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## Ascoviruses (*Ascoviridae*, *Ascovirus*, and *Toursvirus*)

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### Abstract

Members of the family *Ascoviridae* infect larvae and pupae of insects that belong to the order Lepidoptera, and cause a chronic, but fatal disease in the host. They are horizontally transmitted by endoparasitoid wasps during subsequent parasitization events via contamination of the wasp's ovipositor. The virus typically induces a controlled apoptosis in the host cells leading to the cleavage of the host cells into numerous vesicles in which most virions are produced. This leads to a milky-white colouration of the hemolymph, that is a unique diagnostic feature of ascovirus infection. Ascoviruses have a circular double stranded DNA genome and are closely related to iridoviruses, from which they apparently evolved.

### Keywords

Apoptosis, *Ascoviridae*, *Ascovirus*, caspase, *Iridoviridae*, *Iridovirus*, Hymenoptera, Lepidoptera, *Megavirales*, Noctuidae, parasitism, parasitoid, *Toursvirus*, Yponomeutidae.

### Glossary

**Apoptosis** Genetically programmed cell death.

**Apoptotic bodies** Cell vesicles resulting from apoptosis.

**Caspase** Protease that activates a major portion of programmed cell death.

**Endoparasitic wasps** Species of insect parasites belonging to the order Hymenoptera, which lay their eggs in insects where the wasp larvae develop.

**Per os infection** Infection by feeding.

**Programmed cell death** Genetically programmed cascade proteases and nucleases that cleave DNA and proteins within a cell leading to its death.

**Reniform** Shaped like a kidney.

**Transovarial transmission** Transmission of virus inside the egg.

**Virion-containing vesicles** Vesicles containing virions formed by ascoviruses by rescue of apoptotic bodies induced by ascovirus infection.

### Introduction

The family *Ascoviridae* is one of the newest families of viruses, established in 2000 to accommodate several species of a newly recognized type of DNA virus that attacks larvae of insects of the order Lepidoptera. It consists of two genera, *Ascovirus* and *Toursvirus*; for practicality, here we refer to the collective members of the family *Ascoviridae* as ascoviruses. Viruses of this family produce large, enveloped virions, measuring 130 nm in diameter by 300–400 nm in length, and when viewed by electron microscopy have a reticulated surface appearance. They are typically bacilliform or reniform in shape, and contain a circular double-stranded DNA genome that, depending on the species, ranges from 119-200 kbp. Whereas the virions of ascoviruses are structurally complex like those of other large DNA viruses that attack insects, such as those of iridoviruses (family *Iridoviridae*) and entomopoxviruses (family *Poxviridae*), they differ from these in two significant aspects. First, ascoviruses are transmitted from diseased to healthy lepidopteran larvae or pupae by female endoparasitic wasps when these lay eggs in their hosts. Second, ascoviruses have a unique cell biology and cytopathology in which shortly after infecting a cell, they induce apoptosis and then rescue the developing apoptotic bodies and convert these into virion-containing vesicles. This aspect of viral reproduction apparently evolved to disseminate virions to the larval hemolymph (blood) where they could contaminate the ovipositors of female wasps so that the virus could be transmitted to new hosts. Ascoviruses appear to occur worldwide, wherever there are endoparasitic wasps and larvae of species belonging to the lepidopteran family Noctuidae. However, as these viruses have been discovered relatively recently and their signs of disease are not commonly known in the scientific community, relatively few ascovirus species have been described.

## History

The first ascoviruses were discovered during the late 1970s in southern California where they were found causing disease in larvae of moths belonging to the lepidopteran family Noctuidae. Diseased larvae were recognized by the presence of hemolymph that was very white and opaque, in marked contrast to the hemolymph of healthy larvae which is translucent and slightly green (**Figure 1**). The color and opacity of the hemolymph in diseased larvae was shown to be due to the presence of high concentrations of vesicles that contained virions (**Figure 2**). The white hemolymph and virion-containing vesicles are diagnostic for the disease, and the name for this group, ascoviruses (derived from the Greek *asco* meaning 'sac'), was chosen on the basis of the latter characteristic. Since the discovery of the first ascovirus, ascoviruses have been isolated as the cause of disease in many species of noctuid larvae. In addition, an ascovirus that attacks the pupal stage of a species belonging to the family Yponomeutidae was discovered in the 1990s in France.

## Distribution and Taxonomy

With respect to distribution, ascoviruses have been reported from the United States, Europe, Australia, and Indonesia, and it is highly probable that they occur worldwide. This is because their most common hosts, larvae of lepidopteran species belonging to the family Noctuidae, the largest family of the order Lepidoptera, as well as their most common vectors, endoparasitic wasps of the families Braconidae and Ichneumonidae, are distributed throughout the world. Although only a few ascovirus species have been described to date, there are probably many, including variants, that occur worldwide. Thus, given the common occurrence of their hosts and vectors, it is possible that ascoviruses are very common insect viruses. That they have not been discovered in large numbers, for example like baculoviruses (family *Baculoviridae*), is probably because they cause a chronic disease with few easily detectable signs, making it difficult for individuals not familiar with the disease to recognize diseased larvae in field populations.

At present, five species of ascoviruses are officially recognized based on a combination of properties including the relatedness of 28 key genes coding among others for the DNA polymerase and major capsid protein, the percent identity between their sequenced genomes, their lepidopteran host range, and their tissue tropism (**Table 1**). They are split into two genera, *Ascovirus* and *Toursvirus*. The type species of the *Ascovirus* genus is

*Spodoptera frugiperda ascovirus 1a* (SfAV-1a), with the other species being *Trichoplusia ni ascovirus 2a* (TnAV-2a) and *Heliothis virescens ascovirus 3a* (HvAV-3a). To date, the *Toursvirus* genus contains only one virus species originally called *Diadromus pulchellus ascovirus 4a* (DpAV-4a), and now named *Diadromus pulchellus toursvirus* (DpTV-4a). The Arabic numeral reflects the order in which each species was formally recognized, whereas the lower case letter indicates variants of the type species. Variants from the type species are recognized by different consecutive lower case numbers; for example, TnAV-2b and 2c would represent two different variants of TnAV-2a recognized subsequently. Herein, these viruses are referred to by their acronyms without the numerical and lower case suffix.

Members of the *Ascovirus* genus have been isolated from many more insect species than those listed above, but these isolates have turned out to be variants of known ascoviruses, and therefore they have not been named after the host from which they have been isolated. For example, ascoviruses related to TnAV and HvAV have been isolated from noctuid species such as *Autographa precationis*, *Helicoverpa zea*, *Helicoverpa armigera*, and *Helicoverpa punctigera*; however, they do not bear the name of their host of isolation. What this implies is that ascoviruses belonging to the TnAV and HvAV species have a broad and overlapping host range among different noctuid species, although this has only been tested experimentally to a limited extent.

### Virion Structure and Composition

Depending on the species, the virions of ascoviruses are either bacilliform or reniform in shape, with complex symmetry, and very large, measuring about 130 nm in diameter by 300–400 nm in length. Although variations occur in the virion structure among various ascovirus species, their structural components appear to be essentially the same. Ascovirus virions consists of an inner particle surrounded by an outer envelope (**Figure 2**). The inner particle is complex containing a DNA/protein core as well as an apparent internal lipid bilayer surrounded by a distinctive layer of protein subunits. Thus, the virion appears to contain two lipid membranes: one associated with the inner particle and the other forming the lipid component of the envelope. In negatively stained preparations, virions have a distinctive reticulate appearance, which is thought to be due to superimposition of subunits on the surface of the internal particle with those in the envelope.

As indicated by the size and complexity of the virions, the genome of ascoviruses is large, and consists of a single molecule of double-stranded circular DNA. The genomes of SfAV, TnAV, HvAV, or variants of these, in the genus *Ascovirus*, and TvAV-4a the sole virus in the genus *Toursvirus* have been sequenced (**Table 1**). The SfAV-1a genome is 157 kbp and codes for at least 120 proteins, whereas the TnAV genomes are slightly larger, 174-186 kbp, and codes for at least 165-178 proteins. The genomes of HvAV variants range from 186-200 kbp and code for approximately 180-190 potential proteins. The DpTV-4a genome is 119 kb in size and encodes about 119 proteins. Based on gel analyses, ascovirus virions contain more than 12 structural polypeptides ranging in size from 12 to 200 kDa. Furthermore, proteomic analysis revealed that that SfAV-1a virion is composed of at least 21 proteins, whereas similar analyses identified at least 67 proteins in the virion of HvAV-3i. The marked difference in the virion protein profiles of SfAV-1a and HvAV-3i could be due to inadvertent contamination with non-structural proteins or a higher level of structural complexity in the HvAV-3i virion. Regardless, the two major virion proteins in ascovirus virions are, invariably, the major capsid protein and members of an unusual large cationic protein family (ascovirus P64 homologs) that functions in condensing and encapsidating ascovirus genomic DNA. In addition to proteins and the DNA genome, the presence of an envelope as detected by electron microscopy, as well as experiments with detergents and organic solvents, indicate that virions contain a substantial lipid component. And, as in other enveloped viruses of eukaryotes, it is likely that the virion also contains carbohydrate in the form of glycoproteins, though at present none have been identified.

### Transmission and Ecology

One of the most interesting features of ascoviruses is that their transmission from host to host appears to be dependent on their being vectored by female endoparasitic wasps (order Hymenoptera). Ascoviruses are extremely difficult to transmit *per os*, with typical infection rates averaging less than 15% even when larvae are fed as many as  $10^5$  virion-containing vesicles in a single dose. In contrast to this, infection rates for caterpillars injected with as few as 10 virion-containing vesicles are typically greater than 90%. Moreover, experiments with parasitic wasps show that they can effectively transmit ascoviruses to their noctuid hosts. For example, when females are allowed to lay eggs in ascovirus-infected noctuid caterpillars, thereby contaminating their ovipositor, and then allowed to lay eggs in healthy larvae, the majority of the latter contract ascovirus disease. Interestingly, though the parasitoid eggs hatch in their infected noctuid hosts, the parasitoid larvae die as the ascovirus disease develops in the caterpillar. Under field conditions, the prevalence of ascovirus disease in caterpillars is correlated with rates of parasitization by endoparasitic wasps. When wasps from these populations are collected in the field and allowed to oviposit in healthy caterpillars reared in the laboratory, the latter often exhibit ascovirus disease within a few days. Thus, laboratory and field studies provide sound evidence that the primary mechanism for the transmission of ascoviruses attacking noctuid larvae is through being vectored mechanically by parasitic wasps. No evidence has been found in the lepidopteran hosts for transovum or transovarial transmission.

In the case of DpTV (genus *Toursvirus*), the association of the virus with its wasp and caterpillar hosts is much more intimate. DpTV DNA is carried in wasp nuclei as a circular molecule, and small numbers of virions are produced in the oviducts of females. However, the virus does not cause noticeable pathology in the wasp host. The females lay eggs in the pupal stage of the lepidopteran host, *Acrolepiopsis assectella*, introducing small numbers of toursvirus virions along with the wasp eggs. These virions invade lepidopteran host cells, replicate, and initiate destruction of major host tissues. The wasp larva then emerges from the egg and feeds on the host tissues and toursvirus virions. The DpTV genome is carried extrachromosomally by both male and female wasps, where it is apparently transmitted from generation to generation transovarially. These observations make ascoviruses the only known group of viruses pathogenic to insects that are primarily dependent on vectors for their transmission.

Now that the characteristics of the disease are known, field studies in the southeastern United States and California are beginning to show that ascoviruses are probably the most common type of virus to occur during most of the year in populations of several important noctuid pests, including the cabbage looper, *Trichoplusia ni*, fall armyworm, *Spodoptera frugiperda*, and the corn earworm, *Helicoverpa zea*. Prevalence rates range from 10% to 25%, depending on the species and time of the year, with the highest rates of infection, as noted above, being correlated with high levels of parasitization. In South Carolina, ascovirus infection rates as high as 60% have been reported in populations of noctuid larvae at the end of summer.

### **Host Range**

The experimental host range of ascoviruses varies with the viral species. TnAV, HvAV, and SeAV have a broad host range and are capable of replication in a variety of noctuid species, as well as in selected species belonging to other families of the order Lepidoptera. Alternatively, the experimental host range of SfAV is limited to other species of the genus *Spodoptera*. DpTV can replicate in hymenopteran and lepidopteran hosts closely related to its natural host species, *A. assectella*. To propagate virus in the laboratory, all ascoviruses can be grown in their larval or pupal hosts. To infect caterpillars, they are injected with virus in the third, fourth, or early fifth instar, and virion-containing vesicles are harvested from the hemolymph 5–7 days later. While ascoviruses can be propagated in lepidopteran host cell lines, virus pathology usually is not as distinct as that of *in vivo*. In addition, virus replication *in vitro* does not appear to be as high as that *in vivo*.

### **Pathology and Pathogenesis**

#### **Signs of Disease**

In both natural and laboratory environments, the initial signs of ascovirus disease are very subtle, and this probably accounts for why ascoviruses were discovered only recently. The most obvious sign of disease within 24 h of infection is a decrease in the normal rate of feeding. The feeding rate continues to slow as the disease progresses, and as a result larvae fail to gain weight or advance in development. Healthy larvae, particularly in the early stages of development, will easily quadruple their weight and size in a period of 3 or 4 days, whereas ascovirus-infected larvae cease to grow and may actually lose weight. This feature of ascovirus disease is almost impossible to detect in infected larvae in the field. However, it is easily noticed under laboratory conditions when infected and healthy larvae are reared side by side over a period of a few days. A second feature easily noted in the laboratory is that ascovirus diseases are chronic, though usually fatal. When infected during early stages of development, ascovirus-diseased larvae often survive for 2 or 3 weeks beyond the time at which most healthy larvae have completed their development and pupated. Signs of disease other than these are minor, but include the inability to completely cast the molted cuticle, a bloated thoracic region, and a white or creamy discoloration and hypertrophied appearance of the larval body at advanced stages of disease development.

### **Cytopathology and Cell Biology**

In comparison to all other known viruses, the most unique property of ascoviruses is the unusual cytopathology that leads to the formation of the virion-containing vesicles. This process resembles apoptosis, and previous studies of the SfAV-1a genome have shown that it encodes a functional executioner caspase, synthesized 9 h after infection, which by itself is capable of inducing apoptosis (**Figure 3**); at present, no other virus is known to encode a functional caspase. Although *in vitro* studies have demonstrated that knock-down of SfAV-1a caspase by RNA interference significantly reduced the formation of apoptotic vesicles, it is unclear whether caspase homologs in other ascoviruses participate in this process. For example, in similar studies a caspase-like gene in HvAV-3e was shown not to be implicated in apoptosis but was essential for virus replication.

At the cellular level *in vivo*, the disease begins with extraordinary hypertrophy of the nucleus accompanied by invagination of sections of the nuclear envelope, followed by a corresponding enlargement of the cell. Cells typically grow from 5 to 10 times the diameter of uninfected cells. As the nucleus enlarges, the nuclear envelope ruptures and disintegrates into fragments. At about this stage, the cell plasmalemma begins to invaginate along 'planes' toward the now anucleate cell center. Concomitantly, sheets of membrane form closely adjacent to mitochondria that accumulate along the planes. As this process continues, the membrane sheets coalesce and join the invaginating plasmalemma, thereby cleaving the cell into a cluster of 20 to more than 30 vesicles, ranging in size from 5 to 10  $\mu\text{m}$  in diameter. This aspect of ascovirus cellular pathology resembles the formation of apoptotic bodies during apoptosis. However, rather than dissipate as the cell dies, the developing apoptotic bodies are rescued by the virus and progress to form vesicles in which virions continue to assemble. These virion-containing vesicles, also referred to as viral vesicles, typically remain in the tissue until the basement membrane ruptures, though on occasion cell hypertrophy can be so great that the enlarging cell erupts out through the basement membrane of the infected tissue, releasing large fragments of the infected cell directly into the hemolymph. Analysis of ascovirus genomes shows that, unlike many other large DNA viruses, ascoviruses encode several lipid-metabolizing enzymes that are likely involved in the process of converting developing apoptotic bodies into virion-containing vesicles. Recent transcriptome analysis have shown that genes coding for a patatin-like phospholipase, a PlsC phosphate acyltransferase, a fatty acid elongase, and an esterase/lipase in SfAV-1a are highly expressed during later stages of virus infection *in vivo*. These enzymes are known to modify fatty acid chain length and the degree of lipid saturation, presumably generating novel lipid components in the virion and/or the membranes of viral vesicles. Regarding the latter, it is possible that these lipids, in part, function in providing stability to viral vesicles as they circulate in hemolymph for an extended period encompassing several days or weeks, and

when these vesicles are disseminated through oviposition by female parasitic wasps. Another interesting aspect of this cytopathology is that the mitochondria, apparently under ascovirus control, are reprogrammed to assist synthesis of membranes that cleave the cell into the viral vesicles in which most replication occurs. Although the process by which viral vesicles are cleaved from cells varies among different ascoviruses, the histopathology is similar among virtually all ascoviruses. Vesicles accumulate in the tissues where they are formed, but as these tissues degenerate during disease progression, the basement membrane of infected tissues deteriorates and ruptures, allowing the vesicles to spill out into the hemolymph. There they accumulate reaching concentrations as high as  $10^7$ – $10^8$  vesicles per ml within 3–4 days of infection. There is some evidence that viral replication proceeds within the vesicles as they circulate in the hemolymph, and thus this tissue must also be considered one of the tissues attacked by ascoviruses. In fact, because such high concentrations of viral vesicles are found in the hemolymph, this tissue could be considered a major site of infection, particularly if it is eventually shown that these viruses continue to replicate in the vesicles as they circulate in the hemolymph.

Despite the chronic nature of the disease caused by ascoviruses, virion-containing vesicles are present in the hemolymph within 2 or 3 days of infection. When the virus replicates in cells *in vitro*, the vesicles are formed within 12–16 h of infection. The rapid development and circulation of the viral vesicles in the hemolymph probably evolved to enhance transmission of the virus by parasitic wasps.

### **Tissue Tropism**

The cytopathology of ascoviruses is consistent among different viral species; however, considerable variation occurs with respect to the tissues attacked, that is, in which replication occurs. TnAV, HvAV, and SfAV exhibit a relatively broad tissue tropism infecting the tracheal matrix, epidermis, fat body, and connective tissue. Differences exist between these species in that some HvAV variants infect the epidermis much more extensively than TnAV variants, whereas some of the latter can also replicate more extensively in fat body cells, but appear only to do this when larvae are infected early in their development. Alternatively, the type species, SfAV, and its variants have a very narrow tissue tropism, with the fat body being the primary site of infection. DpTV occurs in the nuclei of all tissues of its wasp host, but appears to only produce progeny in ovarian tissues. In its lepidopteran pupal host, it attacks and replicates in a wide variety of tissues.

### **Replication and Virion Assembly**

Although there have been few biochemical studies of viral DNA replication or protein synthesis, studies carried out with ascoviruses *in vivo* and *in vitro* show that progeny virions first appear about 12 h after infection. Virion assembly is initiated after the nucleus ruptures, and occurs prior to and during the cleavage of the cell into viral vesicles. The first recognizable structural component of the virion to form is the multilaminar layer of the inner particle. Based on its ultrastructure, this layer consists of a unit membrane and an exterior layer of protein subunits. As the multilaminar layer assembles, a dense nucleoprotein core aggregates on the interior surface. This process continues until the inner particle is complete. After formation, the inner particle is enveloped by membranes within the cell or vesicle. These membranes are apparently synthesized *de novo*. Thus, the assembly of the virions is reminiscent of that in other viruses with complex virions, such as the iridoviruses, herpesviruses, and poxviruses, where the virions differentiate after association of the precursors of virion structural components.

After formation, the virions of the TnAV ascovirus accumulate toward the periphery of the vesicle where they often form inclusion bodies, that is, aggregations of virions (**Figure 2**). In SfAV, occlusion bodies are formed in which the virions are actually occluded in a 'foamy' vesicular matrix that consists of a mixture of protein and minute spherical vesicles. When viewed with phase microscopy, these viral inclusion and occlusion bodies are phase bright, and are largely responsible for the highly refractile appearance of the vesicles.

Ascoviruses do not typically form the types of occlusion bodies characteristic of other types of DNA insect viruses, such as baculoviruses and entomopoxviruses.

### Origin and Evolution

The subject of viral evolution over millions of years has received relatively little study due to the lack of a fossil record. Moreover, viruses are considered polyphyletic, and thus most of the more than 70 families of viruses are thought to have originated independently. In this regard, ascoviruses may provide a unique opportunity to obtain insights into virus evolution over long periods. Phylogenetic comparisons of ascovirus genes sequenced to date including those coding DNA polymerase and major capsid protein as well as 20 other proteins indicate that these viruses evolved from a lepidopteran iridovirus (family *Iridoviridae*). Iridoviruses, in turn, appear to share a common ancestor with two families of *Megavirales*, *Marseilleviridae* and *Pithoviridae* which attack certain aquatic unicellular organisms.

### Future Perspectives

At present, too little is known about ascoviruses to assess whether they are or will turn out to be of economic importance. Their poor infectivity *per os* makes it highly unlikely they will ever be developed as viral insecticides, especially given the successful advent of insect-resistant transgenic crops. However, as more entomologists become familiar with the disease caused by ascoviruses, it may be shown that in habitats rarely treated with chemical insecticides, such as transgenic crops, these viruses are responsible for significant levels of natural pest suppression, particularly where parasitic wasps are abundant. Such findings would encourage even greater emphasis on the development of biological control and other more environmentally sound methods of pest control. With respect to the cell biology of viral vesicle formation, ascoviruses provide an interesting model for how apoptosis can be manipulated at the molecular level. Additionally, study of the unusual process by which ascoviruses rescue the developing apoptotic bodies to form viral vesicles could lead to insights into how cells manipulate the cytoskeleton and mitochondria. Finally, it is possible that viral vesicles will provide a unique anucleate cellular system for studying the replication of a complex type of enveloped DNA virus *in vitro*.

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**Table 1** Members in the genus *Ascovirus* and *Toursvirus* belonging to the family *Ascoviridae*

| Species name <sup>1</sup>                        | Virus abbreviation | Genome (bp) <sup>2</sup> /ORFs <sup>3</sup> | Accession number    |
|--|--------------------|---|---------------------|
| <i>Ascovirus</i>                                 |                    |   |                     |
| <b><i>Spodoptera frugiperda ascovirus 1a</i></b> | <b>SfAV-1a</b>     | <b>156,922/123</b>                          | <b>[AM3988432]</b>  |
| <i>Trichoplusia ni ascovirus 2a</i>              | TnAV-2a            | N/A <sup>4</sup>                            | [AJ312707]          |
|  | TnAV-6a            | 174,059/165                                 | [DQ517337.1]        |
|  | TnAV-6b            | 185,664/178                                 | [KY434117]          |
| <i>Heliothis virescens ascovirus 3a</i>          | HvAV-3a            | N/A <sup>4</sup>                            | [AJ279817.1]        |
|  | HvAV-3e            | 186,262/180                                 | [EF133465.1]        |
|  | HvAV-3f            | 198,157/190                                 | [KJ755191.1]        |
|  | HvAV-3g            | 199,721/194                                 | [JX491653.1]        |
|  | HvAV-3h            | 190,519/181                                 | [KU170628.1]        |
|  | HvAV-3i            | 185,650/188                                 | [MF781070.1]        |
|  | HvAV-3j            | 191,718/189                                 | [LC332918.1]        |
| <i>Toursvirus</i>                                |                    |   |                     |
| <b><i>Diadromus pulchellus toursvirus</i></b>    | <b>DpTV-4a</b>     | <b>119,343/119</b>                          | <b>[CU469068.1]</b> |
| Tentative species                                |                    |   |                     |
| <i>Spodoptera exigua ascovirus 5a</i>            | SeAV-5a            |   |                     |
| <i>Spodoptera exigua ascovirus 6a</i>            | SeAV-6a            |   |                     |
| <i>Helicoverpa armigera ascovirus 7a</i>         | HaAV-7a            |   |                     |
| <i>Helicoverpa punctigera ascovirus 8a</i>       | HpAV-8a            |   |                     |

<sup>1</sup> Recognized type species (bold text) and variants of type species (plain text)

<sup>2</sup> Complete genome sequence; bp, base pairs

<sup>3</sup> Putative number of open reading frames (ORFs)

<sup>4</sup> Complete genome sequence not available

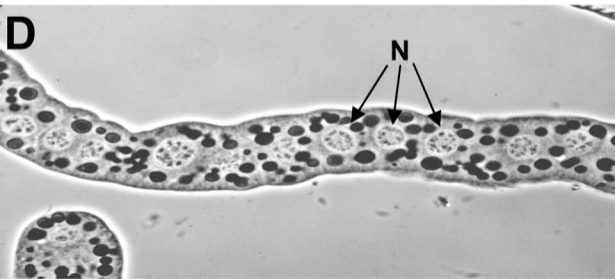
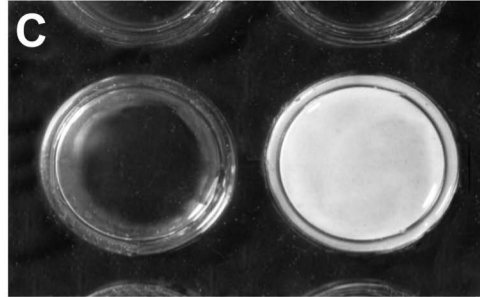
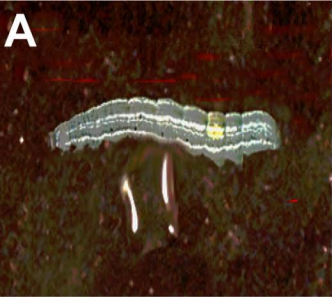
## Figures

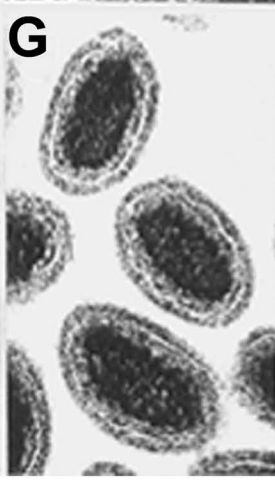
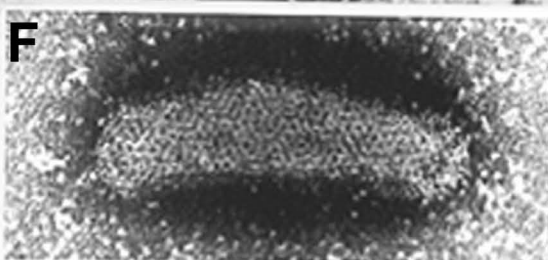
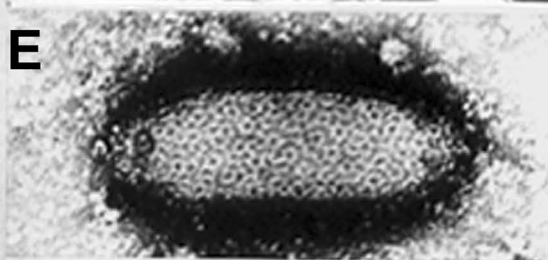
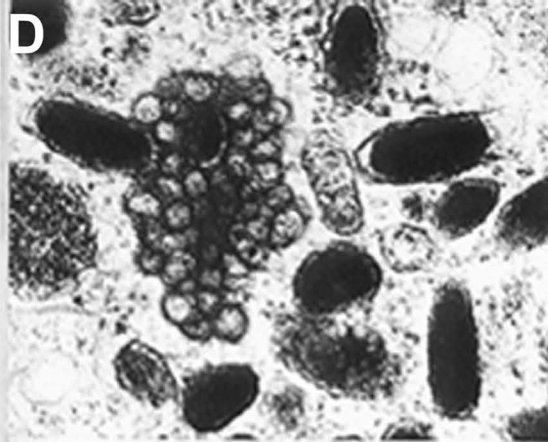
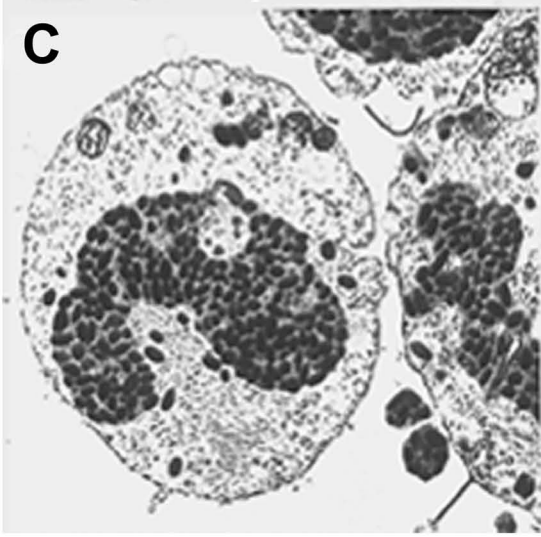
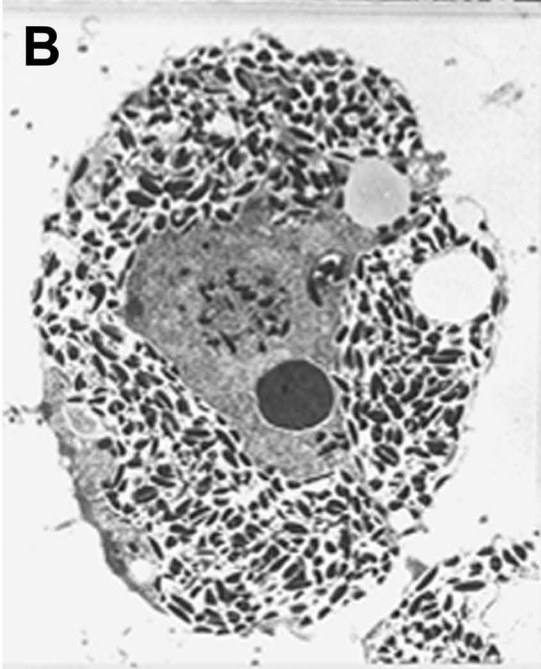
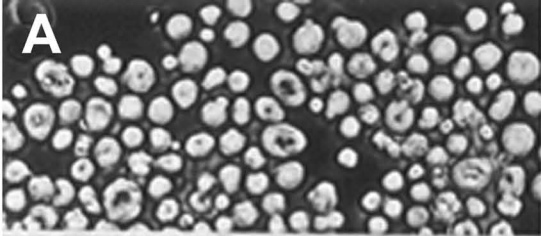
**Figure 1** Major characteristics of the disease typically caused by ascoviruses in lepidopteran larvae. (A) and (B) Healthy and ascovirus-infected larvae, respectively, of the cabbage looper, *Trichoplusia ni*, infected with TnAV. Note the opaque white hemolymph in the infected larva. (C) Spot plate containing hemolymph from healthy (left) and infected larvae (right). (D, E) Sections through lobes of fat body from a healthy and infected larva, respectively, of the fall armyworm, *Spodoptera frugiperda*, infected with SfAV ascovirus. Note the greatly hypertrophied cells in the fat body of the infected larva. The cells in most of this tissue have already cleaved into viral vesicles. N, nuclei.

**Figure 2** Structural and morphological characteristics of ascovirus virions and virion-containing vesicles. (A) Wet mount preparation viewed with phase microscopy of hemolymph from a *Spodoptera frugiperda* larva infected with *Spodoptera frugiperda* ascovirus (SfAV). The spherical refractile bodies are virion-containing vesicles. (B, C) Electron micrographs of ultrathin sections through viral vesicles produced by *Trichoplusia ni* ascovirus (TnAV) and SfAV, respectively. (D) Matrix of the occlusion body produced by SfAV. The occlusion consists of virions, protein, and small spherical vesicles. (E, F) Negatively stained virions of SfAV and TnAV, respectively. Note the reticulate appearance of the virions. (G, H) Electron micrographs of ultrathin cross sections through inner particles of SfAV after formation (G) and during envelopment (H). (I) Ultrathin cross section through a fully developed virion of TnAV.

**Figure 3** Major stages of cellular pathogenesis caused by a typical ascovirus, a process that resembles apoptosis. After infection, the nucleus enlarges and the nuclear membrane invaginates, and then lyses.

Subsequently, the plasmalemma of the cell invaginates and coalesces with cytoplasmic membranes, apparently formed *de novo*, thereby dividing the cell into a cluster of virion-containing vesicles. These vesicles dissociate and are liberated into the hemolymph as the basement membrane of infected tissues degenerates. Virion assembly becomes apparent as the nuclear membrane lyses, and continues throughout all subsequent stages of vesicle formation.





# MAJOR STAGES OF ASCOVIRUS CYTOPATHOLOGY

