The cypovirus *Diadromus pulchellus* RV-2 is sporadically associated with the endoparasitoid wasp *D. pulchellus* and modulates the defence mechanisms of pupae of the parasitized leek-moth, *Acrolepiopsis assectella*

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*Diadromus pulchellus* is a solitary endoparasitoid wasp that parasitizes the pupae of the leek-moth, *Acrolepiopsis assectella* (Lepidoptera). Hitherto, every individual *D. pulchellus* from France that has been investigated was infected by an orthoreovirus, DpRV-1, and an ascovirus, DpAV-4. Recently, a new strain of *D. pulchellus*, established from a French field population, was found to be able to develop on leek-moth pupae, but lacked both DpRV-1 and DpAV-4. However, all these wasps were infected with a new cypovirus, DpRV-2. This cypovirus is transmitted to the *A. assectella* pupae at each wasp oviposition and is replicated mainly in the gut cells of the parasitized pupae. DpRV-2, like the ascovirus DpAV-4, is able to inhibit the defence reaction of *A. assectella* pupae and so contributes to the parasitic success of *D. pulchellus* wasps.

**INTRODUCTION**

Insects are infected by many types of DNA and RNA viruses, as diverse as the baculoviruses (Bilimoria, 1991), entomopoxviruses (Arif & Kurstak, 1991), iridoviruses (Ward & Kalmakoff, 1991), reoviruses (Rabouille et al., 1994; Stoltz & Makkay, 2000), ascoviruses (Federici et al., 2000) and polydnaviruses (Summers & Dib-Hajj, 1995; Beckage, 1998). Most of these viruses are pathogenic towards their insect hosts, but some behave as symbionts, mutualists or non-pathogenic opportunists and can be indispensable for maintaining certain hymenopteran species of parasitoid wasps. The most studied symbiotic viruses belong to the Polydnaviridae family, which has been divided into two genera, *Bracovirus* and *Ichnovirus*, depending on the wasp species from which they were isolated (Beckage, 1998). These viruses are specifically produced in the calyx region located at the base of the lateral oviducts of female wasps and are transmitted to the parasitized host at each oviposition. Although polydnaviruses do not replicate in the host cells, they are indispensable for the parasitic success of the wasp. Indeed, the expression products of their genes interfere with the development and defence mechanisms of the host parasitized by the wasp (Summers & Dib-Hajj, 1995; Asgari et al., 1997; Cui et al., 1997; Beckage, 1998).

Other viruses vectored by parasitoid wasps have also been shown to be involved in suppression of the defence mechanism of the parasitized hosts. The ascovirus DpAV-4 in the lepidopteran host/parasitoid system *Diadromus pulchellus/Acrolepiopsis assectella* has properties similar to those of the *Polydnaviridae* (Bigot et al., 1997a, b). Indeed, DpAV-4 is able to regulate the host immune response specifically by inhibiting the melanization processes of *A. assectella* pupae (Renault et al., 2002). However, unlike the polydnaviruses, DpAV-4 is not an obligate symbiotic virus as it can successfully infect the host in the absence of the parasitoid wasp (Bigot et al., 1997b). Many viruses are probably opportunists, simply using the parasitoid wasps as vectors. However, infections with some of these viruses do not seem to occur by chance and their vectorization may always or frequently depend on a few or even a single wasp species. This is the case for two asco-like viruses, CmV2 and MaV, that are found associated with two *Brachonidae* wasp species, *Cotesia melanoscela* (Stoltz et al., 1988) and *Microctonus aethiopoides* (Barratt et al., 1999), respectively. It also seems to be true of two opportunist reoviruses, DpRV-1 and HeRV, that infect and replicate in two *Ichneumonidae* parasitoid wasps, *D. pulchellus* (Rabouille et al., 1994) and *Hyposoter exiguae* (Stoltz & Makkay, 2000), respectively. In each of these systems, the reoviruses are transmitted to the parasitized hosts at each oviposition, but their physiological relationships with their wasp vectors...
have not yet been elucidated. Recently, an entomopoxvirus (DIEPV) has been shown to be associated with the braconid wasp *Diachasmimorpha longicaudata*, which transmits the virus to its host, *Aphasia suspensa*, during parasitism. The exact role of DIEPV in the physiological relationship between the wasp and its host is not known (Lawrence, 2002).

Our findings have indicated that for more than 10 years DpAV-4 and DpRV-1 have always been present in every individual *D. pulchellus* wasp from all French populations so far investigated (Rabouille et al., 1994; Bigot et al., 1997b). In this paper, we describe a new cypovirus, DpRV-2, which was found in a *D. pulchellus* strain recovered in 1999 from a French population and shown to lack DpAV-4 and DpRV-1. Our results show that DpRV-2 plays a role similar to that of DpAV-4 and that, acting alone, it is able to regulate the defence mechanisms of *A. assectella* pupae.

**METHODS**

**Insects.** The hymenopteran *D. pulchellus* (*Yponomeutidae*) was collected from a leek field in *La Ville aux Dames* (east of Tours, France) during the summer of 1999. The laboratory strain of *D. pulchellus* was established on the laboratory strain of *A. assectella*; the breeding conditions used in these experiments have been described previously (Bigot et al., 1997a, b).

**Preparation of nucleic acids.** Nucleic acids were extracted with guanidium thiocyanate, as described by Laulier et al. (1995), from parasitized or host pupae infected for 48 h. Proteins were removed with phenol/chloroform/isooamyl alcohol (24:23:1) and nucleic acids were precipitated with ethanol. The nature of the different nucleic acids in these extracts was checked by treating with nuclease P1 in TE buffer (Furuichi & Miyura, 1975) or with RNase A at a low salt concentration (0.1 x SSC, pH 8) for 30 min at 37°C (Maniatis et al., 1982).

The dsRNA segments were separated by electrophoresis on 0.8% agarose gel in 1 x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8-1) and stained with ethidium bromide. The sizes of the dsRNA segments were estimated by comparing their migration rates with those of the DpRV-1 segments (Rabouille et al., 1994). dsRNA segments were also separated by 5% PAGE in 1 x TBE and stained with ethidium bromide.

**PCR and hybridization.** The major capsid protein (MCP) of DpAV-4 (accession no. AJ312705; nt 224–1528) was detected by PCR on 10 ng total nucleic acids with primers MCP-1 (5’-GGATATTCCCATATGGCGGCAAAACACT-3’) and MCP-2 (5’-GGGATCCTTAATGATGAAAGGGGCTAC-3’) at 58°C for 30 cycles with 0.5 U Taq polymerase (Promega), under the conditions recommended by the manufacturer. We expected to find a 1305 bp fragment. An internal 0.84 kb fragment of the 1.6 kb segment of DpRV-1 (accession no. X82048) was detected by reverse transcription of 1 µg nucleic acids with 10 pmol of primer 5’-TGAAGCTTGGTGATAGATAGGG-3’, according to the manufacturer’s instructions (Promega). Reverse-transcribed nucleic acids (50 ng) were amplified over 30 cycles at 50°C with primers 5’-TCACTGGTGGGCAAC-3’ and 5’-GATCGAGTATGGGAGGAGC-3’. The PCR products were analysed on an agarose gel, transferred to nylon membrane and hybridized with the 1.6 kb segment cloned in pGEM-T (Bigot et al., 1995).

**Isolation of DpRV-2 virions.** DpRV-2 virions were isolated from 0.8 g of frozen, parasitized *A. assectella* after extracting the parasitoid larvae (Rabouille et al., 1994). They were then purified by the Freon procedure (Chen & Ramiq, 1992).

**Transmission electron microscopy (TEM).** A 10 µl drop of a diluted solution containing the purified DpRV-2 virions was deposited on to a carbon-coated grid. The drop was blotted on to filter paper and, just before drying, stained with uranyl acetate. This negative staining was repeated three times. TEM observations were made with a JEOL electron microscope 1010.

Sections of *A. assectella* pupae that had been parasitized for 24 or 72 h were placed in modified Karnovsky’s fixative at 4°C (MacDowell & Trump, 1976). Fixed tissues were stored in 1-butanol for several days, dehydrated in ethanol and embedded in Epon resin. Ultrathin sections (80 nm) were contrasted with uranyl acetate and lead citrate.

**Implantation of nylon monofilaments.** To test the immune response during parasitism, nylon monofilaments were implanted in *A. assectella* pupae as described previously (Renault et al., 2002). Pupae that were 24 h old were used in all experiments and a nylon monofilament was injected between the 3rd and 4th terminal segments, where the cuticle is thin. This stage was chosen because it is when the female wasps lay their eggs. Forty-eight hours post-implantation, the host pupa was dissected and two characteristics of immunocompetence were checked: formation of a capsule of haemocytes around the nylon monofilament (encapsulation) and melanization of the capsule.

Three categories of *A. assectella* pupae were assayed: non-parasitized pupae, pupae parasitized by the 1999 laboratory strain of *D. pulchellus* and artificially infected pupae. Pupae (24 h old) were exposed to *D. pulchellus* female wasps for a period of 2 h and then collected for implantation. After 48 h, the presence of parasitoid larvae in the pupae stung by the female wasp was also checked by dissection and only the results obtained from those that had actually been parasitized were taken into account. Negative control experiments were performed by ‘stinging’ non-parasitized pupae with a pin dipped in sterile Ringer’s solution.

The DpRV-2 virion extracts used to infect the pupae were crude extracts, obtained by crushing parasitized pupae (72 h old) in sterile Ringer’s solution after dissecting them to remove the parasitoid larvae. The DpRV-2 virion extract was exposed to UV light (λ = 250 nm, 20 min) to obtain extracts containing inactivated DpRV-2 virions as described in Edson et al. (1981). Artificial infection of pupae (24 h old) was performed using a glass pin, which had been dipped in DpRV-2 extract or UV-treated extract and then inserted into the pupae. DpRV-2 development was allowed to proceed for 6 h before the nylon monofilaments were injected. At the end of the experiment and after dissection, DpRV-2 infection of each pupa was confirmed by DpRV-2 genome extraction. Statistical analysis of the experimental data was done with a χ² test.

**RESULTS**

**Presence of a new virus in *A. assectella* parasitized by *D. pulchellus***

A new laboratory strain of *D. pulchellus* was established from adults collected in a leek field to the east of Tours, France, in July 1999. Tests were performed to check for the presence of the previously described DpAV-4 ascovirus and DpRV-1 reovirus (Bigot et al., 1995, 1997a, b). Nucleic acids were extracted from individuals of the 1990 and 1999 laboratory strains of *D. pulchellus*, from *A. assectella* and from *A. assectella* parasitized with the 1999 laboratory strain of *D. pulchellus*. The presence of the ascovirus DpAV-4 was checked by a PCR search for the MCP gene. A 1.3 kb
The genome of the reovirus DpRV-1 is composed of ten segments (Rabouille et al., 1994). The occurrence of the reovirus DpRV-1 was checked using reverse-transcribed nucleic acids with a primer specific to the 1-6 kb segment of DpRV-1, followed by PCR with primers designed to amplify the 1-6 kb segment. As several PCR products were observed, they were analysed on an agarose gel, blotted and hybridized with the 1-6 kb segment. The expected 0-84 kb fragment was observed only in the 1990 strain of D. pulchellus (Fig. 1b, lane 1), but not in the 1999 strain of the wasp (Fig. 1b, lane 2), in non-parasitized A. assectella pupae or in those parasitized by the wasps (Fig. 1b, lanes 3 and 4). Thus, the reovirus DpRV-1 was not present in the 1999 strain of D. pulchellus, in A. assectella or in A. assectella parasitized with the 1999 strain of D. pulchellus.

As the percentage success of parasitism was not reduced, searches for traces of genome material from other types of virus were carried out using total nucleic acid extracts purified from non-parasitized and parasitized A. assectella pupae. On agarose gels stained with ethidium bromide, several discrete nucleic acid segments were detected in the extracts from parasitized A. assectella pupae (Fig. 2a, lane 2). These segments were absent from the nucleic acid samples extracted from non-parasitized pupae (Fig. 2a, lane 1). Further analyses revealed that all seven segments were sensitive to RNase A under conditions of low salt concentration (0-1 x SSC) (Maniatis et al., 1982) (Fig. 2b, lane 2) and resistant to P1 nuclease (Furuichi & Miura, 1975) (Fig. 2c, lane 3), indicating that these segments corresponded to dsRNA molecules. The increased intensity of the two bands indicated by arrows in Fig. 2(c), lane 2, probably indicates that they correspond to several dsRNA segments. These results suggest that a virus, probably a reovirus, was injected into the host pupae by the female wasp at oviposition. Comparison of the pattern of DpRV-1 (Fig. 2c, lane 1) and of the new reovirus (Fig. 2c, lanes 2 and 3) shows clearly that they are two distinct reoviruses.

Virion and genome features

Virus purification was carried out to characterize the reovirus particles in the extracts obtained from parasitized A. assectella pupae in a two-step procedure involving Freon extraction and particle purification by ultracentrifugation on a 1-37 g CsCl ml⁻¹ gradient, as previously described (Rabouille et al., 1994). The virus particles in the purified fractions were finally examined by TEM, using negative staining on carbon-coated grids (Fig. 3a). The particles in the positive fractions were virions that were isomeric in shape and consisted of single-shelled capsids (Fig. 3b). The diameter of the capsid was 55 nm and that of the inner core 30 nm. These virions were very sensitive to the purification treatment and/or plating on the grid, as icosahedral particles consisting of only the inner core of 30 nm with spikes about 12 nm long and 12 nm wide were frequently observed (Fig. 3c). Some loose mulberry-like particles, resulting from broken virions, were also frequent in samples plated on the grids for TEM (Fig. 3a). Particles with an electron-dense core were also found in the plated extracts and probably corresponded to virions with negatively stained nucleic acids (Fig. 3d). To determine the number of dsRNA segments in the new reovirus genome, the nucleic acids contained in these virions were extracted. PAGE analysis of the dsRNA genome revealed that it consisted of ten fragments, ranging from 1-25 to 3-90 kb (Fig. 4, lane 2), including two segments identical in size (Fig. 4, lane 2, indicated by an arrow). The densitometric analysis of this fragment indicated that it had a brightness double that of the next highest fragment, showing that it corresponded to two different segments. The complete genome was 27-5 kb in size.
All the features of the virion and the genome of these particles indicated that the virus purified from the parasitized A. assectella pupae was a cypovirus. Because of the way it is transmitted to A. assectella pupae by D. pulchellus, we named this new cypovirus DpRV-2.

**Location of DpRV-2 in the D. pulchellus wasp and A. assectella pupa**

TEM analysis of ultrathin sections of the gut, abdominal adipocytes, venom glands and genitalia of the female wasp failed to detect any cells infected with DpRV-2. This finding suggested that DpRV-2 virions probably replicate at a very low rate in D. pulchellus tissues. Molecular analysis by agarose gel electrophoresis did not reveal visible dsRNA segments in total nucleic acid extracts purified from female wasps (data not shown). A bioassay similar to that previously used for DpAV-4 (Bigot et al., 1997b; Renault et al., 2002) was therefore used to confirm the presence of infectious traces of DpRV-2 in the genitalia of the female wasps. Briefly, the genitalia of three female wasps were carefully dissected and crushed in a drop of sterile Ringer’s solution. Sterile glass pins dipped in this extract were used to infect ten non-parasitized pupae. Two days post-infection, total nucleic acids from each of the ten inoculated pupae were extracted. The presence of the DpRV-2 dsRNA segments on agarose gels after electrophoresis of samples from each pupa showed that infection by DpRV-2 had occurred (data not shown). A control experiment was performed by simply injecting ten non-parasitized pupae with sterile Ringer’s solution. Two days post-injection, nucleic acids were extracted and no segments of DpRV-2 were detected in the control experiments (data not shown). The data obtained by this procedure suggested that a few DpRV-2 virions were present in the female genitalia of this strain of D. pulchellus.

In parasitized A. assectella, DpRV-2 developed mainly in the gut cells during a 72 h period. After 4 days, the larvae of D. pulchellus had filled the whole of the abdominal cavity of the A. assectella pupa and no further production of DpRV-2 could occur. The cells lysed 48 h after infection and the A. assectella chrysalid died. These findings showed that when A. assectella chrysalids were exposed to DpRV-2 that had not been injected by D. pulchellus wasps at the same time as their eggs, the virus behaved like a pathogen.

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**Fig. 2.** Analysis of dsRNA segments contained in nucleic acid extracts of parasitized pupae. (a) Nucleic acids were extracted from non-parasitized A. assectella (lane 1) and A. assectella parasitized by the 1999 strain of D. pulchellus (lane 2), analysed on a 0.8 % agarose gel and stained with ethidium bromide. The asterisk indicates traces of double-stranded genomic DNA in the extracts. (b) dsRNA was not treated (lane 1) or treated with RNase A under low salt conditions (0.1 × SSC) (lane 2). The nucleic acids were separated on a 1 % agarose gel and stained with ethidium bromide. The sizes of the dsRNA segments of DpRV-2 are indicated on the left. (c) dsRNA was not treated (lane 2) or treated with P1 nuclease in TE (lane 3). The nucleic acids were separated on a 1 % agarose gel and stained with ethidium bromide. dsRNA molecular masses from genomic segments of DpRV-1 are shown in lane 1 (Rabouille et al., 1994). The white arrowheads indicate fragments consisting of several segments.
The progress of infection by DpRV-2 in *A. assectella* tissues was analysed on grids obtained from parasitized pupae 24 and 72 h post-oviposition (p.o.). TEM observation revealed that DpRV-2 replication usually started in gut cells, where virogenic stroma were observed (compare Fig. 5b with Fig. 5a). At 24 h p.o., TEM examination of ultrathin sections of the pupal tissues revealed that the cytoplasm of gut cells was dissociated and already contained free virions and inclusion bodies consisting of virions occluded in a protein matrix, probably corresponding to polyhedrin (Fig. 5c, d). The cell cytoplasm was hypertrophic, due to the size of the virogenic stroma, the quantity of virions and the size of the inclusion bodies (Fig. 5c). After this infection stage, traces of DpRV-2 infection were observed in every tissue of the parasitized pupae. By 72 h p.o., most of the gut cells had lysed and large inclusion bodies mixed with paracrystalline arrays of free virions could be seen (Fig. 5e, f).

**Impact of DpRV-2 on the cell defences of the parasitized host**

In our 1990, 1991 and 1992 laboratory strains of *D. pulchellus* wasps, the ascovirus DpAV-4 was shown to be an essential co-factor for successful parasitism by the wasp by inhibiting the melanization processes of the pupal host (Bigot et al., 1997b; Renault et al., 2002). Although this ascovirus was not found in the wasps of our 1999 laboratory strain, we observed a 96% success rate of wasp parasitism, very similar to the rates achieved by the 1990, 1991 and 1992 strains. In the *D. pulchellus/A. assectella* system, the reimplantation of eggs alone and their complete development was never achieved due to interactions between the co-injected virus and ovary secretions (Bigot et al., 1997a; Renault et al., 2002). Indeed, we observed that when the wasp eggs were implanted alone, they never hatched because they were trapped in melanized capsules (unpublished research).

**Fig. 3.** (a) Virus particles purified from parasitized *A. assectella* pupae by Freon treatment and CsCl gradient centrifugation. (b–d) Details of the icosahedral single-shelled virion (b), inner core with spikes (c) and empty particle (d) negatively stained with uranyl acetate. Bar, 50 nm.

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results). So, in an attempt to confirm the role thought to be played by DpRV-2 in regulating the host's immune response, we used an experimental procedure similar to that used for DpAV-4 (Renault et al., 2002) to investigate the encapsulation and melanization reactions in the A. assectella pupae under three different physiological conditions: pupae that had not been parasitized, pupae that had been parasitized by wasps and pupae that had been artificially infected with DpRV-2.

Positive controls consisting of nylon monofilaments inserted into non-parasitized pupae were found to be encapsulated and melanized 48 h post-implantation (p.i.). Encapsulation was detected in 94.4% of the pupae and melanization in 88.8% (Table 1). These data suggested that 5–10% of the pupae of our A. assectella strain were unable to produce a complete encapsulation reaction.

Only 64.9% of the parasitized pupae displayed encapsulation of the nylon monofilaments and 27.0% of them were melanized (Table 1). This showed that being parasitized by D. pulchellus impaired the ability of A. assectella pupae to react to the introduction of foreign materials. Since DpRV-2 is injected by female wasps at each wasp oviposition and then replicated within the parasitized pupae, we investigated how this virus contributes to the inhibition of the melanization reaction. Extracts containing DpRV-2 virions were injected simultaneously with the nylon monofilaments into the host pupae. At 48 h p.i., we found that the encapsulation rate (94.8%) was the same as that of non-parasitized pupae, but that the melanization rate (37.9%) was significantly lower than that in non-parasitized pupae and was similar to that found in the parasitized pupae (Table 1). Injection of Ringer's solution alone gave the same encapsulation rate (95.7%) and melanization rate (87.2%) as non-parasitized pupae, showing that injection of a solution did not stimulate the immune reactions (Table 1). Rather, the melanization was specifically inhibited by injection of DpRV-2.

DpRV-2 infection of each individual was checked by extracting total nucleic acids and analysing them by agarose gel electrophoresis. No dsRNA segments were found in the non-parasitized pupae (Fig. 6, lane 1), whereas they were detected in all the nucleic acid extracts purified from the parasitized (Fig. 6, lane 2) or artificially infected (Fig. 6, lanes 3 and 4) pupae. These findings suggest that replication of DpRV-2 inhibits the melanization processes of A. assectella pupae. Experiments to confirm this role of DpRV-2 were carried out using UV-inactivated extracts of DpRV-2. At 48 h p.i., the nylon monofilaments recovered from those pupae infected with the UV-inactivated extracts showed 84.8% encapsulation and 72.7% melanization rates. These values were not significantly different from those obtained with non-parasitized pupae (Table 1). Molecular tests were also carried out in an attempt to confirm that no DpRV-2 replication had occurred in these pupae. No dsRNA segments of DpRV-2 were detected in nucleic acid extracts, showing that these virions were inactivated by UV irradiation and did not replicate in the pupae (Fig. 6, lanes 5–9). Overall, our data demonstrate that DpRV-2 is able to inhibit capsule melanization in the parasitized pupae.

**DISCUSSION**

We have described a new reovirus, DpRV-2, and shown it to interfere with the host/parasitoid system in which the pupae of A. assectella are parasitized by the D. pulchellus wasp. The morphology of the virion, the genome of ten dsRNA segments, the histopathology and the features of the inclusion bodies in the parasitized pupae all indicate that DpRV-2 probably belongs to the Cypovirus genus (Hill et al., 2000). Several dsRNA segments will have to be sequenced to confirm this classification, as has been done for Bombyx mori cypovirus (Hagiwara & Matsumoto, 2000; Hagiwara et al., 2001; Ikeda et al., 2001).

Two hypotheses can be proposed to explain the presence of DpRV-2 in certain field areas in which D. pulchellus and A. assectella populations occur. The first is related to a feature of the Allium species that A. assectella infects. In the field, these plants are infected by phytoreoviruses similar to those reported in garlic, Allium sativum (Lot et al., 1994). It is therefore tempting to propose that A. assectella larvae and D. pulchellus wasps could be infected per os by such reoviruses.

If this is the case, this virus would replicate in the parasitized pupae immunomodulated by wasp parasitism. However, this hypothesis is not supported by the particle and genome
features of DpRV-2, which are very different from those of phytoreoviruses.

The second hypothesis is that DpRV-2 could be an opportunist cypovirus that, depending on the environmental conditions, ensures its maintenance in the field by infecting parasitoid wasps, but without producing any pathogenic effects. In the present case, the relationships between the cypovirus and the *D. pulchellus* wasp are probably closer, because our results revealed that DpRV-2 could be advantageous for the parasitic success of the wasp when it is transmitted at oviposition. This seems to be the most likely scenario and it explains the features observed in our 1999 laboratory strain of *D. pulchellus*. However, our previous studies had indicated that two other viruses, the ascovirus DpAV-4 and the reovirus DpRV-1, were present in numerous French field populations and were transmitted to the parasitized host at each wasp oviposition (Rabouille et al., 1994; Bigot et al., 1997b). Our previous data also indicated that the ascovirus DpAV-4 replicates in the

![Fig. 5. TEM images of ultrathin sections of gut cells from non-parasitized pupae (a) and from pupae parasitized for 24 h (b–d) or 72 h (e, f). Bar, 500 nm. mv, Microvilli; l, lumen; ib, inclusion bodies; n, nucleus; vs, viral stroma; pa, pseudocrystalline array; v, virion.](image-url)
parasitized pupae and plays an essential role in the parasitic success of the wasp by modulating the regulation of the defence systems of the parasitized host (Renault et al., 2002). Here, we have shown that in the 1999 strain of wasp, DpRV-2 can perform a role similar to that of DpAV-4. Although we cannot explain why DpRV-1 and DpAV-4 are associated with D. pulchellus in the field populations and in our 1990–1998 D. pulchellus laboratory strains, our present data indicate that the relationship between these two viruses and D. pulchellus is a labile association that maintains this parasitoid wasp in its natural environment. The existence of these two virus alternatives for D. pulchellus (DpRV-1/DpAV-4 versus DpRV-2) therefore suggests that these wasps are able to use viruses that boost their parasitic success. This hypothesis is supported by results obtained with a sibling species, Diadromus collaris, which considerably increased its parasitic success when it acquired DpRV-1 and DpAV-4 under laboratory breeding conditions (Bigot et al., 1997b).

Finally, this is the first report describing a single parasitoid species having associations with different viruses in field populations to trigger its parasitism. In the other parasitoid/virus associations so far described, a parasitoid species has always been associated with just one polydnavirus, reflecting prolonged co-evolution between wasps and viruses (Whitfield, 2002).

The relationship between D. pulchellus and several viruses is therefore quite different as D. pulchellus seems to be more opportunistic in adopting several different ‘viral solutions’. It is possible that the infection induced by the viruses in the host promotes the development of D. pulchellus by modifying several different physiological pathways. However, when DpAV-4 or DpRV-2 are injected directly into the host, these viruses are lethal. Indeed, the factors co-injected at oviposition are indispensable for the inhibition of encapsulation (Renault et al., 2002). Thus, the relationship between D. pulchellus and DpRV-2 clearly cannot be a completely chance association.

It has recently been shown that viruses other than polydnaviruses, such as reoviruses or entomopoxviruses, can be found in wasps and transmitted during oviposition, although their effects on parasite success have not been determined (Stoltz & Makkay, 2000; Lawrence, 2002). In the next few years, it can therefore be reasonably expected that other virus/wasp/host associations presenting similarities to those found in D. pulchellus will be discovered.

Table 1. Percentage encapsulation and capsule melanization around nylon monofilaments implanted for 48 h in A. assectella pupae

<table>
<thead>
<tr>
<th>Pupae*</th>
<th>Encapsulation</th>
<th>Melanization</th>
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<tr>
<td></td>
<td>Presence (no.) Absence (no.) %</td>
<td>Presence (no.) Absence (no.) %</td>
</tr>
<tr>
<td>Non-parasitized (n=4)</td>
<td>51 3 94-4†</td>
<td>48 6 88-8†</td>
</tr>
<tr>
<td>Parasitized (n=2)</td>
<td>24 13 64-9</td>
<td>10 27 27-0*</td>
</tr>
<tr>
<td>Infected with DpRV-2 (n=3)</td>
<td>55 3 94-8*</td>
<td>22 36 37-9*</td>
</tr>
<tr>
<td>Injected with Ringer’s solution (n=3)</td>
<td>45 2 95-7*</td>
<td>41 6 87-2b</td>
</tr>
<tr>
<td>Infected with inactivated DpRV-2 (n=2)</td>
<td>28 5 84-8*</td>
<td>24 9 72-7b</td>
</tr>
</tbody>
</table>

*Each experiment used batches of about 15 pupae; n represents the number of batches in each experiment.
†The same letter indicates that there is no statistical difference between the indicated percentages.

Fig. 6. Analysis of DpRV-2 infection showing the presence of DpRV-2 genomic segments in extracts of non-parasitized pupae (lane 1), parasitized pupae (lane 2), pupae infected with DpRV-2 extract (lanes 3 and 4) and pupae infected with UV-treated DpRV-2 extract (lanes 5–9) after agarose gel electrophoresis and staining with ethidium bromide. All extracts were from individuals of the laboratory strain collected after 1999. The arrow indicates traces of double-stranded genomic DNA in extracts. Lane M contained λ DNA digested with HindIII and was used as a size marker. The fragment sizes (kb) of the markers are indicated.
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