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Common themes in three independently derived endogenous nudivirus elements in parasitoid wasps

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Endogenous Viral Elements (EVEs) are remnants of viral genomes that are permanently integrated into the genome of another organism. Parasitoid wasps have independently acquired nudivirus-derived EVEs in three lineages. Each parasitoid produces virions or virus-like particles (VLPs) that are injected into hosts during parasitism to function in subversion of host defenses. Comparing the inventory of nudivirus-like genes in different lineages of parasitoids can provide insights into the importance of each encoded function in virus or VLP production and parasitism success. Comparisons revealed the following conserved features: first, retention of genes encoding a viral RNA polymerase and infectivity factors; second, loss of the ancestral DNA polymerase gene; and third, signatures of viral ancestry in patterns of gene retention.

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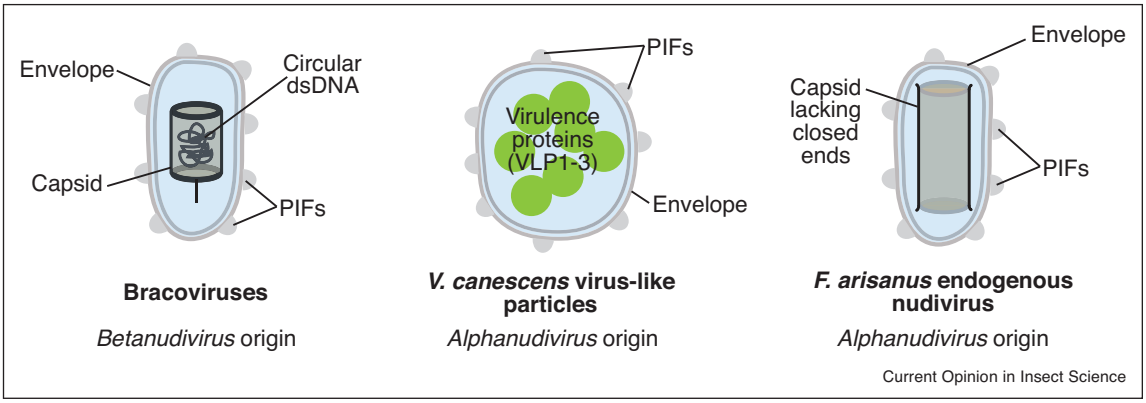
Nudivirus-derived EVEs have been acquired independently by at least three lineages of parasitoid wasps

Endogenous Viral Elements (EVEs) are remnants of viral genomes that are permanently integrated into the genome of another organism [1,2]. While many EVEs degrade over time, others are retained and are co-opted for new functions. Perhaps the most remarkable examples occur in parasitoid wasps, which have acquired functional EVEs several times in independent lineages [3–5]. Parasitoid wasps lay their eggs in or on a host insect where their progeny feed for the immature stages of their development, eventually resulting in the host's death. This lethal interaction between species has led to several innovative strategies that wasps use to promote parasitism, including the use of venom, teratocytes (cells derived from the egg serosal membrane that dissociate and secrete products while circulating in hosts), and the production of virions or virus-like particles in wasps' reproductive tracts in a developmentally controlled fashion [4–7]. To date, EVEs have been genetically characterized in five lineages of parasitoid wasps from two families of large DNA viruses [8••,9••,10••,11•,12,13]. Other examples of non-integrated persistent viral associations are also known [14].

Three lineages of parasitoid EVEs derive from ancestors in the family Nudiviridae (Figure 1). Nudiviruses are non-occluded viruses with circular dsDNA genomes related to baculoviruses and hytrosaviruses of insects [15,16]. Pathogenic nudiviruses can infect many orders of insects as well as crustaceans [17]. These viruses can infect all developmental stages and have varied tissue tropism. Nudiviruses can cause disease (lethality in larvae), can remain asymptomatic in both immature and mature insect life stages, or can become chronic in adults and cause body malformations or sterility [18]. Notably, some pathogenic nudiviruses can integrate into the genomes of host cells, forming a latent infection or becoming endogenized, as in parasitoid wasps and also in the brown planthopper *Nilaparvata lugens* [19,20]. Nudiviruses can be divided into *Alphanudivirus* and *Betanudivirus* genera [21]. 33 genes are shared between all currently sequenced nudivirus genomes (Figure 2, [9••,10••,16,20]). Twenty-one of these genes are also present in all baculovirus genomes ([22], Figure 2). The functional roles of many of these genes have been characterized in baculoviruses, and can be categorized as contributing to: transcription; infectivity; DNA replication; packaging, assembly and morphogenesis; and nucleotide metabolism [23].

The first and best-studied lineage of nudivirus-derived EVEs in parasitoid wasps are polydnaviruses in the genus *Bracovirus* [24]. This lineage arose an estimated 100 mya by integration of a nudivirus into a wasp ancestor in the family Braconidae [25]. This ancestor has since diversified into an estimated 50 000 species named the 'microgastroid complex' [8••,26,27]. All bracoviruses are transmitted vertically and replicate in specialized cells within the calyx region of wasp ovaries to produce a paste-like 'calyx fluid' which comprises virions that contain circular double-stranded DNAs. Wasps inject virions into hosts (along with eggs and venom), and virions infect different host tissues and express gene products that are required for successful development of wasp offspring [4].

Figure 1



Types of nudivirus-derived EVEs in parasitoid wasps and their features. Bracoviruses originate from nudiviruses belonging to the genus *Betanudivirus,* while *V. canescens* VLPs and *F. arisanus* ENV are derived from nudiviruses in the *Alphanudivirus*. Bracovirus virions have enveloped cylindrical nucleocapsids that contain circular double-stranded DNAs. *V. canescens* VLPs have a less defined shape, with envelopes that package wasp-derived virulence proteins (VLP1-3), but lack DNAs or capsids. *F. arisanus* ENV particles are enveloped and have elongated capsids lacking DNA and closed ends. All nudivirus-derived EVEs produce virions or VLPs with envelopes that contain *per os* infectivity factors (PIFs, encoded by *pif0-8* genes).

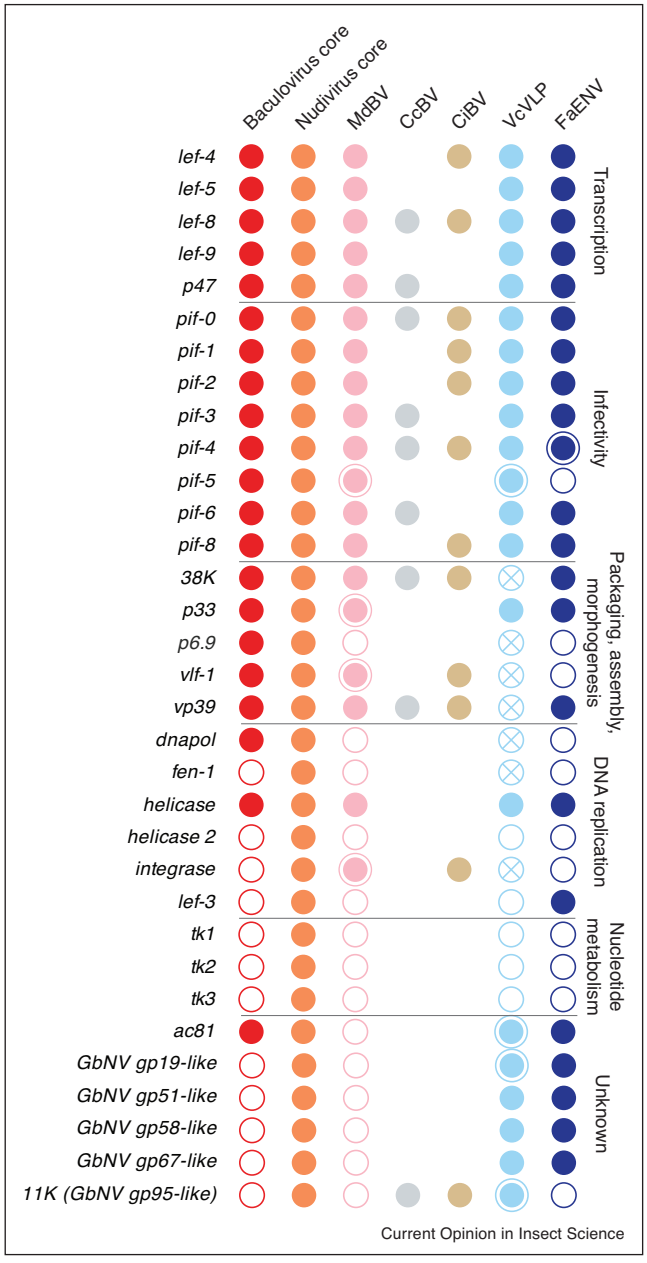
Bracovirus virions share several morphological features with nudiviruses, including the presence of enveloped cylindrical nucleocapsids [28]. Bracovirus genes with functions in virion formation share clear homology with nudiviruses (see below), but the DNAs packaged into virions bear very little sequence similarity to the genomes of nudiviruses or relatives [29•,30–33]. The second lineage is found in the ichneumonid wasp *Venturia canescens*, which produces virus-like particles (VLPs) which comprises gene products that derive from a nudivirus ancestor, but do not contain nucleic acids [10••]. *V. canescens* VLPs (VcVLPs) consist of an envelope with an amorphous round to oblong shape lacking a capsid. VcVLPs deliver wasp-derived proteins into hosts and prevent encapsulation of the parasitoid egg [10••,34–37]. The third lineage is found in braconid wasps from the genus *Fopius*, which are distantly related to species in the microgastroid complex. *Fopius arisanus* produces virus-like particles known as Fopius arisanus Endogenous Nudivirus (FaENV) that consist of enveloped, empty capsids that also lack DNA [9••]. FaENV particles further morphologically differ from bracoviruses and *V. canescens* VLPs by exhibiting long capsids that lack closed ends and are associated with large amounts of extra membranous material. The *V. canescens*, *Fopius*, and *N. lugens* EVEs are derived from alphanudiviruses, while the bracoviruses are derived from betanudiviruses [9••,10••,20].

Current data suggests that whole nudivirus genomes integrated one or more times into wasp genomes, and subsequently gene loss occurred so that only genes important for the production of virions or VLPs, and by extension, parasitism, are retained [8••,9••,10••,38,39••]. Comparing the inventory of nudivirus genes in different lineages of parasitoids can provide insights into the importance of each encoded function in the production of virus (or virus-like particles) on parasitism success via interactions with hosts.

All nudivirus-derived EVEs of parasitoids have retained genes involved in transcription

The first category of genes comprises the transcription machinery: genes that encode the RNA polymerase subunits *p47*, *lef-4*, *lef-8*, *lef-9*, and the initiation factor *lef-5* [40]. In baculoviruses, the RNA polymerase genes are some of the earliest transcribed in the replication cycle. The RNA polymerase holoenzyme recognizes specific promoters and functions to transcribe genes that encode the structural components of virions later in the replication cycle [41,42]. These genes are thus far universally conserved in parasitoid EVEs. In bracovirus-producing *Microplitis demolitor* wasps, late gene transcription is dependent upon the viral RNA polymerase; a phenomenon that is likely conserved in other nudivirus-derived EVEs in parasitoids [43••]. Most nudivirus-derived genes in parasitoid genomes do not contain introns, a feature shared with baculovirus genes, which are transcribed by the baculovirus-encoded RNA polymerase. However, introns have been detected in several of the RNA polymerase subunit genes present in parasitoid genomes, making it likely that RNA polymerase holoenzymes of viral origin are not involved in their own production and are instead controlled by wasp transcriptional machinery [43••,44]. This is perhaps a point where the viral genes interface with wasp processes to constrain the production of virions or VLPs to specific developmental stages and tissues.

Figure 2



Conservation of genes among baculoviruses, nudiviruses, and endogenous nudivirus-like viruses of parasitoid wasps, for 33 core nudivirus genes. Complete parasitoid wasp genomes are available for *Microplitis demolitor* (MdBV), *Venturia canescens* (VcVLP), and *Fopius arisanus* (FaENV), while only partial genomic data are available for *Cotesia congregata* (CcBV) and *Chelonus inanitus* (CiBV). Filled circles indicate the universal presence of a gene in baculoviruses or nudiviruses, or the identification of a gene in an endogenous virus of parasitoid wasps. Open circles indicate the absence of a gene in the baculovirus or nudivirus core gene set, or the lack of detection of a gene in a parasitoid wasp genome, while the absence of a circle indicates incomplete data. An expanded outer circle indicates expansion into a gene family in a wasp genome. A circle filled with a cross indicates the presence of a pseudogene in a wasp genome; no information is available on the presence of pseudogenes for bracoviruses or FaENV.

Per os infectivity factors are almost universally present in nudivirus-derived EVEs of parasitoids

Genes that encode *per os* (oral) infectivity factors (PIFs, *pif-0* through *pif-8*) make up the second functional category of viral genes [45,46]. The presence of *pif* genes in diverse invertebrate DNA viruses suggest they encode components of an ancient virus entry pathway [46]. All of the proteins involved in infectivity listed in Figure 2 form a complex located on virion envelopes in occlusion-derived baculoviruses except Pif-5, which functions independently [47]. PIFs are essential for infection of midgut cells after oral infection of lepidopteran larvae, but are not required for cell to cell spread of the virus thereafter [48–50]. In endogenous parasitoid viruses, these genes are also almost universally conserved (only *pif-5* is missing from FaENV). The retention of *pif* genes in parasitoid EVEs is suggestive of their importance for infection of host cells, despite the delivery of virions or VLPs via injection during oviposition into hosts (rather than oral infection). RNAi knockdown of the *pif-0* and *pif-1* genes in *M. demolitor* wasps resulted in similar amounts of viral DNA delivered into or onto host cells, but the presence of the virulence gene product Glc1.8 on cell surfaces was significantly reduced [43••]. These data demonstrate that bracovirus PIFs do play an important role in infecting host cells (albeit through unknown mechanisms), a process that may be important for EVEs generally due to their known or hypothesized interactions with host immune cells.

None of the nudivirus-derived EVEs of parasitoid wasps possess a DNA polymerase of viral origin

In nudiviruses and baculoviruses, the DNA polymerase functions to replicate the viral double-stranded DNA genome [51,52]. This gene of key importance, *dnapol*, is consistently missing from nudivirus-derived endogenous viruses of parasitoids [8••,9••,10••,53••]. The loss of *dnapol* in parasitoid viruses might purely be related to the fact that these viruses are endogenous and no longer have a need for a polymerase separate from host machinery. The proviral segments of bracoviruses are amplified before excision from the wasp genome in a process presumed to be orchestrated by host DNA polymerases due to the absence of a viral DNA polymerase [54•]. Alternatively, the loss of *dnapol* genes may be an essential early step in the establishment of EVEs that produce virions or VLPs, preventing the replication of any non-integrated viral genomes present in wasp cells and tying the fitness of viral genes to the survival of wasps via their germline. Other genes encoding DNA replication functions including *fen-1*, *helicase 2*, *integrase*, and *lef-3* are variably absent from parasitoid genomes, indicating that retention could be advantageous for the effective production of virions or VLPs in some but not all parasitoid genomes.

Genes that are not universally present in nudivirus-derived EVEs of parasitoids

In contrast to the previous two gene categories, genes involved in producing and assembling viral nucleocapsids are not well conserved among endogenous parasitoid viruses. These encode P33 (Ac92, encoding a sulfhydryl oxidase), 38K (a phosphatase involved in dephosphorylating P6.9), P6.9 (a DNA binding and packaging protein), Vp39 (the major capsid protein) and Vlf-1 (a multi-functional protein that functions as a transcription factor, a capsid protein that packages DNA, and a recombinase that resolves replicated DNA) [43••,55–60]. Perhaps only *p33* is conserved (Figure 2). Bracoviruses have retained *38K*, *p33*, *vlf-1* and *vp39*, but *p6.9* could not be identified in the *M. demolitor* genome to date [53••]. All but one of these genes (*p33*) were found as pseudogenes in the *V. canescens* genome [39••]. These gene remnants contained multiple inactivating mutations and were expressed at negligible levels in ovaries. This is consistent with the lack of viral capsids and packaged DNAs in *V. canescens* VLPs. The *F. arisanus* genome contains *38K*, *p33*, and *vp39*, all of which are expressed in ovaries [9••]. However, *p6.9* and *vlf-1* could not be detected in the *F. arisanus* genome. The extremely long, DNA-negative capsids of *F. arisanus* are reminiscent of *vlf-1* knockout mutants in AcMNPV [58,61]. These patterns of gene loss demonstrate that the production of intact capsids is not a feature required of all parasitoid-produced virions or VLPs for successful interactions with hosts. Instead, the lack of an intact capsid (and underlying genes) could explain differences in strategies used by virions or VLPs to allow parasitoid eggs or larvae to avoid host defenses. Core nudivirus gene homologs of unknown function exhibit a similar pattern of variable retention in parasitoid genomes (Figure 2).

Collections of nudivirus-derived genes in parasitoid wasp genomes have clear signatures of viral ancestry

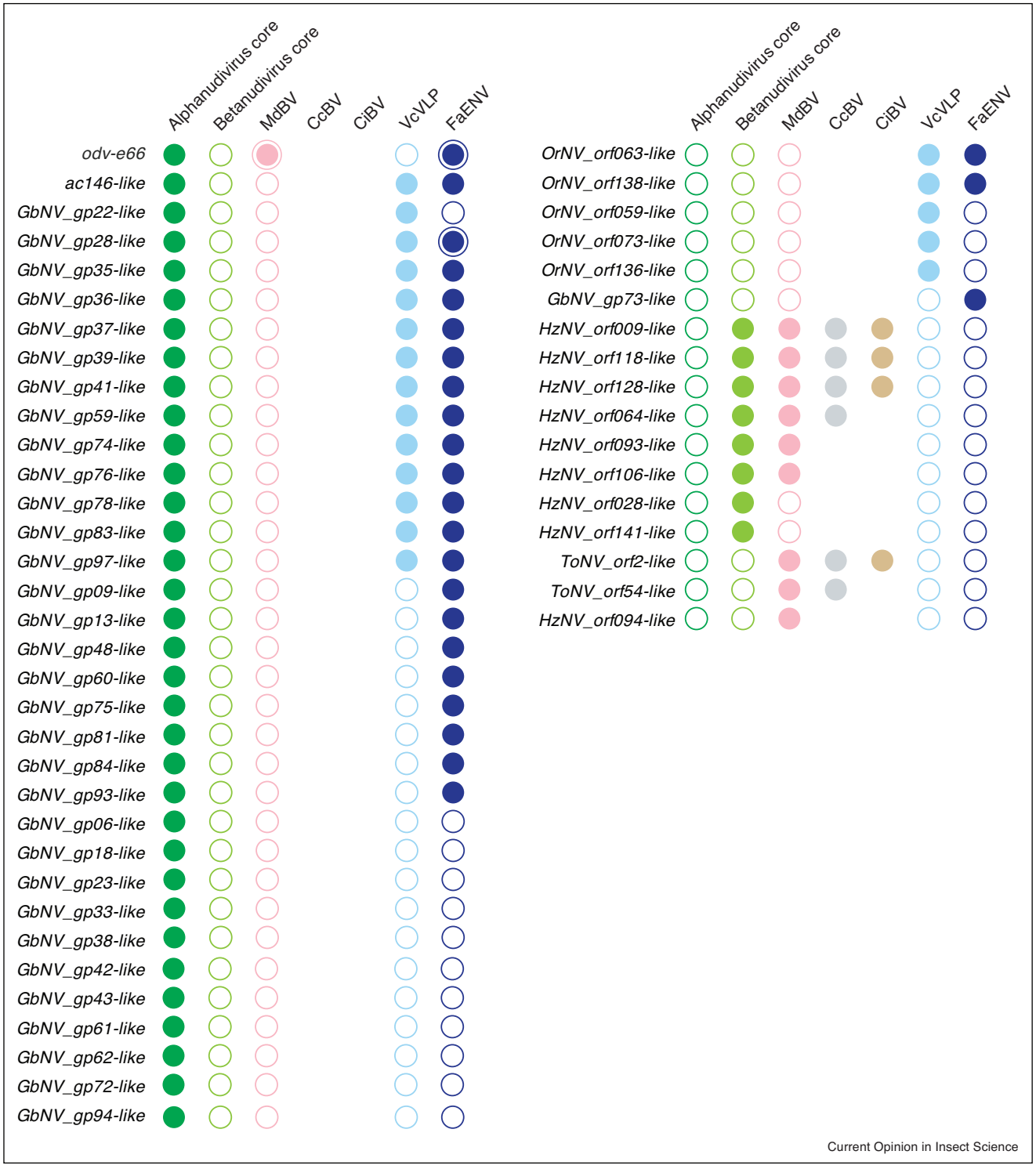
While the pathogenic nudiviruses share a number of key biological features, the alphanudiviruses and betanudiviruses diverged an estimated >200 million years ago and may each have features that are characteristic of each genus [62]. Here, I examine whether the identity of the viral ancestors of EVEs have any impact upon the genes that are now present in parasitoid wasp genomes. Although the nudiviruses share 33 genes, the alphanudiviruses (OrNV, GbNV, DiNV, Kallithea virus) share an additional 33 genes [63], while the betanudiviruses (HzNV-1, HzNV-2, PmNV, ToNV) share an additional eight genes not universally present in alphanudiviruses [64•] (Figure 3). I refer to these genes as 'alphanudivirus or betanudivirus-specific genes' henceforth. The non-core genes retained by each parasitoid EVE is reflective of the nudivirus genus from which they are derived. 22/25 FaENV non-core genes or gene families and 14/19 VcVLP non-core genes are homologous to alphanudivirus-specific genes, while no genes homologous to betanudivirus-specific genes were found. Similarly, 6/9 non-core nudivirus-like genes in *M. demolitor* were related to betanudivirus-specific genes while none were homologous to alphanudivirus-specific genes. The lack of functional information about the majority of these gene products in nudiviruses prevents any strong conclusions about their roles in parasitoid wasp biology. However, the retention of specific genes in all members of a nudivirus genus is suggestive of their functional importance, and could help to prioritize functional characterization of homologous genes retained in parasitoid wasp genomes.

Conclusions

Comparison of inventories of genes retained by EVEs derived from nudiviruses in diverse parasitoid wasp lineages has revealed the following common themes: first, genes encoding a viral RNA polymerase and infectivity factors are important conserved components in the production of functional virions or VLPs; second, all have lost their ancestral DNA polymerase gene; and third, patterns of gene retention have clear signatures of viral ancestry. Although not described in this review, the dispersal of virus-derived genes within wasp genomes is also a shared characteristic of all parasitoid EVEs. The events leading to the evolution of bracoviruses have been difficult to reconstruct because the ancestral integration event occurred so long ago. In contrast, the apparent restriction of *V. canescens* and *Fopius* EVEs to only one or a few species suggests that these integration events occurred more recently in evolutionary time. This indicates that the themes described above can be considered early events in EVE evolution [9••,10••].

The existing body of literature focusing upon parasitoid viruses suggests that symbiosis has evolved in the Hymenoptera only a handful of times in evolutionary history. However, the discovery of parasitoid associated viruses has lagged behind other types of insect microbial symbionts due to a lack of universally conserved genes or high-throughput, inexpensive molecular techniques for their discovery until very recently. As parasitoid wasps represent one of the most astonishing radiations of species on Earth [65], the existing examples of EVEs in parasitoids and their accelerating discovery hint toward the existence of massive untapped diversity of independently derived associations between viruses and parasitoid wasps [9••]. The synthesis presented here not only reveals differences and similarities between independently derived EVEs in parasitoid wasps, but it provides a list of genes that are perhaps likely to be retained in parasitoid EVEs that are yet undiscovered. Using this logic, it should be possible to target these genes in a screen of diverse parasitoid lineages to identify more examples of nudivirus-derived EVEs. The currently described EVEs of parasitoid wasps represent remarkable, integrated

Figure 3



Presence or absence of non-core nudivirus-like genes in endogenous viruses of parasitoids. All gene products have unknown function except for *odv-e66* (involved in infectivity [66]) and *ac146-like* (essential for the production of budded virus [67]). Filled circles indicate the universal presence of a gene in alphanudiviruses or betanudiviruses, or the identification of a gene in an endogenous virus of parasitoid wasps. Open circles indicate the absence of a gene in the alphanudivirus or betanudivirus core gene set, or the lack of detection of a gene in a parasitoid wasp genome, while the absence of a circle indicates incomplete data. An expanded outer circle indicates expansion into a gene family in a wasp genome.

examples of extended phenotypes that warrant further exploration to uncover their diversity and processes that allow their inception.

Conflict of interest statement

Nothing declared.

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