8

Interactions Between Nucleopolyhedroviruses and Polydnaviruses in Larval Lepidoptera

Vincent D'Amico[1] and James Slavicek[2]
[1]*USDA Forest Service, University of Delaware, Newark, DE,*
[2]*Delaware, OH,*
USA

1. Introduction

The field dynamics of some insect populations are strongly influenced by two types of insect viruses: the nucleopolyhedroviruses (NPVs) and the polydnaviruses (PDVs). Although greatly different in origin and mode of infection, both viruses produce considerable mortality directly and indirectly in the field, and have evolved reproductive strategies that use the same life stage of the same host insects. The life histories of these host insects are such that there are many opportunities for coinfection and competition between baculoviruses and polydnaviruses, although these relationships remain largely theoretical and unexplored.

The hosts of both these viruses are found primarily in the insect order Lepidoptera, the moths and butterflies. The viruses are members of two large and diverse families: the Baculoviridae, pathogens of insects that fit the common conception of disease-causing organisms, and the Polydnaviridae, genome-integrated wasp symbionts with a unique natural history. Although the Baculoviridae are known from several insect orders, they occur in their greatest variety as the nucleopolyhedroviruses in the larval Lepidoptera. This is probably because the relatively long, plant-feeding caterpillar stages of moths and butterflies give ample opportunities for *per os* infection, as we will describe in detail below. Many nucleopolyhedroviruses known from the Hymenoptera typically occur in families feeding on leaves during the larval stage, as well, but they will not be included here. The ease of rearing lepidopteran larvae, and their place as pests of human crops, has also led to an accumulation of data on their viral diseases, and the many nucleopolyhedroviruses infecting Lepidoptera make this insect order a good starting point for exploring interactions with other viruses known to occur within them, polydnaviruses in particular. As symbionts of parasitoid wasps, polydnaviruses are also found in lepidopteran larvae. There they produce products that abrogate the larval immune system. Larval hosts immunosuppressed by a polydnavirus fail to muster the encapsulation response that would otherwise kill the parasitoid eggs and larvae; the wasp mutualist, or *carrier*, brings the polydnavirus to its host to ensure the survival of its own progeny. Our interest comes from past research in the field on population and disease dynamics of outbreaking lepidopteran forest pests, although we do not limit ourselves to these here.

2. Nucleopolyhedroviruses

Baculoviruses are a large group of viruses pathogenic to arthropods, primarily insects from the order Lepidoptera and also insects in the orders Hymenoptera and Diptera (Moscardi 1999; Herniou & Jehle, 2007). These viruses have been isolated from over 300 insect species (David, 1975; Tinsley & Harrap, 1978; Harrap & Payne, 1979). Baculoviruses have been used to control insect pests on agricultural crops and forests around the world (Moscardi, 1999; Szewczk et al., 2006, 2009; Erlandson 2008). The *Baculoviridae* are divided into four genera: the Alphabaculovirus (lepidopteran-specific nucleopolyhedroviruses, NPV), Betabaculovirus (lepidopteran specific Granuloviruses, GV), Gammabaculovirus (hymenopteran-specific NPV), and Deltabaculovirus (dipteran-specific NPV) (Jehle et al., 2006). Baculoviruses are arthropod-specific viruses with rod-shaped nucleocapsids ranging in size from 30-60 nm x 250-300 nm.

NPVs initiate infection when a susceptible host ingests virus in the form of occlusion bodies (OBs) present on host plants (Fig. 1A). OBs are composed primarily of the protein polyhedrin, which forms a paracrystalline matrix into which occlusion derived virus (ODV) is embedded. The polyhedron provides the embedded ODV protection from environmental elements such UV light. NPVs produce another form of virus, termed the budded virus, that is produced in the early stages of viral replication (Rohrmann, 2011). Within the alkaline environment of the larval midgut, OBs dissolve, thereby releasing ODV. To initiate an infection, ODV must first traverse a physical structure termed a peritrophic membrane (Fig. 1B), which is composed of proteins, mucopolysaccharides, and chitin (Pritchett et al., 1984; Hegedus, et al., 2009). The peritrophic membrane provides a barrier to gut cells to bacteria, viruses, fungi, and physical damage from ingested plant material. The peritrophic membrane is in a constant state of regeneration from epithelial cells as larvae feed, and the movement of food material through the insect gut also causes loss of the peritrophic membrane.

After penetration of the peritrophic membrane, ODV gains entry into midgut cells by a type of fusion process (Fig. 1C), although this has resisted definitive characterization (Granados & Lawler, 1981). The type NPV, *Autographa californica* multiple NPV (AcNPV), initiates the infection cycle by infecting columnar epithelial cells within the midgut and regenerative epithelial cells in *Trichoplusia ni* (Keddie et al., 1989) or *Spodoptera exigua* larvae (Flipsen et al., 1995). Several factors are involved with the initial act of infection that includes ODV binding to midgut cells at cell receptors, and viral entry into the cells. All sequenced baculoviruses contain genes that code for *per os* infectivity factors (PIFs) that are associated with ODVs but not budded virus (Faulkner et al., 1997; Kikhno et al., 2002; Fang et al., 2006; Harrison et al., 2010; Fang et al., 2009). The pif genes include p74-pif, and pif genes 1-5, Ac119, Ac22, Ac115, Ac96, and Ac148, respectively. Deletion of any of the genes from a viral genome significantly decreases but does not eliminate *per os* infectivity (d'Alencon et al., 2004; Crouch et al., 2007). The PIFs, with the exception of PIF3, are thought to be involved in binding or interacting with the midgut cells that leads to infection (Ohkawa, et al., 2005; Li et al., 2007; Peng et al., 2010; Horton & Burand, 1993).

Upon entry into midgut cells the nucleocapsids are actively transported to nuclear pores in a process that uses actin polymerization (Ohkawa et al., 2010) (Fig. 1C). Viral DNA is then released into the cell nucleus and viral replication ensues (Rohrmann, 2011) (Fig. 1C).

During the early phase of viral replication, BV are produced that bud from midgut cells and infect tracheal epidermal cells, which penetrate the basal lamina (Volkman, 2007). Infection spreads via the tracheal system and haemocytes until many different cells are infected (Engelhard et al., 1994) (Fig. 1D). During the later phase of viral replication, ODV are produced and packaged within OBs. Upon the host's death, liquefaction occurs, releasing OBs into the environment to infect another host (Reardon, 1996; Riegel & Slavicek, 1997).

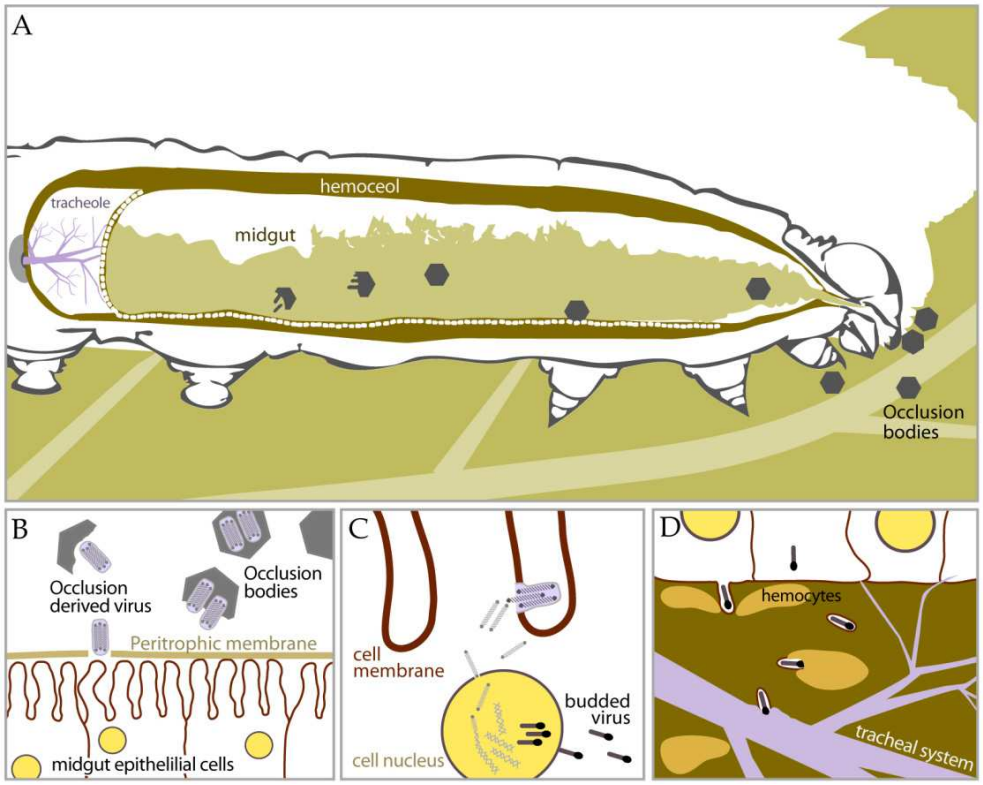


Fig. 1. Nucleopolyhedrovirus transmission. (A) NPV infections in larval Lepidoptera are initiated when caterpillars ingest viral occlusion bodies on foliage. (B) These dissolve in the alkaline conditions of the midgut and release occlusion derived nucleocapsids, which penetrate the peritrophic membrane. (C) Nucleocapsids fuse with the membrane of epithelial cells and pass through nuclear pores to begin production of the budded form of NPV. (D) Budded virus leave the midgud cells and infects hemocytes and other cell types.

Insect hosts have developed several mechanisms to thwart baculovirus infections including the peritrophic membrane physical barrier, developmental resistance (Engelhard & Volkman, 1995), melanization of infected cells, and apoptosis of infected cells. The *Lymantria dispar* MNPV (LdMNPV) exhibits a LD50 (74 OBs) in *L. dispar* (Hoover et al., 2002); however, by the middle of the fourth instar, the LD50 of LdMNPV is 18-fold higher than in newly molted larvae. The majority of cases of developmental resistance within an instar are

midgut-based (Haas-Stapleton et al., 2003). As the virus infects midgut cells the cells are in a continual process of renewal and sloughing. Consequently, if BV are produced and escape the midgut cell before it sloughs off and is excreted, the virus can infect the tracheal cells and usually generate a systemic infection (Engelhard et al., 1994). However, loss of tracheal epidermal cell infections can occur (Haas-Stapleton et al., 2003), and consequently the host can escape viral infection. In addition, larval hosts are less susceptible to viral infection between and within larval instars, due to a process termed developmental resistance (Engelhard & Volkman, 1995).

Because the main way in which polydnaviruses might affect NPV pathogenesis is via immunosuppression, it is important to note that the immune response is a vital part of larval response to infection. Hoover et al., (2002) hypothesized that immune responses play a role in systemic resistance in *L. dispar* to LdMNPV. In non- or semi-susceptible hosts, cellular immune responses to AcNPV-infected tissues can occur (Rivkin et al., 2006; Trudeau et al., 2001; Washburn et al., 2000). Haemocytes of some hosts are refractory to AcNPV infection and replication, which slows or stops the spread of the virus within the haemocoel (Rivkin et al., 2006). Apoptosis of AcNPV-infected cells can also serve as an antiviral response (Clarke & Clem, 2003; da Silveira et al., 2005; Zhang et al., 2002). In a recent study using LdMNPV, McNeil et al. (2010a) found that encapsulation and apoptosis of infected tissues are mechanisms of host defense. Elimination of infected tissue through encapsulation and melanization can occur in lepidopterans infected with AcNPV, but has only been reported in non-susceptible hosts such as *Helicoverpa zea* and *Manduca sexta* (Trudeau et al., 2001; Washburn et al., 2000) and did not differ in effectiveness at different times within the instar. Apoptosis of tracheal epidermal cells was hypothesized to contribute to AcNPV resistance in *Spodoptera frugiperda* (Haas-Stapleton et al., 2003) and it appears to be an effective defense against AcNPV infection in the fat body and epithelium of IH-injected *S. frugiperda* (Clarke & Clem, 2003) and *S. exigua* (Clem, 2005). Removal of midgut infections in *L. dispar* probably involves apoptosis of infected midgut epithelial cells (Dougherty et al., 2006) and is considered a conserved mechanism for midgut based resistance to NPVs in most lepidopterans (Volkman, 2007).

A recent study by McNeil et al. (2010b) found that mid-instar *L. dispar* larvae exhibited a higher degree of hemocyte immunoresponsiveness, a greater potential hemolymph phenoloxidase (PO) activity at the time the virus is escaping the midgut to enter the hemocoel, greater FAD-glucose dehydrogenase (GLD) activity, and more targeted melanization of infected tissue, which correlate with reduced viral success in the host. PO and GLD are components of the humoral immune response associated with melanized encapsulation (Lovallo & Cox-Foster, 1999; Nappi & Christensen, 2005). Phenoloxidase has potent anti-microbial properties, and its activation is tightly controlled within the hemolymph to prevent non-specific activation from harming the host (Jiravanichpaisal et al., 2006). PO activity in the hemolymph of *Heliothis virescens* larvae has been shown to have virucidal effects in vitro (Shelby & Popham, 2006) and previous research has supported the role of PO and cellular immunity in immune defenses against viruses (Stanley & Shapiro, 2007, 2009; Shrestha & Kim, 2008). GLD is induced during encapsulation responses to pathogens (Cox-Foster & Stehr, 1994; Lee et al., 2005). GLD is also involved in the production of free radicals derived from quinones during melanization in insects, and has been hypothesized to strengthen melanized capsules (Cox-Foster & Stehr, 1994), which could also negatively impact viral success.

3. Polydnaviruses

Polydnaviruses are multipartite double-stranded DNA viruses originating in koinobiont endoparasitic Hymenoptera, commonly called parasitoid wasps. The polydnavirus genome is integrated into that of the "carrier" wasp species, and is transmitted through the germ line (Stoltz, 1990; Belle et al., 2002; Wyler & Lanzrein, 2003; Bezier et al., 2009). The origin, replication, and function of polydnaviruses, and their symbiotic relationship with their carriers, make this system unique among members of any taxon. What follows is a short review of the discovery of the polydnaviruses, and our current understanding of their reproduction and functions. We will use the term *carrier* to refer to wasps bearing an genome-integrated polydnavirus. This avoids the connotations of *host* and possible confusion with the host of the wasp – here a lepidopteran larva, or caterpillar. Similarly, *polydnavirus infection* is used to refer to the polydnavirus in the larval host of the carrier wasp, not in the wasp itself.

The discovery of polydnaviruses began with the work of George Salt. Salt had a career that spanned more than a half-century, beginning in the 1920s. His research on the underlying mechanisms of insect immunity culminated in the treatise, *The Cellular Defence Reactions of Insects* (Salt, 1970). This synthesis included the results of earlier work, the most pertinent of which was an experiment involving placing washed eggs of the parasitoid *Venturia* (*Nemeritis) canescens* into living larvae of its host *Ephestia kuehniella* (Salt, 1965; see also Rotheram, 1973). Washed eggs were encapsulated by hemocytes within the larvae, while intact unwashed eggs were not. Salt used simple light microscopy to find the origin of this resistance; a "coating" on the eggs which originated in the calyx of the female parasitoid. This work certainly informed the subsequent seminal research of Stoltz, Vinson, and Mackinnon (Stoltz et al., 1976; Stoltz & Vinson, 1977, 1979). Surveys and research led by these and other researchers in the 1970s and 1980s led to a preliminary understanding of the origin and function of the baculovirus-like particles seen in the calyx fluid of some ichneumonids and braconids (Stoltz et al., 1981; Cook & Stoltz 1983; Fleming & Summers 1986). These particles were injected by carrier wasps into larval hosts along with eggs and toxins, and expressed genes while in that host, but did not replicate in host cells. Parasitoid eggs from such wasps, when placed in a host without other calyx-derived products such as the virus-like particles and wasp toxin, were invariably recognized as foreign, encapsulated, and destroyed (Edson et al., 1981; Stoltz & Guzo, 1986). The particles were clearly necessary for successful reproduction of the carrier wasp, but the emergent question was how these baculovirus-like particles were acquired or produced. The advent of modern molecular analyses allowed researchers to answer this question definitively.

The baculovirus-like particles discovered in braconid and ichneumonid wasps were determined to be a new form of virus, integrated into the genome of the carrier wasp as proviral genetic sequences (Stoltz, 1990; Bezier et al., 2009). Although these sequences were found in both male and female wasp genomes, they were excised to form free viral particles only in the ovary calyx cells of maturing female wasps. This is in stark contrast to a typical baculovirus life cycle (Cory & Myers, 2003). In the polydnavirus life cycle, replication and vertical transmission occur in the carrier wasp, while a form of horizontal transmission and deleterious infection occurs in the carrier wasp's larval host. This has also been previously defined as the two "arms" of the PDV life cycle (Turnbull & Webb, 2002). The larval host takes the brunt of the effects of the polydnavirus in the form of immunosuppression caused by polydnavirus DNA expression - but not through polydnavirus replication in and subsequent destruction of its cells.

Most of the known polydnaviruses are carried by wasps that parasitize larval Lepidoptera, in subfamilies of the Ichneumonidae (these polydnaviruses are termed ichnoviruses) and Braconidae (the bracoviruses) (Stoltz & Vinson, 1977; Fleming & Summers, 1991; Stoltz & Whitfield, 1992; Webb & Strand, 2005). At this time, known polydnavirus carrier wasps occur only in the braconid subfamilies Cardiochilinae, Cheloninae, Mendesellinae, Khoikhoiinae, Miricinae and Microgastrinae, and ichneumonid subfamilies Campopleginae and Banchinae (Table 1) (Stoltz et al., 1981; LaPointe et al., 2007; Whitfield & O'Connor, 2012). Even if only currently named species are considered, these subfamilies contain a total of almost 30,000 wasp species, with many expected to harbor polydnaviruses. Although these braconid and ichneumonid PDVs are at least as diverse as the wasp families themselves and are apparently unrelated to each other, we may use the braconid lineage as an example of how this association originated (Whitfield, 1997; see the excellent review by Whitfield & O'Connor, 2012). Between 85-100 million years ago, an early braconid acquired an integrated nudivirus in a manner not fully understood (Banks & Whitfield, 2006; Murphy et al., 2008). This virus has evolved along with the carrier wasps to the present day, with greater or lesser diversity as a function of the diversification of the carrier wasp (Murphy et al., 2008; Bezier et al., 2009). Modern inexpensive sequencing techniques continue to provide ever-greater detail of these phylogenies.

Polydnavirus	**Wasp family**	**Subfamilies**	**Representative wasp genera**
Bracovirus (BV)	Braconidae	Cardiochilinae	*Cardiochiles*, *Toxoneuron*
		Cheloninae	*Adelius*, *Chelonus*, *Ascogaste*r, *Phanerotoma*
		Khoikhoiinae	*Sania*, *Khoikhoia*
		Mendesellinae	*Epsilogaster*, *Mendesella*
		Microgastrinae	*Apanteles*, *Cotesia*, *Diolcogaster*, *Dolichogenidea*, *Glyptapanteles*, *Hypomicrogaster, Microgaster*, *Microplitis, Pholetesor*
		Miracinae	*Mirax*
Ichnovirus (IV)	Ichneumonidae	Campopleginae	*Campoletis, Campoplex, Dusona, Hyposoter*, *Sinophorus, Venturia*
Banchovirus	Ichneumonidae	Banchinae	*Banchus, Glypta*

Table 1. Wasp families and genera known to harbor polydnaviruses. From Whitfield & O'Connor (2012).

A description of the life cycle and transmission process of a typical PDV can be started with the developing wasp pupa as well as at any other point in the cycle. As noted, PDVs have a transmission cycle unlike that of any other pathogen, and it has even been argued that they may not truly fit the definition of an insect virus (Whitfield & Asgari, 2003; Stoltz & Whitfield, 2009). They exist as proviral DNA integrated into the genome of the carrier wasp species (both male and female), and are "free-living" only during reproduction in female wasp ovarian calyx tissue, or after injection into the host of the carrier wasp. The injected PDV does not reproduce; it appears that some PDVs even lack the genes needed for self-assembly (Bézier et al., 2009). The PDV is at a virtual dead end, except that it allows for the

reproduction of its genome in the developing wasp larva by blocking host immune responses that would otherwise encapsulate and kill it. By the same token, for most if not all carrier wasps, it is likely that successful parasitism is impossible if the polydnavirus is removed from the system: indeed this has been shown explicitly in a few systems (Edson et al., 1981; Stoltz & Guzo, 1986). The large, segmented, double-stranded PDV is excised from the wasp genome only in later pupal and adult stages, and only in female wasps (Gruber et al., 1996; Pasquier-Barre et al., 2002; Kroemer & Webb, 2006). The exact process by which this occurs has only recently been elucidated for some polydnaviruses (Annaheim & Lanzrein, 2007; Bezier et al., 2009), although, as stated previously, it is believed that some PDVs, particularly the bracoviruses, rely on the wasp host for assembly. Virus particles are assembled in the nuclei of calyx cells and accumulate in the lumen of the oviduct.

When the female wasp has emerged and is sufficiently mature, she searches for a suitable host to parasitize (Fig. 2A). During a sting event, the PDV is injected into the wasp's host along with additional proteins, toxin, and parasite eggs (Fig. 2B) (Pennachio & Strand, 2006). Once in the larval host, polydnavirus genes produce proteins that degrade the host's immune response and prevent encapsulation of the parasitoid egg or larva (Fig. 2C) (Stoltz et al. 1988). This is done in concert with other injected materials such as toxins. *Cotesia melanoscela* bracovirus (CmeBV), for example, acts in concert with wasp-produced venom on hemocytes in parasitized gypsy moth larvae (Guzo & Stoltz, 1985; Stoltz et al., 1988; Summers & Dib-Hajj 1995; Nalini et al., 2008). Among the best understood effects of PDVs on lepidopteran larvae are those occurring within the hemocytes of parasitized larvae.

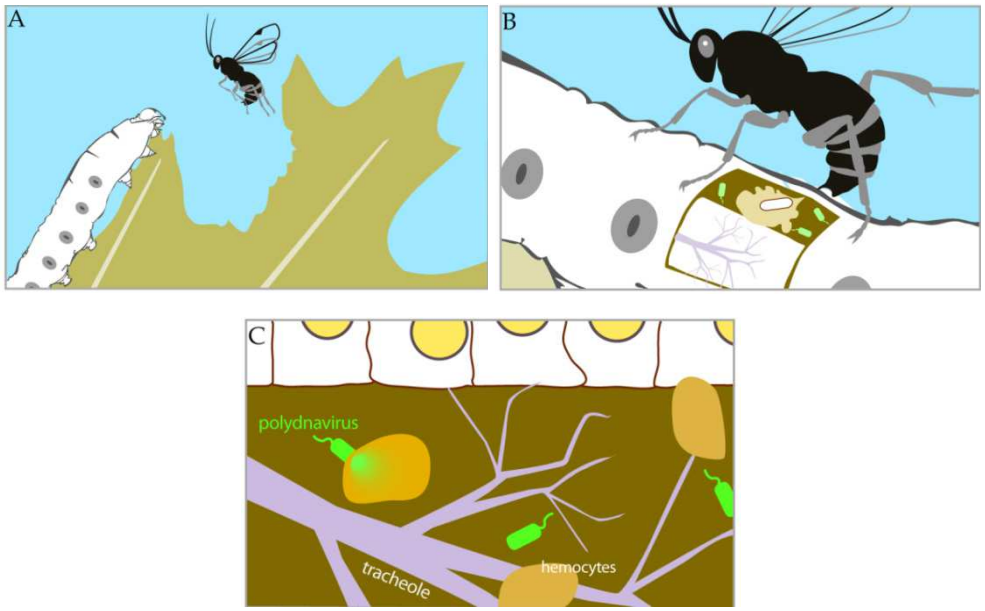


Fig. 2. Polydnavirus transmission. (A) Parasitoid wasps use series of complex cues to find and assess the condition of a host. (B) Larval Lepidoptera are injected with polydnavirus, egg, and toxin. (C) Immunosuppression of larval host occurs, primarily through PDV action on hemocytes.

If PDVs were known only for their ability to prevent encapsulation of foreign bodies, they would rightfully be the subjects of intense research. However, the immunosuppressive activity of polydnaviruses has been shown to extend to effects on baculovirus pathogenesis. This was first shown explicitly by Washburn et al. (1996), who showed that parasite injection of the *Campoletis sonorensis* ichnovirus led to immunosuppression that resulted in a more rapid spread of the AcMNPV in *H. zea* larvae. This effect was attributed to PDV-mediated abrogation of the hemocytic response as pertains to recognition of altered-self: *H. zea* is believed to clear some AcMNPV infections by encapsulating its own infected cells. This phenomenon, of special interest to the microbial ecologist, will be explored in greater detail below. So far as naturally-occurring interactions between the viruses are concerned, they are the most likely to be relevant.

4. Field interactions between polydnaviruses and nucleopolyhedroviruses

We have seen that parasitoids both produce and transmit polydnaviruses in order to suppress the immune system of their caterpillar host, and are thus able to successfully reproduce within it. In many cases baculoviruses and polydnaviruses require equivalent and possibly incompatible resources from the host, if they occur in the same host species (Caballero et al., 1991; Hochberg, 1991a). For example, LdMNPV and *C. melanoscela* are commonly encountered mortality agents of the gypsy moth in field populations in eastern United States. The timing of mortality caused by these agents overlaps completely (Woods et al., 1991; Crossman, 1922). Both enter a host during its larval stage and convert some or most of the host biomass into virus particles (baculoviruses), or aid in its conversion to a parasitoid (polydnaviruses). If a baculovirus kills or physiologically degrades a larva past a certain point, it will no longer sustain a larval parasitoid. If a parasitoid kills its host outright, as most do, baculovirus pathogenesis will be stopped at the point of host death. This would result in reduced or absent production and release of occluded virus, which is necessary to transmit the baculovirus to new hosts. Theoretically, then, a conflict arises whenever polydnaviruses and baculoviruses share a host. How may these organisms interact? Key to our exploration of this question is an analysis of the steps and barriers facing baculoviruses and polydnaviruses, and where these stages of infection overlap and are subject to interference or assistance from each other. To aid in this we will again refer to previous figures (Figs. 1, 2) mapping out both the baculovirus and polydnavirus infection processes, and dividing each process into distinct stages. At some of these stages the viruses are unlikely to influence the progression of one another, while there is great potential for them to do so at other stages. At each stage, the possibilities for interference from the other infection process will be discussed, if applicable. Where necessary for illustrative purposes we use a generalized MNPV, and a bracovirus such as those known from the braconid genus *Cotesia*. This will allow a reasonably cohesive discussion while we clearly recognize the diversity of both virus types.

4.1 Parasitoid selection of suitable host and injection of polydnavirus

Infection with a polydnavirus requires selection of a host by a wasp carrier and an attempt at parasitization of a larvae. Whether or not this selection process is affected by prior infection with a baculovirus is a question that has been explored a number of times in the

past. Some studies have shown parasitoid avoidance of baculovirus infected hosts (e.g. Levin et al., 1983), while others found no effects on parasitoid preference (e.g. Sait et al., 1996). Raimo et al. (1977) showed that *C. melanoscela* readily parasitizes LdMNPV-infected larvae, at least in a laboratory setting. This particular braconid is of some importance in the literature, having also been used in some of the earliest studies of PDVs (Stoltz et al., 1976; Stoltz & Vinson, 1977; Guzo & Stoltz, 1985). In all cases, the length of time since infection began is an important component of parasitoid discrimination. For example, it is unlikely that *C. melanoscela* can detect infection before it produces overt behavioral or physiological changes, by which time the larvae in the field may be three or four days into the infection process. Indeed, in their work on the question of parasitoid discrimination using *C. melanoscela*, Versoi & Yendol (1982) felt it necessary to use infected larvae that were so moribund that they "could not right themselves when turned over".

4.2 Ingestion of polyhedra and release of virions

If full-blown infection by an NPV is likely to deter a parasitoid and thereby prevent PDV infection, a similar phenomenon can occur in the opposite direction. The first stage of infection of a lepidopteran host by an NPV is ingestion of virus-contaminated plant material. It may be argued that this process can be influenced by polydnavirus infection before it has a chance to occur. Larvae that have been stung by a carrier wasp suffer from a range of physiological effects caused by wasp-injected materials. One of these materials is the polydnavirus, and it has been shown that polydnavirus effects on hosts may occur in the presence or absence of a parasitoid egg (Strand & Dover, 1991; Beckage et al. 1994; Fathpour & Dahlman, 1995; Shelby & Webb, 1997). Most relevant of these effects is the arrested development and changes in eating habits seen in parasitized larvae; an unsurprising side effect of toxification, immunosuppression, and hormonal manipulation. The chance of baculovirus infection subsequent to parasitization will be reduced if food consumption decreases, in that the risk of infection for nucleopolyhedroviruses is directly related to the amount (leaf area) of foliage eaten (Dwyer & Elkinton, 1993; Dwyer et al., 2005), and the converse is also true. The amount of food present in the midgut could have an impact on the release of virions from the polyhedral occlusion body as well. Dissolution of the polyhedron is a pH-dependent event that occurs in the highly alkaline larval midgut. Polydnavirus infection is unlikely to *directly* influence midgut pH or the release of virions from polyhedra, but the presence or absence of plant material can do so (Keating et al., 1988; Rossiter et al., 1988). There may be no true competitive component to this aspect of polydnavirus interference with baculovirus transmission in evolutionary terms. However, it significantly influences subsequent infection risk for the parasitized larvae, and thus the parasitoid larvae as well. This is important when assessing the overall probability of both viruses occurring in the same host at the same time.

Might carrier wasps themselves increase the risk of baculovirus ingestion and infection? Parasitoid wasps have been acknowledged as having a role in the field dissemination of baculoviruses. The limited research that exists on this topic has focused particularly on the baculoviruses that infect lepidopteran hosts (Kurstak & Vago, 1967; Hochberg 1991a; 1991b; reviewed by Cossentine, 2009) for reasons of practicality and economic importance already discussed. These baculovirus infections in the field typically occur via ingestion of OBs on foliage, although Raimo et al. (1977) and others have noted that parasitoids can themslves

act as a physical vectors of NPVs. These studies found that contamination of the ovipositor occurs when a wasp stings larvae, resulting in NPV infections in subsequently stung larvae. Early work (e.g. Raimo et al., 1977) did not always compare NPV-induced mortality between NPV + parasitization and NPV-only treatments, partly because such comparisons would have been of special interest only if polydnaviruses had been recognized as causing immunosuppression of the host at that time. When a nucleopolyhedrovirus is present in caterpillar larvae or on their food, parasitoid-mediated transmission of the NPVs could also occur in a number of other ways through contamination of and transfer from the parasitoid's body (Raimo et al., 1977; Young & Yearian, 1989; 1990; Hochberg, 1991a, 1991b). Yet another possibility was raised by Stoltz & Makkay (2003), who found strong circumstantial evidence that latent viruses in *T. ni* larvae could be activated after parasitism by the ichneumonid wasp *Hyposoter exiguae*, which is also known to harbor a polydnavirus (Stoltz & Makkay, 2000). None of these except for the last can be considered direct viral interactions, but they are nevertheless noteworthy.

4.3 Penetration of the peritrophic membrane

As described in Section 2, virions freed from the dissolved occlusion body must penetrate the peritrophic membrane in order to initiate infection of the insect by a baculovirus. Movement through the peritrophic membrane is thought to be facilitated by metalloproteinases termed enhancins (Slavicek, this volume). Enhancins are located within the ODV membrane in the LdMNPV (Slavicek & Popham, 2005) or are co-occluded in the granule of granuloviruses (Wang et al., 1994; Lepore et al., 1996). However, most baculoviruses lack enhancin genes; consequently other means that have yet to be elucidated are used to traverse the peritrophic membrane. Effects of ingestion-related changes to the midgut discussed in 4.2, such as changes in pH, could also influence the penetration of the membrane by virions. This hypothetical effect of gut contents or pH on the post-release penetration of the peritrophic membrane has not been explored and may be noteworthy; otherwise we feel that this stage of infection is not likely to be directly influenced by polydnavirus infection.

4.4 Spread of infection in the larval host

Once through the peritrophic membrane, NPVs infect midgut epithelial cells by binding to the cell membrane and releasing nucleocapsids into the cytoplasm. At this stage of infection, one of the strategies used by larvae to avoid infection is through apoptosis of infected midgut cells (Clarke & Clem, 2003; da Silveira et al., 2005). It has been shown that the PDV associated with *Micropletis demolitor*, the bracovirus MdBV, induces apoptosis in primary hemocytes (Strand & Pech, 1995). Recent research has shown that in some cases, however, PDV-derived proteins can inhibit baculovirus-induced apoptosis of insect cells (Kroemer & Webb, 2006), apparently via suppression of caspase activity. This is currently of interest to researchers wishing to explore methods of improving baculovirus efficacy via PDV genes. Inhibited apoptosis of midgut epithelial cells is not likely to be altering transmission probabilities in the field; however, expression of PDV has not been shown in this cell type. Budded virus leaving infected midgut cells infects other cells, and this stage is the most likely point at which the nucleopolyhedrovirus infection process could be be influenced by the action of a polydnavirus. At this point in the infection process the immune response is

most likely to have a chance to clear an NPV infection, although by no means has this been shown to occur in all NPV-host systems studied.

Experiments done using injections of budded virus have produced different results. Three recent studies using budded virus injection (Rivkin et al., 2006; McNeil et al., 2010a; 2010b) all found some evidence for increased virulence when larvae were previously infected with a polydnavirus. This can be interpreted as resulting from the inability of compromised hemocytes to encapsulate foci of infection. Failed encapsulation of virus-infected cells may explain why some normally refractory larvae exhibit different pathogenesis when infected with a PDV. However, Trudeau et al. (2001) reported that a more important mechanism of resistance in *H. zea* was the unsuitability of hemocytes for infection; budded virus (BV) entered hemocytes but was unable to replicate within them, so that they served as "sinks" for BV. A similar result was obtained by Rivkin et al. (2006), who showed that the hemocytes of *S. littoralis* larvae are particularly resistant to infection by AcMNPV, and that this is an important component of resistance in this insect.

One possibility explaining these results is that unimpaired larval hemocytes could effectively encapsulate a very small number of NPV-produced foci as would be produced in the study by McNeil et al. (2010a), which used only a few budded virus particles to initiate infection, but could not encapsulate the far greater number that occur in an infection initiated *per os*. While exact numbers of foci created in typical *per os* infections are not known, analysis of budded virus production has shown that Ld652Y (*L. dispar*) cells in culture produce from approximately 15 to 125 budded virus particles per infected cell, depending on the viral isolate used (Slavicek et al., 1996; Slavicek et al., 2001). This would quickly result in an enormous number of budded virus particles in the hemocoel. The McNeil et al. (2010a) study methodology may have an analog in the field; however, if a wasp transmits NPV on a contaminated ovipositor as suggested by Raimo et al. (1977), it is likely by transferring very small amounts of budded virus from previously parasitized larvae that were already infected.

Research has been also done in granulovirus-parasitoid systems (Matthews et al., 2004; Santiago-Alvarez & Caballero, 1990), and in studies that used NPVs that are normally not found in the hosts used (Washburn et al., 2000). These are not considered here. In the experiments on normally permissive hosts (Beegle & Oatman, 1974; Raimo et al., 1977; Eller et al., 1988; Murray et al. 1995; Escribano et al., 2000; 2001) that explored polydnavirus interactions with baculovirus challenge, or can be inferred as having done so, none found clear positive effects of coinfection on baculovirus-caused mortality. All of those studies used a *per os* challenge, and are described in greater detail in Section 5 below.

5. PDV-NPV experiments using *per os* nucleopolyhedrovirus

Of primary interest to researchers of field interactions between PDVs and NPVs, are those few experiments that have used *per os* techniques to infect parasitized larvae with NPVs. This technique can be considered the most accurate representation of naturally-occurring exposure to both viruses, and we therefore have given more detailed information on these experiments below. All parasitoid names are followed by parentheses containing the name of the associated PDV.

In an early comparison of NPV mortality in parasitized and unparasitized larvae, Beegle & Oatman (1974) used the *T. ni* MNPV, the parasitoid *Hyposoter exiguae* (+HeIV), and *T. ni* caterpillar larvae. In this experiment, larvae were given a range of doses of TnMNPV *per os* immediately following parasitization. Ld_{50} values for parasitized larvae were approximately twice as high as those for unparasitized larvae (3.16×10^3 vs 1.58×10^3), and LD_{95} values five times higher (7.18×10^4 vs. 1.43×10^4). The format of this experiment can be considered a reasonable representation of field conditions: *per os* acquisition of NPV after infection with a PDV via a stinging event. Subsequent experiments will be shown to bear out such results for *per os* experiments; no increase in pathogenicity of NPVs coincident with PDVs, and typically some effect in the opposite direction. In another experiment conducted before PDVs were fully recognized, Raimo et al. (1977) explored the ability of a parasitoid wasp to physically vector an NPV. This experiment, using *C. melanoscela* (+CmBV), *L. dispar*, and LdMNPV, was not designed to look at the effects of parasitization on NPV mortality. However, in the course of the study a small amount of data was collected that allows for a comparison of virus-caused mortality of unparasitized and parasitized larvae. These larvae were reared from surface-sterilized field-collected eggs. Natural mortality caused by virus in larvae not exposed to parasites was 3.8%. Of larvae exposed to uncontaminated parasites for 2 hours and 24 hours, 9.5 % and 10.0%, respectively, died of virus. We note that this evidence is suspect, considering the possibility of contamination inherent in the use of field-collected material. We also note, however, the possibilities raised by Stoltz & Makkay (2003), of a PDV producing overt infections of a latent baculovirus. This phenomenon has been observed more often in field-collected insects than in laboratory colonies.

The study by Eller et al. (1988) compared mortality in *M. croceipes* (+McBV) parasitized and unparasitized *H. zea* larvae challenged *per os* with HzMNPV. A dose of 15,000 OBs of virus was delivered 0, 1, 2, and 4 d after parasitization (and 6, 7, 8, and 10 days after hatching). In all treatments, parasitization had no significant effects on the HzMNPV-caused mortality of larvae.

In a study that used *Chelonus insularis* (+CinsBV) to parasitize *S. frugiperda* larvae that were then challenged with SfMNPV, Escribano et al. (2000) compared SfMNPV mortality in parasitized larvae to that in virus-challenged unparasitized larvae. The LC_{50} for all instars was higher for parasitized larvae; 2nd instars (1.93×10^5 vs. 1.46×10^5 OBs/ml), 3rd instars (1.15×10^6 vs. 6.03×10^5 OBs/ml), and 4th instars (5.34×10^6 vs. 3.24×10^6 OBs/ml). The parasitized larvae in this case were exposed to *C. insularis* as eggs, which raises serious questions as to the continued presence of CinsBV in the 2nd, 3rd, and 4th instars. In Escribano et al., (2001), using the same agents as in Escribano et al. (2000), parasitized and unparasitized larvae were given the previously determined LC_{90} of SfMNPV. Although not designed to compare virus mortality, the experiment showed no significant differences in mortality between the groups (greater than 95% in both).

Three different parasitoid treatments were used in an experiment by Murray et al., (1995). In this experiment, *M. demolitor* (+MdBV), *C. kazak* (+CkBV) *H. didymator* (+HdIV), were used to parasitize larvae of *Helicoverpa armigera*. At 0 or 2 d post-parasitization these larvae were dosed with HzMNPV applied to the surface of artificial diet, in a 5-dose range from 2 to 6,000 OB/mm2 of diet. In all cases, LD_{50} values were either the same (0 d treatment) or greater (2 d treatment) for parasitized larvae.

D'Amico et al., in review. In a small experiment designed to explore the effects of *C. melanoscela* (+CmBV) parasitization on LdMNPV mortality in gypsy moth larvae, we dosed larvae *per os* with LdMNPV 2 d pre- and 2 d post-parasitization with a range of doses. In all cases, no significant differences were found between larvae that were parasitized and those that were not. We consider it unlikely that changes in CmBV treatments to longer times pre- or post- *per os* LdMNPV challenge would change the amount of LdMNPV-caused mortality in this type of experiment. Both the LdMNPV and CmeBV infection processes have passed critical stages after 48 hours or sooner, if movement of budded LdMNPV out of midgut cells (McNeil et al., 2010a) or the presence of CmeBV-coded products (Guzo & Stoltz, 1985) are considered indicators of success for these viruses in this host. Changes in timing could, however, negatively affect the success of *C. melanoscela* parasitism as the quality of the gypsy moth larval host is compromised by LdMNPV infection.

6. Conclusions

For many larval Lepidoptera, parasitism by a polydnavirus-carrying wasp or infection by a nucleopolyhedrovirus are among the top mortality factors in the field. It is also clear that by necessity, parasitoid wasps and their PDVs compete with NPVs for the larval resource. It will require focused research in this area, however, to elucidate the role played by interactions between polydnaviruses and nucleopolyhedroviruses. In the few studies that have been done, there is no obvious indication that concurrent PDV and NPV-infection leads to greater NPV mortality in lepidopteran larvae challenged with NPVs with which they are normally associated. This is true despite the widely-accepted range of immunosuppressive effects of PDV on the host insect. There is some evidence for the opposite effect, which makes good competitive sense: if a parasitized host continues to be exposed to risk of NPV infection, it is in the interest of the parasitoid to reduce the chance of host death prior to successful maturation of the parasitoid progeny.

7. Abbreviations

NPV - nucleopolyhedrovirus
OB – occlusion body
ODV – occlusion-derived virus
PO - phenoloxidase
GLD - glucose dehydrogenase
PDV - polydnavirus
BV – bracovirus
IV - ichnovirus

8. References

Annaheim, M, & Lanzrein, B. 2007. Genome organization of the *Chelonus inanitus* polydnavirus: excision sites, spacers and abundance of proviral and excised segments. J. Gen Virol., 88: 450-457.

Banks, J.C. & Whitfield, J.B. 2006. Dissecting the ancient rapid radiation of microgastrine wasp genera using additional nuclear genes. Mol. Phylogenet. Evol., 41: 690–703.

Beckage, N. B., Tan, F., Schleifer, K. W., Lane, R. D. & Cherubin, L. L., 1994. Characterization and biological effects from *Cotesia congregata* polydnavirus on host larvae of the tobacco hornworm, *Manduca sexta*. Arch. Insect. Biochem., 26: 165-195.

Beegle, C.C. & Oatman, E.R. 1974. Differential susceptibility of parasitized and nonparasitized larvae of *Trichoplusia ni* to a nuclear polyhedrosis virus. J. Invertebr. Pathol. 24: 188-195.

Belle, E., Beckage, N.E., Rousselet, J. Poirié, M., Lemeunier, F. & Drezen, J.-M. 2002. Visualization of polydnavirus sequences in a parasitoid wasp chromosome. J. Virol., 76: 5793–5796

Bézier, A, Herbinière, J, Lanzrein, B. & Drezen, J.M. 2009. Polydnavirus hidden face: The genes producing virus particles of parasitic wasps. J. Inv. Pathol., 101: 194-203

Cabellero, P., Vargas-Osuna, E. & Santiago-Alvarez, C. 1991. Parasitization of granulosis virus-infected and noninfected *Agrotis segetum* larvae and the virus transmission by three hymenopteran parasitoids. Entomol. Exp. Appl. s58: 55-60.

Clarke, T. E. & Clem, R. J. 2003. *In vivo* induction of apoptosis correlating with reduced infectivity during baculovirus infection. J. Virol., 77: 2227–2232.

Clem, R. J. 2005. The role of apoptosis in defense against baculovirus infection in insects. Curr. Top. Microbiol. Immunol., 289: 113–129.

Cook, D.I. & Stoltz, D.B., 1983. Comparative serology of viruses isolated from ichneumonid parasitoids. Virology 130: 215–220.

Cory, J.S. & Myers, J.H., 2003. The ecology and evolution of insect baculoviruses, Annu. Rev. Ecol. Syst. 34: 239–272.

Cossentine, J.E. 2009. The parasitoid factor in the virulence and spread of lepidopteran baculoviruses. Virologica Sinica 24: 305-314.

Cox-Foster, D.L. & Stehr, J.E. 1994. Induction and localization of FAD-glucose dehydrogenase (GLD) during encapsulation of abiotic implants in *Manduca sexta* larvae. Journal of Insect Physiology 40: 235–249.

Crossman, S. S., 1922. *Apanteles melanoscelus*, an imported parasite of the gipsy [sic] moth. USDA Bulletin 1028, Washington, D. C.

Crouch, E.A., Cox, L.T., Morales, K.G. & Passarelli, A.L. 2007. Inter-subunit interactions of the *Autographa californica* M nucleopolyhedrovirus RNA polymerase. Virology 367: 265–274.

da Silveira, E. B., Cordeiro, B. A., Ribeiro, B. M. & Bao, S. N. 2005. In vivo apoptosis induction and reduction of infectivity by an *Autographa californica* multiple nucleopolyhedrovirus p352 recombinant in hemocytes from the velvet bean caterpillar *Anticarsia gemmatalis* (Lepidoptera: Noctuidae). Res Microbiol 156: 1014–1025.

d'Alencon, E., Piffanelli, P., Volkoff, A.N., Sabau, X., Gimenez, S., Rocher, J., Cerutti, P. & Fournier, P. 2004. A genomic BAC library and a new BAC-GFP vector to study the holocentric pest *Spodoptera frugiperda*. Insect Biochem Mol Biol. 34: 331–41.

David, W.A.L. 1975. The status of viruses pathogenic for insects and mites. Annu. Rev. Entomol. 20: 97-117.

Dougherty E.M., Narang N., Loeb M., Lynn D.E. & Shapiro M. 2006. Fluorescent brightener inhibits apoptosis in baculovirus-infected gypsy moth larval midgut cells. Biocontrol Sci. Technol. 16: 157–168

Dwyer, G. & J.S. Elkinton. 1993. Using simple models to predict virus epizootics in gypsy-moth populations. J. of Anim. Ecol., 62: 1-11.

Dwyer, G., Firestone J., & Stevens T.E. 2005. Should models of disease dynamics in herbivorous insects include the effects of variability in host-plant foliage quality? Am. Nat., 165: 16-31.

Edson, K.M., Vinson, S.B., Stoltz, D.B. & Summers M.D. 1981. Virus in a parasitoid wasp: suppression of the cellular immune response in the parasitoid's host. Science 211: 582-583.

Eller, F. J., Boucias, D. G. & Tumlinson, J. H. 1988. Interactions between *Microplitis croceipes* (Hymenoptera:Braconidae) and a nuclear polyhedrosis virus of *Heliothis zea* (Lepidoptera: Noctuidae). Environ. Entomol., 17: 977- 982.

Engelhard, E. K. & Volkman, L. E. 1995. Developmental resistance in 4th instar *Trichoplusia ni* orally inoculated with *Autographa californica* M nuclear polyhedrosis virus. Virology 209: 384–389.

Engelhard, E. K., Kammorgan, L. N. W., Washburn, J. O. & Volkman, L. E. 1994. The insect tracheal system: a conduit for the systemic spread of *Autographa californica* M nuclear polyhedrosis virus. Proc Natl Acad Sci USA 91: 3224–3227.

Erlandson, M. 2008. Insect pest control by viruses. Encyclopedia of Virology, Third Edition, 3: 125-133.

Escribano, A., Williams, T., Goulson, D., Cave, R.D. & Caballero, P., 2000. Parasitoid–pathogen–pest interactions of *Chelonus insularis*, *Campoletis sonorensis*, and a nucleopolyhedrovirus in *Spodoptera frugiperda* larvae. Biol. Control, 19: 265–273.

Escribano, A., Williams, T., Goulson, D., Cave, R.D., Cha peritrophic membrane an, J.W. & Caballero, P. 2001. Consequences of interspecific competition on the virulence and genetic composition of a nucleopolyhedrovirus in *Spodoptera frugiperda* larvae parasitized by *Chelonus insularis*. Biocontrol, 11: 649-662.

Fang, M., Nie, Y., Harris, S., Erlandson, M.A. & Theilmann, D.A. 2009. *Autographa californica* multiple nucleopolyhedrovirus core gene ac96 encodes a *per os* infectivity factor (PIF-4). J. Virol. 83: 12569–12578.

Fang, M., Nie, Y., Wang, Q., Deng, F., Wang, R., Wang, H., Wang, H., Vlak, J.M., Chen, X. & Zu, Z. 2006. Open reading frame 132 of *Helicoverpa armigera* nucleopolyhedrovirus encodes a functional per os infectivity factor (PIF-2). J. Gen Virol. 87: 2563–2569.

Fathpour, H. & Dahlman, D.L. 1995. Polydnavirus of *Microplitis croceipes* prolongs the larval period and changes hemolymph protein content of the host, *Heliothis virescens*. Arch Insect Biochem Physiol 28: 33–48.

Faulkner, P., Kuzio, J., Williams, G.V. & Wilson, J.A. 1997. Analysis of p74, a PDV envelope protein of Autographa californica nucleopolyhedrovirus required for occlusion body infectivity in vivo. J. Gen. Virol. 78: 3091–3100.

Fleming, J.G.W. & Summers, M.D., 1991. Polydnavirus DNA is integrated into the DNA of its parasitoid host wasp. Proc. Natl. Acad. Sci. 88: 9770-9774.

Fleming, J.G.W. & Summers, M.D., 1986. *Campoletis sonorensis* endoparasitic wasps contain forms of *C. sonorensis virus* DNA suggestive of integrated and extrachromosomal polydnavirus DNAs. J. Virol. 57: 552–562.

Flipsen, J.T., Martens, J.W., vanOers, M.M., Vlak, J.M. & van Lent, J.W. 1995. Passage of *Autographa californica* nuclear polyhedrosis virus through the midgut epithelium of *Spodoptera exigua* larvae. Virology 208: 328–35.

Granados, R.R. & Lawler, K.A. 1981. In vivo pathway of *Autographa californica* baculovirus invasion and infection. Virology 108: 297-308.

Gruber, A., Stettler, P., Heineger, P., Schumpli, D., & Lanzrein, B. 1996. Polydnavirus DNA of the braconid wasp *Chelonus inanitus* is integrated in the wasp genome and excised only in later pupal and adult stages of the female. J. Gen. Virol. 77, 2873–2879.

Guzo, D. & Stoltz, D. B., 1985. Obligatory multiparasitism in the tussock moth, *Orgyia leucostigma*. Parasitology 90, 1-10.

Haas-Stapleton, E. J., Washburn, J. O. & Volkman, L. E. 2003. Pathogenesis of *Autographa californica* M nucleopolyhedrovirus in fifth instar *Spodoptera frugiperda*. J. Gen Virol 84: 2033–2040.

Harrap, K.A. & Payne, C.C. 1979. The structural properties and identification of insect viruses. Adv. Virus Res. 25:273-355.

Harrison R.L., Sparks W.O. & Bonning B.C. 2010. *Autographa californica* multiple nucleopolyhedrovirus ODV-E56 envelope protein is required for oral infectivity and can be substituted functionally by *Rachiplusia ou* multiple nucleopolyhedrovirus ODV-E56. J. Gen Virol. 91:1173–1182.

Hegedus, D., Erlandson, M., Gillott, C. & Toprak, U. 2009. New insights into peritrophic matrix synthesis, architecture, and function. Annu Rev Entomol. 54: 285–302.

Herniou, E.A. & Jehle, J.A. 2007. Baculovirus phylogeny and evolution. Curr. Drug Targets 8: 1043-50.

Hochberg, M.E., 1991a. Intra-host interactions between a braconid endoparasitoid, *Apanteles glomeratus*, and a baculovirus for larvae of *Pieris brassicae*. J. Anim. Ecol., 60: 51-63.

Hochberg, ME. 1991b. Extra-Host interactions between a braconid endoparasitoid, *Apanteles glomeratus*, and a baculovirus for larvae of *Pieris brassicae* J. Anim. Ecol. 60: 65-77.

Hoover, K., Grove, M. J. & Su, S. Z. 2002. Systemic component to intrastadial developmental resistance in *Lymantria dispar* to its baculovirus. Biol Control 25: 92–98.

Horton H.M. & Burand J.P. 1993. Saturable attachment sites for polyhedron-derived baculovirus on insect cells and evidence for entry via direct membrane fusion. J. Virol. 67: 1860–1868.

Jehle, J.A., Lange, M., Wang, H., Zhihong, H., Wang, Y. & Hauschild, R. 2006. Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera. Virology 346: 180-193.

Jiravanichpaisal, P., Lee, B.L. & Soderhall, K. 2006. Cell-mediated immunity in arthropods: hematopoiesis, coagulation, melanization and opsonization. Immunobiology 211: 213–236.

Keating, S. T., Yendol, W.G. & Schultz, J.C.. 1988. Relationship between susceptibility of gypsy moth larvae (Lepidoptera: Lymantriidae) to a baculovirus and host plant foliage constituents. Environmental Entomology 17: 952-958.

Keddie B.A., Aponte G.W. & Volkman L.E. 1989. The pathway of infection of *Autographa californica* nuclear polyhedrosis virus in an insect host. Science. 243: 1728–1730.

Kikhno, I., Gutierrez, S., Croizier, L., Croizier, G. & Ferber, M.L. 2002. Characterization of pif, a gene required for the per os infectivity of *Spodoptera littoralis* nucleopolyhedrovirus. J. Gen Virol. 83: 3013–3022.

Kroemer, J.A. & Webb, B.A. 2006 Divergences in protein activity and cellular localization within the *Campoletis sonorensis* ichnovirus Vankyrin family. J. Virol. 80: 12219–12228.

Kurstak, E. & Vago, C., 1967. Transmission dun virus de la densonucleose par la parasitisma d'un hymenoptere. Rev. Can. Biol. 26: 311-316.

LaPointe, R., Tanaka, K., Barney, W.E., Whitfield, .JB., Banks, J.C., Beliveau, C., Stoltz, D., Webb, B.A. & Cusson, M. 2007. Genomic and morphological features of a banchine polydnavirus: Comparison with Bracoviruses and Ichnoviruses. J. Virol 81: 6491–9501.

Lee,M., Yoon, C.S., Yi, J., Cho, J.R. & Kim, H.S. 2005. Cellular immune responses and FAD-glucose dehydrogenase activity of *Mamestra brassicae* Lepidoptera: Noctuidae) challenged with three species of entomopathogenic fungi. Physiological Entomology 30: 287–292.

Lepore, L. S., Roelvink, P.R. & Granados, R.R. 1996. Enhancin, the granulosis virus protein that facilitates nucleopolyhedrovirus (NPV) infections, is a metalloprotease. *J. Invertebr. Pathol.* 68: 131-140.

Levin, D. B., Laing, J. E., Jaques, R. P. & Corrigan, J. E., 1983. Transmission of the granulosis virus of *Pieris rapae* (Lepidoptera: Pieridae) by the parasitoid *Apanteles glomeratus* (Hymenoptera: Braconidae). Environ. Entomol. 12: 166–170.

Li, X., Song, J., Jiang, T., Liang, C. & Chen, X. 2007. The N-terminal hydrophobic sequence of Autographa californica nucleopolyhedrovirus PIF-3 is essential for oral infection. Arch Virol. 152: 1851–1858.

Lovallo, N. & Cox-Foster, D.L. 1999. Alteration in FAD-glucose dehydrogenase activity and hemocyte behavior contribute to initial disruption of *Manduca sexta* immune response to *Cotesia congregata* parasitoids. Journal of Insect Physiology 45: 1037–1048.

Lovallo, N.C., McPherson, B.A. & Cox-Foster, D.L., 2002. Effects of the polydnavirus of *Cotesia congregata* on the immune system and development of non-habitual hosts of the parasitoid, J. Insect Physiol. 48: 517–526.

Matthews H J, Smith I, & Bell H A. 2004. Interactions between the parasitoid *Meteorus gyrator* (Hymenoptera:Braconidae) and a granulovirus in *Lacanobia oleracea* (Lepidoptera: Noctuidae). Environ. Entomol., 33: 949-957.

McNeil, J., Cox-Foster, D., Slavicek, J. & Hoover, K., 2010a. Contributions of immune responses to developmental resistance in *Lymantria dispar* challenged with baculovirus. J. Insect Physiol., 56: 1167-1177.

McNeil, J., Cox-Foster, D., Gardner, M., Slavicek, J., Thiem, S, & Hoover, K. 2010b. Pathogenesis of *Lymantria dispar* multiple nucleopolyhedrovirus in *L. dispar* and mechanisms of developmental resistance. J. Gen. Virol. 91: 1590-1600.

Murphy, N., Banks, J.C., Whitfield, J.B. & Austin, A.D., 2008. Phylogeny of the microgastroid complex of subfamilies of braconid parasitoid wasps (Hymenoptera) based on sequence data from seven genes, with an improved estimate of the time of origin of the lineage. Mol. Phylogen. Evol. 47: 378–395.

Moscardi, F. 1999. Assessment of the application of baculoviruses for control of Lepidoptera. Annu. Rev. Entomol. 44: 257-289.

Murray, D.A., Monsour, C.J. & Teakle R.E., 1995. Interactions between nuclear polyhedrosis virus and three larval parasitoids of *Helicoverpa armigera* (Hübner). J. Aust. Ent. Soc., 34: 319-322.

Nalini, M. et al., Choi, J.Y., Je, Y.H., Hwang, I. & Kim, Y., 2008. Immunoevasive property of a polydnaviral product, CpBV-lectin, protects the parasitoid egg from hemocytic encapsulation of *Plutella xylostella* (Lepidoptera: Yponomeutidae). J. Insect Physiol. 54: 1125-1131.

Nappi, A.J. & Christensen, B.M. 2005. Melanogenesis and associated cytotoxic reactions: Applications to insect innate immunity. Insect Biochemistry and Molecular Biology 35: 443–459.

Ohkawa, T., Volkman, L.E. & Welch M.D. 2010. Actin-based motility drives baculovirus transit to the nucleus and cell surface. J. Cell Biol.190: 187–95.

Ohkawa, T., Washburn,J.O., Sitapara, R., Sid, E. & Volkman, L.E. 2005. Specific binding of *Autographa californica* M nucleopolyhedrovirus occlusion-derived virus to midgut cells of *Heliothis virescens* larvae is mediated by products of pif genes Ac119 and Ac022 but not by Ac115. J. Virol. 79: 15258–15264.

Pasquier-Barre, F., Dupuy, C., Huguet, E., Moneiro, F. & Moreau, A. 2002. Polydnavirus replication: The EP1 segment of the parasitoid wasp *Cotesia congregata* is amplified within a larger precursor molecule. J. Gen. Virol. 83: 2035–45

Peng, K., van Oers, M.M., Hu, Z., van Lent, J.W. & Vlak, J.M. 2010. Baculovirus per os infectivity factors form a complex on the surface of occlusion-derived virus. J. Virol. 84: 9497–9504.

Pennacchio, F. & Strand, M. R. 2006. Evolution of developmental strategies in parasitic Hymenoptera. Annu. Rev. Entomol. 51: 233 -258.

Pritchett, D. W., Young, S. Y. & Yearian, W. C. 1984. Some factors involved in the dissolution of *Autographa californica* nuclear polyhedrosis virus polyhedra by digestive fluids of *Trichoplusia ni* larvae. J. Invertebr. Pathol. 43: 160–168.

Raimo, B., Reardon R. C. & Podgwaite, J. D., 1977. Vectoring gypsy moth nuclear polyhedrosis by *Apanteles melanoscelus*. Entomophaga, 22: 207-215.

Reardon, R. C. 1996. Gypchek, the Gypsy Moth Nucleopolyhedrosis Virus Product. Morgantown, WV: USDA Forest Service, Northeastern Area, Forest Health Technology Enterprise Team.

Riegel, C. I. & Slavicek, J. M. 1997. Characterization of the replication cycle of the Lymantria *dispar* nuclear polyhedrosis virus. Virus Res. 51: 9–17.

Rivkin, H., Kroemer, J.A., Bronshtein, A., Belausov, E., Webb, B.A. & Chejanovsky, N. 2006. Response of immunocompetent and immunosuppressed *Spodoptera littoralis* larvae to baculovirus infection. J. Gen. Virol. 87: 2217–2225.

Rohrmann, G.F., 2011. Baculovirus Molecular Biology. National Library of Medicine (US), NCBI, Available at [http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=bacvir].

Rossiter, M. C., Schultz, J.C. & Baldwin, I.T. 1988. Relationships among defoliation, red oak phenolics and gypsy moth growth and reproduction. Ecology 69: 267-277.

Rotheram, S.M. 1973. The surface of the egg of a parasitic insect. II. The ultrastructure of the particulate coat on the egg of *Nemeritis*. Proc R Soc Lond Series B, 183: 195 – 204.

Sait, S. M., Begon, M., Thompson, D. J. & Harvey, J. A., 1996. Parasitism of baculovirus-infected *Plodia interpunctella* by *Venturia canescens* and subsequent virus transmission. Funct. Ecol. 10: 586-591.

Salt, G. 1965. Experimental Studies in Insect Parasitism. XIII. The Haemocytic Reaction of a Caterpillar to Eggs of its Habitual Parasite. Proc. Royal Society of London. Series B, 162: 303-318.

Salt, G. 1970. Cellular defense reactions of insects. Cambridge University Press, London and New York.

Santiago-Alvarez, C. & Caballero, P., 1990. Susceptibility of parasitized *Agrotis segetum* larvae to a granulosis virus. J. Invertebr. Pathol. 56: 128-131.

Schmidt, O., Theopold, U. & Strand, M.R., 2001. Innate immunity and its evasion and suppression by hymenopteran endoparasitoids. BioEssays 23: 344–351.

Shelby, K.S., & Webb, B.A. 1997. Polydnavirus infection inhibits translation of specific growth-associated host proteins. Insect Biochem. Mol. Biol., 27: 263–270.

Shelby, K.S. & Popham, H.J.R. 2006. Plasma phenoloxidase of the larval tobacco budworm, *Heliothis virescens*, is virucidal. J. of Insect Sci. 6: 13.

Shrestha, S. & Kim, Y.G. 2008. Eicosanoids mediate prophenoloxidase release from oenocytoids in the beet armyworm *Spodoptera exigua*. Insect Biochem. Mol. Biol. 38: 99–112.

Slavicek, J. M., Mercer, M. J., Kelly, M. E. & Hayes-Plazolles, N., 1996. Isolation of a baculovirus variant that exhibits enhanced polyhedra production stability during serial passage in cell culture. J. Invertebr. Pathol. 67: 153–160.

Slavicek & Popham. 2005. The *Lymantria dispar* nucleopolyhedrovirus enhancins are components of occlusion-derived virus. J. Virol. 79: 10578-10588.

Slavicek, J.M., Hayes-Plazolles, N. & Kelly, M.E., 2001. Identification of a *Lymantria dispar* nucleopolyhedrovirus isolate that does not accumulate few-polyhedra mutants during extended serial passage in cell culture. Biol. Con., 22: 159-168.

Stanley, D. & Shapiro, M. 2007. Eicosanoid biosynthesis inhibitors increase the susceptibility of *Lymantria dispar* to nucleopolyhedrovirus LdMNPV. Journal of Invertebrate Pathology 95: 119–124.

Stanley, D.W. & Shapiro, M. 2009. Eicosanoids influence insect susceptibility to nucleopolyhedroviruses. Journal of Invertebrate Pathology 102: 245–249.

Stoltz, D.B., Vinson, S.B. & Mackinnon, E.A., 1976. Baculovirus-like particles in the reproductive tracts of female parasitoid wasps. Can. J. Microbiol. 27: 1013–1023.

Stoltz, D. B. & Vinson, S. B., 1977. Baculovirus-like particles in the reproductive tracts of female parasitoid wasps. II. The genus *Apanteles*. Can. J. Microbiol. 23: 28-37.

Stoltz, D.B. & Vinson, S.B., 1979. Viruses and parasitism in insects. Advances in Virus Research 24: 125–171.

Stoltz, D. B., Krell, P.J. & Vinson, S.B. 1981. Polydisperse viral DNAs in ichneumonid ovaries: a survey. Can. J. Microbiol. 27: 123-130.

Stoltz, D. B., Guzo, D. & Cook, D. 1986. Studies on polydnavirus transmission. Virology 155: 120-131.

Stoltz, D. B. & Guzo, D., 1986. Apparent haemocytic transformations associated with parasitoid-induced inhibition of immunity in *Malacosoma disstria* larvae. J. Insect Physiol. 32: 377-388.

Stoltz, D.B., Guzo D., Belland, E.R., Lucarotti, C.J. & MacKinnon, E.A., 1988. Venom promotes uncoating in vitro and persistence in vivo of DNA from a braconid polydnavirus. J. Gen. Virol. 69: 903-907.

Stoltz, D. B. 1990. Evidence for chromosomal transmission of polydnavirus DNA. J. Gen. Virol. 71: 1051-1056.

Stoltz, D. B. & Whitfield, J. B., 1992. Viruses and virus-like entities in the parasitic Hymenoptera. J. Hymenopt. Res. 1: 125–139.

Stoltz, D. & Makkay, A., 2000. Co-replication of a reovirus and a polydnavirus in the ichneumonid parasitoid, *Hyposoter exiguae*. Virology 278: 266-275,.

Stoltz, D. & Makkay, A., 2003. Overt viral diseases induced from apparent latency following parasitization by the ichneumonid wasp, *Hyposoter exiguae*. J. Insect Phys. 49: 483-489.

Stoltz, D.B. & Whitfield, J.B., 2009. Virology. Making nice with viruses. Science 323: 884–885.

Strand, M. R. & Dover, B. A. 1991. Developmental disruption of *Pseudoplusia includens* and *Heliothis virescens* larvae by the calyx fluid and venom of *Microplitis demolitor*. Arch. Insect Biochem. Physiol. 18: 131-145.

Strand, M.R. & Pech, L.L. 1995. *Microplitis demolitor* polydnavirus induces apoptosis of a specific haemocyte morphotype in *Pseudoplusia includens*. J. Gen. Virol. 76: 283-291

Summers, M.D. & Dib-Hajj., S.D. 1995. Polydnavirus-facilitated endoparasite protection against host immune defenses. Proc. Natl. Acad. Sci., 92: 29-36.

Szewczyk, B., Hoyos-Carvajal, L., Paluszek, M., Skrzecz, I. & Souza, M.L. 2006. Baculovirus – re-emerging biopesticides. Biotechnol. Adv. 24: 143-160.

Szewczyk, B., Rabalski, L., Krol, E., Sihler, W., & Souza, M.L. 2009. Baculovirus biopesticides – a safe alternative to chemical protection of plants. J. Biopestic. 2: 209-216.

Tinsley, T.W. & Harrap, K.A. 1978. Viruses of invertebrates. Compr. Virol. 12: 1-101.

Trudeau, D., Washburn, J. O. & Volkman, L. E. 2001. Central role of hemocytes in *Autographa californica* M nucleopolyhedrovirus pathogenesis in *Heliothis virescens* and *Helicoverpa zea*. J. Virol. 75: 996–1003.

Turnbull, M.W. & Webb, B.A., 2002. Perspectives on polydnavirus origin and evolution. Adv. Virus Res. 58: 203– 254.

Versoi, P.L. & Yendol, W.G., 1982. Discrimination by the parasite, *Apanteles melanoscelus*, between healthy and virus-infected gypsy moth larvae. Environ. Entomol. 11, 42-45.

Volkman, L. E. 2007. Baculovirus infectivity and the actin cytoskeleton. Curr Drug Targets 8, 1075–1083.

Wang, P., Hammer, D.A., & Granados, R.R. 1994. Interaction of *Trichoplusia ni* granulosis virus-encoded enhancin with the midgut epithelium and peritrophic membrane of four lepidopteran insects. J. Gen. Virol., 75: 1961–1967.

Washburn J.O., Haas-Stapleton E.J., Tan F.F., Beckage N.E. & Volkman L.E., 2000. Co-infection of *Manduca sexta* larvae with polydnavirus from *Cotesia congregata* increases susceptibility to fatal infection by *Autographa californica* M Nucleopolyhedrovirus. J. of Insect Physiol. 46: 179–190.

Washburn, J. O., Kirkpatrick, B. A. & Volkman, L.E., 1996. Insect protection against viruses. Nature 383: 767.

Washburn, J. O., Haas-Stapleton, E. J., Tan, F. F., Beckage, N. E. & Volkman, L. E. 2000. Co-infection of *Manduca sexta* larvae with polydnavirus from Cotesia congregata increases susceptibility to fatal infection by *Autographa californica* M nucleopolyhedrovirus. J. Insect Physiol. 46: 179–190.

Webb, B. A. & Strand, M. R. 2005. The biology and genomics of polydnaviruses, in Gilbert, I., Iatrou, K., Gill S. (Eds.) Comprehensive Molecular Insect Science, San Diego, CA: Elsevier pp. 260–323.

Whitfield, J. B. 1997. Molecular and morphological data suggest a single origin of the polydnaviruses among braconid wasps. Naturwissenschaften 84: 502–507.

Whitfield, J.B., & Asgari, S. 2003. Virus or not? Phylogenetics of polydnaviruses and their wasp carriers. J. Insect Physiol., 49: 397–405.

Whitfield, J.B. & O'Connor, J.M. 2012. Molecular systematics of wasp and polydnavirus genomes and their coevolution. *In* Parasitoid Viruses: Symbionts and Pathogens, edited by Nancy E. Beckage, Jean-Michel Drezen, Elsevier Inc., in press.

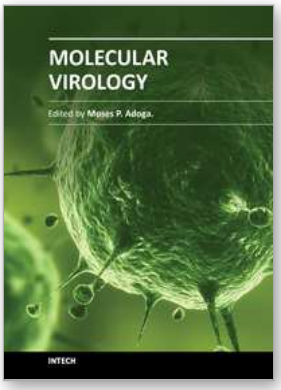
Woods, S., Elkinton, J.S., Murray, K.D., Liebhold, A.M., Gould, J.R., & Podgwaite, J.D., 1991. Transmission dynamics of a nuclear polyhedrosis virus and predicting mortality in gypsy moth (Lepidoptera: Lymantriidae) populations. J. Econ. Entomol., 84: 423-430.

Wyler, S. & Lanzrein, B., 2003. Ovary development and polydnavirus morphogenesis in the parasitic wasp *Chelonus inanitus*. II. Ultrastructural analysis of calyx cell development, virion formation and release. J. Gen. Virol. 84: 1151–1163.

Young, S.Y. & Yearian W.C., 1989. Nuclear polyhedrosis virus transmission by *Microplitis croceipes* (Hymenoptera:Braconidae) adult females reared in infected *Heliothis virescens* (Lepidoptera: Noctuidae) larvae. J. Entomol. Sci., 24: 500-506.

Young, S.Y. & Yearian W.C., 1990. Transmission of nuclear polyhedrosis virus by the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae) to *Heliothis virescens* (Lepidoptera: Noctuidae) on soybean. Environ. Entomol., 19: 251-256.

Zhang, P., Yang, K., Dai, X. J., Pang, Y. & Su, D. M. 2002. Infection of wild-type *Autographa californica* multicapsid nucleopolyhedrovirus induces in vivo apoptosis of *Spodoptera litura* larvae. J. Gen. Virol. 83: 3003–3011.



Molecular Virology

Edited by Mr. Moses Adoga

ISBN 978-953-51-0369-1
Hard cover, 168 pages
Publisher InTech
Published online 21, March, 2012
Published in print edition March, 2012

This book covers various aspects of Molecular Virology. The first chapter discusses HIV-1 reservoirs and latency and how these twin phenomena have remained a challenge to eradication. Aspects regarding the molecular evolution of hepatitis viruses including their genetic diversities with implications for vaccine development are treated in the second chapter. Metabolic disorders that are a consequence of hepatitis C virus infection are discussed in the succeeding chapter. The following two chapters discuss influenza C virus and the applications of viral vectors in therapeutic research. Avian influenza is handled in the sixth chapter and the therapeutic potential of belladonna-200 against japanese encephalitis virus infection is discussed in the succeeding chapter. The last two chapters discuss baculoviruses and their interaction with polydnaviruses. Researchers, lecturers and students will find this book an indispensable companion.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Vincent D'Amico and James Slavicek (2012). Interactions Between Nucleopolyhedroviruses and Polydnaviruses in Larval Lepidoptera, Molecular Virology, Mr. Moses Adoga (Ed.), ISBN: 978-953-51-0369-1, InTech, Available from: http://www.intechopen.com/books/molecular-virology/interactions-between-baculoviruses-and-polydnaviruses

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.