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Review

50-plus years of fungal viruses

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ABSTRACT

Mycoviruses are widespread in all major taxa of fungi. They are transmitted intracellularly during cell division, sporogenesis, and/or cell-to-cell fusion (hyphal anastomosis), and thus their life cycles generally lack an extracellular phase. Their natural host ranges are limited to individuals within the same or closely related vegetative compatibility groups, although recent advances have established expanded experimental host ranges for some mycoviruses. Most known mycoviruses have dsRNA genomes packaged in isometric particles, but an increasing number of positive- or negative-strand ssRNA and ssDNA viruses have been isolated and characterized. Although many mycoviruses do not have marked effects on their hosts, those that reduce the virulence of their phytopathogenic fungal hosts are of considerable interest for development of novel biocontrol strategies. Mycoviruses that infect endophytic fungi and those that encode killer toxins are also of special interest. Structural analyses of mycoviruses have promoted better understanding of virus assembly, function, and evolution.

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Contents

Introduction and historical highlights . . . 357
Diversity and taxonomic considerations . . . 358
Totiviridae (dsRNA) . . . 358
Partitiviridae (dsRNA) . . . 358
Megabirnaviridae (dsRNA) . . . 358
Chrysoviridae (dsRNA) . . . 358
Quadriviridae (dsRNA) . . . 359
Reoviridae (dsRNA) . . . 359
Endornaviridae (dsRNA/ssRNA) . . . 359
Alphaflexiviridae (+RNA) . . . 359
Barnaviridae (+RNA) . . . 359
Gammaflexiviridae (+RNA) . . . 360
Hypoviridae (+RNA) . . . 360
Narnaviridae (+RNA) . . . 360
Mycomononegaviridae (−RNA) . . . 360
Reverse-transcribing mycoviruses (+RNA-RT) . . . 360
Unclassified mycoviruses . . . 360
Structural features . . . 361
Totiviridae . . . 361
Partitiviridae . . . 362
Chrysoviridae . . . 362
dsRNA and RdRp packaging within mycovirus capsids . . . 363

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Virus-induced hypovirulence 363
Viruses of chestnut blight fungus *Cryphonectria parasitica* and recent advances in related fields 363
Hypovirulent strains of white mold fungus *Sclerotinia sclerotiorum* 364
Hypovirulent strains of white root rot fungus *Rosellinia necatrix* 365
Concluding remarks 365
References 365

Introduction and historical highlights

Knowledge of fungal viruses (mycoviruses) has expanded exponentially during the past 53 years, since the first definitive report of viruses infecting the cultivated button mushroom *Agaricus bisporus* (Hollings, 1962). A perception that all mycoviruses are cryptic dsRNA viruses has substantially changed as we have learned more about their genome diversity and potential impacts on their fungal hosts. In the age of a catastrophic Ebola outbreak, it is refreshing to write instead about mycoviruses and their potential benefits to humans; yes, not all viruses are evil, and some viruses may even enhance the value, beauty, or health of their hosts (Kernbauer et al., 2014; Lesnaw and Ghabrial, 2000). Mycoviruses in particular could conceivably be exploited for biological control of their natural fungal hosts that are pathogenic for plants. In the past, such applications of mycoviruses were markedly curtailed by technical difficulties in gaining an insight into their biology and structure, but these limitations have been decreasing with the advent of new research approaches (Dawe and Nuss, 2013; Xie and Jiang, 2014).

The economically important dieback disease of *A. bisporus*, a basidiomycete, was first identified in 1948 in a mushroom house owned by the La France brothers of Pennsylvania (Sinden and Hauser, 1950). The disease hence was called La France disease, and similar diseases were reported soon afterward from Europe, Japan, and Australia. In 1962, Hollings observed and isolated at least three types of virus particles from the sporophores of diseased mushrooms and demonstrated disease transmission to symptomless mushrooms by the isolated particles, marking the dawn of modern mycovirology. There were, of course, prior clues to the existence of mycoviruses, including the discovery in 1959 of the transmissible disease of *Helminthosporium* (*Cochliobolus*) *victoriae*, the filamentous ascomycete that causes Victoria blight of oats and other grains (reviewed by Ghabrial et al. (2013)). It is important to note, however, that despite their relatively recent discovery, fungal viruses are believed to be of ancient origins.

The discovery that viral double-stranded (ds)RNA was responsible for the interferon-inducing activities of culture filtrates from several species of ascomycetous molds in genus *Penicillium*, including *Penicillium chrysogenum*, greatly stimulated the search for mycoviruses and reflected the economic and medical importance of fungi in the 1960s (Ellis and Kleinschmit, 1967; Kleinschmit et al., 1964; Lampson et al., 1967). Both the particles and genomic dsRNAs of these Penicillium viruses are potent stimulators of interferon production in animals (Buck et al., 1971). Penicillium chrysogenum virus (PcV), the only known mycovirus to infect *P. chrysogenum*, has now been well characterized (Castón et al., 2013; Jiang and Ghabrial, 2004). The discovery of PcV particles in many *P. chrysogenum* strains used for industrial production of penicillin raised concerns about the stability of these strains. *P. chrysogenum* has been considered asexual for more than 100 years, and in the absence of sexual reproduction, it has been difficult to improve penicillin yield and strain stability. Recently, however, with knowledge of mating-type (MAT) gene organization, it has been possible to induce a sexual cycle, yielding meiotic ascospores of *P. chrysogenum* (Böhm et al., 2013). Evidence of recombination was obtained and the identified heterothallic sexual cycle was used to generate offspring with novel combinations of traits relevant to penicillin production. Because mycoviruses with possible exception of mitoviruses are reportedly eliminated from some higher ascomycetes by sexual reproduction (Khalifa and Pearson, 2013; McFadden et al., 1983; Xie et al., 2006), some of the ascospore-derived progenies of *P. chrysogenum* are likely to be virus-free, thus potentially improving strain stability.

The discovery of toxin-secreting strains of the ascomycetous yeast *Saccharomyces cerevisiae*, and their phenotypic association with the presence of dsRNA viruses (Bevan et al., 1973), marked the beginning of research into yeast virology in the early 1970s. It was then shown that certain yeast strains secrete protein toxins that are lethal to sensitive strains. The toxin-secreting strains were designated “killer yeasts” and the secreted proteins, “killer toxins” (reviewed by Schmitt and Breinig (2006) and Wickner et al. (2013)). Shortly after this discovery, it became apparent that toxin-producing killer strains are not limited to *S. cerevisiae*, but are also found among other yeast and fungal taxa, including *Hanseniaspora uvarum*, *Ustilago maydis*, and *Zygosaccharomyces bailii* (Park et al., 1996; Schmitt and Neuhausen, 1994). The killer phenotype is in some cases associated with dsRNA mycoviruses but can also be encoded by linear dsDNA plasmids (in *Kluyveromyces lactis* and *Pichia acacia*) or chromosomally (in *Williopsis californica* and *Pichia farinose*). The killer toxins from some yeast and smut strains are encoded by satellite dsRNAs, which are dependent on helper dsRNA viruses from family *Totiviridae* for replication and encapsidation. Although no satellite dsRNAs have been reported in association with virus infection of the filamentous ascomycete *H. victoriae* (Ghabrial and Nibert, 2009), the secreted protein toxin victoriocin, encoded by host chromosomal gene *vin*, is structurally similar to killer toxin-encoding genes (de Sá et al., 2010a, 2010b). Establishing that secreted killer toxins are expressed as preprotoxins has substantially strengthened our knowledge in many areas of biology and provided deeper understanding of essential cellular mechanisms such as posttranslational processing along the secretory pathway.

Fungal viruses are often associated with symptomless infections of their hosts. They are not known to have natural vectors (e.g., arthropods or annelids), are commonly transmitted horizontally in nature by intracellular means (hyphal anastomosis), and are transmitted vertically in nature by disseminating spores (mitotic and sometimes meiotic). Thus, although lacking an extracellular phase to their life cycles, they nevertheless have efficient means for both horizontal and vertical transmission and are clearly very successful, being prevalent in all major taxa of fungi. Estimates of mycovirus incidence suggest that 30–80% of fungal species may be infected (Ghabrial and Suzuki, 2009).

Mycoviruses are of common occurrence in endophytic fungi (fungal endosymbionts of plants), with potentially mutualistic roles in the complex interactions between the two organisms (Bao and Roossinck, 2013; Herrero et al., 2009, 2011). For example, mycoviruses might represent mobile elements that afford their partners greater flexibility for rapid adaptation, a promising trait during environmental changes. Greater understanding of the viruses of fungal endophytes may therefore be helpful for practicing sustainable agriculture, particularly against the backdrop of changing global climate. Moreover, recent phylogenetic studies

have revealed that the fungal and plant virus members of family *Partitiviridae* are not always segregated into distinct phylogenetic clades, but instead are intermixed in some clades (genera *Alpha*- and *Betapartitivirus*), suggesting occasional exchange of these viruses between fungal and plant hosts, possibly involving endophytic fungi in particular (Nibert et al., 2014).

In the remaining sections of this paper, we provide a general review of mycovirology but focusing on recent findings in three particular areas: diversity, structure, and hypovirulence; defined as reduction in virulence of phytopathogenic fungi.

Diversity and taxonomic considerations

Fungal viruses have diverse genomes including ones made of linear dsRNA (currently classified into seven families: *Chryso*-, *Endorna- Megabirna*-, *Quadri*-, *Partiti*-, *Reo*-, and *Totiviridae*), linear positive-sense ssRNA (+RNA) (currently classified into five families: *Alphaflexi*-, *Barna*-, *Gammaflexi*-, *Hypo*-, and *Narnaviridae*), linear negative-sense ssRNA (−RNA) (proposed family Mycomononegaviridae), and circular ssDNA (unclassified). Mycoviruses with dsDNA genomes are missing from the list, but might yet be found since dsDNA viruses of water molds, now classified as protists not fungi, have been reported (Dawe and Kuhn, 1983). Mycoviruses for which three-dimensional (3D) structures have been reported are further described in “Structural features”. For an updated list of all mycoviruses, the reader is referred to ICTV master species list (http://talk.ictvonline.org/files/ictv_documents/m/msl/default.aspx).

Totiviridae (dsRNA)

Members of this family have monosegmented (i.e., nonsegmented) bicistronic genomes, 4.6–7.0 kbp in length and usually encompassing two large, partially overlapping open reading frames (ORFs) on one strand. The 5′-proximal ORF encodes the capsid/coat protein (CP) and the 3′-proximal ORF encodes the RNA-dependent RNA polymerase (RdRp). Members that infect fungi are currently grouped in two genera: *Toti*- and *Victorivirus* (Ghabrial, 2008; Wickner et al., 2011). Viruses in genus *Totivirus* have been found to infect the yeasts *S. cerevisiae*, *Scheffersomyces segobiensis*, and *Xanthophyllomyces dendrorhous*; the smut fungus *U. maydis*; and the subterranean fungus *Tuber aestivum*, the black summer truffle, representing the first evidence for mycoviruses in ectomycorrhizal fungi (ectophytes of plant roots) (Baeza et al., 2012; Stielow and Menzel, 2010; Taylor et al., 2013). Mycoviruses belonging to genus *Victorivirus*, in contrast, have been found to infect only filamentous fungi (Ghabrial and Nibert, 2009).

At least three different strategies for RdRp expression appear to be used among members of this family: (1) as a fusion with CP (CP/RdRp) following ribosomal frameshifting, as in *Saccharomyces cerevisiae* virus L-A (ScV-L-A) and also among certain viruses that infect parasitic protozoa (Dinman et al., 1991); (2) as a fusion with CP within the same, single ORF, as in *Ustilago maydis* virus H1, in which case the RdRp is putatively released from the fusion by proteolysis (Kang et al., 2001); and (3) as a separate nonfused protein consequent to translationally coupled termination–reinitiation (stop/restart translation), as in *Helminthosporium victoriae* virus 190S (HvV190S) and apparently for other victoriviruses (Huang and Ghabrial, 1996; Li et al., 2011, 2015; Soldevila and Ghabrial, 2000). An H-type pseudoknot-containing small RNA cassette, 38 nt upstream of the AUGA motif containing the CP stop codon and the RdRp start codon are sufficient for stop/restart translation in this system.

Partitiviridae (dsRNA)

Members of this family have bisegmented genomes, 1.4–2.4 kbp in length and encompassing one large ORF per segment. Generally the smaller segment (dsRNA2) encodes the CP and the larger segment (dsRNA1) encodes the RdRp. These two genome segments are packaged into separate virus particles. Following a recent reorganization of this family to reflect phylogenetic relationships, members that infect fungi are now grouped in three genera: *Alpha*-, *Beta*-, and *Gammapartitivirus* (Nibert et al., 2014). Alpha- and betapartitiviruses infect not only filamentous fungi but also plants (as discussed briefly in “Introduction and historical highlights”), whereas gammapartitiviruses infect only filamentous fungi. In general, partitivirus infections are largely symptomless (though see “Virus-induced hypovirulence” below).

Megabirnaviridae (dsRNA)

Rosellinia necatrix megabirnavirus 1 (RnMBV1) is the prototype of genus *Megabirnavirus*, the only current genus in this family. It has two genome segments separately encapsidated in isometric particles of ~50 nm diameter. dsRNA1 spans 8.9 kbp encompassing two partially overlapping ORFs on one strand. ORF1 encodes the CP and ORF2 encodes the RdRp, which is expressed as a fusion product with CP, probably via ribosomal frameshifting (Chiba et al., 2009; Salaipeth et al., 2014). dsRNA2 spans 7.2 kbp encompassing two non-overlapping ORFs in the same frame on one strand. The ORF3 product appears to be proteolytically processed into smaller proteins in infected mycelia (Kanematsu et al., 2014), whereas expression of the predicted ORF4 product has yet to be shown. The 5′ untranslated region (UTR) of each genomic plus strand is extremely long (~1.6 kb), suggesting it is likely to include an internal ribosomal entry site (IRES) for translational initiation. Characterization of viral mutants that appear during laboratory passage, particularly after transfection of fungal host strains with purified virions, suggests that dsRNA2 is dispensable for viral replication but required for efficient replication, maintenance in culture, and hypovirulence induction (Kanematsu et al., 2014).

Chrysoviridae (dsRNA)

Penicillium chrysogenum virus (PcV) is the prototype of genus *Chrysovirus*, the only current genus in this family (Ghabrial and Castón, 2011). It has four monocistronic genome segments, 2.4–3.6 kbp in length and separately encapsidated in virus particles. dsRNA1 encodes the RdRp, and dsRNA2 encodes the major CP. Although proteins P3 and P4, respectively encoded by dsRNA3 and -4, are of unknown function, the P3 sequence contains a “phytoreovirus S7 domain” found in viral proteins with nucleic acid binding activities, and the P4 sequence contains motifs that form the conserved core of a known superfamily of cysteine proteases. Interestingly, the N-terminal regions of PcV P3 (and corresponding P3 proteins of other chrysoviruses) share significant sequence similarity with comparable N-terminal regions of the chrysovirus RdRp. The 5′ UTRs of most chrysovirus dsRNAs are relatively long, 140–400 nt in length, suggesting the possible presence of an IRES. In addition to strictly conserved 5′- and 3′-terminal sequences, the 5′ UTR of all four dsRNAs contain a 40- to 75-nt region with high sequence identity followed by a 30- to 50-nt region with strong sequence similarity and including “CAA” repeats similar to translational enhancer elements in the 5′ UTR of tobamoviruses (Jiang and Ghabrial, 2004).

At least two divergent clades of “chryso-like” viruses with either 3 or up to 5 genome segments have also been described, but not yet recognized as distinct taxa by the ICTV (Li et al., 2013; Urayama et al., 2010, 2012; Wang et al., 2014). One of the viruses

associated with La France disease of *A. bisporus* (Van der Lende et al., 1996) appears to belong to one of these clades (Urayama et al., 2012), and indeed some other of these chryso-like viruses appear to alter colony morphology and reduce virulence of their host fungi, unlike recognized chrysoviruses.

Quadriviridae (dsRNA)

This family (and genus *Quadrivirus*) accommodates a single species to date, *Rosellinia necatrix quadrivirus 1*, two strains of which have been well characterized: W1075 and W1118, isolated from different locations in Japan (Lin et al., 2012, 2013). Virus particles are isometric, ~45 nm in diameter, and separately package each of the four monocistronic genome segments, each 3.7–4.9 kbp in length. The function of the dsRNA1 translation product remains unknown, but both dsRNA2 and dsRNA4 encode CPs that co-assemble capsids, and dsRNA3 encodes the RdRp. A case for capsid assembly by more than one CP has been made for two other divergent groups of fungal dsRNA viruses that remain unclassified to date: botybirnaviruses (Wu et al., 2012) and one clade of the chryso-like viruses described above (Urayama et al., 2012; 2014). As also found in chrysoviruses, the 5′ UTR of each quadrivirus genome segment contains “CAA” repeats.

Reoviridae (dsRNA)

The presence of “reo-like” virus particles in a fungus was noted first in 1994 (Enebak et al., 1994). Genus *Mycoreovirus* was then created to accommodate three species, *Mycoreovirus 1* to *Mycoreovirus 3* (including well-characterized representative isolates MyRV1 to MyRV3, respectively). MyRV1 and -2 were isolated from *Cryphonectria parasitica*, and MyRV3 from *Rosellinia necatrix* (Hillman and Suzuki, 2004; Wei et al., 2004). All mycoreoviruses confer hypovirulence to their respective natural hosts.

Mycoreovirus genome segments are monocistronic with 5′ caps on their positive strands. MyRV1 and -2 have 11 genome segments (S1–S11) whereas MyRV3 has 12 segments, 0.7–4.1 kbp in length for each virus. Interestingly, an MyRV3 mutant lacking S8 and thus possessing only 11 segments emerges during passage of the field isolate originally infected with MyRV3 and shows comparable replication levels (Kanematsu et al., 2004). Protein assignments have been established for some of the MyRV1 genome segments including the RdRp, the putatively “$T=2$” CP, the capping enzyme, and a putatively myristoylated outer capsid protein (Hillman and Suzuki, 2004; Supyani et al., 2007).

MyRV1 also undergoes spontaneous mutations during passage, including large internal deletions of S4 (Eusebio-Cope et al., 2010). Intriguingly, other MyRV1 segments including S10 are prone to rearrangements when the host antiviral RNA silencing machinery is suppressed (Sun and Suzuki, 2008; Tanaka et al., 2011, 2012), as also discussed in “Virus-induced hypovirulence”. Despite deletions of most of the protein-coding regions of S4 and S10 in the preceding mutants, these mutant viruses show comparable replication levels to wild-type MyRV1, suggesting that both encoded proteins (VP4, the putatively myristoylated protein, and VP10) are dispensable for viral replication. The mutant viruses with a deleted form of S4, however, are impaired in vertical transmission via conidia. In addition, the mutant viruses with a deleted form of S4 and/or S10 induce distinct symptoms in *C. parasitica.*

Endornaviridae (dsRNA/ssRNA)

Although mycoviruses assigned to family *Endornaviridae* are classified with dsRNA viruses in the 9th ICTV Report, they are phylogenetically more closely related to alpha-like ssRNA viruses (Ghabrial and Suzuki, 2009; Hacker et al., 2005). Endornaviruses do not form true virions; the linear dsRNA replicons, 14–17 kbp long, are found in cytoplasmic vesicles in infected plants, fungi and oomycetes. Each characterized genome encodes a single long polypeptide that includes aa sequences typical of viral RNA helicases, UDP glucosyltransferases and RdRps.

Alphaflexiviridae (+RNA)

Botrytis virus X, which infects the phytopathogenic fungus *Botrytis cinerea* is the only known member of genus *Botrexvirus* in this family (Howitt et al., 2006; Pearson and Bailey, 2013). Its genome consists of a single linear molecule of +RNA, ~7.0 kb in length excluding the 3′-poly(A) tail and encompassing five ORFs. ORF1 encodes a large protein with methyltransferase (capping enzyme), helicase, and RdRp motifs, and ORF3 encodes the CP (the only structural protein). The functions of the other predicted translation products (ORF2, -4, and -5) are unknown. The genome lacks a “triple gene block” module, an evolutionarily conserved set of genes involved in cell-to-cell and long-distance movements of the plant-infecting members of this family. Virions are flexuous filaments of ~720 nm modal length and ~13 nm diameter. Botrytis virus X was discovered co-infecting the same fungal isolate of *B. cinerea* as gammaflexivirus Botrytis virus F (see below).

Sclerotinia sclerotiorum debilitation-associated RNA virus (SsDRV) is the prototype of genus *Sclerodarnavirus* in this family and was originally isolated from hypovirulent strain Ep-1PN of the phytopathogenic fungus *Sclerotinia sclerotiorum* (Xie et al., 2006), along with an unassigned +RNA virus, *Sclerotinia sclerotiorum* RNA virus L, which appears related to hepatitis E and rubi-like viruses (Liu et al., 2009). The SsDRV genome consists of a single linear molecule of +RNA, ~5.4 kb in length excluding the 3′ poly(A) tail and encompassing a single ORF that encodes a large protein with methyltransferase, helicase, and RdRp motifs. A virus similar to SsDRV was recently isolated and characterized from another hypovirulent strain of *S. sclerotiorum*, SX247 (Hu et al., 2014). Both viruses appear notable for not encoding a CP, unlike other members of this family.

Barnaviridae (+RNA)

Mushroom bacilliform virus (MBV), is the prototype of genus *Barnavirus*, the only known genus in family *Barnaviridae*. Virions are bacilliform and non-enveloped and are, typically, 19 x 50 nm, in width and length, respectively. Virions contain a single linear molecule of a positive sense ssRNA, 4.0 kb in size, and a single major CP of 21.9 kDa. MBV RNA (4009 nt ; GenBank U07551) comprises four major and three minor ORFs. The deduced aa sequence of ORF2 contains putative serine protease motifs related to chymotrypsin. ORF3 encodes a putative RdRp and ORF4 encodes the CP. The polypeptides potentially encoded by ORFs 1, 5, 6 and 7 show no sequence similarity to known polypeptides. Virions accumulate singly or as aggregates in the cytoplasm. The virus infects the common cultivated button mushroom (*Agaricus bisporus*). Transmission is horizontal via mycelium and possibly vertical by basidiospores. Distribution of MBV in nature coincides with that of the commercial cultivation of *A. bisporus* and occurs in most major mushroom-growing countries. MBV commonly occurs in mixed infection with a dsRNA virus (LaFrance isometric virus, LFIV) in mushrooms afflicted with La France disease. MBV is not required in pathogenesis involving LFIV, and does not share sequence similarity with LFIV. The amino acid sequences of the putative chymotrypsin-related serine protease and RdRp suggest an evolutionary relationship with some ssRNA positive sense plant viruses, particularly poleroviruses, sobemoviruses and enamoviruses (Revill, 2011; Revill et al., 1994, 1998, 1999).

Gammaflexiviridae (+RNA)

Botrytis virus F is the prototype of genus *Mycoflexivirus* in this family (Howitt et al., 2001; Pearson and Bailey, 2013). Its genome consists of a single linear molecule of +RNA, ~6.8 kb in length excluding the 3′-poly(A) tail and encompassing two major ORFs. ORF1 encodes a large protein (153 kDa) with methyltransferase and helicase motifs and terminating with a UGA stop codon. Read-through of this stop codon is expected to yield an even larger protein (212 kDa), which also includes RdRp motifs. ORF2 encodes the CP. Virions are flexuous filaments of ~720 nm modal length and ~13 nm diameter. As mentioned above, Botrytis virus F was discovered co-infecting the same fungal isolate of *B. cinerea* as alphaflexivirus Botrytis virus X.

Hypoviridae (+RNA)

Cryphonectria hypoviruses 1 to 4 (CHV1 to -4) are currently grouped in sole genus *Hypovirus* within this family (Nuss and Hillman, 2011). They infect the chestnut blight fungus *C. parasitica* throughout chestnut-growing areas of Europe, North America, and Asia, each virus with possible exception of CHV4 resulting in hypovirulence on chestnut trees and altered fungal morphology in culture. Infection of fungal mycelium is known to occur only through hyphal contact. Transmission rate through conidiospores varies greatly but could be as high as 100% in some cases, whereas transmission through ascospores is not known to occur. No true virions are associated with members of this family. Instead, pleomorphic vesicles containing viral RNAs and replication-associated proteins can be isolated from infected mycelia.

The hypovirus genomes were originally thought to be dsRNA but are now considered to be +RNA, ~9–13 kb in length excluding the 3′ poly(A) tail and encompassing two minimally overlapping ORFs in CHV1 and -2 but only one ORF in CHV3 and -4. CHV3 and -4 are also distinct in encoding a putative glycosyltransferase domain. Based on these differences as well as phylogenetic analyses, it seems proper to divide the Cryphonectria hypoviruses into two new genera: Alphahypovirus containing CHV1 and -2 and Betahypovirus containing CHV3 and -4.

The ORFA polyprotein product of CHV1 and -2 includes papain-like protease p29 followed by basic protein p40, which are separated by p29-mediated cleavage. p29 also contributes to suppression of host pigmentation, reduced sporulation, and reduced laccase accumulation; functions as a suppressor of host RNA silencing; and serves to promote RNA recombination of a co-infecting mycoreovirus. The ORFB polyprotein product of CHV1 and -2 appears to be expressed via translationally coupled termination–reinitiation (Guo et al., 2009). It includes second papain-like protease p48 followed by a large RdRp/helicase protein, which are separated by p48-mediated cleavage. The single polyprotein product of CHV3 and -4 contains functional domains in the following order: papain-like protease related to p29, glycosyltransferase, permuted papain-fold peptidase distinct from p48 (such peptidases function as de-ubiquitinating and de-SUMOylating enzymes in other systems), RdRp, and helicase.

In addition to CHV1 to -4, isolates of five other species have been reported: *Fusarium graminearum* hypovirus 1 (FgHV1), *Phomopsis longicolla* hypovirus 1, *Sclerotinia sclerotiorum* hypoviruses 1 and 2 (SsHV1 and -2), and *Valsa ceratosperma* hypovirus 1 (Hu et al., 2014; Khalifa and Pearson, 2014; Koloniuk et al., 2014; Wang et al., 2013; Xie et al., 2011; Yaegashi et al., 2012). Three of these are closely related to CHV3 and -4 and would be grouped with them in proposed new genus Betahypovirus; however, FgHV1 and SsHV2 are distinct. SsHV2 (genome ~15 kb, one ORF) represents a separate phylogenetic clade, suggesting creation of a third new genus in this family (Gammahypovirus). FgHV1 (genome ~13 kb, two ORFs), on the other hand, may be a recombinant in that most of its ORFB product sequences are closely related to CHV1 and -2 while its ORFA product sequences are closely related to CHV3 and -4.

Narnaviridae (+RNA)

Members of this family contain the simplest genomes of any autonomous RNA virus, each a single linear molecule of +RNA, 2.3–3.6 kb in length and encompassing a single ORF that encodes the RdRp (reviewed by Hillman and Cai (2013) and Wickner et al. (2013)). The family comprises two genera based on subcellular location. Members of genus *Narnavirus* have been found in the yeast *S. cerevisiae* as well as in the protistan water mold *Phytophthora infestans*, and are confined to the cytosol. Members of genus *Mitovirus*, in contrast, have been reported only in filamentous fungi to date and are localized to the mitochondria. Lacking a CP, their genomes are confined within intracellular lipid vesicles, as in the case of several other RNA viruses of "lower" eukaryotes including hypoviruses.

Mycomononegaviridae (−RNA)

Sclerotinia sclerotiorum negative-stranded RNA virus 1 (SsNSRV1) is the prototype of this proposed family (Liu et al., 2014). Its genome consists of a single linear molecule of −RNA, ~10 kb in length and encompassing six consecutive, non-overlapping ORFs. The largest ORF (ORF5, L) encodes a large protein with RdRp and methyltransferase motifs, closely related to that of other viruses in order *Mononegavirales*. Conserved gene-junction sequences of other mononegaviruses were also identified in SsNSRV1, although the genome organization of SsNSRV1 is distinct. Other mononegaviruses typically contain five main ORFs, arranged in the order N-P-M-G-L. In SsNSRV1, however, ORF2 has been identified as nucleoprotein (N), and an additional ORF (ORF6) is found downstream of the L gene. The functions of the other four SsNSRV-1 gene products remain unassigned at present. SsNSRV1 appears to form enveloped filamentous virions, ~1000 nm in length and 25–50 nm in diameter, with long flexuous nucleocapsids. Purified virus particles are competent to transfect virus-free *S. sclerotiorum* protoplasts, conferring hypovirulence. Incomplete genome sequences with similarity to SsNSRV-1 were recently assembled from other fungi (Kondo et al., 2013a).

Reverse-transcribing mycoviruses (+RNA-RT)

In addition to ssRNA and dsRNA mycoviruses, the 9th Report of ICTV lists the families *Metaviridae* and *Pseudovirida* under Reverse-transcribing RNA Viruses (Eickbush et al., 2011; Boeke et al., 2011). These descriptions were reproduced from the 8th ICTV Report because members of these families are no longer referred to as viruses in recent literature but rather as retrotransposon. ICTV is currently considering a new structure for classification of retrotransposons within the Report similar to that used for satellites.

Unclassified mycoviruses

A number of mycoviruses that remain to be formally classified are described at several places in this paper, and others have not been discussed due to space limitations. As the pace of viral discovery has quickened with new sequencing methods, the next several years will undoubtedly see a great deal of activity in this regard. Unclassified mycoviruses that deserve special comment here, however, are those with circular ssDNA genomes, including the prototype *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV1) (Yu et al., 2010, 2013). The genome of SsHADV1 is only ~2.2 kb in length but encompasses two ORFs. One ORF encoding CP is on one DNA strand, and another ORF encoding a replication initiation protein (Rep),

with sequence similarity to those of geminiviruses and conserved motifs for rolling-circle replication, is on the complementary strand. Unlike geminiviruses, however, the virus particles of SsHADV1 are isometric (i.e., non-twinned) and only ~21 nm in diameter; the SsHADV1 CP is divergent from those of geminiviruses; and SsHADV1 does not encode a movement protein, which is key for cell-to-cell movement of geminiviruses in plants. See "Virus-induced hypovirulence" for further discussion of this virus.

Structural features

Despite the broad diversity of fungal viruses described in "Diversity and taxonomic considerations", 3D structural analyses have focused to date on those mycoviruses with dsRNA genomes and single-layered icosahedral capsids. All appear to lack an extracellular phase in their life cycles, and virus particles accumulate in the fungal cytoplasm. These particles can usually be purified in large amounts with good structural preservation, enabling their characterization by 3D cryo-electron microscopy (cryo-EM) and/or X-ray crystallography.

Structural analyses of dsRNA mycoviruses have shown ubiquitous features in a broad spectrum of dsRNA viruses, including those that infect prokaryotes and complex eukaryotes. The totiviruses L-A and P4, which infect the yeast *S. cerevisiae* and the smut fungus *U. maydis*, respectively, were the first unambiguously described viruses with an unusual $T=1$ capsid formed by 12 decamers (rather than 12 pentamers) (Cheng et al., 1994). These capsids are an exception to the quasi-equivalence theory introduced by Caspar and Klug (1962), as they would correspond to the forbidden "$T=2$" layer or, formally, $T=1$ with a capsid protein (CP) dimer as the asymmetric unit.

Notably, the "$T=2$" capsids of dsRNA viruses, referred to as the inner or core capsid in the multilayered capsids of *Reoviridae* members (Grimes et al., 1998; Reinisch et al., 2000) as presumably also for mycoreoviruses, are commonly designed to remain structurally intact throughout the viral life cycle, thereby sequestering the dsRNA genome and avoiding induction of dsRNA-signaled host defense mechanisms that operate in some hosts. Moreover, and probably more fundamentally, conservation of this unusual capsid stoichiometry and architecture is thought to be related to its RNA synthesis activities, including organization of the packaged dsRNA molecule(s) and capsid-bound RdRp complex(es) for replication and transcription reactions; extrusion of the positive-strand transcripts for protein synthesis or packaging into new virions; and, in some viruses, addition of 5′ caps to those transcripts. Thus, the "$T=2$" capsids are not simply inert containers for sequestering and protecting the dsRNA genome, but instead dynamic assemblies or nanomachines that mediate multiple activities.

Totiviridae

3D structures have been reported to date for three fungal totiviruses: ScV-L-A, prototype of genus *Totivirus*; *Ustilago maydis* virus P4; and HvV190S, prototype of genus *Victorivirus* (Castón et al., 1997, 2006; Cheng et al., 1994; Dunn et al., 2013; Naitow et al., 2002,

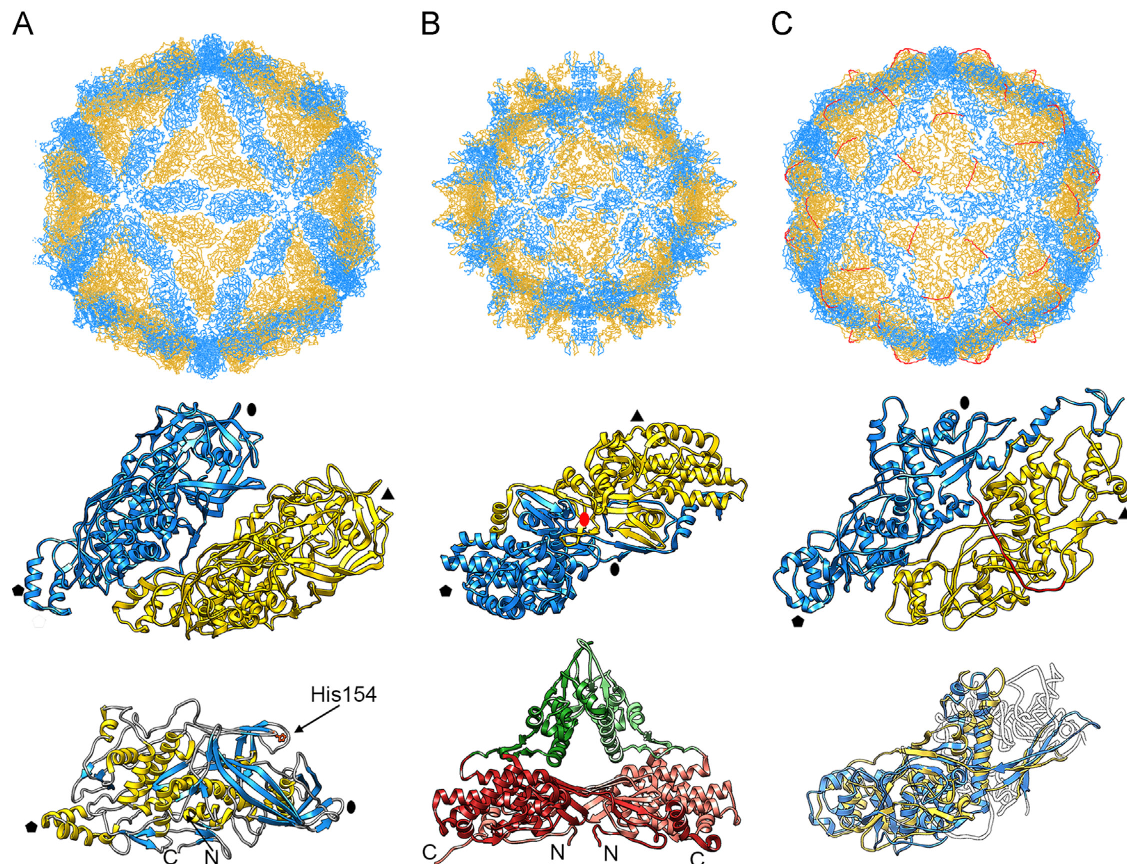

Fig. 1. 3D structures of mycovirus capsids. (A) "$T=2$" capsid of totivirus ScV-L-A viewed along a 2-fold axis of icosahedral symmetry, showing the CP subunits A (blue) and B (yellow) (top). Atomic model of a CP dimer (PDB 1m1c; 680 residues); symbols indicate icosahedral symmetry axes (middle). Side view of a CP monomer (capsid exterior at top) (bottom). (B) "$T=2$" capsid of partitivirus Penicillium stoloniferum virus F (top). Atomic model of a CP dimer (PDB 3es5; 420 residues); red oval indicates a local twofold symmetry axis (middle). Side view of a CP dimer; the arch (green) and shell (red) domains are indicated (bottom). (C) Authentic $T=1$ capsid of chrysovirus PcV (top). Atomic model of CP (PDB 3j3i; 982 residues) showing the N-terminal domain A (1–498, blue), the linker segment (499–515, red), and the C-terminal domain B (516–982, yellow) (middle). Superimposed A and B domains (white segments indicate poorly matching regions for both domains) (bottom).

including X-ray crystallography at 3.4-Å resolution). 3D structures have also been reported for two protozoal members of this family, *Trichomonas vaginalis* virus 1 and *Giardia lamblia* virus, and show strong similarities to the fungal virus structures (Janssen et al., 2015; Parent et al., 2013). The single-layered, 120-subunit capsids of all these totiviruses are 40–49 nm in diameter and exhibit a "$T=2$" organization in which the asymmetric unit is a CP (sometimes called Gag) dimer (Fig. 1A, top). The CP monomer can adopt two related conformations, termed A and B, which have notable structural differences especially on the subunit surfaces and reside in different bonding environments (i.e., make non-equivalent contacts) within the capsid. These subunits are arranged in two sets of five: five A subunits directly surround each icosahedral 5-fold axis and five B subunits intercalate between the A subunits, forming a decamer. Twelve such $(A{:}B)_5$ decamers then constitute the complete capsid. Adjacent A and B subunits within each decamer are oriented approximately in parallel (Fig. 1A, middle), suggesting an asymmetric A:B dimer as a possible intermediate for capsid assembly.

In the case of ScV-L-A and putatively other members of genus *Totivirus*, CP plays another, notable role in the viral life cycle. CP region Gln139–Ser182, which contributes to the capsid outer surface, mediates decapping of cellular mRNAs and attachment of m^7GMP to His154 (Tang et al., 2005). This activity was originally proposed to promote survival and translation of the viral positive-strand transcripts, which lack 5′ caps and 3′ poly(A) tails, by providing decapped cellular mRNAs to act as decoys for the cellular 5′→3′ exoribonuclease that degrades uncapped transcripts (Masison et al., 1995) (Fig. 1A, bottom). Recent studies, however, have suggested that this activity is also the first half of a more complete "cap snatching" activity by the capsid, as a result of which the 5′ caps removed from cellular mRNAs are then transferred onto the nascent 5′ ends of the viral transcripts (Fujimura and Esteban, 2011, 2012). Interestingly, members of genus *Victorivirus* (and other genera in family *Totiviridae*) have much longer 5′ UTRs than do members of genus *Totivirus*, suggesting that the 5′ UTRs of their positive-strand transcripts fold to form IRES structures that promote cap-independent translational initiation, obviating the need for cap snatching. Thus, to date, cap snatching is thought to be unique to members of genus *Totivirus* in this family.

Partitiviridae

3D structures have been determined to date for four fungal partitiviruses: betapartitiviruses *Fusarium poae* virus 1 and *Sclerotinia sclerotiorum* partitivirus 1, and gammapartitiviruses Penicillium stoloniferum virus F and Penicillium stoloniferum virus S (Ochoa et al., 2008; Pan et al., 2009, including X-ray crystallography at 3.3-Å resolution; Tang et al., 2010a, 2010b; Xiao et al., 2014) (Fig. 1B, top). The single-layered, 120-subunit capsids of these viruses are 35–42 nm in diameter and distinctive in having "arch-like" surface features that protrude above the main capsid shell. These "$T=2$" capsids also exhibit a distinctive organization, as the CP dimer has almost perfect local twofold symmetry (Fig. 1B, middle) and is stabilized by domain swapping within the shell region of the A and B subunits and also by intradimeric interactions between the protruding "arch" domains (Fig. 1B, bottom). A very similar organization has been found in a picobirnavirus (Duquerroy et al., 2009), a bisegmented dsRNA virus that infects humans and other vertebrates. Based on their 3D structures, partiti- and picobirnaviruses are proposed to be assembled from dimers of CP dimers (i.e., tetramers) as intermediates, whereas some other "$T=2$" capsids such as those of family *Toti*- and *Reoviridae* members are proposed to be assembled from pentamers of CP dimers (i.e., decamers).

Chrysoviridae

3D structures have been determined to date for two chrysoviruses: Penicillium chrysogenum virus (PcV) (Fig. 1C, top) and *Cryphonectria nitschkei* chrysovirus 1 (Castón et al., 2003; Gómez-Blanco et al., 2012; Luque et al., 2010). Stoichiometric estimates as well as mass determinations by scanning transmission electron microscopy had previously indicated that the PcV capsid comprises only 60 CP subunits, not 120 as found in most other dsRNA viruses. 3D structures have now confirmed that the ~40-nm diameter capsids of these viruses are distinctive in being authentic $T=1$ shells formed by only 60 copies of CP. Notably, however, the ~105-kDa CP of each virus encompasses two α-helical domains, which are divergent in sequence but structurally conserved such that each CP subunit appears to constitute a genetic duplication (Fig. 1C, middle). Moreover, these two domains in each CP subunit occupy comparable positions within the chrysovirus capsid as do the A and B subunits in the "$T=2$" capsids of totiviruses and others, with the adjacent A and B domains within each CP pentamer in the chrysovirus capsid oriented approximately in parallel. The duplicated domain (~350 residues) appears to have a single "hotspot", located on the capsid outer surface, at which variations are introduced by insertion of peptide segments in different chrysoviruses.

Comparisons of CP structural folds suggest that viral evolution has yielded only a small number of successful CP lineages (Abrescia et al., 2012). The chrysovirus duplicated domain (i.e., that formed by half of the full CP) seems to provide information regarding the primordial fold of a single, dominant lineage of dsRNA virus CPs, which share a long, spinal α-helix tangential to the capsid surface, as also found in the CPs of tailed dsDNA bacteriophages and herpesviruses (Luque et al., 2014). In addition, the unique structural details of the chrysovirus capsid reinforce the idea that a $T=1$ layer with a dimer (of either intrasubunit domains or separate subunits) as the asymmetric unit provides an optimal framework for managing dsRNA metabolism.

Table 1
Genome packaging densities in fungal dsRNA viruses.

Virus family	dsRNA features			Capsid features		
	No. segments	Size (kbp)	MW[a] (MDa)	RdRp	ϕ^b/r^c (nm)	dsRNA density (bp/100 nm^3)[d]
Reoviridae						
Orbivirus	10	~19.2	13.1	12	~52/22	43
Totiviridae, L-A	1	~4.6	3.1	1–2	~43/17	22
Partitiviridae, PsV-S	1 (2)[e]	~1.7 (3.3)	1.2 (2.2)	1	~35/12	23
Chrysoviridae, PcV	1 (4)[e]	~3.2 (12.6)	2.2 (8.6)	1	~40/16	19

[a] MW were calculated assuming a mass of 682 Da/bp.
[b] Outer diameter.
[c] Inner radius.
[d] Densities when volume of a perfect sphere is assumed and any other internal components are ignored.
[e] PsV-S and PcV dsRNA features: the genome is formed by two or four dsRNA molecules, respectively, but a mean value was calculated for each column as there is one dsRNA molecule/particle.

dsRNA and RdRp packaging within mycovirus capsids

Fungal dsRNA viruses have spacious capsids compared to the inner cores of complex eukaryotic dsRNA viruses (Table 1). Whereas reoviruses have 9–12 genome dsRNA segments packed with high density at $\sim$40 bp/100 nm^3, fungal virus capsids (including LA, PcV and PsV-F) contain a single dsRNA molecule, loosely packed at $\sim$20 bp/100 nm^3. The looser packing of the dsRNA probably improves template motion in the more spacious transcriptional and replicative active particle. Although the 3D capsid structures of representative *Toti*-, *Partiti*-, and *Chrysoviridae* members have been reported as discussed above, no 3D structures for their RdRps, either as isolated proteins or as packaged inside virions, have been reported to date. For members of each of these families, the RdRp molecules appear to be incorporated in only 1 or 2 copies per virion. For partiti- and chrysoviruses, the RdRp is expressed as a physically separate protein, from a separate genome segment, and must therefore be incorporated into virions via noncovalent interactions with the capsid and/or genome. The same is true for totiviruses such as HvV190S in the genus *Victorivirus*, except that the RdRp is expressed as a physically separate protein from the single genome segment of those viruses via a coupled termination–reinitiation mechanism (Huang and Ghabrial, 1996; Li et al., 2011, 2015; Soldevila and Ghabrial, 2000). For totiviruses such as ScV-L-A in the genus *Totivirus*, in contrast, the RdRp is expressed as a C-terminal fusion product with the CP (i.e., as a CP/RdRp protein) via programmed ribosomal frameshifting (Dinman et al., 1991). As a result, in these totiviruses, the 1 or 2 RdRp domains per virion are covalently tethered to the capsid via the fused CP domain, which occupy 1 or 2 subunit positions in the capsid.

Due to the extensive interactions between the inner capsid surface and the underlying packaged RNA, in partiti- and chrysoviruses the outermost RNA layer is ordered, whereas the innermost RNA shells are more diffuse. These contacts have been defined at the atomic level in PcV and PsV-F virions (Luque et al., 2014; Pan et al., 2009). The lower density at the central region and the associated slight increase in dsRNA mobility might be necessary for maximum RdRp activity in the context of a nonfused RdRp complex.

Virus-induced hypovirulence

Mycovirus-induced hypovirulence (reduction in virulence of phytopathogenic fungi) is best appreciated in reflections on the devastating chestnut blight pathogen *C. parasitica*, which destroyed the beloved American chestnut tree at the turn of the twentieth century. The hopeful quotation from Robert Frost's “Evil Tendencies Cancel” comes to mind:

“Will the blight end the chestnut?
The farmers rather guess not.
It keeps smoldering at the roots
And sending up new shoots
Till another parasite
Shall come to end the blight.”

The success in applying hypovirulent strains of *C. parasitica* to combat chestnut blight in Europe has provided the impetus for exploiting mycoviruses as biocontrol agents. Significant progress has been made in identifying and characterizing mycoviruses that confer hypovirulence on their economically important plant pathogens as discussed below.

Viruses of chestnut blight fungus Cryphonectria parasitica and recent advances in related fields

Viruses that have been shown to confer high levels of hypovirulence to *C. parasitica* under laboratory conditions include CHV1, -2, and -3 and MyRV1 and -2 as introduced in “Diversity and taxonomic considerations” (*Hypoviridae* and *Reoviridae*, respectively) (Hillman and Suzuki, 2004). Notably, the level of hypovirulence conferred by a particular virus is strain specific, as in the case of diseases caused by plant or animal viruses (Chen and Nuss, 1999). Viral biocontrol of chestnut blight is so far the only successful example of large-scale field-level suppression of a phytopathogenic fungal disease, in which CHV1 is believed to play the dominant role. How effectively hypovirulent strains have suppressed chestnut blight seems to correlate with geography. In the US, they have been generally unsuccessful, with potential exceptions in a few areas (Milgroom and Cortesi, 2004), while in Europe, they have been shown to suppress the disease and disseminate. It is well accepted that one of the major factors governing effectiveness of hypovirus transmission via hyphal anastomosis in the field is the level of vegetative compatibility group diversity of *C. parasitica*. Recent ecological studies have contributed to better understanding of the dynamic interactions between this fungus and its viruses in forests (Bryner and Rigling, 2012; Bryner et al., 2014; Dawe and Nuss, 2013).

C. parasitica and its viruses have contributed a great deal to enhance our understanding of basic aspects of mycovirology such as virus replication, antiviral RNA defense at both cellular (RNA silencing) and population (vegetative incompatibility) levels, viral evasion of antiviral defense, and silencing-associated RNA recombination (Choi et al., 2012; Nuss, 2011; Zhang et al., 2014a, 2014b). RNA silencing is an RNA-mediated gene regulation mechanism conserved across eukaryotes, but with the main RNA-silencing components—Dicer-like proteins (DCLs), Argonaute-like (AGLs), and RNA-dependent RNA polymerase proteins (RDRs)—varying in numbers between organisms. *C. parasitica* has two *dcl* genes, four *agl* genes, and four *rdr* genes. Among them, only *dcl2* and *agl2* are required for RNA silencing (Segers et al., 2007; Sun et al., 2009) and indeed high-level induction of *dcl2* and *agl2* mRNAs is observed upon infection by some viruses or transgenic expression of exogenous dsRNAs (Zhang et al., 2008; Sun et al., 2009). As an evasion response, CHV1 protein p29 suppresses this induction. Strains of *C. parasitica* defective for RNA silencing allow for enhanced virus replication and altered symptoms, whether or not the virus originated from *C. parasitica* (Fig. 2). For example, RnMBV1 originating from *R. necatrix* accumulated to 20-fold higher levels and manifested more severe symptoms in defective strain Δ*dcl2* than in wild-type strain EP155 (Salaipeth et al., 2014).

In at least some cases, RNA silencing in *C. parasitica* is also involved in viral genome rearrangements. CHV1 is often associated with defective interfering (DI) RNAs smaller than the genomic RNA, which appear spontaneously during subculturing of infected wild-type fungal strains, but not silencing-defective strain Δ*dcl2* or Δ*agl2*; moreover, the DI-RNAs from wild-type strain EP155 are readily transmitted via hyphal anastomosis to other strains

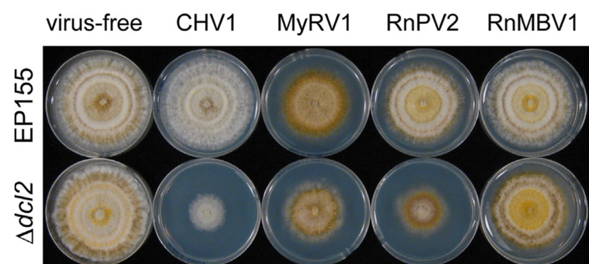

Fig. 2. Colony morphologies of *C. parasitica* strains. Wild-type strain EP155 and RNA silencing-deficient strain Δ*dcl2* were infected or not by different mycoviruses as indicated (RnPV2, *Rosellinia necatrix* partitivirus 2; see text for other abbreviations). All cultures were grown on potato dextrose agar for 6 days at room temperature before photography.

competent for RNA silencing, but not to the defective strains (Zhang et al., 2008; Sun et al., 2009). These and other findings suggest a requirement for the RNA silencing machinery in generation and/or maintenance of the CHV1 DI-RNAs. For a different *C. parasitica* virus, MyRV1, genome rearrangements involving large internal deletion appear spontaneously in genome segments S4 and S10. Expression of the CHV1-derived RNA-silencing suppressor p29, however, results in much more frequent occurrence of these and other rearranged genome segments of MyRV1 (Tanaka et al., 2012). In this case, then, it appears that RNA silencing in *C. parasitica* reduces viral genome rearrangements, opposite the case for CHV1 but again suggesting a role for the silencing machinery in affecting generation and/or maintenance of altered viral genome RNAs.

Plant virologists historically developed *Nicotiana benthamiana* as a model host for a number of plant viruses (Bombarely et al., 2012; Goodin et al., 2008). *C. parasitica* has been comparably developed as a model host for a number of fungal viruses (Eusebio-Cope et al., 2015). Fundamental technologies for introduction of homologous and heterologous viruses, and transformations including targeted gene disruptions, have promoted the development of both systems. To date, hypo-, megabirna-, mycoreo-, partiti-, and victoriviruses, originating from *C. parasitica* or not, have been shown to infect the wild-type EP155 strain. Among many important findings has been the identification of host factors involved in symptom induction (Faruk et al., 2008). Advantages of *C. parasitica* as a virus host over *N. benthamiana* include the availability of haploid genetics (allotetraploid for *N. benthamiana*) and instant gene disruption technologies for multiple targets, which are still difficult in plants. Inversely, transient expression systems such as agro-infiltration in plants (including RNA silencing suppressor assay) are not as yet established in *C. parasitica* or other filamentous fungi. Agrobacterium-mediated stable transformation, however, has been used in fungi (Michielse et al., 2005).

Hypovirulent strains of white mold fungus Sclerotinia sclerotiorum

Ascomycete *S. sclerotiorum* has a broad host range including many important field and vegetable crops such as rapeseed, beans, and lettuces. The diseases caused by this fungus are often called white mold or stem rot. *S. sclerotiorum* usually kills host plants quickly (in a week or less) and produces sclerotia (dormant fungal bodies) on diseased parts remaining in the soil. These sclerotia may later germinate myceliogenically to produce infectious hyphae that can infect the bases of nearby plants or carpogenically to produce ascospores that can disseminate more widely. It is a very common fungus and can reach massive populations in the field, promoted by rainfall and high humidity. Control is usually not efficient since resistant crop cultivars are not available. Exploiting mycoviruses to reduce its virulence thus seems to offer promise. Prospects for exploiting mycoviruses to control fungal diseases of field crops were recently discussed (Xie and Jiang, 2014). Field crops usually have short life spans, necessitating quick action, which mycoviruses might be able to provide. Furthermore, field crops often have a small and uniform canopy, facilitating the application of viral biocontrol agents.

S. sclerotiorum strains host various distinct types of mycoviruses (Jiang et al., 2013; Xie and Jiang, 2014), several of which confer hypovirulence in the laboratory, including SsPV1 mentioned in “Diversity and taxonomic considerations” (*Partitiviridae*), SsDRV described in “Diversity and taxonomic considerations” (*Alpha¯exiviridae*), SsHV1 and -2 mentioned in “Diversity and taxonomic considerations” (*Hypoviridae*), *Sclerotinia sclerotiorum* mitovirus 1, SsNSRV1 described in “Diversity and taxonomic considerations” (Mycomononegaviridae), and SsHADV1 described in “Diversity and taxonomic considerations” (Unclassified mycoviruses) (Hu et al., 2014; Khalifa and Pearson, 2014; Liu et al., 2014; Xie et al., 2006, 2011; Xie and Ghabrial, 2012; Yu et al., 2010). Hypovirulence-associated mycoviruses of *S. sclerotiorum* thus appear to be abundant in nature (Fig. 3). Unfortunately, many of these viruses cannot be used directly to control disease in the field because their spread is restrained by vegetative incompatibility among resident *S. sclerotiorum* strains, which can be numerous and complicated under field conditions (Attanayake et al., 2013; Kohn et al., 1990).

On the other hand, a few of these mycoviruses from *S. sclerotiorum* are endowed with robust infectivity (used here to mean virus ability to invade, replicate, and spread through host tissue), particularly SsHADV1 and SsPV1 (Xiao et al., 2014; Yu et al., 2010). Recent findings suggest that viruses similar to SsHADV1 are widespread in nature (Du et al.,

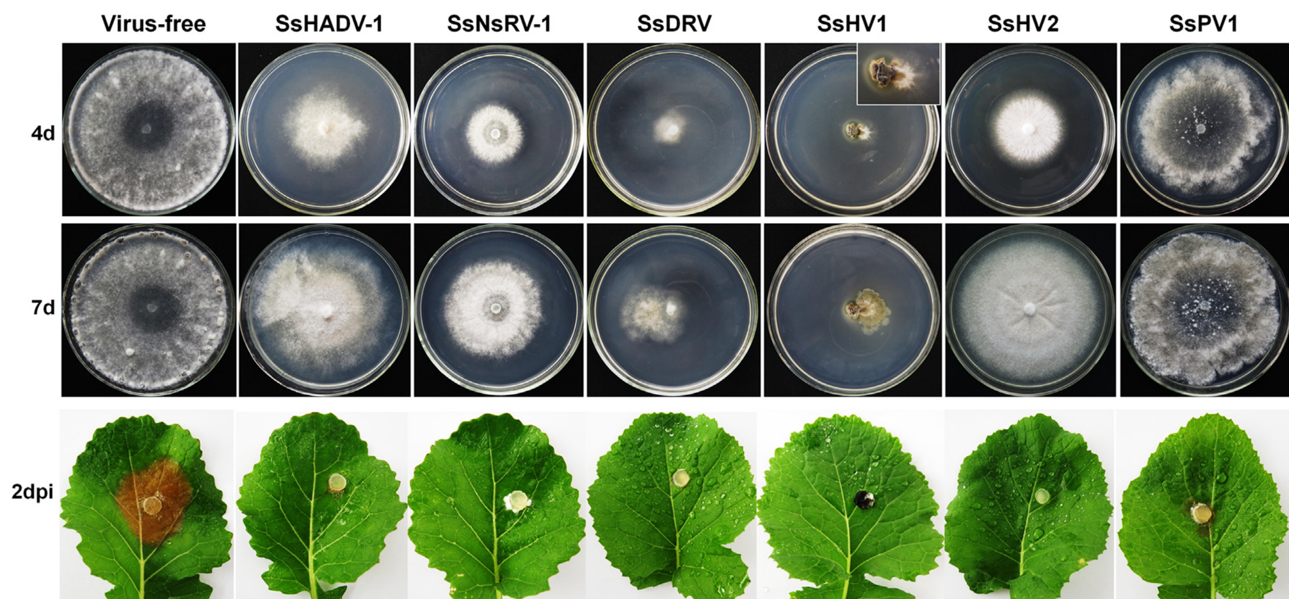

Fig. 3. Colony morphologies and virulence levels of *S. sclerotiorum* strains. Mycovirus-infected hypovirulent strains used are SsHADV-1-infected strain DT-8, SsNsRV-1-infected strain Ep-1PNA367, SsDRV-infected strain Ep-1PN, SsHV-1-infected strain SZ-150, SsHV2-infected SX247, and SsPV1-infected strain WF-1. Virus-free strain 1980 was inlcuded as a control. All cultures were grown on potato dextrose agar for 4 or 7 days at 20 °C before photography. Hyphal agar discs were taken from the margin of an actively-growing colony of each strain and placed on detached leaves of *Brassica napus* (rapeseed). The inoculated leaves were incubated for 2 days at 20 °C with 100% relative humidity before photography.

2014; Ng et al., 2014; Sikorski et al., 2013; Yu et al., 2013). Importantly, purified SsHADV1 particles can directly infect hyphae of *S. sclerotiorum*, indicating that host vegetative incompatibility should not be a limiting factor for transmission of this virus, and indeed field tests have shown that spraying hyphal fragments of SsHADV1-infected strain DT-8 on aerial parts of rapeseed can successfully control stem rot caused by *S. sclerotiorum* (Yu et al., 2013). Although partitiviruses are often associated with symptomless infections, SsPV1 has strong impact on the growth and pathogenicity of *S. sclerotiorum* (Xiao et al., 2014). Moreover, SsPV1 can be easily transmitted via hyphal contact regardless of vegetative incompatibility. These properties of SsPV1 suggest that it may also have a potential for controlling stem rot in the field.

S. sclerotiorum is a conidiospore-less fungus, and as in the case of some other fungus/mycovirus systems, its mycoviruses are inconsistently transmitted via ascospores. Instead, the sclerotia described above play a critical role in virus transmission, meaning that hyphal contact is the main conduit for transmission of *S. sclerotiorum* mycoviruses in the field and that sclerotial maintenance of virus-infected, hypovirulent *S. sclerotiorum* strains might promote their survival well after the time of initial application to a field. To explore SsHADV1, SsPV1, or other mycoviruses as potential biocontrol agents of *S. sclerotiorum* diseases, it will be necessary to better understand such ecological characteristics of these viruses.

Hypovirulent strains of white root rot fungus Rosellinia necatrix

White root rot is prevalent in perennial crops worldwide, and particularly in Japan. This disease is difficult to control, partly because the causal pathogenic fungus *R. necatrix* inhabits the soil. The fungus spreads in soil in the form of mycelia and shows relatively simple population structure in infested fields, with limited vegetative incompatibility. This feature may allow for efficient transmission of viruses with biocontrol potential. A large-scale screen of over 1000 field isolates of *R. necatrix* revealed an overall virus incidence of $\sim$20% in the collected samples.

Most known viruses of *R. necatrix* have dsRNA genomes and cause symptomless infections (Kondo et al., 2013b). A few viruses, however, induce hypovirulence, including RnMBV1 and MyRV3 described in “Diversity and taxonomic considerations” (*Megabirnaviridae* and *Reoviridae*, respectively). Importantly, these viruses are infectious as particles in transfection assays and reduce virulence of all tested strains of the host fungus (Chiba et al., 2009). In general, transfection of desired fungal isolates with any virus of interest and the release of transfected isolates in the infested fields represent an efficient means of virocontrol. RnMBV1 is superior to MyRV3 as a biocontrol agent because the former is maintained stably under both laboratory and field conditions, while the latter is lost relatively easily. Furthermore, RnMBV1 virions stored at $-80\,^{\circ}$C for years remain competent for transfection and induction of hypovirulence. dsRNA viruses of *R. necatrix* that induce symptomless infections include partitiviruses, quadriviruses, and victoriviruses. *Rosellinia necatrix* fusarivirus 1 is the only known positive-strand RNA virus that infects this host fungus, and asymptomatically at that (Zhang et al., 2014c).

R. necatrix has emerged as a good model system for studying virus–virus and virus–host interactions. As observed for viral studies in *C. parasitica* (Eusebio-Cope et al., 2015), many similar techniques and tools are available for viral studies in *R. necatrix* (Kondo et al., 2013b). *R. necatrix* has some disadvantages compared to *C. parasitica*, in that transformation and virion transfection of *R. necatrix* are not as easy, molecular tools and biological resources available for *R. necatrix* are not as many, and reverse genetics is not yet available for any of the known *R. necatrix* viruses. In addition, conidia of *R. necatrix* scarcely germinate to generate mycelia under laboratory conditions. A strong point of *R. necatrix*, on the other hand, is applicability of a zinc-mediated method for horizontal transmission of viruses between mycelially incompatible strains (Ikeda et al., 2013), which is worth testing in other filamentous fungi.

Concluding remarks

Recent studies have identified a diverse group of mycoviruses capable of promoting distinct hypovirulence in their phytopathogenic fungal hosts and thus excellent candidates for implementation of biocontrol strategies. Vegetative incompatibility is a key factor in mycovirus transmission and natural spread. With the availability of genome sequences of several important phytopathogenic fungi, it is now possible to identify and characterize the pertinent *vic* genes allowing potential modulation of incompatible reactions. This is exemplified by the recent identification of six vic loci in the chestnut blight fungus (Choi et al., 2012; Zhang et al., 2014b). Use of virus particles as a novel fungicide appears feasible with the geminivirus-related ssDNA mycovirus SsHADV1 since its purified particles can directly infect fungal hyphae and confer hypovirulence. Structural studies of dsRNA fungal viruses have revealed novel features and contributed to a better understanding of the structure, function and evolution of these viruses.

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