

Review

Virus or not? Phylogenetics of polydnaviruses and their wasp carriers

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Abstract

Our current, still limited, understanding of the comparative biology and evolution of polydnaviruses (PDVs) is reviewed, especially in the context of the possible origins of these parasitoid viruses and of their coevolution with carrier wasps. A hypothetical scenario of evolution of PDVs from ascovirus (or ascovirus-like) ancestors is presented, with examples of apparent extant transitional forms. PDVs appear, in the case of bracoviruses, to show phylogenetic relationships that mirror those of their wasp carriers: with ichnoviruses, the picture is less clear. Ongoing sequencing studies of entire PDV genomes from diverse wasp species are likely to greatly contribute to our understanding of PDV evolution.

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1. Introduction

Endoparasitoid wasps live most or all of their larval lives inside the bodies of other animals, typically other

insects. They thus face a number of serious physiological challenges, among them the immune responses of their hosts. It is not surprising that they have evolved mechanisms to overcome host immune responses. Many of these mechanisms are remarkable in their origins and surprising in their variety. Maternal secretions produced in the female wasp reproductive organ or accessory glands enable parasitic wasps to escape the cellular defense reactions of their habitual host caterpillars

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(Stoltz, 1986). Virus-like particles, containing or lacking nucleic acids, have been isolated from reproductive systems of female wasps that actively or passively interfere with host immune responses when introduced into the host. *Venturia canescens* virus-like particles (VcVLPs) were amongst the first particles discovered and have been shown to confer protection to parasitoid eggs against host encapsulation reactions, as became apparent under conditions where the particles were removed from the egg surface (Salt, 1973; Rotheram, 1973a, b). Molecular analysis of these particles has shown that they do not contain nucleic acids and may protect parasitoid eggs by local interactions with the host (Schmidt et al., 2001) of egg surface-bound components (Kinuthia et al., 1999) or concentration-dependent immune-inhibitors (serpins) from the calyx fluid (Beck et al., 2000).

Exploration of other parasitoid wasps systems led to the discovery of virus-like particles containing DNA molecules (Stoltz and Vinson, 1979) that were later shown to express genes in infected host cells and actively to suppress the host immune system. Due to morphological appearance, the particles found in braconid wasps were initially thought to be related to baculoviruses (Stoltz et al., 1976; Stoltz, 1981; Stoltz et al., 1984). Although the morphological similarity of the two virus groups continues to be noted (Webb et al., 2000; see also Blissard et al., 2000), genetic and biochemical analyses have failed to demonstrate any close relationship. The particles from braconid wasps, called bracoviruses, are cylindrical in shape with a uniform diameter but are variable in length and have a tail-like structure (Stoltz and Vinson, 1979). A second group of particles, found in ichneumonid wasps, contain polydisperse, double-stranded, circular DNA molecules. These particles, called ichnoviruses, are morphologically distinct from the braconid particles, having a lenticular and uniform shape enveloped with two membranes. A new family of insect viruses, *Polydnaviridae*, was proposed that originally comprised only the ichneumonid particles, the braconid viruses being then still classified as baculoviruses (Stoltz et al., 1984). In recognition of the fact that both braconid and ichneumonid particles had a similar production mechanism and mode of action, they were later both classified under *Polydnaviridae* comprising two genera: *Bracovirus* and *Ichnovirus*, respectively (see Webb et al., 2000 for current summary).

Polydnaviruses (PDVs) are produced in the calyx tissue of the endoparasitoid female reproductive organ, situated in the upper part of the lateral oviduct (Stoltz and Vinson, 1979). Particles are released from the calyx cells into the lumen either by cell lysis (bracoviruses) or by budding through the membrane (ichnoviruses). At parasitization, the female wasp injects the particles together with egg(s) into the host hemocoel. When eggs are artificially injected into caterpillars without the particles or with UV-inactivated particles, they are encapsu-

lated by the host, which is an indication that the particles are essential for successful parasitism (Edson et al., 1981). The particles do not replicate in the host caterpillar but viral genes are expressed in host tissues (Fleming, 1992). Expression of these genes, which is either transient (Asgari et al., 1996) or persistent throughout parasitism (Fleming et al., 1983; Strand et al., 1992), interferes with host physiology including suppression of the cellular immune system (Shelby and Webb, 1999).

The genome or nucleic acid content of PDVs consists of several double-stranded DNA segments ranging from 2 to 28 kbp (Krell, 1991). DNA segments are integrated into the wasp genome and are vertically transmitted to the next generation in the same manner as wasp genes (Fleming and Summers, 1986; Stoltz et al., 1986; Stoltz, 1990). Belle et al. (2002) have recently located the polydnavirus DNA on chromosome 5 of *Cotesia congregata* using in situ hybridization; in most groups we do not know the location. Bracoviruses seem to contain only one DNA segment per particle (Albrecht et al., 1994), but various segments with different size classes collectively comprise the observed heterodisperse population of particles. Various studies have demonstrated sequence homologies between the segments, due to segment nesting or similarity among the genes (Webb, 1998). Segment nesting is implicated as a mechanism to increase gene copy numbers of functional genes in parasitized insects (Webb and Cui, 1998). Genome structure in PDVs is thus complex and confusing, but appears to be highly sophisticated in terms of function.

In this review, we would like to discuss aspects of the evolution of a mutually beneficial relationship between PDVs and their carrying wasps with an emphasis on the phylogenetics of bracoviruses, given that there is more information available on the taxonomy of the braconid carriers compared to that of ichneumonids.

2. An endosymbiotic origin for polydnaviruses

Symbiosis, once regarded as a rare biological event, has been more recently revealed to be a widespread phenomenon of central importance in the light of discoveries during the last 30 years in the evolution of eukaryotic cell organelles (summarized in Margulis, 1993). Many processes may be involved in the establishment of symbiotic relationships between complex organisms, including the selection of appropriate partner organisms. For millions of years, parasitoids have evolved strategies to manipulate the immune defense of their hosts, which in turn have no doubt adapted to counter the challenge. The general implication is that for the developing parasitoid to be successful, the host's counter adaptations remain one step behind those of the parasitoid. To overcome the host defense system, many parasitoids encode cellular homologues that mimic or counter-

act key molecules of host regulatory cascades in immunity and development. PDVs may represent sophisticated examples of highly adapted gene systems that interact with host functions in a concerted fashion. Whereas typical viruses are pathogenic and engage in parasitic associations with their infected organisms, in multi-specific interactions such as parasitoid-host interactions mutualism might exist between viruses and their carrier organisms. Apart from one instance in which a baculovirus has detrimental effects on the parasitoid (Hamm et al., 1988), all the other reported viruses carried by parasitoid wasps have no serious pathogenic effects on the wasp. In the cases where the overall relationship may be advantageous for both the virus and the parasitoid, it can be regarded as a mutualistic symbiosis (Whitfield, 1990; Fleming, 1992).

3. Ascoviruses and other parasitoid viruses

Ascoviruses that cause chronic/fatal diseases in the larvae of noctuid lepidopterans are transmitted among hosts by parasitoid wasps at oviposition (Federici et al., 1991, 2000) and are less infectious when orally inoculated (Govindarajan and Federici, 1990). The viral particles are bacilliform in shape and have a double-stranded DNA about 130–200 kbp in length. *Diadromus pulchellus* (Ichneumonidae) ascoviruses (DpAVs) are found in most tissues of the male and female wasps and are transmitted to the host *Acrolepiopsis assectella* due to contamination of the wasp genitalia with the virions at oviposition (Bigot et al., 1997). DpAV particles are most likely vertically transmitted to the parasitoid eggs along the germ cell-line in an episomal form. Although the genome of the virus is not integrated into the wasp genome, some data suggest that most *D. pulchellus* wasps carry the virus (Bigot et al., 1997). The virus seems to have become adapted to the parasitoid host and to have established a mutualistic relationship. The association is specific. When another parasitoid species, *Itoplectis tunetana*, harbors DpAV, rapid virus amplification in the lepidopteran host precludes the development of the hymenopteran larvae. In addition, DpAV viral products seem to modulate host physiology in favor of the developing parasitoid. This is similar to the way in which PDV genes are expressed inside the host. In turn, the wasp also seems to modulate amplification of the virus.

In addition to ascoviruses, other known or unclassified viruses have been reported from hymenopteran endoparasitoids. *Diachasmimorpha longicaudata* (Braconidae) parasitizes the tephritid fruit fly, *Anastrepha suspensa* and normally injects entomopoxviruses (DIEPVs) into the host together with egg(s). A 24 kDa parasitism-specific protein was found to be encoded by both the host and the parasite but its expression is induced by DIEPV

(Shi et al., 1999). Other virus-like particles were found in the poison glands of *D. longicaudatus* that resemble plant rhabdoviruses (Lawrence and Akin, 1990). The particles seem to help the parasitoid suppress host defence mechanisms (Rolle and Lawrence, 1994).

Virus-like particles produced in the accessory glands of the parasitoid *Leptopilina heterotoma* specifically attack lamellocytes of the host *D. melanogaster* (Rizki and Rizki, 1984; Rizki and Rizki, 1990). Stoltz and Makay (2000) reported co-replication of a reovirus and a polydnavirus in a colony of the ichneumonid wasp, *Hyposoter exiguae*. In addition to the wasp's reproductive system and calyx cells, the reovirus was detected in most other tissues. Unusual virus-like particles (CmV2) were described in *Cotesia melanoscela* which apparently replicate in both the parasitoid and host tissues (Stoltz and Faulkner, 1978). Further investigations indicated that these described particles do not belong in Polydnaviridae since they lack polydisperse DNA (Stoltz et al., 1988b). It appears that CmV2 also replicates in non-reproductive tissues of the female and male parasitoid. However, in this case, no pathogenicity toward parasitoids is apparent.

4. A scenario for the evolution of PDVs

In most cases mentioned above, the viruses have limited or no effect on the wasp but are pathogenic to the parasitoids' hosts. Therefore, it is quite tempting to speculate that they originated from lepidopteran pathogens rather than hymenopteran. The isolated viruses are all capable of replicating in host (lepidopteran) cells in which they have pathologic effects, although they are transmitted by the apparently unaffected wasps. During the course of evolution, these viruses might have been "domesticated" by certain wasps; in some cases, viral genetic material may have become integrated into the wasp genomes and tissue-specific replication mechanisms evolved that are regulated by the wasps (Fig. 1). This may have been accompanied by the deletion of virus-specific genes required for replication. PDVs, in which complete integration of the genome into the chromosomes of the carrying wasps has occurred, apparently only contain genes that are necessary to modulate physiological processes of the host caterpillar so as to advance the developing endoparasite. Ascoviruses are, in functional terms, arguably the closest possible relative to at least some PDVs, appearing to be a hypothetical ancestral form. DpAV, with its genome being transmitted vertically to the wasp offspring in an episomal form (Bigot et al., 1997, 2000), is perhaps in a transitional phase from a fully independent to a semi-dependent virus (Fig. 1). Bracoviruses and ichnoviruses, in which there is integration of the genome into wasp chromosomes, with single and multiple DNA molecules


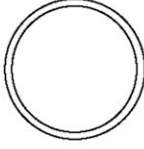
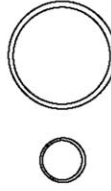
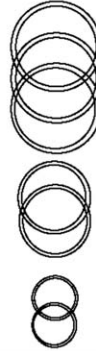
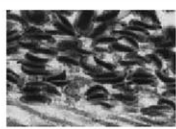
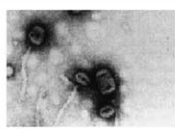
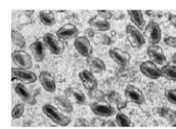
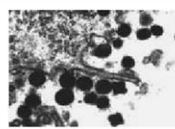
Virus				
	Ascoviruses	Bracoviruses	Ichnoviruses	Virus-like particles
Integration into Wasp genome	-	+	+	+++
Extrachromosomal viral DNA				none
Segment packaging per virion	Single	Single	multiple	-
Example	 <i>Diadromus pulchellus</i>	 <i>Chelonus inanitus</i>	 <i>Campoletis sonorensis</i>	 <i>Venturia canescens</i>

Fig. 1. A speculative evolutionary process of viral symbiogenesis. PDVs and VcVLPs might have been derived from an ancestral host-specific pathogenic virus which become exposed to an ancestral parasitoid. Thus, some viruses associated with endoparasitic hymenopterans might represent transitional stages between pathogenic and mutualistic PDVs. In this regard, an ascovirus (AV)-like progenitor might perhaps be considered as ancestral to both PDVs and VcVLPs. DpAV particles are not pathogenic to the carrying wasp but when injected into the host caterpillar at oviposition they replicate and cause a fatal disease. The genome, consisting of a circular dsDNA molecule, is vertically transmitted to the offspring in an episomal form. Bracovirus and ichnovirus particles singly or multiply encapsidate circular DNA molecules, respectively, and their DNA content is carried by the wasp chromosome in an integrated form. The particles do not replicate in the host caterpillar but viral proteins expressed in infected host cells interfere with host physiology. VcVLPs confer protection to the developing parasitoid by VLP-proteins that are exclusively produced in the wasp ovary.

incorporated into particles (Albrecht et al., 1994; Stoltz et al., 1981), may represent the next stage of an evolutionary process (operating independently within the two wasp groups) towards complete dependence of virus functions on the wasp. In this context, *V. canescens* VLPs, lacking DNA molecules, may be viewed as relying on immune regulative properties performed by particle proteins produced exclusively as maternal protein secretions. In a recent study, it has been shown that coat proteins from VcVLPs, *Campoletis sonorensis* PDVs, *Spodoptera frugiperda* AV-1a and DpAV-4a have sequence and structural similarities (Federici and Bigot, this issue). This study also suggests that VcVLPs and PDVs may have been derived from AVs. While bracoviruses are not obviously derived from AVs, we suggest that similar early stages in development of the virus/host interaction to those we have described above are likely to also have been true of bracovirus evolution.

The relationship between PDVs and parasitoid wasps might be regarded as *integration*—‘the display of structures, functions, etc which are more than and different from, those of which the participants are capable as individuals’ (Lewis, 1985). Considering their current status,

the question is whether PDVs should be regarded as viruses or not. With the accumulation of molecular data, it is apparent that these particles are an integrated part of the parasitoid survival strategy. In the analogous cases of the endosymbiogenesis of eukaryotic cells and the evolution of eukaryotic organelles (eg. mitochondria and chloroplasts) from prokaryotes, some properties have clearly evolved from simple bacteria, while others can only be understood in the context of their status as organelles that are an integral part of eukaryotic cells. In their current forms, PDVs are just as highly integrated a component of their carrier wasps.

5. True viruses? Or maternal protein secretions?

Although PDVs have presumably evolved from more conventional pathogenic viruses, it is probably useful, in functional terms, to regard these particles as wasp protein secretions or wasp-mediated gene delivery systems. In other words, the functional properties of maternal secretions encoded by the parasitoid genome may also be regarded as part of the extended phenotype (sensu

Dawkins, 1990) by which the parasitoids are enabled to modify the physiology of their hosts. In fact, if we consider these particles as wasp delivery tools, some mysteries and peculiarities can be resolved without imposing virological concepts onto biological observations. True viruses replicate, producing progeny within infected hosts. The so-called “genome” of PDVs relies for reproduction on the wasp chromosomal mode of replication. The nucleic acid content of PDVs contains non-coding sequences, which is more in line with the wasp genome structure. Introns are usually found in PDV genes (as in wasp genes) but are rarely found in viral genes. In addition, polydnal gene expression is not typical of viruses, since gene expression does not coincide with virus replication. As suggested by Stoltz and Xu (1990), PDVs do not participate in the host-parasitoid interaction as genetically independent entities since the extra-chromosomal viral DNA plays no role in the genetic transmission of virus-DNA RFLP polymorphism (Stoltz and Xu, 1990). Polymorphisms that exist in viral genomes between and within populations of wasps might simply relate to the ability of the parasitoid to adapt to new hosts.

Production of bracovirus and ichnovirus particles and VcVLPs is developmentally regulated (Albrecht et al., 1994; Webb and Summers, 1992; Reineke et al., 2002) and seems to be induced by ecdysteroid molting hormones. In a recent study, it was shown that the production of excised circles from the genome is also developmentally regulated (Savary et al., 1999). Although the mechanism of excision and circularization seems to be due to recombination of homologous repeat regions flanking the segments, the mechanism of DNA amplification is largely unknown. In this regard, a rolling circle amplification has been suggested, although it remains to be experimentally demonstrated. Another mechanism could be local amplification of DNA fragments similar to the case of chorion genes in follicle cells during *Drosophila* oogenesis (Spradling, 1993). Chorion genes are amplified above the copy number of the remainder of the follicle cell genome before and during a time of high-level transcription. This phenomenon is also developmentally regulated and influenced by ecdysteroids.

PDV genes that are expressed in the host are not known to show significant sequence similarity to other virus genes (although it appears that some have no known similarity to genes from other wasp groups either). What little information exists on structural proteins of PDVs shows that the coding sequence for some of these proteins is not encapsidated (p44 from CsPDVs (Deng et al., 2000) and Crp32 from CrPDVs (Asgari et al., 1998)) but is instead encoded by the wasp genome. Studies also indicate that not only certain PDV structural proteins (Strand et al., 1994; Webb and Luckhart, 1994; Asgari, unpublished data) but also at least some viral expression products (Webb and Summers, 1990) show

similarity with venom proteins. There is also evidence that viral genes show tissue-specific regulation with venom proteins. The open reading frame (ORF) encoding a small venom protein from *Cotesia rubecula* is located upstream in opposite direction to the ORF for Crp32 and is part of 5'-untranslated region of Crp32 cDNA (Asgari et al., 2003), causing the two genes to be expressed in calyx and venom tissues in a mutually exclusive fashion. In some cases, PDVs are only effective when they are accompanied by venom proteins (Kitano, 1986; Tanaka, 1987; Stoltz et al., 1988a). The three isolated VcVLP proteins are closely related to proteins associated with basic cellular wasp functions, which raises the question whether some of these proteins may be of insect origin (Reineke et al., 2002; Hellers et al., 1996; Asgari et al., 2002). These observations further support the idea that PDVs/VLPs are perhaps wasp-generated delivery systems that are parts of an extended phenotype of the parasitoid wasp.

6. Phylogenetics of polydnalviruses: co-phylogeny with hosts?

From very early on in the identification and understanding of the roles of polydnalviruses in parasitoid/host interactions, it was realized that related groups of wasps tended to have morphologically similar viruses (Stoltz and Vinson, 1977, 1979). This realization arose not only due to the morphological types characterized above as ichnoviruses and bracoviruses, but also due to trends within each group, especially within the bracoviruses. Serological comparisons (Cook and Stoltz, 1983), as well as Southern blots of DNA circlets from various species versus PDV DNA from a reference species (e.g., Stoltz et al., 1981; Stoltz and Whitfield, 1992), suggested that more closely related wasps carried more genetically similar viruses. From these early perceived trends, as well as from the knowledge that PDVs were inherited as integrated chromosomal DNA in Mendelian fashion, several phylogenetic predictions were made.

First, it was predicted that those wasps that carry the PDVs would form monophyletic lineages (Whitfield, 1990; Stoltz and Whitfield, 1992). This prediction has been borne out convincingly at least within the Braconidae. Whitfield (1997) demonstrated that the subfamilies of wasps that carry bracoviruses all belong to a monophyletic “microgastroid assemblage”, based on both morphological data from Whitfield and Mason (1994) and DNA sequence data from the mitochondrial 16S rRNA gene. These relationships have been confirmed by subsequent studies incorporating additional taxa and an additional gene, the nuclear 28S rRNA gene. While it has not yet been demonstrated that all subfamilies and genera within this group carry bracoviruses (several rare groups have not yet been tested), all species so far tested

within this lineage have been found to harbor them. Recently, Whitfield (2002) estimated that this lineage is approximately 74 (± 11) million years old, by using molecular clock calculations from three genes, calibrated by available braconid fossil ages. The general picture as we currently understand it is depicted in Fig. 2.

In ichneumonids, there is also a tendency for ichnovirus-bearing wasps to be confined to the subfamily Campopleginae (Stoltz and Whitfield, 1992; Webb et al., 2000). There are exceptions, however, including ichnoviruses known from the subfamily Banchinae (Stoltz et al., 1981). The Banchinae, while relatively closely related to the Campopleginae, do not appear to on the basis of either morphological evidence (Gauld, 1985; Wahl, 1988, 1991) or of DNA sequence data (Belshaw et al., 1998), to be their sister-group. It is also not clear that all Campopleginae carry ichnoviruses, even if one counts the virus-like particles of *Venturia* (Rotheram, 1967; 1973a, b; Feddersen and Schmidt, 1986) and

Bathyplectes (Hess et al., 1980; Stoltz et al., 1981) as ichnoviruses. We are not yet in a position to say definitively whether or not the ichnovirus-bearing wasps form a monophyletic lineage, but current evidence suggests that perhaps the situation may be more complicated.

A second phylogenetic prediction is that, within each wasp lineage carrying the viruses, phylogeny of the PDVs should mirror the phylogeny of their wasp carriers (cophylogeny). Any deviations from this pattern would suggest that horizontal transmission of PDVs occurs between wasps sharing the same hosts, either currently (there is no direct evidence for this) or historically. The prediction of co-phylogeny has been much more difficult to test than that of wasp carrier monophyly, for several reasons. First, for statistical testing of co-phylogeny, wasp phylogenies (as well as viral phylogenies) must be estimated with relatively high confidence. This is currently true at the subfamily level for the bracovirus-bearing wasp lineage (Whitfield, 1997; Dowton and Austin,

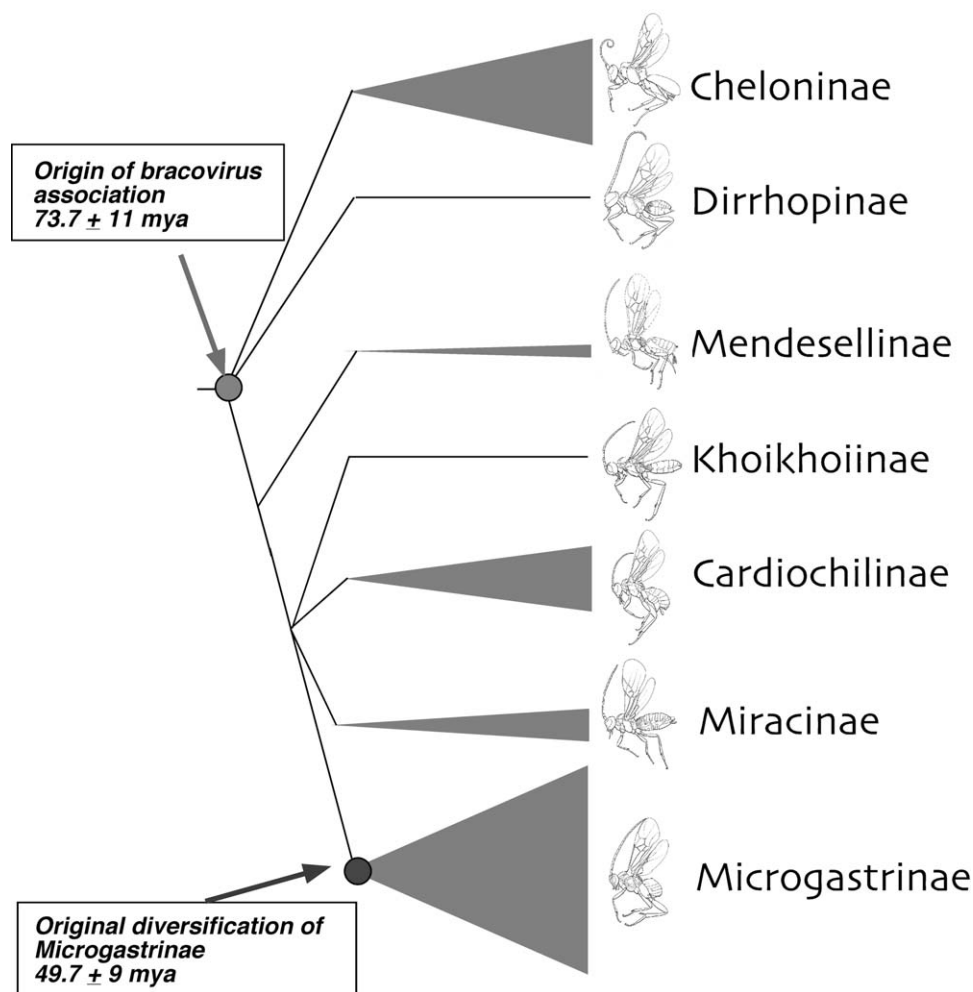


Fig. 2. Summary of relationships among the bracovirus-bearing wasps (based on Whitfield, 1997; Dowton and Austin, 1998), indicating estimated times of origin of the virus-bearing clade and of the subfamily Microgastrinae (dates from Whitfield, 2002). The uncertainty of the branching order among Khoikhoiinae, Cardiochilinae, Miracinae and Microgastrinae stems from some conflict among data sets, and from the absence of molecular data for Khoikhoiinae. Line drawings of taxa are modified from Wharton et al. (1997).

1998; Whitfield, 2002) but not yet very confidently so for the ichnovirus-bearing groups. Knowledge of PDV phylogeny is still in its infancy (but see below). Second, knowledge of PDV genes is very recent and extremely incomplete, especially at the comparative level. Current efforts in several laboratories to sequence entire genomes of multiple PDVs will ultimately “fill in the blanks” here, although it is not yet clear that gene content is highly conserved among PDVs. At this point we have only a few isolated PDV genes that are known from multiple wasp species, and we are dependent upon the patterns of variability within those genes for estimation of PDV phylogeny.

Nevertheless, some progress has been made in testing co-phylogeny between the bracovirus-bearing wasps and their viruses. Whitfield (2000) found essentially perfect (and statistically significant) correspondence (Fig. 3) between phylogenetic relationships among several *Cotesia* species (based on the mitochondrial 16S rRNA and ND1) and their bracoviruses (based on the *CrVI* gene originally characterized in *C. rubecula*). Current studies

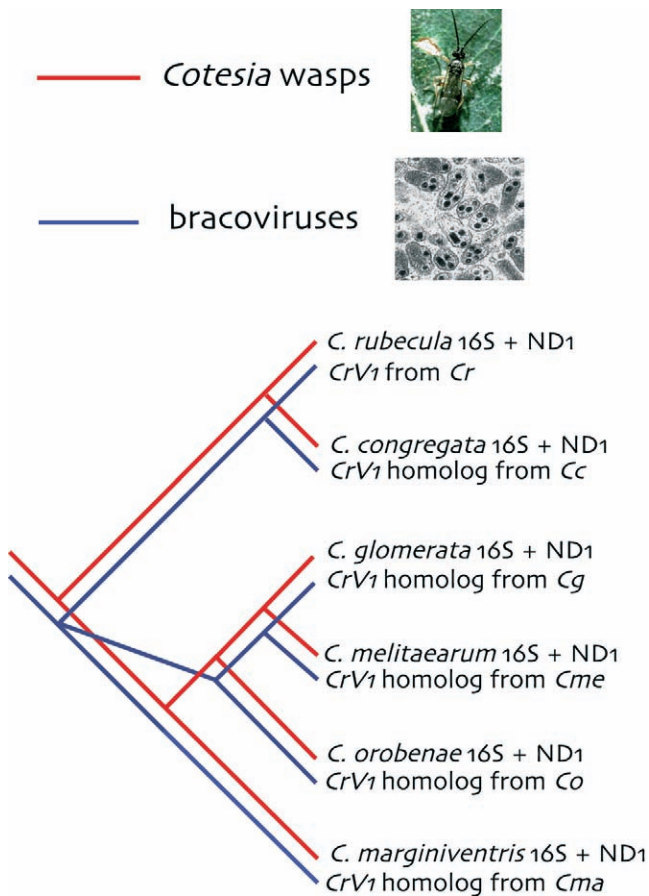


Fig. 3. Co-phylogenetic pattern of relationships between several species of *Cotesia* (red lines) based on 16S and ND1 DNA sequences, and the PDVs they carry (blue lines), based on sequences from *CrVI* gene homologs. This degree of correspondence is statistically unlikely to have arisen by chance (Whitfield, 2000), and instead is likely to reflect the hereditary mode of transmission.

(Michel-Salzat and Whitfield, unpublished) appear to confirm this trend, at least within microgastrinae wasps, based on the *CrVI* gene (Asgari et al., 1996) and the *EPI* gene characterized from *C. congregata* (Harwood and Beckage, 1994; Savary et al., 1997).

7. Future prospects

It is evident that the PDV/parasitoid wasp integrated system, as we currently understand it, represents one of the most amazing examples of endosymbiosis known. From what we know already, what would appear to have arisen as pathogenic associations between viruses and insects has evolved into a number of coevolved, tightly linked functional complexes of genes no longer dissectable into separate biological entities. Phylogenetic studies reinforce this view of mutual tight interrelationship in evolutionary time, indeed to the degree that PDVs cannot only be identified from, but also more or less defined as parts of, their wasp carriers. It is thus at least possible that PDVs are entirely wasp in origin (or nearly so), although such an origin would require the evolution of virus-like packaging mechanisms *de novo*.

We are still profoundly ignorant of much of the evolution and function of PDV genes. Our understanding of this system, both from a comparative physiological and from an evolutionary viewpoint, will become vastly richer as the genomes of multiple PDVs are fully sequenced, and as the comparative structure and function of additional PDV genes is explored. At this point we can see only the tip of the iceberg.

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