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Many evolutionary roads led to virus domestication in ichneumonoid parasitoid wasps☆

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The investigation of endogenous viral elements (EVEs) has historically focused on only a few lineages of parasitoid wasps, with negative results consistently underreported. Recent studies show that multiple viral lineages were integrated in at least seven instances in Ichneumonoidea and may be much more widespread than previously thought. Increasingly affordable genomic and bioinformatic approaches have made it feasible to search for viral sequences within wasp genomes, opening an extremely promising research avenue. Advances in wasp phylogenetics have shed light on the evolutionary history of EVE integration, although many questions remain. Phylogenetic proximity can be used as a guide to facilitate targeted screening, to estimate the number and age of integration events and to identify taxa involved in major host switches.

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Introduction

The use of endogenous viral elements (EVEs) by several lineages of parasitoid wasps represents a remarkable example of convergent evolution [1]. EVEs in the form of virions (polydnaviruses, or PDVs) or virus-like particles (VLPs) are used to deliver virulence proteins, suppress the immune system of the wasp's host, and/or modify its growth and development [2,3]. Recent studies have shown that viral acquisition has occurred in more hymenopteran lineages than previously thought [4••,5••,6••,7,8,9••]. They furthermore suggest that multiple viral ancestors were integrated through varied pathways [10–12,13•,14,15]. Understanding the phylogenetic history of EVE occurrence in parasitoid wasps is crucial to unraveling the multiple processes leading to viral domestication. Here we review the recent progress in the field, focusing on the Ichneumonoidea, where the most well-characterized EVEs are found, outline the major gaps in our knowledge, and identify a few challenges for future research.

Overview of endogenized viruses in Ichneumonoidea

Types of evidence for EVE presence or absence

The first evidence for EVEs in parasitoid wasps was based on morphological and histological analyses of wasp ovaries (see Ref. [16] for an overview). Morphological evidence involves detection of an enlarged, cup-shaped section just below the ovary, called the calyx, in which virion production takes place [17]. Its conclusiveness can be strengthened by selective staining of DNA, which is typically present in large amounts in virion-rich tissues (but note that certain VLPs only contain proteins; [14]). Histology, especially ultra-thin sectioning combined with transmission-electron microscopy (TEM) of the calyx tissue, goes a step further by also elucidating virion morphology, which can differ considerably even in a single type of EVE carried by different wasp species [18•]. Beyond morphology, early studies demonstrated that it was the virus particles themselves that suppressed the hosts immune response [19]. Proteomics, transcriptomics or the preparation of cDNA libraries from calyx tissue can be used to identify genes involved in the production

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of the virions, which are typically of viral origin [15,20]. In contrast, isolating the virions and sequencing the DNA molecules that they contain yields virulence genes of very diverse, but usually non-viral genomic origins [21]. Finally, sequencing of the whole genome of an EVE-carrying parasitoid wasp allows the study of the genomic architecture of the integration [4••,5••,13•, 14].

Phylogenetic distribution of EVEs

A review of the available evidence for EVE presence across Ichneumonoidea shows that there has been a clear emphasis on screening species across just a handful of the ~84 subfamilies within Ichneumonoidea (Table 1, Supplementary Table 1). Evidence for the absence of EVEs may not have been consistently reported across the literature but is essential to fully understand EVE distribution and evolution. Recent studies have shown that virus domestication is more common than previously thought [4••,5••]; thus a wider taxonomic search using both histological and genomic approaches is warranted.

A phylogenetic perspective on EVE distribution among taxa of Ichneumonoidea not only facilitates targeted screening, but also allows estimating the likely number of integration events. The last decade has seen a considerable overhaul of our knowledge of the phylogenetic history of Braconidae and Ichneumonidae [22–26,27•,28], resulting in an improved assessment of the phylogenetic history of EVE occurrences (Figures 1 and 2).

In the *Bracovirus* case, it is likely that all microgastroid wasps bear bracoviruses: this is a clearly monophyletic lineage and no species yet investigated in the group have definitely been found to lack them (although PCR amplification of nudivirus-origin genes failed in a questionably preserved Khoikhoinae; [10]). In totality, the evidence indicates that a single integration event took place at the base of the microgastroid clade (Figure 1). For the remaining Ichneumonoidea, however, the phylogenetic picture is not as clear-cut. Recent genomic studies considerably expanded our knowledge of EVE evolution, suggesting a more complex story in Campopleginae than previously assumed and even revealing entirely new discoveries of EVEs in Opiinae, Microgastrinae and Pimplinae [4••,5••].

For Campopleginae, there has been historically a tacit understanding that ichnoviruses were found in all members of the subfamily, but such an assumption depends on mapping the existing records on a phylogenetic tree (Figure 2). The recent discovery of a lack of ichnovirus genes in a species of *Dusona* [4••] suggests either a later acquisition in a subset of campoplegine taxa or multiple loss events in the evolution of the subfamily. Furthermore, the recent characterization of the *Venturia canescens* genome confirmed that the genes responsible for the formation of VLPs in this species are integrated, of viral origin, and related to nudiviruses [14], thus certainly representing a separate integration event. The authors also found some potential remnants of ichnovirus genes in the genome, perhaps suggesting an EVE replacement in this species, but the matches were not strong enough to be confident in this hypothesis. Until further evidence demonstrates ichnovirus presence in members of the clade that includes *Venturia*, domestication of an *Ichnovirus* in a more restricted subset of Campopleginae should be considered possible.

In Banchinae, recent molecular and genomic characterizations of the EVEs in two species revealed several genes homologous to the ichnoviruses in Campopleginae, as well as similarities in the architecture of the genome integration [29,30]. While the phylogenetic evidence [22,27•] and existing genome screening [4••] suggest two parallel domestication events from the same viral group in Campopleginae and Banchinae, we cannot completely rule out the possibility that this pattern is due to a single domestication followed by multiple losses. Extensive taxon sampling within Banchinae as well as in the subfamilies that are phylogenetically in between them and the Campopleginae is required to resolve this question.

Recently, a novel EVE was discovered in the braconid *Fopius arisanus* (Opiinae) [5••], a major biocontrol agent of tephritid fruit flies. In this species, VLPs are produced in the nuclei of calyx cells, lack DNA, and are most closely related to pathogenic *Alphanudivirus* (Nudiviridae) sequences, highlighting a second independent viral integration event in Braconidae. Two additional suspected EVEs were discovered very recently from sequenced genomes [4••,27•]. Both are related to the filamentous dsDNA virus in *Leptopilina boulardi* (LbFV), a cynipoid parasitoid of *Drosophila*, and have some similarity to hytrosaviruses. These viruses were found in divergent taxa, one as a second EVE in *Cotesia vestalis* (Microgastrinae) and the other in *Dolichomitus imperator*, a member of Pimplinae (Ichneumonidae). The latter is a novel subfamily to contain an EVE and represents the first idiobiont ectoparasitoid with an EVE. The role of EVEs in ectoparasitoids has never been examined, but they may have venom-like functions that benefit the wasp, such as increasing nutritional value of the host, maintenance of paralysis, or microbial functions [31].

In total, this represents a minimum of seven integration events in Ichneumonoidea, three identified in Braconidae and four in Ichneumonidae. The viral elements found in *Leptopilina* (Figitidae) and the recently uncovered evidence for additional EVEs in the genome of *Eurytoma brunniventris* (Chalcidoidea: Eurytomidae [9••]) suggest that future genome sequencing may reveal even more massive convergence in EVE uptake across the tree of

Table 1

Taxonomic distribution of EVE presence or absence, as reflected in the literature. Numbers in parenthesis after the name of each taxon represent the number of genera or subfamilies screened for EVEs out of the total number. A plus sign means evidence for presence, a minus sign evidence for absence, and a question mark denotes uncertain evidence for presence. See Supplementary Table 1 for details and literature references

Family/subfamily	EVE type[a]	Ovary morphology[b]	TEM of calyx tissue[c]	Viral machinery genes[d]	Packaged DNA or protein[e]	Wasp genome[f]
Braconidae (11/41)						
Cardiochilinae (1/17)	*Bracovirus*	1+	1+	1+	1+	
Cheloninae (3/23)	*Bracovirus*	3+	3+	1+	2+	1+
Euphorinae (2/59)	VLP	2+	2+		1+	1−
Helconinae (1/18)	None detected					1−
Khoikhoiinae (1/2)	*Bracovirus*?			1?, not amplified		
Macrocentrinae (1/8)	None detected					1−
Mendesellinae (1/2)	*Bracovirus*			1		
Microgastrinae (11/81+)	*Bracovirus*	5+	6+	4	3+	2+
Miracinae (1/1)	*Bracovirus*?	1		1		
Opiinae (4/39)	Nudivirus, rhabdovirus, entomopox-virus (exogenous)	3+	4+	2+	1+	1+ 1−
Rogadinae (1/62)	None detected					1−
Ichneumonidae (8/42)						
Adelognathinae (1/1)	None detected					1−
Banchinae (3/67)	Ichnovirus	3+	1+ 2?	1+	2+	1+
Campopleginae (11/66)	Ichnovirus + VLP (nudiviral origin)	6+ 5?	5+ 3?	4+	3+	3+ 1−
Ctenopelmatinae (2/107)	Unclear / none	1+				1−
Ichneumoninae (1/437)	Ascovirus (exogeneous)		1?		1?	
Mesochorinae (1/12)	None detected					1−
Pimplinae (1/77)	LbFV (similar to hytrosavirus)					1+
Xoridinae (1/4)	None detected					1−

[a]Unclear evidence as indicated by a question mark mostly stems from incomplete information in the literature about the detection method.
[b] Enlarged region below wasp ovarioles found ('calyx').
[c] Transmission electron microscopy identified structure of virions produced in wasp ovaries.
[d] Genes involved in particle formation and/or virus DNA replication sequenced, usually through transcriptomics, proteomics or cDNA library preparation of purified virions or at least ovary tissue.
[e] Packaged genes from within virions isolated and sequenced ('viral genome', but genes usually not of viral origin), or their effect on host cells studied (e.g. through RNA interference).
[f] Wasp genome sequenced and integration of viral machinery genes and/or of packaged virus genome among the wasp genes examined.

parasitoid wasps. Furthermore, the discoveries of exogenous viruses with convergent functionality to EVEs (a poxvirus in an Opiinae and an ascovirus in an Ichneumoninae species [32,33•,34]) suggest that viral mutualisms do not necessarily require genome integration (Figure 2).

Age of domestications of *Bracovirus* and *Ichnoviruses*

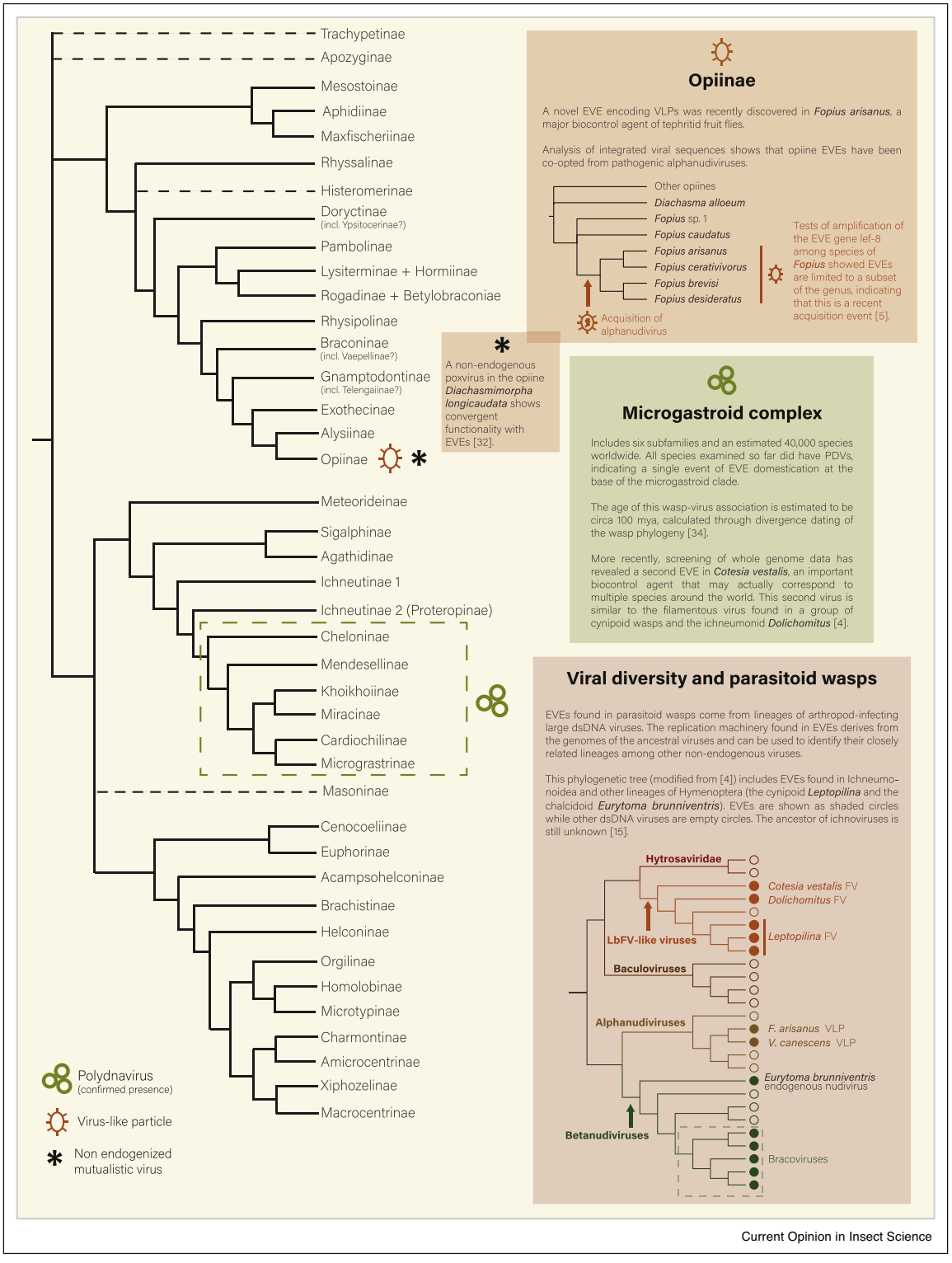
The phylogenetic distribution indicates an old age for both the *Bracovirus* and the *Ichnovirus* mutualisms. The oldest fossil microgastroid is a chelonine from Oise amber (47.8–56 Ma; [35]), but a divergence dating study [36] estimated an age of 103 Ma for the group. Although this result was largely based on an age estimate for Baltic amber fossils that is now considered too high, this number is reasonable given the extensive ghost ranges common in the insect fossil record.

As for ichnoviruses, Campopleginae and Banchinae fossils are known from the Florissant Formation (37–33.7 Ma; [37]). A recent dating study recovered a likely age of 98 Ma for Campopleginae and even 117 Ma for Banchinae [28]. These dates might have to be reviewed if it is shown that not all members of these subfamilies harbour ichnoviruses, and improved fossil sampling may also lead to the revision of these age estimates in the future. In any case, the *Bracovirus* and *Ichnovirus* domestications certainly date back many dozens of million years.

Adding a phylogenetic perspective to EVE discovery

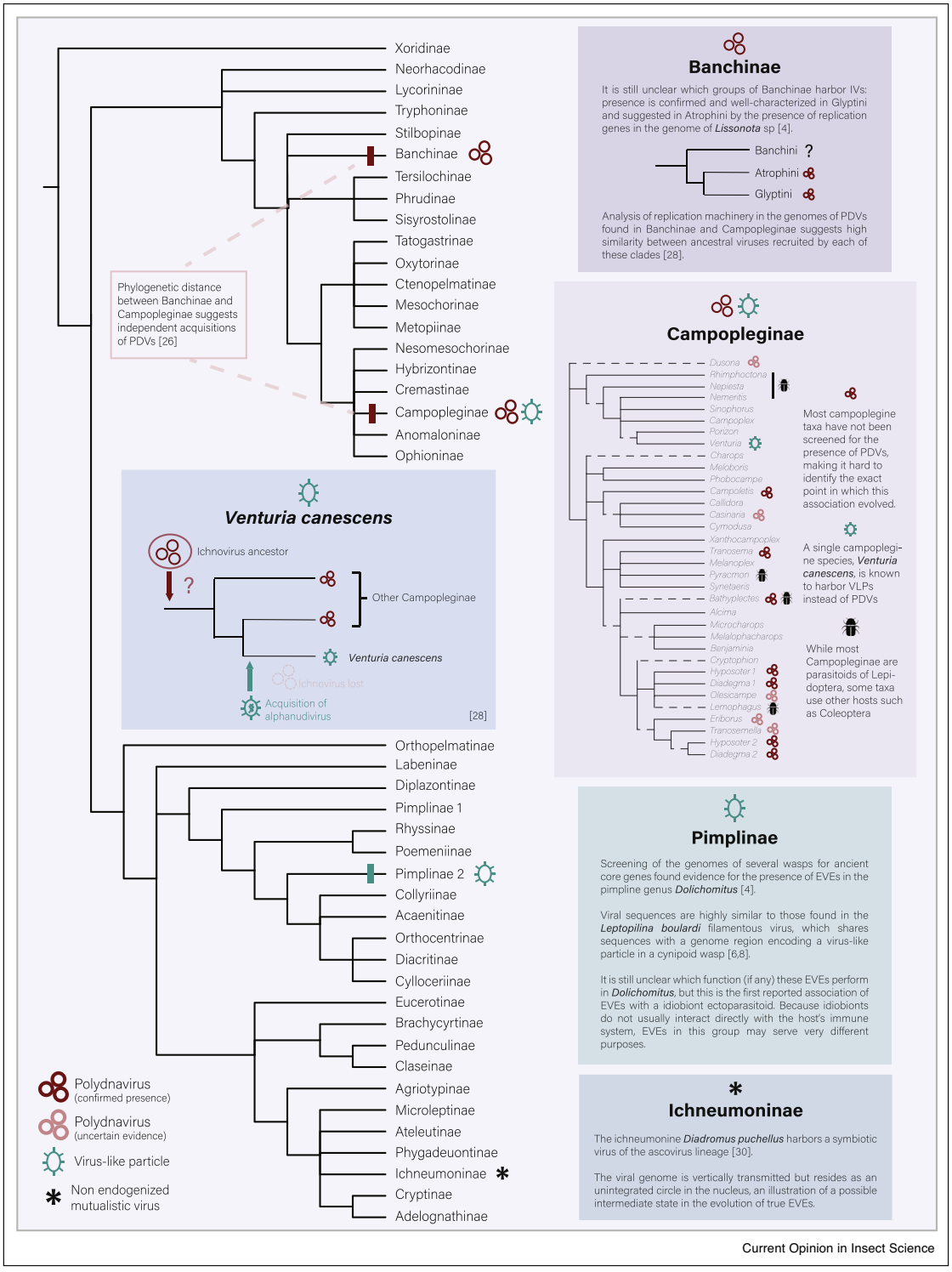
Bracoviruses and the microgastroid wasps harbouring them offer a clear example of an ancient EVE acquisition that persisted in a hyperdiverse group through time, with

Figure 1



Phylogenetic overview of the EVEs found in Braconidae. On the left, consensus phylogenetic tree of the family based on the most recent studies [26,27•]. Uncertain or poorly supported relationships are shown as polytomies and dashed lines. Lineages with EVEs are highlighted with symbols for either PDVs or VLPs. Colored boxes summarize current information about braconid EVEs. Phylogenetic relationships for VLP-carrying species of *Fopius* based on sequences for the *lef-8* gene available in Ref. [5••]. Phylogenetic tree for arthropod-infecting large dsDNA viruses modified from Ref. [4••].

Figure 2



Phylogenetic overview of the EVEs found in Ichneumonidae. On the left, consensus phylogenetic tree of the family based on the most recent studies [22–25,27•] and unpublished data. Uncertain or poorly supported relationships are shown as polytomies and dashed lines. Lineages with EVEs are highlighted with symbols for either PDVs or VLPs. Colored boxes summarize current information about ichneumonid EVEs. Simplified phylogeny of Banchinae depicts the putative relationship among tribes based on current information. Phylogenetic tree for Campopleginae based on unpublished molecular data held by the authors. The box titled '*Venturia canescens*' depicts a hypothesized sequence of events in which ichnoviruses are domesticated earlier in campoplegine evolution and lost in the *Venturia* clade following the acquisition of VLPs derived from an alphanudivirus.

the existing evidence suggesting correspondence between wasp and viral phylogenies (reviewed in Ref. [38]). For other EVEs in parasitoid wasps, it is already clear that presence of endogenous viruses cannot be assumed across entire lineages, especially for Opiinae, Banchinae and Campopleginae (Figures 1 and 2). Thus, taxonomic generalizations should be replaced by systematic screening of individual lineages using phylogenetic proximity as a roadmap. Robust demonstrations of co-phylogeny between wasps and EVEs may be the best non-exhaustive evidence of widespread presence of viral elements across entire lineages.

In many cases it is not clear whether wasp taxa for which EVEs have not been reported actually lack EVEs, possess distantly related undetected EVEs, or have simply not been sufficiently investigated to conclude anything. Current limitations to improving the situation partly revolve around the need to accurately identify and provide voucher specimens for the wasps, while at the same time preserving them in a condition suitable for transmission electroscopy (in fixative such as 2% gluteraldehyde in 0.1 M cacodylate buffer – [18•]), genomic level analysis (ultracold frozen) or preferably both with independent specimens. Ichneumonoid wasps are hyperdiverse and poorly studied, with an estimated several hundred thousand species, and include many small and relatively uncommon species that are near impossible to identify at low taxonomic levels in the field. Obtaining DNA barcodes from field samples before genome sequencing might allow streamlining of the process, although at present faunal inventories and reference barcode libraries remain highly incomplete. Currently, there are approximately 230 000 barcodes for roughly 13 000 species of Ichneumonoidea in the Barcode of Life Database (http://www.barcodinglife.org/), compared to about 47 000 described and many more undescribed species [39].

Another labor-intensive but effective method for obtaining samples is through rearing from host insects as is done at several tropical sites (e.g. Ref. [40]); there is a strong need to pursue this underdeveloped aspect of field collecting both for completeness of sampling and for understanding the natural history of virus/wasp/host insect interactions.

Indeed, very little is known about the impact of EVEs on long-term evolutionary dynamics between parasitoids and their hosts. It has been suggested that the presence of EVEs may increase host specialization and diversification within Ichneumonoidea [27•], and more specifically in the microgastroid complex [41], but specific tests of these hypotheses have not been completed. There is also growing evidence that EVEs impact multi-species interactions and thus may have far wider significance than just between the wasp and the virus. For example, recent studies show that wasp viral particles can reach the salivary glands of their hosts, which then alters the quantity and specificity of volatiles released by the host plant in response to herbivory [42,43]. These volatiles are then used as cues for parasitism for the hyperparasitoids of the EVE carrying wasps, demonstrating complex and interesting evolutionary interactions across ecological networks. The evolution of EVEs seems to occur as a response to the evolutionary need to cope with the immune system of the hosts, an interaction that applies more directly to koinobiont endoparasitoids (see Box 1). Acquisition of EVEs in wasps with other host relationships (e.g. idiobionts) may be less likely or serve very different purposes (Figure 2). Switching among host lineages may also represent a remarkable evolutionary challenge for the wasp-EVE interaction. While all microgastroids are associated with lepidopteran hosts, other discovered EVEs are in parasitoids that attack different insect orders; studying their loss or persistence during major host switches is a promising avenue of research [18•]. In addition, comparing the rate at which the viral machinery evolves with that of the packaged virulence genes would provide unique insights into selective

Box 1 Glossary terms

Endogenous viral elements (EVEs): DNA sequences of viral origin that become permanently integrated into the genome of another organism. EVEs can degrade over time or be retained and assume important roles for the biology of their host organisms.

Virus-like particles (VLPs): molecules that are structurally similar to viruses but lack any genetic material. In parasitoids, VLPs act as delivery systems for virulence factors and other wasp-derived proteins. These molecules can originate as a functional adaptation deriving directly from the metabolism of eukaryotic organisms (in which case they do not constitute EVEs) or from the genomic capture of ancient viruses with subsequent loss of the packaged DNA.

Polydnaviruses (PDVs): Large double-stranded DNA viruses whose packaged genome is composed of multiple molecules of circular DNA (segments) that encode virulence genes. In addition to these proviral segments, the integrated PDV genomes include clusters of replication genes that are expressed in the wasp calyx cells during the process of PDV production, but not packaged in the viral particles. PDVs are found in the parasitoid families Braconidae (bracoviruses, or BVs) and Ichneumonidae (ichnoviruses, or IVs).

Ancient core genes: Loci encoding replication components that are common to most families of double-stranded DNA viruses that infect insects. Targeted search for sequences of ancient core genes in parasitoid genomes is one of the tools that can be used to identify EVEs.

Koinobionts: Parasitoids that allow the host to continue feeding and developing during parasitization. Koinobionts usually live inside the tissues of the host (endoparasitoids), and as such need to cope with the host's immune system and other physiological changes. Almost all parasitoid wasps with EVEs discovered thus far are koinobiont endoparasitoids.

Idiobionts: Parasitoids that permanently interrupt the development of the host at the time of parasitization via the inoculation of an incapacitating cocktail of venom and other substances. Idiobionts are usually ectoparasitoids and likely have little interaction with the internal physiology of their hosts.

pressures caused by host switches on the wasp-virus association. Campopleginae might represent an ideal study system to tackle such questions, given their multiple transitions from Lepidoptera to Coleoptera, Hymenoptera and even Raphidioptera (Figure 2).

Finding viruses using the latest sequencing and bioinformatics techniques

Sequencing advances beyond just purification and electrophoresis [44] led to new approaches to EVE discovery, from clonal or targeted DNA [10] to mRNA amplification of known viral genes [45,46]. However, targeted amplification is complicated by the rapid mutation rate of viral genes, making primer or probe design challenging, especially when screening across diverse lineages [27•]. Genome sequencing allowed for characterization of viral gene dispersion, synteny, phylogenetic history, and other sequence characteristics beyond single genes, and thus has become the best way to validate if viruses are endogenized [10,47–50]. Further refinements in assembly and annotation have better outlined the comparative genomic composition and organization of bracoviruses and ichnoviruses across wasp genomes [12,18•,29,51,52••,53]. Genome sequencing has also led to the discovery of novel viruses, suggesting that virus domestication may be more common than previously thought and the viruses involved are more diverse and have integrated more recently than bracoviruses and ichnoviruses [4••,5••].

In addition to genome sequencing, innovative bioinformatic approaches can lead to viral discovery through targeted searches for ancient core genes that are the most commonly found across all known dsDNA viruses, many of which are arthropod infecting viruses [4••]. However, this may not indicate endogenization and may not find viruses that show little recognizable homology to other known virus lineages, as is the case with ichnoviruses. Endogenization can be inferred through discovery of viral genes dispersed through the wasp genome. If genome quality is poor, integration may be inferred indirectly by using a sliding window approach across wasp scaffolds to search for viral signatures, including intron-less genes, abrupt changes in gene order and homology, transposable elements, small RNAs, or differential gene statistics such as nucleotide composition, codon bias and read coverage [54,55]. These bioinformatic approaches can provide an economical screen, thereby helping to improve taxon sampling without the need for particularly complete genome assemblies. However, their false-positive recovery rate may be rather high, and histological examinations and TEM analysis of reproductive regions may still provide the most efficient approach to novel virus discovery across the broadest range of taxa. This approach can also help focus genomics-based approaches to specific lineages for a cost-effective and well-evidenced screening methodology.

Conclusions

The increased feasibility in generating whole genome sequence data and the recent boost in wasp phylogenetics represent an opportunity for a revolution in our understanding of the distribution of EVEs across parasitoid lineages. This opens the venue for a comprehensive catalogue of EVE-bearing lineages, studies of co-phylogeny and comparisons of genomic architecture among independent viral acquisition events. These advances, in turn, will allow us to investigate major evolutionary questions involving the association between viruses and wasps. What makes certain virus lineages susceptible to integration, while others are not? Does the use of EVEs have a significant impact on the diversification process among wasp lineages? Does the use of EVEs lead to an expansion or to a restriction in host use by parasitoids? What we do know is that the phylogenetic distribution and evolution of EVEs among parasitoid Hymenoptera is only beginning to be explored.

Conflict of interest statement

Nothing declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cois.2021.12.001.

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- • of special interest
- •• of outstanding interest

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Genome assemblies of parasitoid wasps were screened for ancient core genes that indicate the presence of EVEs. Evidence for the presence of novel EVEs was found in a novel lineage (Pimplinae) and a secondary EVE in one species of Microgastrinae. The lack of ichnoviruses in many intervening groups between Banchinae and Campopleginae indicates that viral acquisition evolved separately in the two groups. In addition to showcasing a new bioinformatic approach for EVE discovery, this study highlights that virus acquisition may be a common process in the evolutionary history of parasitoid wasps.

5. •• Burke GR, Simmonds TJ, Sharanowski BJ, Geib SM: **Rapid viral symbiogenesis via changes in parasitoid wasp genome architecture**. *Mol Biol Evol* 2018, **35**:2463-2474

This study was the first to demonstrate a recent and novel EVE type within Braconidae and in a new subfamily (Opiinae). The study annotated the alphanudivirus-related EVE within the *Fopius arisanus* genome. Screens of additional *Fopius* species showed that only a subset of species contained the virus, indicating a recent acquisition. The wide dispersal of viral genes also indicated that gene dispersal may occur relatively quickly after integration.

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The VLPs found in the figitid wasp *Leptopilina boulardi* result from an ancestral virus domestication, possibly originally involving behavioral manipulation of the wasp's host.

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This study sequenced whole genomes for two campoplegine species and compared the genomic architecture of the integrated ichnovirus genomes. Sequences that make up the proviral segments were shown to be highly dispersed in the wasp genome, while the viral replication machinery is clustered and conserved. Differing genomic architectures in ichnoviruses and bracoviruses highlight how different evolutionary trajectories have led to virus domestication.

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