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Chapter 16

RNA Viruses in Parasitoid Wasps

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SUMMARY

This chapter describes the different RNA viruses that have been detected at least once in parasitoid wasps. Four different RNA virus families have been reported in parasitoids: corona-like and picorna-like viruses for the positive-sense, single-stranded RNA viruses, rhabdoviruses for negative-sense, single-stranded RNA viruses, and reoviruses for segmented, double-stranded RNA viruses. They have been found in Ichneumonidae, Braconidae and Pteromalidae, in a total of only 10 hymenopteran species. Their morphology, localization in the parasitoid and host, and transmission are described. Their possible involvement in the success of parasitism and pathogenicity are discussed.

ABBREVIATIONS

AFLP	amplified fragment length polymorphism
AGF	accessory gland filaments
DlEPV	Diaschimimorpha longicaudata entomopoxvirus
DpAV-4	*Diadromus pulchellus* ascovirus-4
DpRIV-1	*Diadromus pulchellus* idnoreovirus-1
DpRV-2	*Diadromus pulchellus* reovirus
EST	expressed sequence tag
HeRIV-2	*Hyposoter exiguae* idnoreovirus-2
hpp	hours post-parasitism
ICTV	International Committee on Taxonomy of Viruses
LbFV	*Leptopilina boulardii* filamentous virus
McSRV	*Microplitis croceipes* small RNA containing virus
MlRVLP	*Meteorus leviventris* reovirus-like particle
OpbuCPV19	*Operophtera brumata* cypovirus 19
OpbuRV	*Operophtera brumata* reovirus
ORF	open reading frame
PcColike-V	*Psyttalia concolor* corona-like virus
PcRVLP	*Psyttalia concolor* reovirus like particle
PDV	polydnavirus
PpSRV	*Pteromalus puparum* small RNA containing virus
RdRp	RNA-dependent RNA polymerase
RT-PCR	reverse-transcribed polymerase chain reaction
TEM	transmission electron microscopy
VcSRV	*Venturia canescens* small RNA containing virus
VLP	virus-like particle

INTRODUCTION

A wide variety of viruses have been reported to infect insects. In the 8th International Committee on Taxonomy of Viruses (ICTV) report and ICTV website (http://www.ictvdb.org/Ictv/index.htm), 15 different families are reported: Ascoviridae, Baculoviridae, Birnaviridae, Coronaviridae, Dicistroviridae, Iflaviridae, Metaviridae, Nodaviridae, Parvoviridae, Polydnaviridae, Poxviridae, Pseudoviridae, Reoviridae, Rhabdoviridae, Tetraviridae) (Büchen-Osmond, 2003, Fauquet *et al.,* 2005, Kapoor *et al.,* 2010, Jacas *et al.,* 1997). They included the different types of genomes: double-stranded circular DNA (Ascoviridae, Baculoviridae, Birnaviridae, Polydnaviridae, Poxviridae), single-stranded DNA (Parvoviridae), positive- and negative-sense, single-stranded RNA (Coronaviridae, Dicistroviridae, Iflaviridae, Metaviridae, Nodaviridae, Picornaviridae, Rhabdoviridae, Tetraviridae), and segmented, double-stranded RNA (Reoviridae).

Surprisingly, only six of these families have been identified in parasitoid wasps: Ascoviridae, Coronaviridae, Iflaviridae, Polydnaviridae, Poxviridae, Reoviridae, and Rhabdoviridae. The absence of other families cannot be definitely concluded, as no systematic search for all types of viruses has been undertaken. It could be noted that the reovirus *Hyposoter exiguae* idnoreovirus-2 (HeRIV-2) was first identified in *Trichoplusia ni* cell cultures infected with calyx fluid from *Hyposoter exiguae*. It was subsequently discovered that the wasp tissues were infected with HeRIV-2. This had not been detected earlier due the abundance of polydnavirus virions in *H. exiguae*, which could have masked the presence of HeRIV-2 (Stoltz and Makkay, 2000). The same phenomenon could occur in the many of the wasps that carry polydnaviruses. Coronaviridae and Polydnaviridae are unusual families of viruses that have not been recovered from any insects other than parasitoid wasps. All the other families are also found in Lepidoptera (Ascoviridae, Poxviridae, Reoviridae), Hymenoptera (Picornaviridae) or Diptera (Rhabdoviridae).

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The family of viruses most often detected in parasitoids is that of the Polydnaviridae, and it is estimated that tens of thousands of species carry these viruses. This is due to the fact that these viruses have been stably integrated in the genome of ancestral species of whole groups. The other families have been less commonly described, partly because they have not been systematically searched for. They infect very few wasp species. Among those infected by DNA viruses, only one species (*Diadromus pulchellus*) carried an ascovirus (DpAV-4), and one an entomopoxvirus (DlEPV in *Diaschimimorpha (=Biosteres) longicaudata*) (Bigot *et al.,* 1995; Lawrence, 1988). The RNA viruses are very rare in parasitoids. Both the Coronaviridae and the Rhabdoviridae have only been identified in one species, *Psyttalia* (=*Opius*) *concolor* (Jacas *et al.,* 1997) and *Diachasmimorpha longicaudata* (Edson *et al.,* 1982; Lawrence, 1988; Lawrence and Akin, 1990; Lawrence and Matos, 2005) respectively. The Picornaviridae have been detected in four species: *Microplitis croceipes* (Hamm *et al.,* 1992), *Venturia canescens* (Reineke and Asgari, 2005) *Nasonia vitripennis* (Oliveira *et al.,* 2010), and *Pteromalus puparum* (Zhu *et al.,* 2008). The Reoviridae are the RNA viruses most commonly reported in parasitoids although only six species have been shown to carry them: *Meteoris leviventris* (Edson, 1981), *P. concolor* (Jacas *et al.,* 1997), *M. croceipes* (Hamm *et al.,* 1994), *H. exiguae* (Stoltz and Makkay, 2000), *D. pulchellus* (Rabouille *et al.,* 1994; Renault *et al.,* 2003) and *Phobocambe tempestiva* (Graham *et al.,* 2006).

Wasps infected with RNA viruses include endoparasitoids: Ichneumonidae (*D. pulchellus*, *V. canescens*, *H. exiguae*, *P. tempestiva*) and Braconidae (*P. concolor*, *D. longicaudata*, *M. croceipes, M. leviventris*), and ectoparasitoids: Pteromalidae (*N. vitripennis* and *P. puparum*).

It must be noted that the RNA viruses are usually detected in association with various other families of viruses such as virus-like particles (VLPs), polydnaviruses (PDVs), or ascoviruses, which makes it difficult to investigate the role of RNA viruses in host/parasitoid relationships. With two exceptions, *Diadromus pulchellus* idnoreovirus-1 and *Diadromus pulchellus* reovirus (DpRV-1 and DpRV-2), the physiological impact of the presence of RNA viruses on successful parasitism remains to be elucidated (Bigot *et al.,* 1997, Renault *et al.,* 2003).

In this chapter, I will describe the different RNA viruses according to their genome: positive-sense, single-stranded linear RNA (Coronaviridae and Picornaviridae), negative-sense, single-stranded linear RNA (Rhabdoviridae), and double-stranded, segmented linear RNA viruses (Reoviridae). A new virus, LbFV, was discovered recently in *Leptopilina boulardi*. It does not resemble any conventional virus and so far the DNA or RNA nature of its genome has not been determined (see Varaldi *et al*., Chapter 17 of this volume; Varaldi *et al.,* 2010).

The localization of the RNA viruses in the wasp and in the lepidopteran host will be described, as well as the type of transmission in wasps, the phylogeny of the viruses, and their role in the host/parasitoid relationships.

POSITIVE-SENSE, SINGLE-STRANDED RNA VIRUSES

Two families of these types of viruses have been detected in parasitoid wasps: coronaviridae-like and picornaviridae-like viruses.

The virions of Coronaviridae are spherical or pleimorphic, and have an envelope. They are 120–160 nm in diameter. The genome consists of a single molecule of linear, positive-sense, single-stranded RNA, and is 25,000–30,000 bases in length. Most of these viruses have been described in mammals, and one of the best known is SARS-CoV, which is responsible for a severe acute respiratory syndrome in humans (Spaan *et al.,* 2005).

As far as I am aware, corona-like viruses have only been reported in a single species of parasitoid, *P. concolor* (PcCo-likeV) (Jacas *et al.,* 1997). They were purified from the venom apparatus of female parasitoids. Transmission electron microscopy (TEM) revealed a virion typical of Coronaviruses, pleomorphic in shape, about 100 nm in diameter, in an envelope with club-shaped projections (15 nm) (Fig. 1A). However, no molecular analysis has been performed to confirm that this virus contains ssRNA or encodes the characteristic genes of the Coronaviridae. At the cellular level, PcCo-likeV was visible as spherical VLPs in the cytoplasmic vesicles of secretory cells (Fig. 2A). However, PcCo-like viruses are always associated with a reovirus, and the VLPs observed could therefore have originated either from the reovirus or the corona-like virus. Unfortunately, nothing is known about the localization of the corona-like virus elsewhere in the parasitoid or in its different tephritid hosts. This makes it impossible to know whether this virus infects the parasitoid or the host, or if it is involved in host–parasitoid interactions. The PcCo-like virus is the only Coronavirus reported in insects and it would be informative to investigate its mechanisms of infection and its evolutionary history.

The virions of Picornavirales consist of an isometric capsid 22–30 nm in diameter. They have no envelope. The 7000–8000-base genome consists of a single molecule of linear, positive-sense, single-stranded RNA. The 5′ extremities are linked to a protein (VpG). They infect a very wide range of hosts, extending from mammals to insects. Two families have been reported in invertebrates, the Dicistroviridae and the Iflaviridae. The type species of the Dicistroviridae is the Cricket paralysis virus. Dicistroviridae are infectious and fatal for many hymenopteran species, such as the honey bee or ants (Christian

et al., 2005a). The Iflaviridae have also been described, and they infect the honey bee, and different moths including silkmoths (Christian *et al.,* 2005b).

The picorna-like virus was first identified in *M. croceipes* (Braconidae) using TEM (Hamm *et al.,* 1992). Their presence has more recently been reported in the ichneumonid *V. canescens* (Reineke and Asgari, 2005), and the pteromalid *N. vitripennis* (Oliveira *et al.,* 2010) by identifying sequences homologous to the RNA-dependent-RNA-polymerase (RdRp) of Picornaviridae using cDNA-amplified fragment length polymorphism (AFLP) analysis and the expressed sequence tag (EST) library, respectively. Some colonies of the Pteromalid *P. puparum* are infected with a pathogenic picorna-like virus (Zhu *et al.,* 2008).

The picorna-like virus found in *V. canescens* (VcSRV) was detected during the cDNA-AFLP analysis of two different lines of wasps (RP and RM) (Reineke *et al*., 2003). This cDNA was expressed differently in the two lines. The analysis of a more complete cDNA has made it possible to identify an open reading frame (ORF) of 515 amino acids displaying homology with the RdRp of other picornaviruses. In *N. vitripennis*, the ESTs showing no homology to the recently sequenced genome of the wasp (The Nasonia working group, 2010) were analyzed, and two sequences of 2789 bp (NvitV-1) and 1523 bp (Nvitv-2), respectively, were identified. The putative ORFs had the eight characteristic domains of the RdRp, and could be used for phylogenetic analysis. Analysis of VcSRV, NvitV-1 and NvitV-2 RdRp has revealed that they are more closely related to the new Iflaviridae family of Picornavirales superfamily than to the other families (Reineke and Asgari, 2005; Oliveira *et al.,* 2010).

The presence of virions in the parasitoid was confirmed in *V. canescens* calyx region and in the muscle of *M. croceipes*. The 30–36-nm particles are isometric, non-enveloped, arranged in a para-crystalline array typical of picornaviruses (Fig. 1B) (Reineke and Asgari, 2005).

The *M. croceipes* picorna-like virus (McSRV-like) has been detected in deteriorating muscles of larvae, but also in other tissues of pupae and adults. These tissues were too severely damaged to be clearly identified (Hamm *et al.,* 1992). Recently, infections of *P. puparum* with a picorna-like virus (PpSRV) were detected from the abnormal morphology of the venom apparatus in about 5% of the females (Zhu *et al.,* 2008). The same percentage of infected females was observed over a two-year period, and there was no detectable disease phenotype. The molecular analysis of a partial sequence of PpSRV made it possible to carry out the phylogenetic analysis of this virus. PpSRV is similar to the Dicistroviridae, and clearly different from the Iflaviridae detected in *V. canescens* and *N. vitripennis*. Dicistroviridae are pathogens that affect several Hymenoptera, but essentially the Apidae and Formicidae. The honey bee can be infected by 18 different ssRNA viruses, and four have been detected in the same strain. McSRV and PpSRV are picorna-like viruses that have been described as pathogens of parasitoid hymenoptera (Hamm *et al.,* 1994, Zhu *et al.,* 2008).

In contrast, even though reverse-transcribed polymerase chain reaction (RT-PCR) fragments from VcSRVs

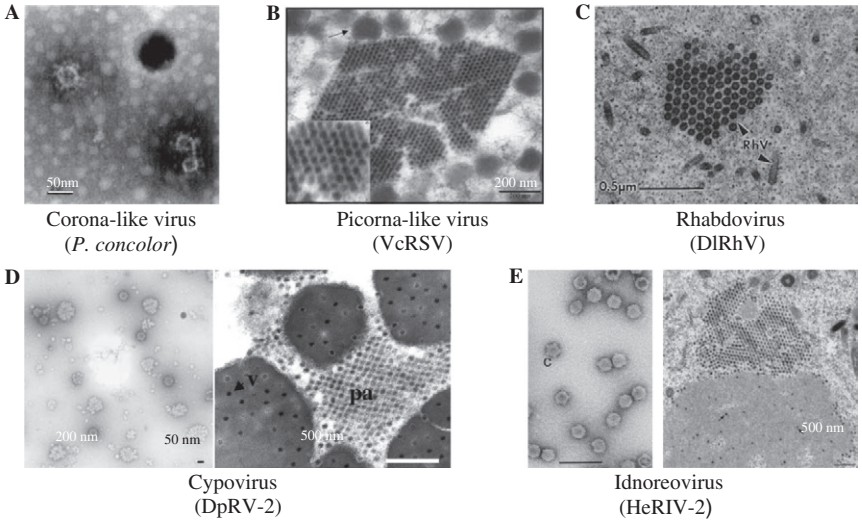

FIGURE 1 Morphology of virions of the various viral genera detected in parasitoids. A: Corona-like virus in *P. concolor* (Jacas *et al.,* 1997) (with permission of *Ann. Appl. Biol.*). **B:** Picorna-like virus (VcSRV) in *V. canescens* (Reineke and Asgari, 2005) (with permission from *J. Ins. Physiology*). **C:** Rhabdovirus in *D. longicaudata* (DlRhV) (Lawrence and Akin, 1990) (with permission of *Can. J. Zool.*). **D:** Cypovirus (DpRV-2) in *D. pulchellus* (Renault *et al.,* 2003) (with permission from *J. Gen. Virol.*) **E:** Idnoreovirus (HeRIV-2) in *H. exiguae* (Stoltz and Makkay, 2000) (with permission from *Virology*).

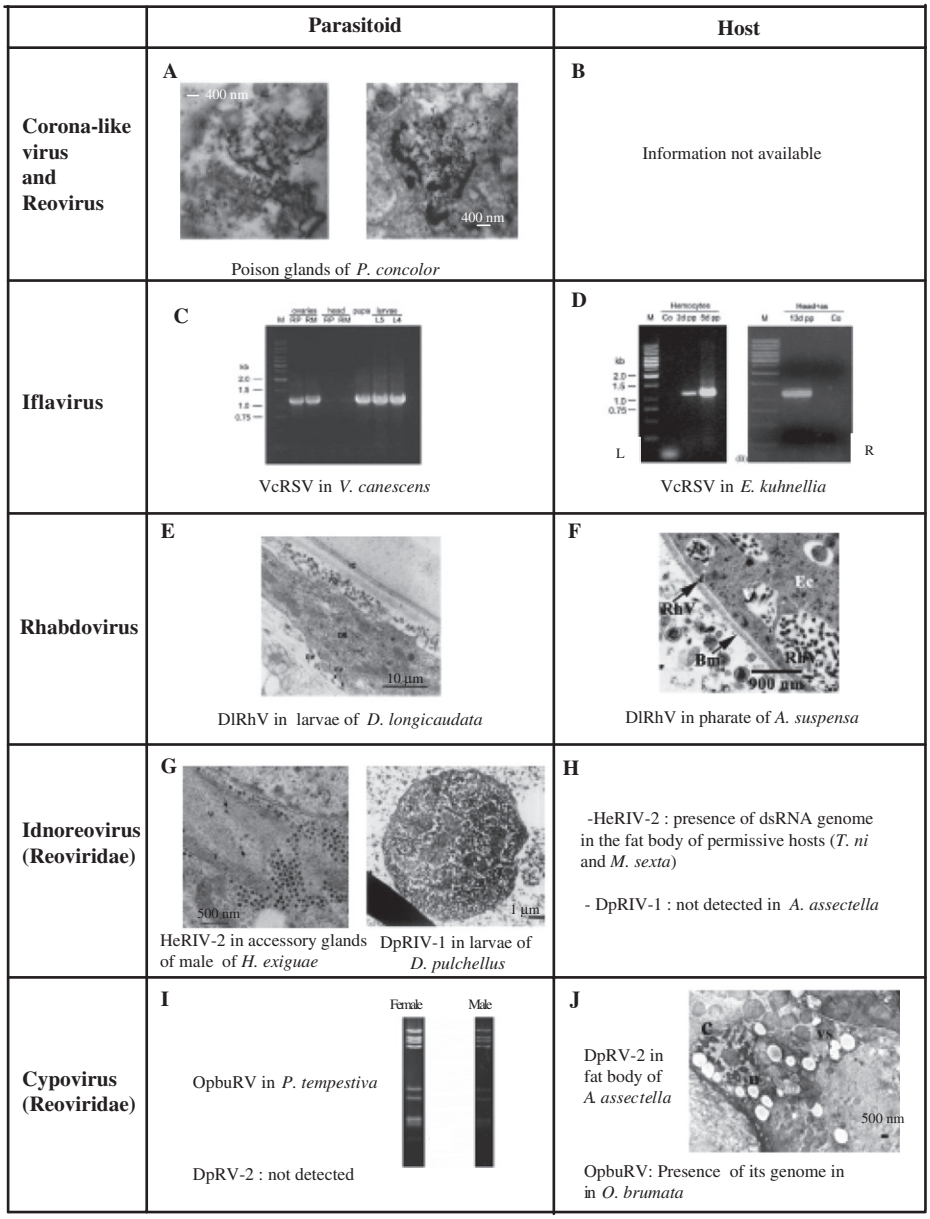

FIGURE 2 Presence and localization of parasitoid RNA viruses in the lepidopteran host and in the parasitoid. A, B: TEM has revealed the corona-like virus in the venom glands of *P. concolor* (A), but provided no information about its lepidopteran host (B) (Jacas *et al.,* 1997) (with permission from *Ann. Appl. Biol.*). Localization of VcRSV in different tissues and different developmental stages of two strains of *V. canescens* (RP and RM) by RT-PCR of RdRp (C) (Reineke and Asgari, 2005) (with permission from *J. Ins. Physiology*). Presence of VcRSV in hemocytes at various days post-parasitization (dpp) (left), and in the head and terminal abdominal segments (as) (right) of the host *E. kuhnellia* 13 dpp. The controls (Co) were unparasitized caterpillars. (D) (Reineke and Asgari, 2005) (with permission from *J. Ins. Physiology*). **E, F:** Localization by TEM of the rhabdovirus DlRhV in the eggs and larvae of the parasitoid *D. longicaudata* (E), and in the pharate of its host, *A. suspensa* **(F)** (Lawrence and Matos, 2005) (with permission from *J. Ins. Physiology*). **G:** Localization of the idnoreoviruses HeRIV-2 in the accessory glands of *H. exiguae* males, and of DpRIV-1 in the larvae of *D. pulchellus* (Stoltz and Makkay, 2000; Rabouille *et al.,* 1994) **H:** Occurrence of HeRIV-2 in the permissive hosts *T. ni* and *M. sexta* demonstrated by the detection of the genome segments and the absence of DpRIV-1 in *A. assectella* (Stoltz and Makkay, 2000; Rabouille *et al.,* 1994) (with permission from *Virology*). **I:** Detection of the genome segments of the cypovirus OpbuRV in the parasitoid *P. tempestiva,* and the absence of DpRV-2 in *D. pulchellus*. **J:** Localization of DpRV-2 in the fat body of the leek-moth *A. assectella* (Graham *et al.,* 2006; Renault *et al.,* 2005) (with permission from *J. Inv. Pathol.* and *J. Gen. Virol.*).

have been found in the larvae, pupae, and ovaries of *V. canescens*, no deleterious effect was detected on the reproduction of the laboratory colony (Fig. 2C) (Reineke and Asgari, 2005). Similar observations were made for NvitV-1 (Oliveira *et al.,* 2010). VcSRV and NvitV-1 could therefore be considered to be nonpathogenic

commensal viruses of the parasitoids. Moreover, VcSRV is not detected in newly collected colonies, and NvitV-1 is not transmitted to sibling species (*N. giraulti* and *N. longicornis*) that have been reared in close proximity for many years (Oliveira *et al.,* 2010). The Iflaviridae, which include both VcSRV and NvitV-1, seem to correspond to nonpathogenic Picornavirales; however, this will have to be confirmed if any other Iflaviridae are discovered in parasitoids. The Picornaviridae are probably the easiest RNA viruses to detect as they can be identified in the cDNA library due their polyadenylation. The extension of genome- and EST-sequencing in Hymenoptera will almost certainly lead to the discovery of new Picornaviridae.

Virions of the Iflaviridae, VcSRV, are detected in the hemocytes, head and terminal abdominal segments of parasitized host caterpillars, *Ephestia kuhnellia* (Fig. 2D). However, it is not known whether the presence of VcSRV promotes the development of the parasitoid larvae in the caterpillar (Reineke and Asgari, 2005). If so, the relationship must be commensal, as it is not deleterious to the parasitoid or to the host. Nothing is known about the presence of virions in the hosts of *M. croceipes* and *N. vitripennis*. As the virus is found in parasitized *E. kuehniella*, the mode of transmission to infect the host and then parasitoid larvae within the host probably resulted from vertical transmission. The same type of transmission probably occurs in NvitV-1 and NvitV-2.

These three different picorna-like viruses are always detected in parasitoids in association with other types of viruses. McSRV is associated with at least one PDV, and in some strains also with a baculovirus, and VcSRV and NvitV-1 with VLPs containing any nucleic acids (Hamm *et al.,* 1992; Reineke and Asgari, 2005; Oliveira *et al.,* 2010). The PDV and the VLPs could play an essential role in inhibiting the immune response and development of the host, suggesting that the picorna-like viruses could be opportunistic viruses. This fits in with the fact that, like NvitV-2 and McSRV, VcSRV is not detected in every parasitoid strain.

It should be noted that a third type of ssRNA virus has been detected in the ESTs of *N. vitripennis* (Oliveira *et al.,* 2010). The cDNA of NvitV-3 was abundantly detected in both adults and pupae. The phylogeny of its RdRp shows that it is close to the Nora virus of *Drosophila melanogaster*, which is no longer classified as a Picornavirus (Oliveira *et al.,* 2010).

NEGATIVE-SENSE, SINGLE-STRANDED RNA VIRUSES

Among the negative-sense, single-stranded RNA viruses found in insects, only one member of the Rhabdoviridae (DlRhV) has been detected in the braconid, *D. longicaudata*.

The virions of Rhabdoviridae consist of an envelope and a nucleocapsid, and they have a characteristic bullet shape. They measure 45–100 nm in diameter, and 100–430 nm in length. The nucleocapsid is elongated with helical symmetry. The complete genome is 11,000–15,000 bases long, and consists of a single, linear molecule of negative-sense, single-stranded RNA. They infect both mammals and plants. Rhabdoviruses are rare in insects, the best described being the Sigma virus that infects *Drosophila melanogaster* and is implicated in the sensitivity to CO_2 (Tordo *et al.,* 2005; Fleuriet, 1999).

The rod-shaped particles observed in the venom apparatus are 250 nm long and 60–70 nm wide; they are rounded at both ends, and are characteristic of the Rhabdoviridae (Fig. 1C) (Edson *et al.,* 1982; Lawrence, 1988; Lawrence and Akin, 1990). However, no molecular analysis has ever been performed to confirm that the genome is composed of a single segment of negative-sense, single-stranded RNA corresponding to that of the Rhabdoviruses. The phylogenic analysis of DlRhV to find out whether it belongs to one of the six genera identified in the Rhabdoviridae remains to be done (Tordo *et al.,* 2005).

The rhabdovirus DlRhV was first reported during larva–pupae apolysis of *Anastrepha suspensa* parasitized by several *D. longicaudata* braconid wasps. DlRhV was not found in nonparasitized larvae. The virus proliferates in the cells of the cuticular epidermis (Fig. 2E) (Lawrence, 1988; Lawrence and Matos, 2005). The presence of the parasite blocks the migration and exocytosis of vesicles that is observed at the apices of cells of the epidermal tissues of the cuticle in nonparasitized pupae. The rhabdovirus is particularly abundant in these vesicles (Lawrence, 1988). During parasitism, DlRhV virions are first observed in the hemolymph 24–36 h post-parasitism (hpp), before the parasitoid eggs hatch, which occurs at about 48 hpp. The DlRhV particles are more abundant in the hemolymph than in the epidermal cells until 48–52 hpp. From 80 hpp, the particles accumulate in the epidermis and appear to replicate (Lawrence and Matos, 2005).

As the DlRhV particles are only detected in superparasitized *A. suspensa*, they were supposed to originate from the parasitoid and to be transmitted during oviposition. Accordingly, DlRhV virions have been detected in the poison apparatus of *D. longicaudata* females. The particles are located in the middle-third of the accessory glands filaments and in a stroma surrounded by vesicles, similar in morphology to that observed in parasitized *A. suspensa* (Fig. 2F) (Lawrence and Akin, 1990). DlRhV particles are not present in the oviduct of *D. longicaudata,* or in the previtellogenic and chorionated vitellogenic ova. They have been detected in the subchorionic space of oviposited eggs. It can therefore be hypothesized that DlRhVs are deposited at the periphery of the ooplasm during its passage through the oviduct below the junction with the venom

apparatus (Lawrence and Matos, 2005). The micropyles at the extremity of the chorion could be the entry point for DlRhV. DlRhV particles are also found in midgut lumen of parasitoid first-instar larvae. It is not known if these particles are derived from those observed around the egg inside the chorion (Lawrence and Matos, 2005).

The transmission of DlRhV to the wasp could occur as follows: the virions are produced in the poison glands and enter the egg chorion of *D. longicaudata* during its passage through the lateral oviduct. The egg is oviposited with various accessory materials originating from the venom apparatus. DlRhV particles are probably also directly injected into the host larvae as virions, and are detected in the hemolymph 24–36 hpp, i.e., before the wasp hatches. Once the wasp larva has hatched, it can feed on host tissues, thus concomitantly absorbing DlRhV virions that are subsequently found in the midgut lumen. This virus could also be present in other tissues to ensure that it is transmitted into the wasp's venom glands.

DlRhV has always been detected in the wasp in association with an entomopoxvirus (DlEPV). However, these two viruses occupy different tissues, zone II of accessory gland filaments (AGF) for rhabdovirus, and zone III of AGF for the entomopoxvirus. The transmission of these viruses also occurs via different ways: outside the chorion for entomopoxvirus, and inside for rhabdovirus.

The role of DlRhV in the successful development of *D. longicaudata* has not been clearly demonstrated, although viral accumulation in the cell epidermis could block the molting process by inhibiting the migration and apolysis of vesicles (Lawrence, 1988). *A contrario*, the entomopoxvirus is truly beneficial in the development of *D. longicaudata* by inhibiting one of the normal functions of hemocytes: the encapsulation of foreign bodies. The hemocytes show profound alterations after DlEPV infection: blebbing, cytoplasmic fragmentation, and apoptosis (Lawrence, 2005).

SEGMENTED, DOUBLE-STRANDED RNA VIRUSES

The segmented, double-stranded RNA viruses found in the parasitoid wasps all belong to the Reoviridae family. These virions consist of a capsid, a core, and nucleoprotein complex. They are not enveloped. The capsid is isometric and shows icosahedral symmetry. It is 60–80 nm in diameter. The capsids are composed of two shells, and sometimes display surface projections. The genome is monomeric, and segmented (10–12 segments), and consists of double-stranded RNA. The complete genome is 18,000–30,000 bases in length. The Reoviridae have been divided into nine genera. Two are found in parasitoids: the Cypoviridae and the idnoreoviridae. Both these reoviruses have an isometric capsid, with icosahedral symmetry, which is about 55–69 nm in diameter for cypovirus and 30 nm for idnoreovirus (Fig. 1D, DpRV-2; Fig. 1E, HeRIV-2, respectively) (Renault *et al.,* 2003; Stoltz and Makkay, 2000). The main difference at the morphological level is that cypoviruses are occluded in a polyhedrin matrix, whereas idnoreoviruses are not (figs. 1D, E) (Mertens *et al.,* 2005a, b).

Three types of association between the Reovidae and parasitoids have been described: nonpathogen commensal, mutualist, commensal, and mutualist (Renault *et al.,* 2005).

The nonpathogenic commensal reoviruses are found in the tissues of both female and male wasps which do not show any specific signs of infection, and the populations do not display disease-related collapse. They are always associated with other types of viruses. The reoviruses are detected in *M. leviventris*, associated with VLPs (Edson, 1981), those detected in *P. concolor* with corona-like viruses (Jacas *et al.,* 1997), those in *M. croceipes* with nudiviruses and polydnaviruses (Hamm *et al.,* 1994), and those in *H. exiguae* with polydnaviruses (Stoltz and Makkay, 2000). These four reoviruses are probably all idnoreoviruses, as they are not occluded.

The reoviruses in *M. leviventris* (MlRVLP) and in *P. concolor* (PcRVLP) are found in the venom apparatus of females, and so could be transmitted to the host during ovipositing, as has been observed for DlRhV, but no study has so far been performed to investigate the transmission of these reoviruses. Likewise, it is not known whether these viruses are also present in other tissues of the female or male wasp. MlRVLP is probably commensal as it is associated with VLPs, which are implicated in egg masking during parasitism in other species (Edson *et al.,* 1982). PcRVLPs may play a more active role, as they are associated with a corona-like virus, and nothing is known about these two viruses with regard to host/parasitoid relationships (Jacas *et al.,* 1997). This peculiar association could help to elucidate the use of nonconventional viruses in the deregulation of host immunity and development.

HeRIV-2 has been detected in the calyx of their parasitoid hosts. It is also present in various wasp tissues: ovaries, ovarioles, testes, male accessory glands, midgut, and malphigian tubules. McRVLP has been detected essentially in midgut epithelial cells and oenocytes (Fig. 2G) (Stoltz and Makkay, 2000, Hamm *et al.,* 1994). HeRIV-2 virion morphogenesis has been observed in all tissues except male testes. Moreover, wasp tissue larvae have less RNA than young or older infected adults, indicating that HeRIV-2 replicates in larvae and adults. The transmission of HeRIV-2 to the parasitized lepidopteran host has been explored in three permissive hosts (which allow the wasp to develop) (*Trichoplusia ni, Malacosoma disstria*, and *Manduca sexta*) and in two non-permissive hosts (*Orygia leucostigma* and *Lymantria dispar*). Female wasps oviposited on the different hosts, and then the HeRIV-2 RNA

genome was looked for. It was found in two permissive hosts and in one non-permissive host, so its replication must depend solely on the host cells, not on the development of parasitoid larvae. No replication was observed in any host when the infection was performed *per os* in first instar larvae, indicating that the transmission in *H. exiguae* more likely involve stinging (Stoltz and Makkay, 2000). HeRIV-2 is probably transmitted to the host during oviposition, and then either HeRIV-2 replicates inside the host and the wasp is infected by feeding on the host tissue, or it directly infects the wasp larvae and then replicates in various different tissues. Transmission in the wasp is probably also vertical and associated with parasitism for McRVLP. A female *M. croceipes* from a noninfected colony was mated with males carrying McRVLP. Adults resulting from this cross were positive for McRVLP (Hamm *et al.,* 1994).

DpRV-2 and *Operophtera brumata* reovirus (OpbuRV) are mutualist viruses and they are usually the only virus present, without any associated viruses, except in 8% of cases where OpbuRV is found associated with *Operophtera brumata* cypovirus 19 (OpbuCPV19) (Renault *et al.,* 2003, Graham *et al.,* 2006). The case of DpRV-2 is quite unusual because its presence could not been detected either by TEM or by nucleic acid extraction in the parasitoid *D. pulchellus*. However, its presence in the genitalia of *D. pulchellus* female wasps has been confirmed by injection into the host of extracts of wasp genitalia that transmit the infection (Renault *et al.,* 2003). In contrast, extraction of the nucleic acids from female and male *P. tempestiva* revealed the presence of OpbuRV, although its precise localization in the wasp tissues is not known (Fig. 2I) (Graham *et al.,* 2006).

After injection into the lepidopteran host, probably during ovipositing, DpRV-2 is mainly detected in the midgut cells where it replicates in a virogenic stroma (Fig. 2J) (Renault *et al.,* 2003). OpbuRV has been detected in the whole extract of the lepidopteran *Operophtera brumata,* but no localization by TEM analysis has been performed (Graham *et al.,* 2006).

DpRV-2 was shown to inhibit the melanization reaction of the host, allowing the wasp larvae to develop. It plays an indispensable role in the host/parasitoid relationship which is equivalent to those demonstrated for the DpAV-4 virus and for polydnaviruses. No mutualist relationship has been demonstrated between OpbuRV and the wasp; however, it is the only virus that has been detected in the parasitoid wasp *Phobocampe tempestiva* and most wasps are infected, and there is a correlation between the presence of infected wasps and the percentage of infected *O. brumata,* suggesting that the parasitoid is at least involved in the dispersion of the virus. It would be very interesting to explore the exact role of OpbuRV in the parasitic success of *P. tempestiva* in *O. brumata*. DpRV-2 resembles a cypovirus in that it is occluded, whereas OpbuRv is not and probably belongs to the new genus of the idnoreoviruses.

The last type of possible relationship between reoviruses and wasps is commensal and mutualist, and has only been described for the reovirus DpRIV-1 (Rabouille *et al.,* 1994). DpRIV-1 is the type species for idnoreoviruses (Mertens *et al.,* 2005b). This reovirus is present in all the natural French populations of *D. pulchellus* examined up to 1999. In *D. pulchellus*, DpRIV-1 is mainly detected in gut epithelial cells, gut lumen, malphigian tubules, and to a lesser extent in the venom glands of the females. The viruses are concentrated in large vesicles present in the epithelial cells, which are released into the gut lumen without cell lysis (Fig. 2G). DpRIV-1 replicates in *D. pulchellus* because more viruses are detected in older wasps than in newly emerged insects. In contrast, DpRIV-1 has not been detected in the lepidopteran host, *A. assectella*, showing that it had not replicated in the lepidopteran host. DpRIV-1 resembles a commensal virus of *D. pulchellus,* as it has no impact on the fitness of the wasp (Rabouille *et al.,* 1994). However, it seems to play a more subtle role in the host/parasitoid relationship because, even if it does not replicate in the host, it does regulate the replication of the associated ascovirus Dp-AV4 (Bigot *et al.,* 1997). Ascovirus DpAV-4 is indispensable for successful parasitism by down-regulating host immunity at the level of melanization (Bigot *et al.,* 1997, Renault *et al.,* 2002). However, when DpAv-4 was injected alone into the lepidopteran host, infection occurred very rapidly and the host died within 72 h (Bigot *et al*., 1995). In contrast, when co-injected with DpRIV-1 during ovipositing, the replication of DpAV-4 was slower, and allowed *D. pulchellus* larvae to develop (Bigot *et al.,* 1997). In conclusion, DpRIV-1 is commensal but also indispensable for parasitic success, and can therefore be considered as being indirectly mutualist.

Is there any correlation between the genus of reovirus (cypovirus or idnoreovirus) and the type of their relationships with the wasp (commensal, mutualist, commensal and mutualist)? Most studies have only been performed at the morphological level. Only one of the parasitoid reoviruses, DpRV-2, is most probably a cypovirus, because it is the only one to be occluded (Renault *et al.,* 2003) but no genome sequence analysis has been performed to confirm this classification. One other cypovirus, OpbuCPV19, is occasionally detected in the wasp *P. tempestiva* (8% of wasps and always in association with OpbuRV). Most cypoviruses have been detected in Lepidoptera (Mertens *et al.,* 2005a). However it would be very interesting to find out whether some cypoviruses can occur in the wasps that parasitize these Lepidoptera. DpRV-2 is a mutualist reovirus and OpbuRV, which is also suspected of being a mutualist, is an idnoreovirus. Therefore, it looks as though there

is no correlation between the type of association with the wasp and phylogenetic classification of the virus.

All the other types of reoviruses seem to belong to the idnoreoviruses, insect viruses which are not occluded (Mertens *et al.,* 2005a, b). Phylogenetic analyses have been performed of only two idnoreoviruses, DpRIV-1 and OpbuRV for which the sequences of the RdRp are available (Bigot *et al.*, 1995, Renault *et al.,* 2005, Graham *et al.,* 2008). They do not show any similarities with other reoviruses. It would be interesting to sequence the RdRp of all the other idnoreoviruses identified so far. The idnoreoviruses include all types of virus: commensal (HeRIV-2, MvRVLP, MlRVLP), commensal and mutualist (DpRIV-1), and suspected mutualist (OpbuRV), so as for cypoviruses there is no correlation between the type of association with the wasp and the virus type. Interestingly, it must be noted that, except for OpbuRV, all the idnoreoviruses are found associated with various other viruses (PDV, corona-like, ascovirus, VLP).

One last remark about idnoreovirus, in the two cases where the genome has been analyzed, a supernumerary segment was found in the females (DpRIV-1 and OpbuRV). This segment is a marker of diploidy rather than being female specific, as it is also detected in the diploid males of *D. pulchellus* (Rabouille *et al.,* 1994) produced in inbred laboratory populations due to the sex determination mechanism (homozygotes at the sexual locus are males). In DpRIV-1, sequencing of the supplemental fragment has revealed that it is composed of a duplication of part of the longest fragment of DpRIV-1 (Bigot *et al.,* 1995). As diploid individuals are most probably mostly females in wild populations, it might play a role in regulating the replication of DpAV-4 (Renault *et al.,* 2005). Unfortunately, no sequence information about the supplemental segment of OpbuRV is available (Graham *et al.,* 2008), and the systematic search for the presence of the supplementary fragment in the other idnoreoviruses could provide information about the exact role of this segment in the life-cycle of these viruses.

CONCLUSION

So far, only four families of RNA viruses have been detected in parasitoids, although eight families have been described in insects. It could be wondered whether the other families of parasitoids are really absent, or if this is due to the fact that inadequate techniques were used to detect viruses. In fact, several different methods have been used: TEM of the venom glands of females, extraction of nucleic acids, followed by DNAse digestion to eliminate the polydnavirus or ascovirus genome, RT-PCR with primers specific of RdRp or data mining in an EST library. To resolve this problem of detecting RNA viruses, a systematic search for viruses in parasitoids should be carried out using a combination of these different methods.

Only a few hymenopteran species (10) are known to be infected by RNA viruses, although thousands of species are known to carry polydnaviruses or VLPs. This could suggest that the presence of polydnaviruses or VLPs may block infections with other viruses. However, some cases of coinfection and coreplication of RNA viruses and polydnaviruses have been described in *H. exiguae*, *M. croceipes*, and *M. leviventris*. The absence of detection of coinfection with polydnaviruses in most cases is perhaps simply due to the fact that the polydnavirus is present in large quantities in parasitoids, and so masks the presence of the other viruses, as in *H. exiguae* (Stoltz and Makkay, 2000).

However, it must be noted that RNA viruses are most often detected in association with various other viruses, such as picorna-like virus, ascoviruses, polydnaviruses, and entomopoxviruses, except in two cases; the reoviruses DpRV-2 and OpbuRV are the only viruses to have been detected as the sole virus in parasitoids. We could suggest that the RNA viruses are opportunistic viruses, which require preliminary infection with other viruses in order to infect the host. This hypothesis is reinforced by the fact that the association with parasitoids is sporadic. Indeed, most of them are detected in some strains but not in others, for example HeRIV-2 or VcRSV. They resemble conventional viruses, where populations or individuals can be either infected or not infected. With the exception of the reovirus DpRV-2, none is directly implicated in regulating host immunity, or promoting successful parasitism.

Information about the RNA viruses is also limited at the molecular level, as very few of them have been sequenced. More data on their genome composition would allow the determination of their phylogeny and facilitate the study of their life-cycle. We hope that this part of our work will expand in the coming years as genome sequencing of parasitoids advances, as exemplified by the recent discovery of the sequences of picorna-like viruses in the EST library of *N. vitripennis* (Oliveira *et al.,* 2010). The limitation in the number of studies of the RNA viruses in parasitoids is probably also due to the fact that infections with these viruses have little impact on the viability of economically important parasitoids used in pest control.

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