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Viruses and Virus-like Entities in the Parasitic Hymenoptera

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Abstract.— We provide here an overview of viruses and virus-like agents affecting the parasitic Hymenoptera. An amazingly complex variety of such agents is now known. By far the majority are non-pathogenic, replicating primarily in the female reproductive tract, from which they are often if not invariably delivered into host insects during oviposition; most, in fact, would appear to be beneficial to the parasitoids in which they are found. Emphasis is necessarily placed on the better known polydnaviruses, which are carried by perhaps tens of thousands of braconid and ichneumonid species. Polydnavirus DNA appears to be permanently integrated into parasitoid genomes, thereby ensuring transmission to all progeny; in keeping with this observation, these, where present, are known to be required for successful parasitism. Relationships among the polydnaviruses are discussed in terms of their possible relevance to parasitoid phylogeny and classification.

Like other insects, the parasitic Hymenoptera must presumably from time to time be subject to infectious viral disease. There are, to be sure, numerous opportunities. For example, viruses could readily spread horizontally between larval cohorts of those species which are either gregarious or polyembryonic. The host itself could represent a source of infectious virus for developing parasitoid larvae, and possibly as well for adult wasps feeding upon hemolymph exuded from oviposition sites. Finally, infectious disease agents could be transmitted mechanically via contaminated ovipositors of hyperparasites. Yet, oddly enough, viral diseases in the parasitic Hymenoptera are seemingly rare. Reasons for this are not immediately evident. Conceivably, many parasitoids succumb to disease prior to emergence from their hosts; such events might easily go undetected, even in a laboratory situation.

A SURVEY

In fact, we are aware of only one example of a recognized viral pathogen of parasitoids: a recently described baculovirus from the braconid, *Microplitis croceipes* (Cresson) (Hamm et al. 1988); thus far, replication has been observed only in fat body tissue. A baculovirus has also been described from the ichneumonid parasitoid, *Mesoleius tenthredinis* (Morley) (Stoltz et al. 1981). Like most of the viruses and virus-like agents that are described below, this virus appears to replicate only in the female reproductive tract and/or its accessory glands; like *all* of the agents described below, the *Mesoleius* baculovirus is apparently non-pathogenic (at least for the *parasitoid* host).

The best-known of the parasitoid-associated viruses are the polydnaviruses, which now constitute a recognized virus family (Polydnaviridae) within which 2 groups are recognized, namely the bracoviruses and ichnoviruses, which differ in terms of both morphology and host range; the polydnaviruses will be subsequently examined in considerable depth, and so are not discussed further here. Emerging as potentially a second virus family is a growing assemblage of entities having as a distinct common morphology a long, filamentous, enveloped nucleocapsid. Examples of these viruses are known from both braconid (Stoltz and Vinson 1977 and 1979a; Styer et al. 1987; Hamm et al. 1990;) and ichneumonid (Krell 1987) parasitoids; one has been shown to replicate in host tissues (Styer et al. 1987). In terms of nucleocapsid morphology, the filamentous virus observed in the braconid *Cotesia congregata* (Say) (Stoltz and Vinson 1977, 1979a) very closely resembles a filamentous virus from the tsetse fly *Glossina pallidipes* Austen (Odindo et al. 1986). It should be noted that a somewhat similar virus has been described from honey bees (Clark 1978; Bailey et al. 1981).

A variety of other viruses (again all *non*-pathogenic) have been reported from parasitoid species (Table 1). Stoltz et al. (1988a) describe an unusual virus (designated CmV2) which apparently replicates in all individuals of certain *Cotesia*

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Table 1. Viruses and virus-like particles in the parasitic Hymenoptera.

Type	Host species	Site(s) of replication in: parasitoid	host	References
Viruses				
baculovirus	*Mesoleius tenthredinis*	ovary	not known	Stoltz (1981)
baculovirus[1]	*Microplitis croceipes*	fat body	not known	Hamm et al. (1988)
rhabdovirus	*Diachasmimorpha longicaudatus*	venom gland	not known	Lawrence and Akin (1990)
poxvirus	*Diachasmimorpha longicaudatus*	venom gland	hemocytes	Lawrence and Akin (1990)
long, filamentous (unclassified)	*M. croceipes* *Cotesia congregata* *Cotesia hyphantriae* *Diadegma terebrans*	ovary	not known	Stoltz and Vinson (1977, 1979a) Krell (1987), Hamm et al. (1990)
unclassified	*Cotesia melanoscela*	ovary, muscle, hemocytes, tracheal epithelium	fat body, hemocytes	Stoltz et al. (1988a)
polydnavirus	see Table 2	ovary	no	Stoltz and Vinson (1979a) Stoltz et al. (1981) Krell (1991)
Virus-like particles[2]				
	Venturia canescens	ovary	not known	Rotheram (1967, 1973) Feddersen and Schmidt (1986)
	Meteorus leviventris	venom gland	not known	Edson et al. (1982)
	Bathyplectes spp.	ovary	not known	Hess et al. (1980), Stoltz et al. (1981)
	Leptopilina heterotoma	"long gland"[3]	not known	Rizki and Rizki (1990)

[1] The only viral pathogen known thus far in the parasitic Hymenoptera.
[2] So-called either because they do not contain nucleic acid (e.g., *Venturia*), or else because they bear no resemblance to any known viruses.
[3] Convincing evidence for particle morphogenesis has yet to be provided.

melanoscela (Ratzeburg) populations. Having large fusiform nucleocapsids, this virus superficially resembles the ichnoviruses (Stoltz and Faulkner 1978), ascoviruses (Federici 1983), and a virus-like particle observed in fire ants (Avery et al. 1977); however, unlike any of the polydnaviruses, CmV2 does not have a segmented genome (Stoltz et al. 1988a). Replicating in the ovarian calyx, among other tissues, CmV2 is able to gain entry into the oviduct, and is subsequently injected into host larvae during oviposition; replication ensues, but apparently with no ill effect.

Lawrence and Akin (1990) have recently described two "virus-like" agents replicating in the venom apparatus of the braconid, *Diachasmimorpha longicaudatus* (Ashmead). One of these is almost certainly a rhabdovirus, and the other an entomopoxvirus. Both are apparently delivered to host animals during oviposition, with subsequent replication occuring in the case of at least one of them (the poxvirus). It now seems probable that both viruses are present in *all* individuals (Lawrence, personal commununication). No information is yet available concerning the effects, if any, of either virus upon host physiology. Virus-like particles have also been described in the venom apparatus of another braconid, *Meteorus leviventris* (Wesmael) (Edson et al. 1982) but these remain uncharacterized.

Recently, a particulate secretion has been reported by Rizki and Rizki (1990) in the cynipoid wasp *Leptopilina heterotoma* (Thomson). These

particles, which are apparently formed within an accessory gland of the wasp ovary, are immunosuppressive within host *Drosophila* larvae. Specifically, the *Leptopilina* particles can be shown to destroy lamellocytes, which are otherwise capable of encapsulating parasitoid eggs (Rizki and Rizki 1984). At this point, it is not known whether the particles contain nucleic acid, and details of morphogenesis are unavailable. Accordingly, these particles are best referred to as "virus-like". Since the particles can be readily purified, it seems likely that additional information relating to this fascinating biological system will emerge in the near future.

Finally, much of the inspiration leading to the discovery and characterization of the polydnaviruses (see below) has surely been derived from the early studies of Salt and Rotheram on the ichneumonid parasitoid, *Venturia canescens* (Gravenhorst) and its host *Anagasta kuhniella* (Zeller); for details, the reader should consult reviews by Salt (1968, 1970). Briefly, Rotheram (1967) identified a particulate secretion comprised largely of virus-like particles produced in the nuclei of cells found in the calyx, a region of the ovary situated between the ovarioles and the oviducts. Salt (1968, 1970), and more recently, Feddersen and Schmidt (1986), showed that "calyx fluid" conferred resistance on parasitoid eggs to host immune responses. It has now been determined that the virus-like particles share antigenic determinants with one or more host proteins (Feddersen and Schmidt 1986), suggesting that the parasitoid egg surface (which is coated with particles: Rotheram 1973) is recognized as self, hence evading surveillance by the host immune system. Despite their origin in cell nuclei, *Venturia* particles do not contain DNA (Feddersen and Schmidt 1986). A reasonable working hypothesis might posit that *Venturia* carries a "defective" polydnavirus, the genome of which resides within the parasitoid genome, but fails to become packaged into virus particles.

THE POLYDNAVIRUSES

In what follows, we provide a brief overview of what is currently known about the polydnaviruses. In so doing, we will highlight those features which distinguish this group from what may be referred to as "typical" viruses. Finally, considerable emphasis will be placed on the question of why polydnaviruses should be of interest to the hymenopterist community.

Classification.— With regard to viruses replicating in animal hosts, the great majority may be conveniently assigned to a particular family on the basis of three criteria: site of replication (nucleus vs. cytoplasm), morphology, and information concerning the nature of the genome. In the case of the polydnaviruses, the latter has particular significance as a diagnostic tool, since it allows us to readily distinguish these viruses from all others. Briefly, encapsidated polydnavirus genomes consist of a *poly*disperse population of double-stranded circular *DNA* molecules (hence, *polydna*virus; Stoltz et al., 1984); within this population, there exist at least 20-30 classes of molecules differing in terms of molecular size (Krell and Stoltz 1979, 1980, Stoltz et al. 1981, Krell et al. 1982). As will be seen (see: Life Cycle, below), there also exists a linear, chromosomal, copy of the polydnavirus genome. In terms of morphology, the polydnaviruses comprise two quite distinct groups, the so-called bracoviruses and ichnoviruses; as the names suggest, these are found, respectively, in certain braconid and ichneumonid wasps only. Bracovirus particles consist of cylindrical nucleocapsids, of variable length, surrounded either individually or in groups by a single unit membrane; ichnovirus particles consist of fusiform nucleocapsids surrounded by two unit membrane envelopes (Stoltz and Vinson 1979a). Typical examples are given in Figure 1. Bracovirus structure clearly resembles that of the baculoviruses, at least in some respects (Stoltz and Vinson 1979a); ichnovirus particles only superficially resemble a number of large viruses having lenticular nucleocapsids (e.g. Avery et al. 1977; Stoltz and Faulkner 1978; Federici 1983).

Distribution.—Preliminary lists of species carrying polydnaviruses have been provided by Stoltz and Vinson (1979a) and Stoltz et al. (1981); more recent compilations are given by Krell (1991) and in Table 2. Among the Braconidae, polydnaviruses are known from three subfamilies: Cheloninae, Microgastrinae, and Cardiochilinae. Within the same lineage are included several other subfamilies (Miracinae, Khoikhoiinae, Adeliinae and probably also Neoneurinae; see Fig. 2), some or all of which might reasonably be predicted to carry polydnaviruses. Ichnoviruses have been, thus far, detected primarily in the Campopleginae. However, isolates from *Glypta* spp. (Stoltz et al. 1981,

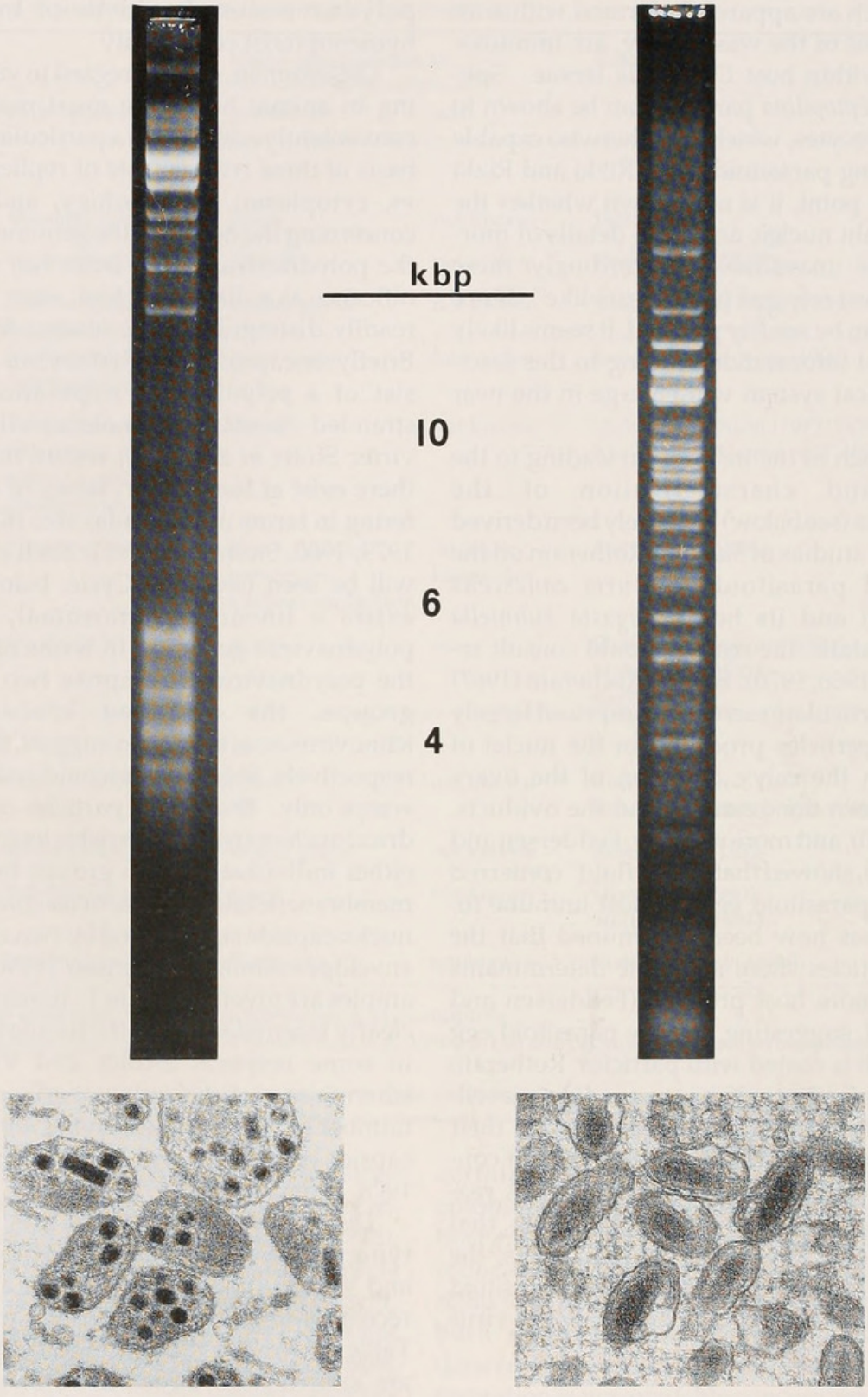

Fig. 1. Typical examples of polydnavirus particles and encapsidated genomes. Below left is shown an electron micrograph of a bracovirus (from *Protapanteles paleacritae*) and, to the right, an ichnovirus (from *Hyposoter exiguae*). Above these, 0.8% agarose gel electrophoretic profiles of DNAs extracted from calyx fluids are seen after ethidium bromide staining; approx. 1 µg of viral DNA is present in each lane. Left, bracovirus DNA from *Microplitis croceipes*; right, ichnovirus DNA from *H. rivalis*. The bands represent different populations of *intact* (i.e., undigested) circular DNA molecules. Size markers (in kilobase pairs) are given for the superhelical forms of viral genome segments. Experience thus far suggests that bracovirus genome segments typically exhibit a relaxed open circular topology, and are for the most part larger than 10 kb. Ichnovirus genome segments are often smaller (e.g., 2-10 kb in the genus *Hyposoter*); superhelical forms tend to predominate, especially when DNAs have been extracted from purified virus particles.

Table 2. Parasitoid species in which polydnaviruses have been found. For references, consult Stoltz and Vinson (1979a), Stoltz et al. (1981), and Krell (1991). Ichnoviruses were not, prior to the present report, known from either *Dusona*, *Lissonota* or *Synetaeris*. *M. demolitor* is also a new listing (see Strand and Dover 1991).

BRACONIDAE

Cheloninae
- *Ascogaster argentifrons* (Provancher)
- *Ascogaster quadridentata* Wesmael
- *Chelonus blackburni* Cameron
- *Chelonus altitudinis* Viereck
- *Chelonus* nr. *curvimaculatus* Cameron
- *Chelonus insularis* Cresson
- *Phanerotoma flavitestacea* Fischer
- *Campoletis sonorensis* (Cameron)

Cardiochilinae
- *Cardiochiles nigriceps* Viereck

Microgastrinae
- *Apanteles crassicornis* (Provancher)
- *Apanteles fumiferanae* Viereck
- *Cotesia congregata* (Say)
- *Cotesia flavipes* (Cameron)
- *Cotesia glomerata* (Linnaeus)
- *Cotesia hyphantriae* (Riley)
- *Cotesia kariyai* Watanabe
- *Cotesia marginiventris* (Cresson)
- *Cotesia melanoscela* (Ratzeburg)
- *Cotesia rubecula* (Marshall)
- *Cotesia schaeferi*(Marsh)
- *Diolcogaster facetosa* (Weed)
- *Glyptapanteles flavicoxis* (Marsh)
- *Glyptapanteles indiensis* (Marsh)
- *Glyptapanteles liparidis* (Bouché)
- *Hypomicrogaster ecdytolophae* (Muesebeck)
- *Microgaster canadensis* Muesebeck
- *Microplitis croceipes* (Cresson)
- *Microplitis demolitor* Wilkinson
- *Pholetesor ornigis* (Weed)
- *Protapanteles paleacritae* (Riley)

ICHNEUMONIDAE

Banchinae
- *Glypta fumiferanae*
- *Glypta* sp.
- *Lissonota* sp

Campopleginae
- *Campoletis aprilis* (Viereck)
- *Campoletis flavicincta* (Ashmead)
- *Campoletis* sp.
- *Casinaria forcipata* Walley
- *Casinaria infesta* (Cresson)
- *Casinaria* sp.
- *Diadegma acronycta* (Ashmead)
- *Diadegma interruptum* (Holmgren)
- *Dusona* sp.
- *Eriborus terebrans* (Gravenhorst)
- *Hyposoter annulipes* (Cresson)
- *Hyposoter exiguae* (Viereck)
- *Hyposoter fugitivus* (Say)
- *Hyposoter lymantriae* (Cushman)
- *Hyposoter rivalis* (Cresson)
- *Olesicampe benefactor* Hinz
- *Olesicampe geniculatae* Quednau & Lim
- *Synetaeris tenuifemur* (Walley)
- *Tranosema* sp.

and unpublished data) are known; this genus is included in a different taxon: Banchinae (Gauld 1984; Wahl 1988). The two subfamilies are fairly closely related, so that surveys of additional related subfamilies (Figure 3) for the presence of polydnaviruses would presumably be worthwhile.

Preliminary observations suggest the following:

— each "affected" parasitoid species carries a different polydnavirus characteristic of that species.

— if one species within a particular genus carries polydnavirus, they all are likely to.

Needless to say, most groups of parasitic Hymenoptera have not yet been examined in any systematic way for the presence of polydnaviruses. Incidental observations, however, suggest that these viruses are not present in *Alysia* (Alysiinae), *Bracon* (Braconinae), or *Aleiodes* (Rogadinae) in the Braconidae (Stoltz, unpublished). Although both *Alysia* and *Aleiodes* are endoparasitic, they appear to comprise a lineage independent from the microgastrine complex of subfamilies (Tobias 1967; Capek 1970; Shaw 1983; Gauld 1988; Gauld and Bolton 1988; Quicke and van Achterberg 1990; Whitfield in press). Among the ichneumonid taxa, again little is known concerning the incidence of polydnavirus carriage, if any, within the majority of subfamilies.

Life Cycle.—Polydnavirus replication is strictly nuclear, and appears to be limited to the ovarian calyx of certain species of parasitic Hymenoptera. Replication begins during the pupal stage (Norton and Vinson 1983; Theilmann and Summers 1986), and has long been assumed to be triggered by hormonal changes occurring during morphogenesis of the ovary; more recently, viral replication in explanted *Campoletis sonorensis* (Cameron) ovaries has been shown to be induced by ecdysone (Webb and Summers in press). Aside from this observation, the molecular details of viral replication remain

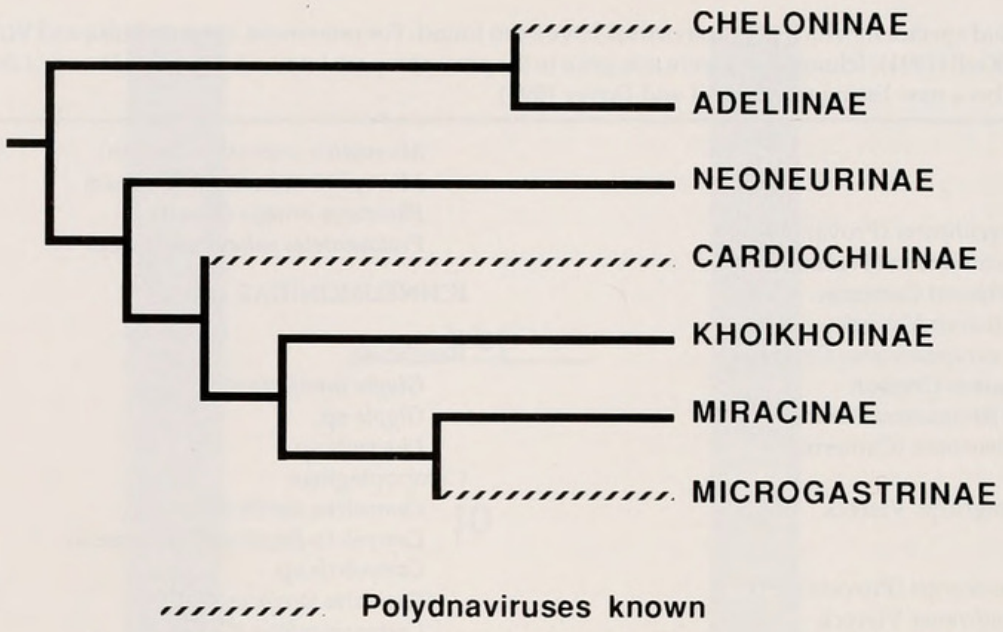

Fig. 2. Phylogenetic hypothesis for the subfamilies of Braconidae that carry polydnaviruses. Adapted from Austin (1990); based also on data from Mason (1983). There is no general agreement that the Ichneutinae also belong in this complex (see, e.g., Quicke and van Achterberg 1990), but the possibility exists.

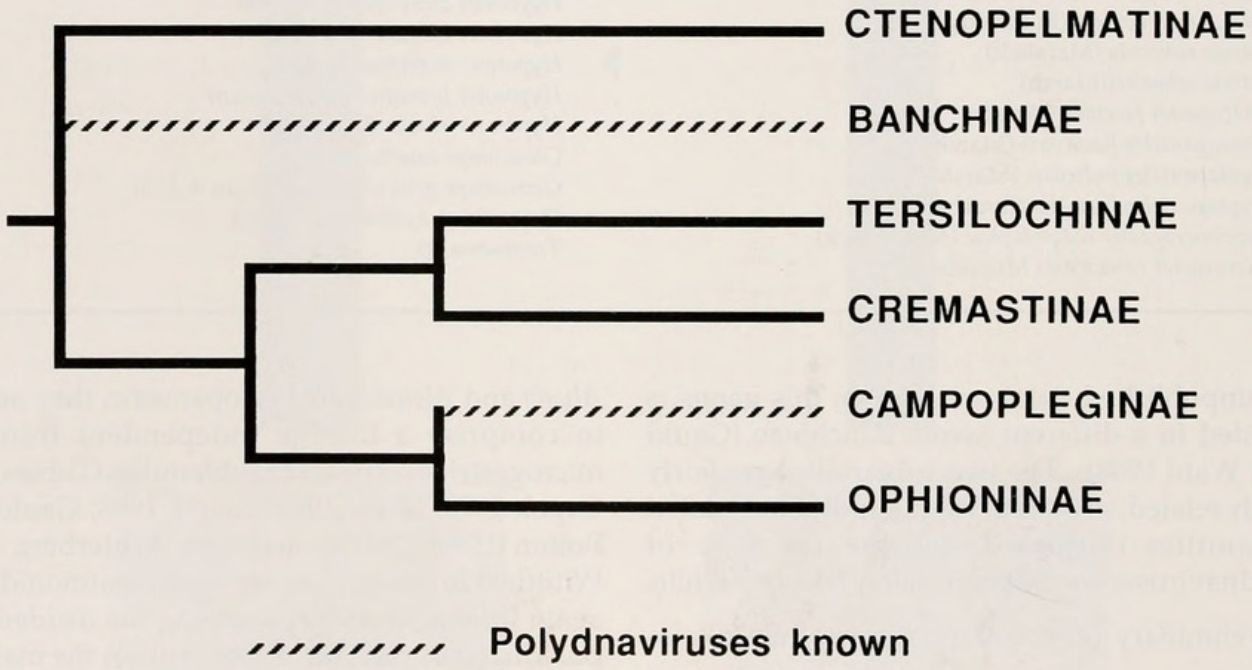

Fig. 3.Phylogenetic hypothesis for the subfamilies of Ichneumonidae that carry polydnaviruses. Adapted from Gauld (1985); the Anomaloninae probably also belong here (Wahl 1988).

obscure. Following replication, mature particles enter the lumen of the reproductive tract where they comprise, as seen previously with *Venturia*, a so-called "calyx fluid". The oviducts, then, contain both eggs and virus; it should therefore come as no surprise to learn that both are injected into host animals during oviposition (Stoltz and Vinson 1977, 1979b). Extensive electron microscopic studies carried out in the mid to late 1970s established that: 1) **polydnavirus replication occurs in the ovaries of *all* females of *all* affected species,** and 2) **polydnaviruses are "designed" for export** (i.e., to the parasitized host). Each of these observations has far-reaching implications. The first indicates that **polydnavirus transmission within parasitoid populations occurs with 100% efficiency**, suggesting further that **polydnaviruses must have an important role to play in the parasitoid life cycle.**

The second observation suggests that **a functional role for polydnavirus particles should be sought in the host.**

We may think of polydnavirus "transmission", and indeed the polydnavirus life cycle itself, as consisting of two pathways (Stoltz in press). In one, polydnavirus *DNA* is transmitted within *parasitoid* populations; in the other, polydnavirus *particles* are transmitted to *host* insects during oviposition, the consequences of which will be discussed below. These two pathways are considered here in the order given, recognizing however that they are mutually interdependent. Within parasitoid populations, polydnaviruses are transmitted genetically in the form of proviruses. This conclusion is based upon two lines of evidence. First, genetic crossing experiments have clearly demonstrated that both ichnovirus and bracovirus genome segments are transmitted to parasitoid progeny according to simple Mendelian rules (Stoltz 1990). Secondly, linear DNA sequences cognate to polydnavirus genome segments are covalently linked to parasitoid chromosomal DNA; this, too, has been shown for both ichnoviruses (Fleming and Summers 1986, 1991; Xu and Stoltz 1991) and a bracovirus (Stoltz et al. unpublished data). It now seems reasonable to assume that the linear, chromosomal, copies of polydnavirus genome segments may serve as templates for the replication of the circular DNAs which ultimately become packaged into virus particles (Stoltz 1990; Fleming and Summers 1991; Xu and Stoltz 1991).

As mentioned previously, polydnavirus particles are designed for delivery into parasitized insects; here, the circular form of polydnavirus DNA establishes what amounts to a genetic colonization of the host animal. Comprising this second arm of the polydnavirus life cycle are the following elements: rapid entry of virions into a variety of host tissues (Stoltz and Vinson 1979a), uncoating of viral nucleic acid either at or within cell nuclei (Stoltz and Vinson 1979a), persistence of viral genome segments throughout the entire course of parasitoid development (Theilmann and Summers 1986; Stoltz et al. 1986), and virus-specific transcription (Fleming et al. 1983; Blissard et al. 1986a, 1986b, Theilmann and Summers 1988; Stoltz et al. 1988b), all in the absence of viral replication. The ultimate purpose of these activities, presumably, is to ensure successful parasitism. This conclusion has been drawn from considerable experimentation, the results of which have conclusively demonstrated that polydnavirus particles are in most cases absolutely required for successful parasitism (Edson et al. 1981; Stoltz and Guzo 1986; Guzo and Stoltz 1987). There is good evidence, albeit circumstantial, that transcriptional activity is also a requirement (Stoltz and Guzo 1986; Guzo and Stoltz 1987). As yet, no biological activity has been associated with any polydnavirus gene product; however, that is surely only a matter of time. Since polydnaviruses are generally immunosuppressive in host insects (Edson et al. 1981; Stoltz and Guzo 1986; Guzo and Stoltz 1987), it can reasonably be assumed that host defenses represent a primary and perhaps continuing target for virus-specific gene expression. In addition, it may be assumed that polydnaviruses may exert profound effects on other aspects of host physiology, so as to make that more compatible with the needs of developing parasitoid eggs and/or larvae. In keeping with this assumption, we note that a bewildering variety of biological events have now been ascribed to the activity (direct or indirect) of polydnaviruses; these are listed in Table 3, and have been described elsewhere in some detail (Stoltz in press).

Being immunosuppressive, the polydnaviruses may provide particularly useful tools with which to examine the nature of invertebrate immune responses, a subject which has in recent years received increasing attention. Immunosuppression appears to be due, at least in part, to changes in hemocyte number, behaviour, and/or viability (Stoltz and Guzo 1986; Davies et al. 1987; Guzo and Stoltz 1987; Wago and Tanaka 1989), but the molecular basis for these effects remains to be elucidated. In addition, it is not yet clear whether similar mechanisms operate to protect both eggs and larvae. In one study, the restoration of an immune response against yeast cells had no apparent effect on developing parasitoid larvae (Stoltz and Guzo 1986); again, the basis for an apparently selective immunosuppression in such systems is not understood. It is of course quite possible that in some systems the larval surface is not seen as foreign, while the egg is (or vice versa). In any case, it is clear that an investigation of host/parasitoid interactions at the cell/molecular level can be expected to shed new light on some fundamental entomological questions.

Origins.— Where did the polydnaviruses come from? At present, one can only speculate. There are two principal possibilities: 1) they arose from the parasitoid genome itself or, 2) they originally ex-

Table 3. Physiological changes in host animals attributed to the presence of polydnaviruses and virus-like agents (modified from Stoltz (in press)).

Activity	References
Suppression of cellular immune response	Salt 1970 (VLP); Vinson 1977; Edson et al. 1981 (V); Rizki and Rizki 1984 and 1990 (VLP); Guzo and Stoltz 1985, 1987; Stoltz and Guzo 1986 (V); Vinson, Stoltz 1986 (V); Feddersen and Schmidt 1986 (VLP); Tanaka 1987; Strand and Wong 1991 (V).
Inhibition of weight gain	Vinson et al. 1979 (V).
Changes in hemocyte count or behaviour	Rizki and Rizki 1984, 1990 (VLP); Stoltz and Guzo 1986; Guzo and Stoltz 1987; Tanaka 1987; Davies et al. 1987 (V); Wago and Tanaka 1989; Strand and Wong 1991 (V); Stoltz and Beckage (V; unpublished data).
Appearance of new hemolymph polypeptides	Cook et al. 1984 (V); Beckage et al. 1987.
Inhibition of phenoloxidase activity	Stoltz and Cook 1983 (V); Beckage et al. 1990 (V).
Inhibition of protein storage in fat body	Tanaka 1986.
Reduction in hemolymph viscosity	Davies et al. 1987 (V); Stoltz (unpublished data).
Change in hemolymph trehalose concentration	Dahlman and Vinson 1977.
Degeneration of hemopoietic tissue	Guzo and Stoltz 1987.
Pigmentation changes	Beckage et al. 1990 (V).
Degeneration of the prothoracic gland	Dover et al. 1988a (V).
Prolongation and/or arrest of development	Tanaka 1987; Dover et al. 1988b (V); Tanaka and Vinson 1991a; Strand and Dover 1991 (V).
Perturbation of hormone levels	Dover et al. 1987, 1988 (V); Tanaka and Vinson 1991b.

V = gradient-purified polydnavirus used (otherwise, calyx fluid). VLP= virus-like particle. It should be noted that venom is required for full activity of some braconid polydnaviruses (e.g. Kitano 1982; Stoltz et al. 1988b).

isted as typical viruses[1] (Whitfield 1990). While there is no easy way to assess the relative merits of these scenarios, the latter is perhaps more satisfying for a number of reasons. First, there are striking parallels between bracoviruses and the well-known insect baculoviruses. For example, the diameter and overall appearance of the capsid is similar (Stoltz and Vinson 1979a); in both cases, the envelope may surround nucleocapsids either individually or in groups; in addition, some bracovirus nucleocapsids are fully as long as baculovirus nucleocapsids (Stoltz et al. 1976); finally, uncoating of both bracovirus and granulosis virus nucleocapsids occurs at nuclear pores (Summers 1969; Stoltz and Vinson 1979b). While these may represent examples of convergent evolution, it is just as reasonable to suppose that some sort of stable relationship arose between a progenitor baculovirus and the parasitoid reproductive tract, and that some such relationship ultimately gave rise to the bracoviruses. In this regard, it is of interest to note that such a relationship apparently exists between a baculovirus and the ichneumonid parasitoid, *Mesoleius tenthredinis* (Stoltz 1981); as with the bracoviruses, all females thus far examined appear to carry virus, and replication is apparently restricted to the parasitoid ovary. Further characterization of this interesting system should be given high priority. Finally, defective interfering (DI) forms of baculoviruses have recently been discovered (Kool et al. 1991; Krell, pers. commun.). DI particles contain sub-genomic nucleic acid molecules, and have been known to ameliorate the severity of viral disease (Dimmock and Barrett 1986). Conceivably, non-pathogenic bracovirus

[1]For the purpose of this discussion, typical viruses are those for which there exist hosts susceptible to productive infection (thus generating progeny virus particles). In the case of the polydnaviruses, however, all hosts permissive for viral replication already carry the viral genome, that being transmitted as a provirus.

genomes could have evolved from defective interfering baculovirus particles.

The bracoviruses and ichnoviruses have been assigned to the same virus family because of similarities in both genome organization and life cycle. It should be stressed, however, that this does not mean that the family is necessarily monophyletic. If monophyletic, then the polydnaviruses must have arisen from a common ancestor, which could have been either a virus or an appropriate collection of parasitoid genes. This hypothesis does not, however, readily account for the major structural differences which define the two polydnavirus subgroups (see Fig. 1). Alternatively, the braco- and ichnoviruses could have arisen from entirely different types of viruses, with extant similarities resulting from convergent evolution. It could be argued that, for the latter to have occurred, the ovary should be a favoured site for *non-cytopathic* virus replication; in fact, as we have seen (Table 1), this would certainly appear to be the case. The establishment of a variety of non-pathogenic viral agents in the parasitoid ovary could then be seen as a prerequisite for the origin of ancestral polydnavirus/parasitoid complexes. Variations on this theme might also have given rise to the *Venturia* and *Leptopilina* particles, among others waiting to be described.

The polydnavirus/parasitoid relationship is undoubtedly ancient, at least in terms of origin(s). Thus it is reasonable to assume that during its establishment, opportunities must have existed for genetic transfer between viral and host genomes as (we suppose) they co-evolved to become a single genome. Indeed, it could generally be argued that the establishment of a genetic fusion between two originally separate entities (e.g., mitochondria and eukaryotes) might require that some functions be transferred from one to the other, and vice versa. One might predict, for example, that some genes required for viral morphogenesis might have lost encapsidation signals: since morphogenesis occurs only in the wasp ovary, there would be no point in packaging such genes for expression in the parasitized host. Similarly, parasitoid genes promoting successful parasitism might more usefully have become incorporated into the viral genome, to be subsequently delivered to parasitized insects. It is of considerable interest in this regard to note that the ichnovirus, CsV, is thought to have acquired a parasitoid venom gland gene, which is duly expressed in parasitized host larvae (Webb and Summers 1990).

Implications for Parasitoid Systematics.— Given that polydnavirus DNA is apparently transmitted in a stable Mendelian fashion, it cannot logically be regarded as differing in any significant way from parasitoid genomic DNA. Put more directly, when we examine polydnavirus DNA, we are also examining parasitoid DNA. It follows that relationships among the polydnaviruses should parallel those among the parasitoids with which they are associated. It may thus be instructive to consider what little is known at present about polydnavirus relationships in the context of parasitoid systematics.

It is of interest in this regard to note an apparent congruence of classical taxonomic determinations with the results of previous electron microscopic studies. Early work clearly established the existence of two bracovirus morphotypes, distinguished on the basis of whether one or several nucleocapsids were enclosed within the viral envelope (see Figure 1). Within members of the (former) genus *Apanteles*, these two morphotypes were represented in roughly equivalent numbers, suggesting—in hindsight—that the genus could well be polyphyletic, or that at least one biologically relevant division might be found within the genus. In Table 4, information on bracovirus morphology is presented in relation to Mason's (1981) reclassification of *Apanteles*. Interestingly, those genera which Mason assigned to the tribe Cotesiini are for the most part characterized by polydnaviruses in which nucleocapsids are not individually enveloped; of 5 such genera for which data are presently available, only one (*Diolcogaster*) seems out of place: unlike all other Cotesiini thus far examined, polydnavirus nucleocapsids in *Diolcogaster* are individually enveloped. It might, accordingly, be suggested that the taxonomic position of *Diolcogaster* could profitably be reconsidered. Alternatively, absence of an individual envelope for each viral nucleocapsid might represent a synapomorphy for a lineage somewhat less inclusive than the Cotesiini as a whole.

Additional information may in future be derived from analyses of viral DNA. For phylogenetic inference, such studies should ideally incorporate an analysis of sequence data from both wasps and viruses (see below). Thus far, our studies have been limited to the identification of shared (or greatly similar) genome segments between wasp taxa, using nucleic acid hybridization/blotting techniques. This approach can provide some indi-

cation of which taxa share genetically similar polydnaviruses, although the data obtained are not directly useful for phylogenetic studies because no distinction can be made between ancestral and derived similarities. Some observations are already available (Figures 4 and 5); these are provided here merely as preliminary indication of a possible correspondence between wasp and virus relationships. In Figure 4, polydnavirus DNA circles (i.e., genome segments) from a variety of braconid parasitoids have been electrophoretically separated and then probed with 32P-labelled DNA from *Cotesia melanoscela*. Hybridization signals were detected only within members of the same genus; no hybridization signal was detected even in the case of *Glyptapanteles*, which, according to Mason (1981), belongs to the same tribe as *Cotesia*. Our results, as far as they go, are therefore entirely in accord with Mason's generic division of *Apanteles*. Parenthetically, it should be noted that while both *C. melanoscela* and *G. flavicoxis* (Marsh) are gypsy moth parasitoids, their respective polydnaviruses are quite dissimilar; thus there is no indication that host-sharing influences the identity of the viruses carried by these two parasitoid species. It is therefore reasonable to assume that their cognate polydnaviruses likely engage the same host milieu in rather different ways. Conversely, where two viruses show significant sequence homology, we might reasonably predict that shared gene products are interacting with common host targets. In any case, it will be of considerable interest to extend the DNA studies so as to include additional genera; in combination with analyses of wasp morphological and molecular data, this may permit a further refinement of microgastrine classification. The development of new data in a timely fashion could prove particularly useful for this group, since Mason's (1981) work on this group is currently undergoing reappraisal (Walker et al. 1990; Mason and Whitfield in preparation).

Preliminary studies involving ichneumonid polydnaviruses (ichnoviruses) are equally interesting, while nevertheless somewhat more difficult to interpret because of uncertainties in the current generic classification (see, e.g., discussion by Wahl 1987). Even so, some inferences may reasonably be drawn from already available, if limited, data: for

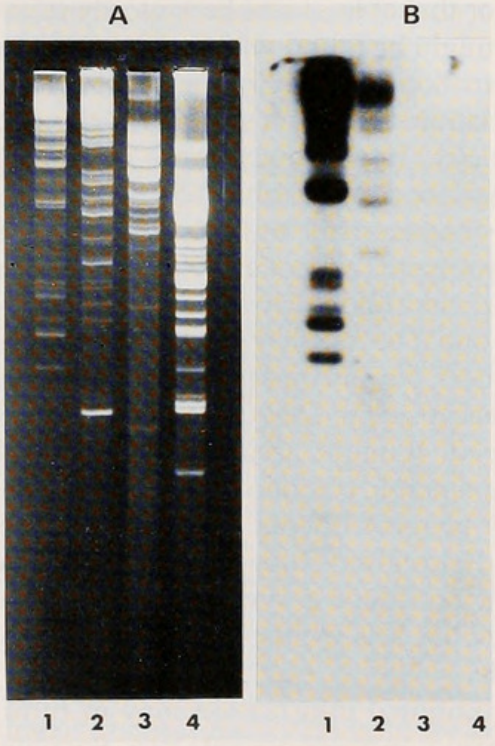

Fig. 4. DNA homologies among polydnaviruses from different braconid genera. A and B represent gel and Southern blot, respectively, of viral DNAs extracted from calyx fluids from the following: *Cotesia melanoscela*, *C. marginiventris*, *Microplitis croceipes*, and *Cardiochiles nigriceps* (lanes 1 to 4, respectively). The blot was probed overnight under conditions of high stringency (68°C., 0.5 M Na-phosphate, pH 7.2, containing 7% SDS) using 2.5 x 105 cpm/ml. of ^{32}P-labelled whole viral DNA from *C. melanoscela*. Exposure was for 24 hrs

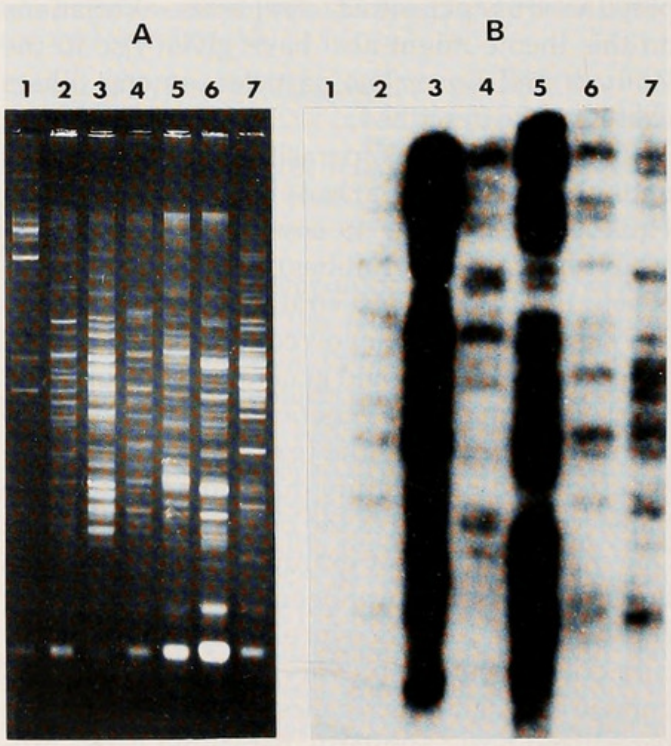

Fig. 5. DNA homologies among polydnaviruses from 5 different ichneumonid genera. A and B represent gel and Southern blot, respectively, of viral DNAs extracted from calyx fluids of the following: *Campoletis* sp., *Diadegma* sp., *Hyposoter fugitivus*, *H. annulipes*, *H. lymantriae*, *Diadegma terebrans*, and *Olesicampe geniculatae* (lanes 1 to 7, respectively). Experimental conditions were as given in Fig. 4, except that the probe used here was ^{32}P-labelled *H. fugitivus* whole viral DNA.

Table 4. An apparent congruence of taxonomic assignments made on the basis of viral and parasitoid morphology in the Microgastrinae. In most cases, only a single species has been examined by electron microscopy. Exceptions are *Apanteles*, *Glyptapanteles* and *Cotesia*, in which respectively two, three and nine species have been examined (see Table 2).

VIRUS	PARASITOID	
# nucleocapsids/ envelope [1]	genus [2]	tribe
	Apanteles	Apantelini
1	*Pholetesor*	Apantelini
1	*Microgaster*	Microgastrini
1	*Hypomicrogaster*	Microgastrini
1	*Clarkinella*	Microgastrini
1	*Microplitis*	Microplitini
several	*Cotesia*	Cotesiini
several	*Glyptapanteles*	Cotesiini
several	*Protapanteles*	Cotesiini
several	*Protomicroplitis*	Cotesiini
1	*Diolcogaster*	Cotesiini

[1] Stoltz and Vinson (1977) and unpublished data.

[2] Genera and tribes here listed are as given by Mason (1981), whose revision of *Apanteles* was based primarily on parasitoid morphology. It should be noted that all of the genera now listed under Cotesiini were formerly included within the single genus *Apanteles*. An additional analysis of the tribes has been published by Walker et al. (1990).

example, probing viral DNA from various wasp taxa with a particular viral genome segment can suggest, at least in certain cases, that some species (within a particular genus) might be more closely related than others. Our admittedly limited studies (Figure 5) would seem to suggest that polydnavirus DNAs from *Hyposoter fugitivus* and *H. lymantriae* are much more similar to each other than to those from some other species assigned to the same genus. Note also that representative viruses from three different campoplegine genera (*Hyposoter*, *Diadegma* and *Olesicampe*) would seem to be related. Viruses from certain other genera (e.g., *Campoletis*) appear to be only distantly related, if at all, to the "Hyposoter group" identified here (i.e., *Hyposoter*, *Diadegma*, *Olesicampe*). Much the same kind of information was developed in an earlier study in which viral antigens were compared by immunoblotting (Cook and Stoltz 1983). Additional work along these lines may ultimately prove useful in helping to refine the difficult taxonomy of ichneumonid (especially campoplegine) parasitoids.

Obviously, more useful molecular data for phylogenetic analysis will be derived from the actual sequencing of viral DNA, which would provide a more direct assessment of homologies or differences in DNA sequence (Hillis et al. 1990; Swofford and Olsen 1990), and would allow polarization of characters via outgroup analysis. Presently, with the single exception of *Campoletis sonorensis* virus (CsV), so little is known about polydnavirus DNA sequences that primers for the polymerase chain reaction (which allow for easy isolation of homologous regions of DNA - see, e.g. White et al. 1989) are not generally available. A substantial number of *H. fugitivus* polydnavirus genome segments have now been cloned (Xu and Stoltz 1991) and many of these have been used as probes vs. other parasitoid taxa (Xu and Stoltz, unpublished data); some cross-hybridize significantly and may therefore contain conserved sequences. We would predict that genes potentially encoding conserved domains will include those required for the replication and encapsidation of polydnavirus genomes. Identification and characterization of such genes will be necessary for an elucidation of viral interrelationships; it is our hope that knowledge concerning the latter will ultimately prove useful in establishing a linkage, assuming one exists, between viral and parasitoid phylogenies.

CONCLUDING REMARKS

We have argued above that the study of polydnaviruses may be relevant, perhaps significantly, to the systematics of at least some groups of the parasitic Hymenoptera. There are additional benefits to be gained from studying these and other parasitoid-associated viruses; these derive from the ways in which they are identified and examined: typically, electron microscopy and agarose gel electrophoresis (of encapsidated viral DNA). Both of these procedures require that the reproductive tract be dissected out from adult female parasitoids, thus affording an opportunity to examine the morphology of the ovary and its accessory glands. This kind of information has already proven extremely useful to systematists (Pampel 1913; D'Rozario 1942; Iwata 1959, 1960; Robertson 1968; Edson and Vinson 1979; Edson et al. 1982; Maeto 1987). Electon microscopy, in particular, may add a new dimension to what is already known.

If it could ever be shown that genetic relationships among the polydnaviruses run parallel to the phylogeny of their parasitoid carriers, if only in part, then perhaps knowledge of one might have

predictive significance for the other. For example, polydnaviruses in *Hyposoter* and *Olesicampe* would appear to be relatively closely related (Cook and Stoltz, 1983, and present report). Yet, the parasitoids which carry them attack quite dissimilar hosts: Lepidoptera and Hymenoptera, respectively. Should we assume that these viruses are nonetheless doing something very similar in these disparate hosts, and if so, what? Answers to such questions could well prove intriguing. This situation may be contrasted with that previously discussed, in which two apparently unrelated viruses (from *C. melanoscela* and *G. flavicoxis*) nevertheless find themselves interacting (how?) with identical host larvae (*L. dispar*).

At this point, it may perhaps suffice to suggest that much more work needs to be carried out on the taxonomic and biological diversity of polydnaviruses (and other viruses and virus-like agents) associated with hymenopteran parasitoids. Coincident with this need is a pressing demand for more work on the systematics of the parasitic Hymenoptera. We predict that these two agendas could be mutually informative.

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