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Bracoviruses, ichnoviruses, and virus-like particles from parasitoid wasps retain many features of their virus ancestors☆

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Animal genomes commonly contain genes or sequences that have been acquired from different types of viruses. The vast majority of these endogenous virus elements (EVEs) are inactive or consist of only a small number of components that show no evidence of cooption for new functions or interaction. Unlike most EVEs, bracoviruses (BVs), ichnoviruses (IVs) and virus-like particles (VLPs) in parasitoid wasps have evolved through retention and interaction of many genes from virus ancestors. Here, we discuss current understanding of BV, IV and VLP evolution along with associated implications for what constitutes a virus. We suggest that BVs and IVs are domesticated endogenous viruses (DEVs) that differ in several important ways from other known EVEs.

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Introduction

Viruses are often viewed as obligate, intracellular parasites because they depend on the biosynthetic machinery of a host cell to produce genome-containing particles (virions). In turn, the function of virions is usually to horizontally transmit the entire viral genome by infecting other host cells that support the production of progeny virus. Studies dating to the 1970s discovered that parasitoid wasps in two families, the Braconidae and Ichneumonidae, produce massive quantities of DNA-containing virions in calyx cells that are located in the ovaries of females [1]. The virions produced in braconids were thereafter named bracoviruses (BVs), while the virions produced in ichneumonids were named ichnoviruses (IVs) [2]. Together, BVs and IVs were also referred to as polydnaviruses [2]. Subsequent work showed that BVs and IVs evolved from different viruses that integrated into the germline of wasps [3••,4••]. However, BVs and IVs have been similarly repurposed through convergence to produce virions that horizontally transfer virulence genes to the hosts that female wasps parasitize, whereas the viral genome components that produce virions in calyx cells remain integrated. The virulence genes virions deliver further have functions in hosts that enable wasp offspring to successfully develop [3••,4••]. BVs and IVs thus differ from most viruses in that virions do not transmit all viral genome components to host cells they infect, but are nonetheless essential for vertical transmission of all the viral genome components that are required to produce virions in wasps. Integration of viruses related to the BV ancestor into two other parasitoids have further resulted in ovary calyx cells producing virus-like particles (VLPs) that also have known or likely functions in parasitism [3••,4••]. In this review, we summarize findings indicating that many types of viruses can be vertically transmitted. We then discuss key features of BVs, IVs and VLPs, the challenges associated with naming these entities, and future study directions.

Viruses commonly integrate into the genome of hosts

Many viruses are only transmitted horizontally while others have evolved latent phases that enable long-term persistence and vertical transmission. Well-known examples of viruses that are both horizontally and vertically transmitted include many large DNA viruses (phages) that infect bacteria, retroviruses that infect vertebrates, and Herpes virus-6 (HHV-6) that infects humans [5–7]. DNA fragments from many types of viruses have also been identified in the genomes of different animals [8].

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These fragments, referred to as endogenous virus elements (EVEs) [8], may derive from all or portions of a viral genome integrating into the germline of a host. Most EVEs are rapidly inactivated and decay under neutral evolution. However, a few genes from retroviruses have been coopted by mammals and insects for new functions [9,10] while some EVEs have known or hypothesized roles in protecting hosts against infection from related viruses [11].

In the case of insects, large DNA viruses with circular genomes in the family *Baculoviridae* produce covert infections through unknown mechanisms, while the circular genome of one member of the family *Nudiviridae*, which is closely related to baculoviruses, has been shown to both integrate and remain circular as an episome in the nuclei of persistently-infected host cells [12]. EVEs from a diversity of RNA and DNA viruses have also been identified in insect genomes. Among these are nudivirus-derived EVEs identified in a planthopper (*Nilaparvata lugens*) [13], a chalcidoid wasp (*Eurytoma brunniventris*) [14], and two aphids (*Aphis glycines*, *Melanaphis sacchari*) [15]. A survey of 240 insect genomes further identified 359 genes of nudivirus origin in 43 other species [16]. The parasitoid *Leptopilina boulardi* is infected by an unclassified virus currently named L. boulardi Filamentous Virus (LbFV) [17]. Recognizable homologs of LbFV genes have been identified in the genomes of several parasitoid wasp species [18•,19•]. Similar to retroviruses, nudiviruses and LbFV-like viruses may be predisposed to integrating into the germline of insects due to life cycle features. The majority of EVEs identified in insects are also inactive or consist of one or a few genes that show no evidence of cooption or interaction [20]. Known exceptions are: (1) BVs that evolved from a nudivirus [21••,22••], (2) IVs that evolved from a still unknown virus ancestor [19•,23••,24•], and (3) VLPs comprised of virion components identified in the parasitoids *Venturia canescens* and *Fopius arisanus* that also evolved from nudiviruses [3••,4••]. BVs, IVs and VLPs are much more complex than other known EVEs because many genes from their virus ancestors have been retained by wasps and still function to produce virions or virion components. We thus suggest domesticated endogenous virus (DEV) more accurately describes these entities than the term EVE.

Nudiviruses and DEVs in parasitoids exhibit a continuum of interacting genes

The circular, double-stranded DNA genomes (80–230 kb) of viruses in the Nudiviridae and its sister family, the Baculoviridae, are comprised of many intronless genes (Table 1). Viruses in both families specifically infect arthropods, are usually pathogenic, and replicate in the nuclei of host cells, which release mature virions comprised of nucleocapsids surrounded by a single envelope [25]. Baculoviruses and nudiviruses also share a partially overlapping set of core genes with functions in replicating the viral genome and producing virions (Table 1) [26]. These include a DNA polymerase that studies of baculoviruses indicate is primarily responsible for replicating the viral genome. It also includes four genes that produce a DNA dependent RNA polymerase that through promoter recognition specifically transcribes the structural genes that produce the nucleocapsid and envelope proteins that form virions [27] (Table 1).

Bracoviruses

BVs assemble in the nuclei of ovary calyx cells that lyse to release mature virions into the lumen of the lateral oviducts. Transcriptome data from ovaries together with the proteomic analysis of virions from two braconids, *Cotesia congregata* and *Chelonus inanitus*, provided the first evidence that proteins in BV virions are homologs of nudivirus genes, which strongly suggested BVs evolved from a nudivirus ancestor [21••,28] (Figure 1). Comparing BVs to nudiviruses indicates they also share several morphological features (Figure 2). Phylogenetic studies support that the nudivirus ancestor of BVs integrated into the genome of a braconid ~100 million years ago (MYA) [29••,30], which was followed by speciation events that have resulted in an estimated ~50,000 BV-producing wasp species existing today [31]. Collectively, these BV-producing wasps form a monophyletic assemblage called the Microgastroid complex that is divided into six subfamilies [32] (Santos *et al.*, this issue).

Insights into BV genome evolution and function primarily derive from studies of two species, *Microplitis demolitor* and *C. congregata*, in the subfamily Microgastrinae [21••,22••,33••,34,35•,36••,37•]. Unlike nudiviruses, genome sequencing of initially *M. demolitor* and then *C. congregata* showed that BV genome components are globally dispersed in the genomes of wasps [33••,34,36••]. However, these components function as two units, which underlies how virions have been repurposed to package only virulence genes. The first of these units consists of nudivirus genes that despite being dispersed are coordinately expressed and interact to produce virions [22••,36••] (Table 1). Included in this gene set are the four nudivirus core genes that produce the RNA polymerase, which are expressed early in calyx cells. This is followed by the expression of several other nudivirus genes that encode nucleocapsid and envelope proteins which the nudivirus RNA polymerase transcribes [22••,36••,38] (Table 1). The fact that almost none of these genes has gained introns after 100 million years suggests strong evolutionary constraints continue to operate in regulating transcription of these viral genes probably in virogenic stroma where virion assembly occurs [39] (Figure 2). Almost half of these genes reside in a very similar ~100 kb domain in the genomes of *M. demolitor* and *C. congregata*, which may be a remnant of the nudivirus that integrated into the wasp ancestor, but the remainder are widely and disparately dispersed [33••,36••]. Notably absent, however, is a

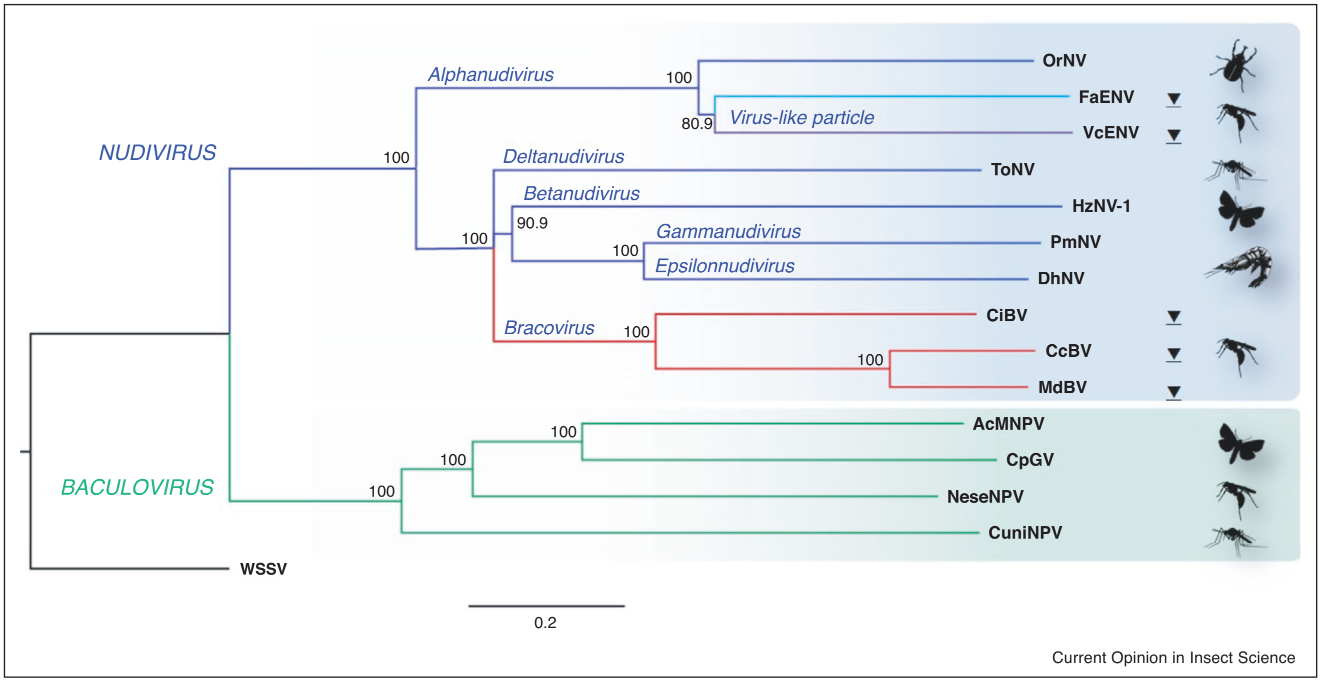
Table 1

Retention of the 32 nudivirus core genes among baculoviruses, nudiviruses and endogenized nudiviruses from parasitoid wasps

	GENE FUNCTION	BACULOVIRUS				NUDIVIRUS					ENDOGENOUS NUDIVIRUS				
		α	β	δ	γ	α	β	δ	γ	ε*	Bracovirus			VLP	
	gene name	Ac MNPV	Cp GV	Nese NPV	Cuni NPV	Or NV	Hz NV-1	To NV	Pm NV	Dh NV	Cc BV	Md BV	Ci BV	Vc ENV	Fa ENV
	DNA replication/processing														
1	***DNA polymerase DNApol***	●	●	●	●	●	●	●	●	●	○	○	/	⊗	/
2	***helicase***	●	●	●	●	●	●	●	●	●	●	●	/	●	●
3	*helicase 2*	○	●	○	○	●	●	●	⬤	⬤	○	○	/	○	○
4	***integrase (int)***	○	○	○	○	●	●	●	●	●	⬤	⬤	●	⊗	/
5	*flap endonuclease fen-1*	○	○	○	○	●	●	●	●	●	⬤	●	/	⊗	/
	Transcription/RNA polymerase														
6	***RNA polymerase p47***	●	●	●	●	●	●	●	●	●	●	●	/	●	●
7	***RNA polymerase lef-4***(1)	●	●	●	●	●	●	●	●	●	●	●	●	●⊗	●
8	*RNA polymerase lef-5*	●	●	●	●	●	●	⬤	●	●	●	●	/	●	●
9	***RNA polymerase lef-8***	●	●	●	●	●	●	●	●	●	●	●	●	●	●
10	***RNA polymerase lef-9***	●	●	●	●	●	●	●	●	●	●	●	/	●	●
	Nucleotide metabolism														
11	*thymidine kinase tk1*	○	○	○	○	●	●	●	●	●	/	/	/	/	/
12	*thymidine kinase tk2*	○	○	○	○	●	●	●	●	●	/	/	/	/	/
13	*thymidine kinase tk3*	○	○	○	○	⬤	●	●	⬤	⬤	/	/	/	/	/
	Structure/Caspid components														
14	***very late factor vlf-1***(2)	●	●	●	●	●	⬤	●	⬤	⬤	⬤	⬤	●	⊗	○
15	***capsid protein vp39***	●	●	●	●	●	●	●	●	●	●	●	●	⊗	●
16	*p6.9*(3)	●	●	●	●	●	●	●	●	●	⬤	⬤	/	⊗	/
17	***phosphatase 38K***	●	●	●	●	●	●	●	●	●	●	●	●	⊗	●
	Per os infectivity factors & envelope components														
18	***pif-0 (p74)***	●	●	●	●	●	●	●	●	●	●	●	●	●	●
19	***pif-1***	●	●	●	●	●	●	●	●	●	●	●	●	●⊗	●
20	***pif-2***	●	●	●	●	●	●	●	●	●	●	●	●	●	●
21	***pif-3***	●	●	●	●	●	●	●	●	●	●	●	/	●	●
22	***pif-4 (19 kDa)***	●	●	●	●	●	●	●	●	●	●	●	●	●	⬤
23	*pif-5 (odv-e56)*	●	●	●	●	●	●	⬤	●	●	⬤	⬤	●	⬤	○
24	***pif-6 (ac68)***	●	●	●	●	●	●	●	●	●	●	●	/	●	●
25	***pif-8 (vp91)***	●	●	●	●	●	●	⬤	●	●	●	●	●	●	●
26	*sulfhydryloxidase p33 (Ac92)*(4)#	●	●	●	●	●	●	●	●	●	⬤	⬤	/	●	●
27	*ac81*	●	●	●	●	●	●	●	●	●	○	○	/	⬤	●
	Other particule components														
28	*11K*	○	○	○	○	●	●	●	●	●	●	●	⬤	⬤	○
29	*alpha/beta hydrolase GbNVorf19-like*(5)	○	○	○	○	●	●	●	●	●	●	●	/	⬤	●
30	*GbNVorf51-like*	○	○	○	○	●	●	●	●	●	○	○	/	●	●
31	*GbNVorf58-like*(6)	○	○	○	○	●	●	●	●	●	○	○	/	●	●
32	*GbNVorf67-like*(7)	○	○	○	○	●	●	●	●	●	●	●	/	●	●

Gene functions were assigned on the basis of homology with baculovirus genes and/or proteomic analysis of virions. Four baculoviruses in green (Autographa californica MNPV, Cydia pomonella GV, Neodiprion sertifer NPV and Culex nigripalpus NPV) and five nudiviruses in dark blue have been used as representatives of the genera currently recognized by ICTV (Oryctes rhinoceros NV, Heliothis zea NV-1, Tipula oleacera NV and Penaeus monodon NV) or that have been proposed as new genera (*) in recently published studies (Dikerogammarus haemobaphes NV). Two complete bracoviruses (Cotesia congregata BV and Microplitis demolitor BV) and one partial bracovirus (Chelonus inanitus BV) are shown in red. Two other endogenized nudiviruses that produce virus-like particles (Venturia canescens ENV and Fopius arisanus ENV) are shown in purple and light blue, respectively. Filled circles (●) indicate the presence of the corresponding gene, whereas expanded filled circles (⬤) indicate the gene has expanded into a multimember family. Open circles (○) indicate the absence of the gene and cross filled circles (⊗) indicate detection of a remnant of the ancestral integrated gene. Finally, slashes (/) indicate either that no information is available from the wasp genome (*Chelonus inanitus*), the gene remnant was not deeply searched for (*Fopius arisanus*), or genes are too closely related to wasp genes to unambiguously determine their origin (e.g. *thymidine kinases*). (1) Newly identified *lef-4* pseudogene within VcENV cluster 6 (accession number KP972600), from position 3,891 to 5,798. (2) For the *vlf-1* family, *vlf-1* and *HzNV-1 orf140-like* were considered to belong to the same recombinase gene family. (3)A thorough search for the *p6.9* core gene revealed a putative candidate from position 61,992 to 62,192 in the DhNV genome and two putative candidates from positions 1,679,579 to 1,679,358 and 1,735,989 to 1,736,246 within *M. demolitor* scaffold_0102 (accession number NW_014463878.1). (4) The presence of two copies of *p33/ac92* within the DhNV genome was not confirmed. # P33/Ac92 is detected in both baculovirus envelope and nucleocapsid fractions but is present in higher quantities within the former. (5)A *GbNVorf19-like* homolog was identified from position 1,730,226 to 1,731,254 within the same *M. demolitor* scaffold_0102. (6)A *GbNVorf58-like* homolog was identified from position 62,198 to 62,365 within the DhNV genome. (7)A *GbNVorf67-like* homolog was identified from position 1,449 to 2,756 within *M. demolitor* scaffold_0267_contig3 (accession number AZMT02018993.1). The core genes conserved for the virus phylogeny are indicated in bold (see Figure 1).

Figure 1



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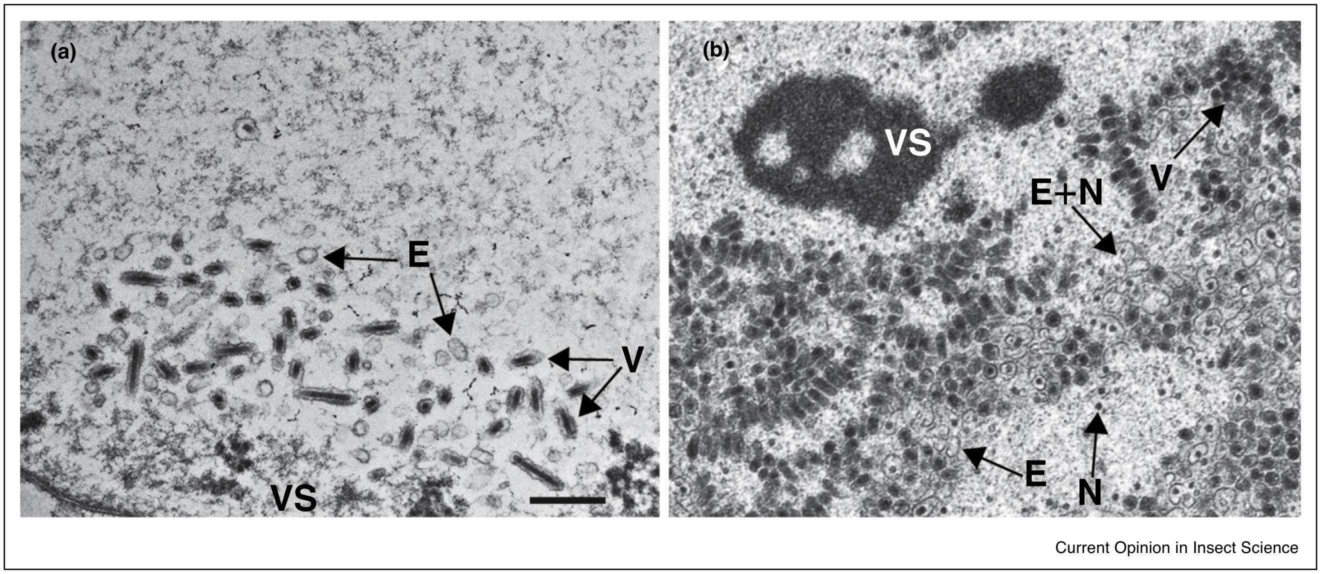
Phylogenetic tree for baculoviruses, nudiviruses and endogenous nudiviruses. The colors and abbreviations are those defined in Table 1. The tree was constructed using previously described sequences [3••], which include 17 nudivirus core genes that are indicated in bold in Table 1. Amino acid alignments were individually performed with default parameters using the MAFFT alignment plugin v7.0450 from Geneious Prime 2019.2.3 software. A consensus tree was then constructed using Geneious Tree builder on concatenated and refined multiple alignments with the following parameters: genetic distance model = Jukes-Cantor, tree build method = Neighbor-Joining, resampling method = bootstrap, number of replicates = 1,000. A nimavirus (white spot syndrome virus (WSSV, AF369029)) was used as outgroup based on gene content. Numbers on tree nodes indicate bootstrap supports (1000 replicates).

nudivirus DNA polymerase that as a core gene would have been present in the ancestor of BVs [33••,36••]. Loss of this gene early in evolution has been suggested as potentially critical in preventing the ancestor from being able to replicate its genome and cause disease in wasps [4••]. The second functional unit is a collection of DNA domains containing virulence genes called proviral segments. Most proviral segments are tandemly arrayed in macroloci with orthology relationships suggesting organization was already established before *M. demolitor* and *C. congregata* diverged 53 MYA [36••]. Proviral segments are strongly amplified and then processed into circular, double stranded DNAs that range from ~6 to >40 kb that are individually packaged into virions but have aggregate sizes of >200 kb [40,41•,42•]. Amplification of proviral segments requires a wasp DNA polymerase that remains unidentified [33••,36••,43•,44]. However, recombinases and conserved sequences flanking proviral segments were both likely inherited from the nudivirus ancestor, and are known to also be required for processing functions that produce the circular, dsDNAs that are packaged into nucleocapsids [22••,40,41•]. Some of the virulence genes on proviral segments originate from wasps [37•,45,46] while others derive from transposable elements [36••,37•,47,48] or potentially the nudivirus ancestor but have diverged to the point that their phylogenetic relationship is no longer detectable. However, most BV virulence genes are of unknown origin [37•]. Females inject eggs and BVs into hosts, which is followed by the former hatching and the latter rapidly infecting different cell types followed by expression of virulence genes. Functional studies further implicate several BV virulence genes in altering host immune defenses and growth in ways that enable wasp larvae to develop [49–51].

Ichnoviruses

Several thousand species in two subfamilies of the Ichneumonidae (Campopleginae, Banchinae) produce IVs in calyx cells that morphologically are very similar to one another but are distinct from BVs [23••,52]. Insights into IV evolution and function derive from largely similar approaches to those used to characterize BVs. Studies that initially focused on the campoplegine *Hyposoter didymator* identified several genes whose products are detected in IV virions, which suggests these genes encode virion structural proteins [23••]. None of these genes

Figure 2



Electron micrographs showing that nudivirus and BV virions exhibit several similarities. (a) Morphogenesis of the betanudivirus Heliothis zea nudvirus -1 (HzNV-1) in the nucleus of an infected host cell. Spheroidal envelopes (E) *de novo* assemble in proximity to virogenic stroma (VS), which is followed by the *de novo* formation of a single electron dense, cylindrical nucleocapsid within an envelope to form a virion (V). Scale bar = 500 μm. (b). Morphogenesis of Microplitis demolitor bracovirus (MdBV) in the nucleus of a calyx cell. Individual, crescent-shaped envelopes (E) and electron dense nucleocapsids (N) *de novo* assemble in proximity to virogenic stroma (VS). This is followed by envelopes individually surrounding cylindrical nucleocapsids (E + N) to produce virions (V) that form parallel arrays before calyx cell lysis releases virions into the lateral oviducts. HzNV-1 nucleocapsids are larger than MdBV nucleocapsids, which likely reflects that the HzNV-1 genome (228 kb) is much larger than any of the viral segments that are individually packaged into MdBV virions (6-40 kb). In contrast, the number of MdBV virions that form in the nuclei of calyx cells is much larger than the number of HzNV-1 virions that form in the nuclei of permissive host cells.

share significant homology with genes in any known virus but they reside in domains of the wasp genome named IV structural protein encoding regions (IVSPERs), which exhibit features that strongly suggest a viral origin [23••,53••]. Whole genome sequencing of *H. didymator* and another campoplegine, *Campoletis sonorensis*, indicate that gene content and order in IVSPERs are conserved while proviral segments containing virulence genes exhibit considerable dispersion and are more variable [54••]. Wasps in the subfamily Banchinae are less studied, but genes in the IVSPERs of campoplegine wasps have been identified in the banchine *Glypta fumiferanae* that also exhibit similar order [24•]. However, phylogenetic data indicate that the Campopleginae and Banchinae are separated by several other subfamilies of ichneumonids that do not produce IVs. These findings thus overall favor the interpretation that IVs arose twice from the same or a closely related virus that is either extinct or undiscovered [19•,32].

Virus-like particles

Endogenized nudiviruses in *V. canescens* and *F. arisanus* have resulted in a different strategy of parasitoid wasp manipulation of host physiology: producing VLPs that contain proteins but no nucleic acid [3••,19•]. Compared to BVs, the nudiviruses associated with *V. canescens* and *F. arisanus* have also been more recently acquired and thus provide additional insights into nudiviruses domestication. Sequencing of *F. arisanus* shows that the nudivirus genes which produce the RNA polymerase and several structural genes that encode envelope and nucleocapsid components are expressed in calyx cells while other nudivirus core genes are pseudogenized or absent, suggesting decay (Table 1). In turn, the VLPs produced in calyx cells consist of a partially formed nucleocapsid that is surrounded by an envelope [4••]. Results from *V. canescens* likewise indicate the four nudivirus genes that encode the RNA polymerase subunits and several nudivirus structural genes that encode predicted envelope components are expressed [3••]. Among the latter are several '*per os* infectivity factor (*pif*)' genes that are also encoded by nudiviruses and baculoviruses. Experimental studies in baculoviruses further indicate several *pif* gene products interact and have functions in infecting midgut cells [55]. In contrast, that nudivirus genes encoding nucleocapsid components are pseudogenized (Table 1) [43•] is consistent with formation of VLPs comprised of an envelope that surrounds at least three proteins of wasp origin [3••]. Assays further indicate the VLPs produced by *V. canescens* bind to the surface of wasp eggs while the proteins inside envelopes have functions in protecting eggs from the host's immune system [56]. The nudivirus genes in the *V. canescens* and *F. arisanus* genomes are much less dispersed than in the wasps that produce BVs; overall suggesting the early stages of domestication after integration of a complete nudivirus genome include pseudogenization and decay of genes that are not selected for, while genes that remain functional progressively disperse via chromosomal rearrangements. Like BV-producing wasps, *F. arisanus* and *V. canescens* also both lack a nudivirus DNA polymerase suggesting loss of this gene is an important early step in nudivirus domestication while retention of the genes that produce the RNA polymerase is key to producing virions or VLPs that have been repurposed for functions in parasitizing hosts.

The viral nature of BVs, IVs and VLPs

Altogether, nudiviruses have commonly given rise to EVEs that in most insects show no evidence of being functional. In contrast, nudiviruses coopted by a number of parasitoids continue to produce virions (BVs), VLPs comprised of a nucleocapsid and viral envelope or VLPs that consist of a viral envelope alone. IVs derived from an unknown virus further exhibit many functional features of BVs. Coming to a consensus about naming BVs, IVs and VLPs is a challenge. At present, the International Committee on Taxonomy of Viruses (ICTV) defines a virus *sensu stricto* as a type of mobile genetic element that encodes at least one protein that is a major component of the virion encasing the nucleic acid of the respective mobile genetic element (https://talk.ictvonline.org/information/w/ictv-information/383/ictv-code). In a broader definition provided in the same text by the ICTV, mobile genetic elements that are of evolutionary descent from a virus can also be considered viruses. BVs and IVs could thus be formally considered viruses given the strong evidence supporting their evolution from viral ancestors. In contrast, we suggest VLP would be a more appropriate term for the particles produced by *V. canescens* and *F. arisanus* which also are of viral origin but are not mobile genetic elements. BVs and IVs are clearly no longer autonomous in an evolutionary sense given that there is no replicating viral genome that is distinct from the wasp genome. However, forgoing the name 'virus' when referring to BVs or IVs detracts from the fact that both produce massive numbers of infectious virions in wasp calyx cells that package many genes through the coordinated interaction of many other genes that were inherited from endogenized virus ancestors. Most BV gene products also continue to interact as they do in baculoviruses and nudiviruses, which further suggests these entities are better described as DEVs than EVEs which are predominantly non-functional sequences of viral origin in the genomes of animals. DEV would be appropriate for VLPs as well if a slightly more flexible definition of viruses was accepted, given that BVs and VLPs both have clear phylogenetic links to nudiviruses (Figure 1). BVs and IVs have also been called 'symbiotic viruses' by analogy to vertically transmitted bacterial symbionts that have given rise to organelles like mitochondria and chloroplasts [57]. While bacterial symbionts still contain remnants of their original genome, BV, IV, and VLP genome components are all integrated in the genomes of wasps. However, the comparison of DEVs to other symbionts of microbial origin does provide another perspective when considering what to call BVs and IVs.

Other challenges in the study of DEVs also exist. For example, while many nudivirus-derived EVEs have been identified in insects, to date all examples of endogenized nudiviruses that continue to produce virions are in parasitoids. Does this reflect more intensive study of parasitoids owing to the discovery of BVs and IVs in the 1970s, or does the life cycle of these insects, which obligately involves the development of offspring in another arthropod and horizontal transfer of many factors that promote offspring development from wasps to hosts, lend itself to coopting viruses for similar purposes? A second need is to deorphanize the function of many BV and IV genes in order to understand how transcription is controlled to assure that virions are only produced in calyx cells. In summary, additional comparative data on BVs and IVs are likely to further enhance insights about genome organization and function. Other comparative studies will also likely identify further examples of endogenized virus domestication.

Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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