline pH and release virions, the persistence of baculoviruses in high pH soils is poor compared to soils of neutral or slightly acid pH (Jaques, 1985). In a study on SeMNPV isolated from soil substrates across four zones of horticultural greenhouses in southern Spain (Murillo et al., 2007), the pH of the soil substrate was found to vary seasonally (Fig. 7.1a), with pH values in the fall (mean pH 8.6) significantly higher than during other periods of the year (pH 7.8-8.1). This was probably due to the application of alkaline substrate disinfection treatments at the end of the summer. Soil substrate pH differed between greenhouse zones in this area, but more significantly the prevalence of mortality in bioassays (an indicator of the abundance of SeMNPV OBs in substrate samples) was negatively correlated with substrate pH (Fig. 7.1b). Moreover, certain genotypes were associated with soil substrates with higher pH and other genotypes were associated with lower pH, suggesting that certain genotypes may be better able to withstand high pH conditions (Fig. 7.1c). Finally, soil substrate pH affected the probability of isolating single or mixed genotype isolates (Fig. 7.1d), suggesting that soil substrates with lower pH harbored larger and more diverse OB populations (Murillo et al., 2007). Although thought-provoking, the possibility that genotypic variants differ in their ability to persist in the environment, perhaps due to the size or robustness of their OBs, has not been the subject of systematic study.

7.12. BIOTIC FACTORS THAT INTERACT WITH VIRUS POPULATIONS

7.12.1. Plant Phenology, Structure and Nutritional Value

Plant species and phenology have marked effects on the persistence of baculovirus OBs on plant surfaces (section 7.6.2.1.), but also influence the nutritional quality of foliage and the physical toughness or other physical defenses against herbivory. As a result, the lethality of baculoviruses is often observed to vary markedly depending on the food plant species (Farrar and Ridgway, 2000; Wan et al., 2016). In some cases this may be due to compensatory feeding, through which the insect consumes greater quantities of foliage on poor quality food plants in an attempt to acquire sufficient nutrients and energy to achieve growth and development. Studies on virus transmission on different species or cultivars of plants should take differential feeding into account to accurately assess the role of food plant on the relationship between OB density and prevalence of infection.

Studies on plant nutritional quality usually focus on key indicators of compounds required for insect growth and development, such as proteins (nitrogen content), carbohydrates, fatty acids and the presence of defensive compounds. Food plant quality can affect different indicators of immune function including hemocyte numbers, phenoloxidase levels and encapsulation responses (Klemola et al., 2007; Shikano et al., 2010). Insects that feed on poor quality plants appear to be at increased risk of infection by baculoviruses compared to insects that feed on high quality plants (Raymond and Hails, 2007; Shikano et al., 2010). The plant can also have a marked effect on the fitness of the pathogen. Winter moth (*O. brumata*) larvae feeding on oak, a good quality food plant, were at lower risk of infection than conspecific larvae that fed on sitka spruce. However, each oak-fed larva produced larger numbers of OBs and the overall production of OBs in each cohort of insects was also significantly greater on oak compared to spruce-fed insects (Raymond and Hails, 2007). Furthermore, phenology-related reductions in leaf nitrogen and phenolic content of silver birch (*Betula pendula*) affected hemocyte numbers in *L. dispar* larvae and the prevalence of lethal nucleopolyhedrovirus disease, although not in a systematic manner. There was also some evidence that larvae feeding on older nitrogen-depleted foliage were more likely to succumb to spontaneous virus disease that was triggered in covertly infected individuals (Martemyanov et al., 2015b).

In the case of physical defensive structures, very little attention has been paid to their role in modulating the primary infection process in baculoviruses. In a comparative study on armyworm (*Mythimna unipuncta*), the prevalence of nucleopolyhedrovirus infection and speed of kill of the virus were similar on spiny and smooth fescue grasses, and the peritrophic membrane remained undamaged despite the presence of grass fragments with spines in the food bolus (Keathley et al., 2012).

7.12.2. Phytochemical-Virus Interactions

Plants produce an enormous diversity of defensive compounds, many of which are designed to reduce feeding by insects or other herbivores. Studies on plant-mediated effects on insect-virus interactions have focused on baculoviruses (reviewed by Duffey et al., 1995). Plant chemistry effects on insect viruses are usually examined following observations that a

baculovirus-based insecticide has poor efficacy on a particular type of crop (Stevenson et al., 2010), or to examine the ecological effects of defoliation on disease dynamics in forest-feeding pests (Elderd et al., 2013).

Compounds on plant surfaces will interact with OBs from insect cadavers or other environmental reservoirs, whereas compounds within plant tissues only interact with OBs in the insect gut following consumption of virus-contaminated foliage. An interesting example of leaf surface chemical effects is that of chickpea (*Cicer arietinum*), which has leaf trichomes that produce abundant organic acids, producing a leaf surface pH <3. However, following the application of nucleopolyhedrovirus OBs, two phenolic compounds (isoflavonoids) are released onto the leaf surface and rapidly inactivate OBs, although details of the chemical mechanism for inactivation are uncertain (Stevenson et al., 2010).

As foliage is consumed and moves through the insect gut a series of interactions occur between phytochemicals, digestive tract enzymes and virus particles (reviewed by Cory and Hoover, 2006). Immediately following ingestion of plant material, salivary gland secretions containing enzymes such as glucose oxidase can produce reactive oxygen species, like hydrogen peroxide that can inactivate the bacterial pathogen, *Bacillus thuringiensis* (Musser et al., 2005), and possibly viruses. Following this, exposure to plant phenolic compounds in the midgut can result in aggregation of baculovirus OBs which then fail to release the ODVs that infect midgut cells (Duffey et al., 1995). ODVs are also likely to be damaged or inactivated by exposure to phenolic compounds or free radicals. For example, the lethality of nucleopolyhedrovirus OBs decreased in the presence of two phenolic compounds, rutin or chlorogenic acid, in *H. zea* larvae (Felton et al., 1987). In a detailed study on the role of induced hydrolyzable tannins in oak, the presence of dietary tannins reduced the susceptibility of *L. dispar* larvae to their nucleopolyhedrovirus and reduced the risk of transmission (Elderd et al., 2013). Tannins are hydrolyzed in the insect gut to release phenolic compounds. The results of this study were used to develop a model to demonstrate that the prevalence of oak trees in mixed forests will effectively predict the severity and periodicity of *L. dispar* outbreaks (see Chapter 12).

The integrity of the peritrophic membrane lining the midgut may also be compromised by interactions with phytochemicals and plant enzymes (Pechan et al., 2002), or physically altered in response to the consumption of different types of foliage, resulting in a negative correlation between peritrophic membrane thickness and the probability of lethal baculovirus infection (Plymale et al., 2008). Midgut cells can also suffer oxidative stress from the presence of reactive species and respond by sloughing off from the midgut wall before the virus has established a systemic infection in the insect (Hoover et al., 2000).

Phytochemicals, or phytochemical-induced signals, may also cross the gut and modify immune function or host physiology, resulting in increased or decreased susceptibility to baculovirus disease in relation to food plant quality, as described in section 7.12.1 (Klemola et al., 2007; Shikano et al., 2010). However, several studies on induced plant defenses have failed

to detect altered susceptibility to nucleopolyhedroviruses (Plymale et al., 2007; Martemyanov et al., 2012; Sarfraz et al., 2013).

7.12.3. Virus Interactions with Alternative Hosts

The host range of invertebrate viruses varies widely from highly host specific viruses, such as SeMNPV (Simón et al., 2004b), to viruses such as invertebrate iridescent virus 6 that can replicate in a wide range of arthropods and even ectothermic vertebrate hosts (Ohba and Aizawa, 1979; Stöhr et al., 2016). This means that viruses with an extended host range can exploit alternative hosts and are less dependent on the presence and density of particular host species than are highly host-specific viruses. Nonetheless, the ability to exploit a range of host species is likely to come at a cost of reduced fitness for the pathogen in terms of the capacity to infect, replicate and produce progeny particles in less than optimal host species. That said, very little attention has been paid to determining the tradeoff between the use of alternative host species and the fitness of invertebrate viruses in nature. There are several virus-invertebrate pathosystems that could be suitable for examining the ecological roles of alternative hosts in agricultural or natural ecosystems. In the case of baculoviruses, the extended host range of viruses, such as AcMNPV and Anagrapha falcifera multiple nucleopolyhedrovirus (AnfaNPV), means they can be used as biological insecticides to control multi-species complexes of noctuid looper pests (Vail et al., 1999). This system offers the intriguing possibility of determining the roles of each of the looper species in the persistence, transmission and genetic diversity of a multi-host baculovirus on *Brassica* spp. or other shared food plants.

Alternative host species also facilitate the survival of viruses in other systems. An iridescent virus that was transmitted through acts of cannibalism and interspecific predation persisted in two species of terrestrial isopods (woodlice) in a grassland ecosystem (Grosholz, 1992). Survival decreased and the prevalence of disease increased in mixed populations compared to the single species population, apparently due to an increased frequency of interspecific aggression in mixed populations. Additionally, ascoviruses that are transmitted between hosts by endoparasitoid wasps, appear capable of infecting numerous species of noctuid larvae, reflecting the oviposition preferences of their parasitoid vectors (Hamm et al., 1998).

Many viruses may be presumed to be host-specific because they have not been looked for in species other than the host from which they were initially isolated (Roy et al., 2009). Given that there is growing evidence that insects frequently harbor covert infections by viruses (Kemp et al., 2011; Virto et al., 2013), it is clear that disease-based estimates of the prevalence of virus pathogens in invertebrate populations represent a major underestimate of their true prevalence. As such, even when opportunities for horizontal transmission are scarce, sublethal disease and the activation of covert infections into lethal viroses (Cooper et al., 2003; Burden et al., 2006) are likely to have an impact on the populations of rare host species and non-pest species that by definition exist at low densities. Moreover covert infections in populations of “unexpected” hosts are likely to be overlooked unless systematic surveys are performed (Roy et al., 2009). Some support for the unexpected-host-hypothesis comes from a molecular study on the presence of a

nucleopolyhedrovirus in populations of the winter moth, *O. brumata*, which fortuitously identified the virus in two sympatric heather-feeding geometrids: the July highflyer (*Hydriomena furcata*) and the grey mountain carpet (*Entephria caesiata*) (Graham, 2005). However, the precise role of these species in the ecology of the winter moth-virus pathosystem remains unknown.

7.12.4. Competition and Facilitation in Virus Interactions with Other Organisms

7.12.4.1. Virus Interactions with Parasitoids

Of the interactions of invertebrate viruses with other parasites and pathogens, those related to parasitoid wasps have attracted by far the most attention, probably due to the abundance and conspicuousness of parasitoids in natural and agricultural ecosystems. However, despite the number and diversity of studies on virus – parasitoid interactions in the laboratory, few consensus principles have emerged on the competitive interactions among these natural enemies. This is probably due to three main factors: (i) a frequent focus on examining the "compatibility" of parasitoids and viruses used in the biological control of pests, (ii) complexity in the outcomes of virus-parasitoid coinfection studies arising from marked interspecific diversity in parasitoid biology, (iii) complicating issues arising from the presence of different polydnaviruses in many of the most common braconid and ichneumonid parasitoids, which suppress host immune function.

Parasitoids, particularly endoparasitoids that oviposit within the body of lepidopteran hosts, can detect physiological changes related to infection status and discriminate against infected hosts in favor of healthy individuals (Kyei-Poku and Kunimi, 1997; Matthews et al., 2004; Jiang et al., 2014). In many cases, parasitism must occur hours or days prior to virus infection for the parasitoid progeny to have any chance of developing in baculovirus-infected insects, otherwise the host is usually killed by the pathogen before wasp larvae can complete their development (Cossentine, 2009). In some baculoviruses and entomopoxviruses, developing wasp larvae are killed by virus-encoded toxic factors that eliminate the competitor (Cossentine, 2009; Okuno et al., 2002), whereas parasitoid larvae in iridescent virus-infected hosts themselves become infected by the virus and die (López et al., 2002).

The outcomes of virus-parasitoid interactions from the perspective of the pathogen range from facilitation to competition. The virus gains opportunities for transmission because parasitized hosts are often more susceptible to infection than non-parasitized individuals (Santiago-Alvarez and Caballero, 1990; Gou et al., 2013), likely due to immune suppression by parasitoid polydnaviruses (Washburn et al., 2000; Rivkin et al., 2006; although see D'Amico et al., 2013a). The presence of the virus is also frequently associated with reduced growth of developing wasp larvae, arising from the stunted growth of the host insect and the rapid sequestering of host resources for virus replication (Nakai et al., 1997; Azam et al., 2016).

Given that viruses replicating in parasitized insects face direct competition for host resources it is not surprising that such conditions are appropriate for the triggering of patent disease in covertly infected insects (Stoltz and Makkay, 2003), or selection for highly virulent

strains of virus with rapid speed of kill (Escribano et al., 2001). Moreover, reduced host growth and the segregation of host resources by the developing parasitoids can result in a reduction in the number of progeny virus OBs produced in each infected and parasitized host (Escribano et al., 2001; Cai et al., 2012). This could impact directly on virus transmission, although a fraction of the parasitoids that emerge from virus-infected hosts may be contaminated and capable of transmitting the virus to other hosts during acts of oviposition (Brooks, 1993).

In a long term study on *L. dispar* populations across different states in the United States, four species of parasitoids, a nucleopolyhedrovirus (LdMNPV), and a fungal pathogen were quantified in samples taken over a 17 year period (Hajek and van Nouhuys, 2016). At sites with outbreak populations of *L. dispar* one braconid parasitoid was found in association with LdMNPV infection far more frequently than expected by chance, possibly due to a polydnavirus-mediated reduction in host resistance to virus infection, whereas other parasitoids appeared to avoid virus-infected gypsy moth larvae, or were killed by the fungus before they could complete their development.

7.12.4.2. Virus Interactions with Other Pathogens

Interactions among different types of invertebrate viruses, or between viruses and other pathogens, depend on the route or mechanism of infection, and the temporal sequence of infection by each entity. Studies focused on biological control tend to consider mixtures of pathogens administered simultaneously to pest insects with the aim of identifying potentiation or antagonism in the insecticidal characteristics of the pathogens (Harper, 1986). Such studies have identified virus factors, such as enhancin in baculoviruses and spheroidin in entomopoxviruses, that degrade the larval peritrophic membrane and facilitate the primary infection of midgut cells by these viruses (Wang and Granados, 1997; Mitsuhashi et al., 1998).

In coinfected hosts, studies have focused on the outcome of within-host competition, which largely depends on the replication rate and speed of kill of each pathogen or the ability of one virus to disrupt the replication of another. Examples come from studies on coinfecting baculoviruses (Hacket et al., 2000; Wennmann et al., 2015), and between baculoviruses and other viruses (Ishii et al., 2002), fungi (Malakar et al., 1999b), and entomopathogenic nematodes (Agra-Gothama et al., 1995).

An alternative approach, and one that is generally more applicable to an ecological context, is the examination of how virus-infected insects respond to superinfection by another pathogen. Superinfection occurs when an individual that is already infected by one pathogen is then infected by a second pathogen. For example, when nucleopolyhedrovirus-infected larvae of the diamondback moth, *Plutella xylostella*, were challenged with different concentrations of *B. thuringiensis*, lower than expected mortality occurred at low doses of *B. thuringiensis*, suggesting a protective effect of virus infection (Raymond et al., 2006). Within-host interactions include the ability of a virus to induce cells to become refractive to superinfection some hours after the initial infection, thereby blocking the systemic infection process for the second virus (Beperet et al., 2014).

At the population level, the gypsy moth nucleopolyhedrovirus (LdMNPV) and a fungal pathogen did not interact to influence the mortality of this insect (Malakar et al., 1999a). The virus continued to act in a density dependent manner, independent of the presence of the fungus, the prevalence of which was not affected by host density (Liebhold et al., 2013). In a long term study, coinfection by LdMNPV and the fungal pathogen decreased with increasing host density for reasons that remain unclear but may be related to the speed of kill and propagule production by each type of pathogen or host responses to population density (Hajek and van Nouhuys, 2016).

The presence of a densovirus in natural populations of *Helicoverpa armigera* reduced the susceptibility of larvae to infection by nucleopolyhedrovirus (HearNPV) and to low doses of Bt toxin. HearNPV replication was also reduced in coinfected hosts indicating an important protective effect by the densovirus (Xu et al., 2014). Similarly, the symbiont *Wolbachia* protected *Drosophila melanogaster* from several RNA viruses but not from a DNA virus, i.e., an iridescent virus (Hedges et al., 2008; Teixeira et al., 2008). Subsequently, increased susceptibility to another DNA virus, a nucleopolyhedrovirus, was observed in *Wolbachia*-infected *S. exempta* (Graham et al., 2012). Although the responses of *Wolbachia*-infected insects differ for RNA and DNA viruses, given the high incidence of *Wolbachia* in many insect populations, the ecological consequences of symbiont-mediated shifts in susceptibility to infection by viruses is likely to provide novel insights into individual and population level processes in *Wolbachia*-infected insect virus pathosystems. This issue has particular implications for the use of *Wolbachia*-infected mosquitoes for the suppression of arbovirus transmission in tropical regions (Lambrechts et al., 2015).

In a more extreme case of facilitation between viruses, the non-occluded Spodoptera exigua iflavirus 1 becomes intimately associated with the OBs of a nucleopolyhedrovirus (SeMNPV) when both viruses replicate in coinfected larvae of *S. exigua* (Jakubowska et al., 2016). Iflavirus particles appeared to be incorporated into the OB protein matrix and were transmitted efficiently in OBs, which also protected the iflavirus from high temperatures and UV radiation during periods in the environment. In essence the iflavirus became a hitchhiker by using OBs as a vehicle to improve its survival and transmission opportunities.

Within host interactions of viruses and other pathogens can be common and can be highly influential to the evolution of virulence (Alizon et al., 2013). However, most studies are limited by their focus on the outcome of mixed infections in individuals, rather than population level effects. Clearly it is necessary to coordinate within-host and between-host studies over multiple cycles of transmission to obtain a useful perspective on the role of pathogen interactions and multiple infections in the evolution of virulence.

7.12.4.3. Virus Interactions with Microbiota

Finally, the role of microbiota on susceptibility to disease is an issue that has begun to attract a great deal of attention following the advent of metagenomics techniques. Non-pathogenic bacteria on the phylloplane of different host plants did not elicit a host immune

response and did not affect susceptibility of *T. ni* larvae to nucleopolyhedrovirus infection (Shikano et al., 2015). In contrast, when the gut microbiota of *S. exigua* larvae was controlled or eliminated using antibiotics, survival of SeMNPV-infected larvae increased and OB production decreased almost 3-fold in insects lacking gut microbiota (Jakubowska et al., 2013). Phylloplane organisms and the host plant can markedly influence the gut microbiota, but the implications of these findings on virus transmission in nature have yet to be determined.

7.13. FUTURE DIRECTIONS

Despite being simple replicating entities, devoid of life *per se*, viruses interacting with their hosts provide a wealth of challenging ecological and evolutionary questions. The diversity of the ecological processes described in this chapter bears testament to the complexity of invertebrate-virus relationships that range from genes and genomes to populations and species. With the recent growth in virus detection, sequence determination and particle visualization techniques our ability to examine invertebrate virus processes at the cell and organism levels are set to provide extraordinary opportunities for the understanding of host-virus relationships. At the other extreme, the development of population models supported by empirical observations continues to advance and offer opportunities to understand the fundamental ecological processes that regulate insect populations and drive particular patterns of population dynamics, best exemplified in phytophagous insects in forest ecosystems.

Of the key issues that need to be addressed over the coming decade, from a personal perspective the following stand out: (i) As we can screen large numbers of insects for covert virus infections quantifying the impact of sublethal disease on invertebrate-virus population dynamics should become increasingly achievable. (ii) As we recognize that many virus populations are highly diverse, determining the selective nature of this diversity in transmission and persistence in natural ecosystems will doubtless provide profitable lines of research. (iii) Given the uncertainty that nations will be able to check global climate change, studies on the effects of predicted rises in temperature on fundamental ecological processes, including invertebrate disease dynamics, will become increasingly relevant.

Additional intriguing issues relate to the role of epigenetic mechanisms in modulating infectious disease processes in invertebrates and unraveling the relationship between virus genotype and phenotype, such as observed in the case of *egt* and *ptp* manipulation of host behavior. Such studies are likely to provide original and exciting insights at the individual and population levels.

Acknowledgments

I thank Jenny Cory, Judy Myers and Rosa Murillo for kind permission to reproduce figures, Miguel López-Ferber, Primitivo Caballero, James Becnel, Sean Moore and Rodrigo Lasa for helpful discussions and Gabriel Mercado for logistical support.

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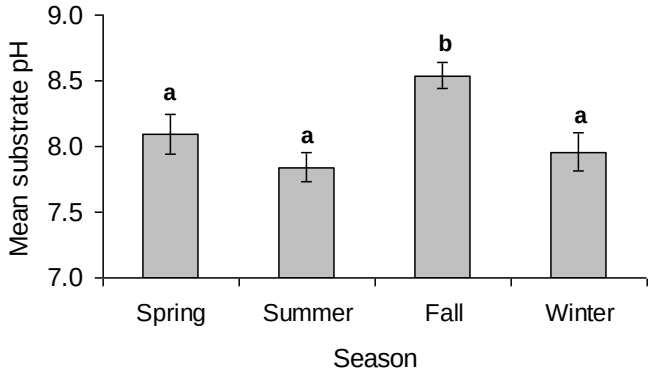
Figure legends

Figure 7.1. Persistence of nucleopolyhedrovirus (SeMNPV) occlusion bodies in greenhouse soil substrate in southern Spain; a) Seasonal fluctuations in substrate pH, b) Influence of substrate pH on mortality of *Spodoptera exigua* larvae in bioassays, an indicator of the abundance of OBs in substrate samples, c) Mean substrate pH from which four genotypic variants of SeMNPV (Se-G24 to Se-G27) were isolated in bioassays, d) Median substrate pH from which single or mixed genotype infections were isolated in bioassays. Vertical bars indicate 95% confidence interval of means or interquartile range about the median. Columns headed by identical letters do not differ significantly ($P>0.05$). Reproduced from Murillo et al. (2007) with permission from Elsevier.

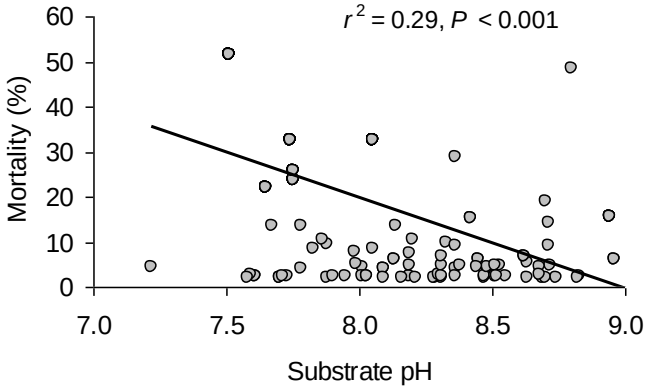
Figure 7.2. Cyclic population dynamics of the Western tent caterpillar (*Malacosoma californicum pluviale*) and its nucleopolyhedrovirus in British Colombia, Canada over a 24 year period; a) Fluctuations in numbers of tents (families) on Galiano Island (columns) and percentages of families that contain diseased insects (black dots), b) Negative correlation between rate of population change (R) and percentage of families containing diseased individuals, where $R = \log(n+1/n)$, indicating the change in population size on Galiano Island from one year (n) to the next (n+1), c) Negative correlation between female fecundity (expressed as mean number of eggs in each egg mass) and the percentage of families containing infected insects. Figures redrawn from Myers and Cory (2016), creative commons attribution license.

Fig. 7.1

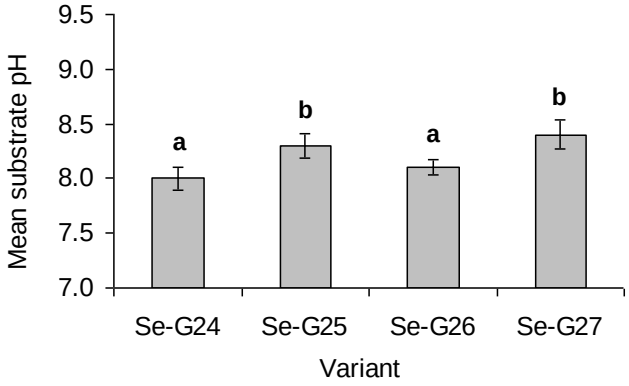
a)


9.0
8.5
8.0
7.5
7.0
Mean substrate pH
a
a
b
a
Spring
Summer
Fall
Winter
Season

b)


$r^2 = 0.29, P < 0.001$
60
50
40
30
20
10
0
Mortality (%)
7.0
7.5
8.0
8.5
9.0
Substrate pH

c)


9.5
9.0
8.5
8.0
7.5
7.0
Mean substrate pH
a
b
a
b
Se-G24
Se-G25
Se-G26
Se-G27
Variant

d)

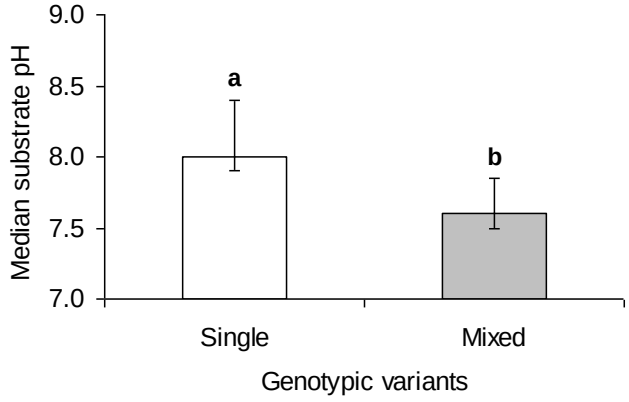
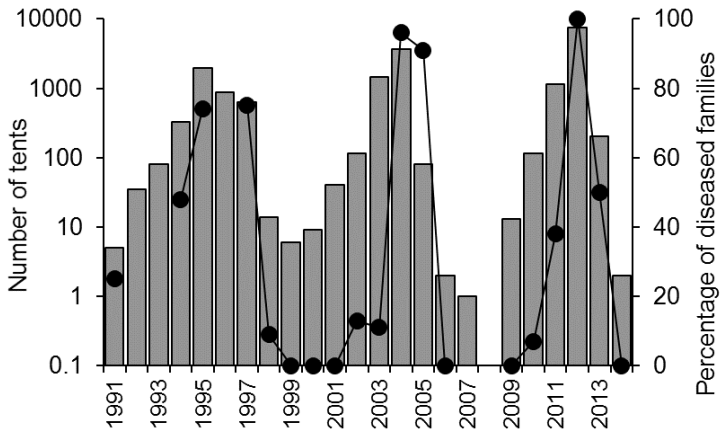
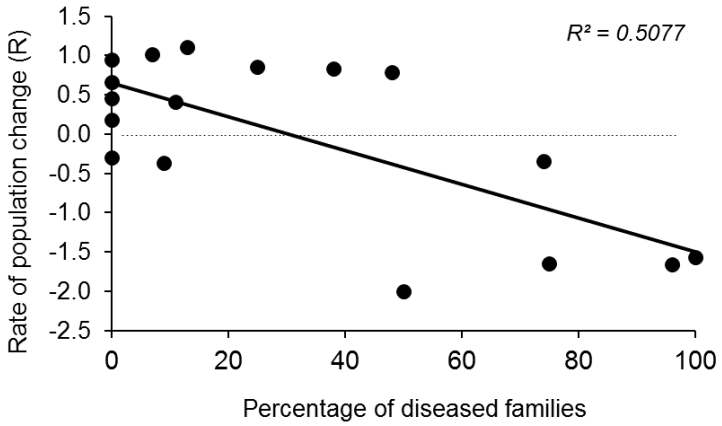

9.0
8.5
8.0
7.5
7.0
Median substrate pH
a
b
Single
Mixed
Genotypic variants

Fig 7.2

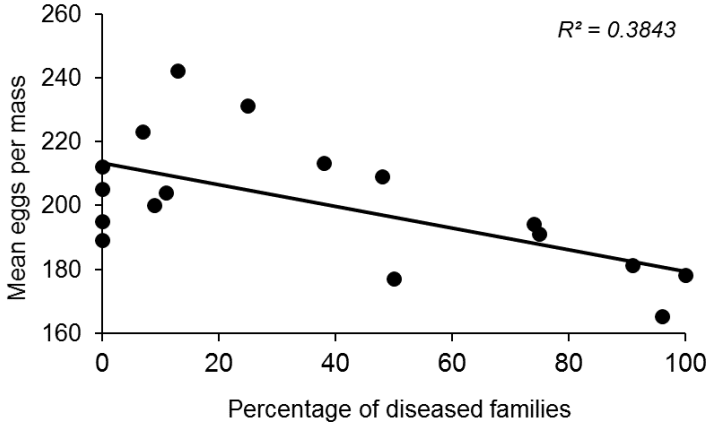
a)


Number of tents
Percentage of diseased families
10000
1000
100
10
1
0.1
100
80
60
40
20
0
1991
1993
1995
1997
1999
2001
2003
2005
2007
2009
2011
2013

b)


R² = 0.5077
Rate of population change (R)
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
0
20
40
60
80
100
Percentage of diseased families

c)


R² = 0.3843
Mean eggs per mass
260
240
220
200
180
160
0
20
40
60
80
100
Percentage of diseased families