

Non-pathogenic reoviruses of leafhoppers and planthoppers

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Reoviruses of hoppers are reviewed with emphasis on their transmission, classification, and evolution. Plant reoviruses multiply in both plants and hopper vectors, whereas three non-pathogenic reoviruses are known; leafhopper A virus, Peregrinus maidis reovirus, and Nilaparvata lugens reovirus (NLRV), that do not appear to cause disease in their insect hosts, and do not multiply in plants but share many properties with plant reoviruses. Genome analysis of NLRV would place it in the genus Fijivirus in the family Reoviridae, and this assignment would be consistent with the biological and physicochemical properties of Fijiviruses, except for host range. The hypothesis that plant reoviruses originate as viruses exclusively of insects is studied.

Key words: reovirus / Fijivirus / virus transmission / reovirus evolution

NEW VIRUSES are often discovered in apparently healthy insects in the course of electron microscope observations of tissues or purified preparations made with other purposes in mind. They are sometimes referred to as latent, persistent, occult, hereditary, symbiotic, asymptomatic, or symptomless viruses,^{1,2} and little is known about many of them. Nault³ observes that non-pathogenic viruses are unfortunately ignored by most plant virologists because they do not cause plant disease, and are also neglected by insect pathologists because of apparent lack of potential for biological control. Our knowledge of their features and taxonomic relationships to economically important plant and animal reoviruses are, therefore, still very limited. Data on host range, mode of transmission, and genome organization could offer invaluable information with which to explore virus-vector interaction, virus pathogenicity toward plants and animals, and virus evolution.

The focus of this chapter is to compare these non-pathogenic reoviruses of leafhoppers and planthoppers with plant reoviruses, which also multiply in these insects and so could be closely related. Such viruses may be the key to the origin and phylogenetics of the

plant reoviruses.³⁻⁶ Since there are some excellent reviews on the subject,^{4,5,7-10} we shall discuss differences and similarities between plant reoviruses and non-pathogenic reoviruses of hoppers with particular attention to a recently discovered non-pathogenic agent, Nilaparvata lugens reovirus (NLRV).

Plant reoviruses and their insect hosts

Plant reoviruses are of course also viruses of insects. To summarize, there are three genera so far, *Phytoreovirus*, *Fijivirus*, and *Oryzavirus*.¹¹ Phytoreoviruses include wound tumor virus (WTV),¹² rice dwarf virus (RDV),^{13,14} and rice gall dwarf virus (RGDV).¹⁵⁻¹⁸ Oryzaviruses are comprised of rice ragged stunt virus (RRSV),¹⁹⁻²⁴ and Echinochloa ragged stunt virus (ERSV).²⁵⁻²⁷ The genus *Fijivirus* contains essentially three species, Fiji disease virus (FDV),^{28,29} a complex including maize rough dwarf virus (MRDV),⁷ rice black streaked dwarf virus (RBSDV),³⁰ and others,³¹⁻³³ and oat sterile dwarf virus (OSDV).^{31,34-37}

All but WTV, which infects dicots,^{8,12} infect monocotyledonous plants, for the most part grasses and cereals. Phytoreoviruses are transmitted by leafhoppers whereas Fijiviruses and oryzaviruses are vectored by planthoppers (Table 1). Economically important leafhoppers are members of the family Cicadellidae, and planthopper vectors belong to the family Delphacidae.⁴⁶

Pathogenic reoviruses of insects

Insects are infected by a group of pathogenic reoviruses, the cytoplasmic polyhedrosis viruses (CPVs). These are often seen in members of the orders Lepidoptera, Diptera and Hymenoptera,^{47,48} and are used as control agents for these insects.⁴⁸⁻⁵⁰ Another type of pathogenic reovirus is reported from the housefly.^{48,51,52} Besides these viruses, insects and some ticks have associations with reoviruses of animals; they are host vectors of members of the genera *Orbivirus* and *Coltivirus*.⁴

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Table 1. Reoviruses of leafhoppers and planthoppers

Virus	Leafhopper (L) or planthopper (P)	
<i>Phytoreovirus</i>		
Wound tumor	<i>Agallia constricta</i> (Van Duzee)	L
	<i>A. quadripunctata</i> (Provancher)	L
	<i>Agalliopsis novella</i> Say	L
Rice dwarf	<i>Nephotettix cincticeps</i> (Uhler)	L
	<i>N. nigropictus</i> (Stål)	L
	<i>N. virescens</i> (Distant) ³⁸	L
	<i>Recilia dorsalis</i> (Motschulsky)	L
Rice gall dwarf	<i>Nephotettix cincticeps</i> (Uhler)	L
	<i>N. nigropictus</i> (Stål)	L
	<i>N. virescens</i> (Distant)	L
	<i>N. malayanus</i> Ishikawa et Kawase	L
	<i>Recilia dorsalis</i> (Motschulsky)	L
<i>Fijivirus</i>		
Fiji disease	<i>Perkinsiella saccharicida</i> Kirkaldy	P
	<i>P. vastatrix</i> (Breddin)	P
	<i>P. vitiensis</i> Kirkaldy	P
Maize rough dwarf MRDV strain	<i>Laodelphax striatellus</i> (Fallén)	P
	<i>Toya propinqua</i> (Fieber)	P
	<i>Sogatella vibix</i> (Haupt)	P
	<i>Javesella pellucida</i> (Fabricius)	P
	<i>Ribautodelphax notabilis</i> (Logvinenko)	P
MRDV Cereal tillering strain	<i>Laodelphax striatellus</i> (Fallén)	P
	<i>Dicranotropis hamata</i> (Boheman)	P
MRDV Mal de Rio Cuarto strain	<i>Delphacodes kuscheli</i> Fennah	P
Pangola stunt	<i>Sogatella furcifera</i> (Horváth)	P
	<i>S. kolophon</i> (Kirk.)	P
Rice black streaked dwarf	<i>Laodelphax striatellus</i> (Fallén)	P
	<i>Ribautodelphax albifascia</i> (Matsumura)	P
	<i>Unkanodes sapporona</i> (Matsumura)	P
Oat sterile dwarf	<i>Javesella pellucida</i> (Fabricius)	P
	<i>J. discolor</i> (Boheman)	P
	<i>J. dubia</i> (Kirschbaum)	P
	<i>J. obscurella</i> (Boheman)	P
	<i>Dicranotropis hamata</i> (Boheman)	P
<i>Oryzavirus</i>		
Rice ragged stunt	<i>Nilaparvata lugens</i> (Stål)	P
	<i>N. bakeri</i> (Muir) ³⁹	P
Echinochloa ragged stunt	<i>Sogatella longifurcifera</i> (Esaki et Ishihara) ²⁵	P
Non-pathogenic viruses		
Leafhopper A	<i>Cicadulina bimaculata</i> (Evans) ^{40, 41}	L
Peregrinus maidis reo	<i>Peregrinus maidis</i> (Ashmead) ^{42,43}	P
Nilaparvata lugens reo	<i>Nilaparvata lugens</i> (Stål) ⁴⁴	P
	<i>Laodelphax striatellus</i> (Fallén) ⁴⁵	P
Graminella nigrifrons reo	<i>Graminella nigrifrons</i> (DeHong and Mohr) ⁶¹	L

Modified from Francki and Boccardo,⁸ Conti,⁴ and Nault.³

Non-pathogenic reoviruses of insects

The features of three non-pathogenic insect reoviruses are rather well documented. One such virus is leafhopper A virus (LAV), found in *Cicadulina bimaculata*.^{40,53} LAV was found during studies of wallaby ear disease of maize, now known to be caused by insect salivary toxins rather than a reovirus, as originally supposed.⁵⁴ It has 10 genome segments of a total molecular weight estimated as 19×10^6 .^{6,40} The second virus is Peregrinus maidis virus (PgMV) from the planthopper of this species.^{42,43} PgMV was also found in apparently healthy hoppers during an investigation of maize mosaic rhabdovirus, which also multiplies in the same hopper. The virus infects many internal organs: the salivary gland, intestine, mycetome, fat body and ovary.⁴² There are 12 genome segments with a total molecular weight estimated as 21.3×10^6 .^{6,43}

The third virus, Nilaparvata lugens reovirus (NLRV), was found during studies of planthopper and leafhopper-borne viruses.⁴⁴ Nilaparvata lugens, which occurs in south-east Asia,⁵² not only seriously damages rice but also propagatively transmits RRSV and rice grassy stunt tenuivirus⁵⁵ to rice crops. Electron microscopy of insect tissues infected with NLRV shows tubular structures typically observed in plant and insect cells infected with plant reoviruses.^{7,8,56,58-60} Immunohistochemistry on paraffin sections of hoppers shows that the virus invades nearly all organs, with highest levels in the fat body, salivary glands, and to a lesser extent, the intestine (H. Noda and N. Nakashima, unpublished results). Incidence of infection in experimental hoppers fluctuates from about 30 % to more than 90 % depending on rearing conditions. The virus can be passed to non-viruliferous hoppers by injection into the hemocoel, and by rearing with viruliferous hoppers. There are 10 genome segments of a total molecular weight of about 18×10^6 .

Another reo-like virus has recently been found in the black-faced leafhopper, *Graminella nigrifrons*,⁶¹ and is believed to be identical to the virus reported by Childress and Harris.⁶² Although the particle structure, physicochemical properties and mode of transmission are unknown, it is of interest as the fourth non-pathogenic reovirus from fulgoroids.

Certain other non-pathogenic reoviruses are also reported from insects or insect cell lines. A grasshopper, *Caledia captiva*, harbors a reovirus with 10 genome segments.⁶³ Electron microscopy of the Mexican bean beetle *Epilachna varivestis* revealed the

consistent presence of reovirus-like particles in the cytoplasm and rarely in nuclei.⁶⁴ The bedbug *Cimex lectularis* carries a reovirus with 11 genome segments.⁶⁵ A reovirus is also reported from a laboratory stock of the braconid parasitoid *Microplitis croceipes*,⁶⁶ this virus lacks spikes and structurally resembles a rotavirus. Reo-like viruses are also found in the dipteran insects *Drosophila melanogaster* and *Ceratitis capitata*.^{67,68} An interesting reovirus has been found in *Drosophila simulans*, that alters the morphology of the infected fly.⁶⁹⁻⁷¹ This *Drosophila* S virus induces the S character, a maternally inherited abnormality of the setae or bristles. *Drosophila* cell lines sometimes contain reoviruses⁷²⁻⁷⁴ but it is not clear whether *Drosophila* is a natural host.

All these apparently non-pathogenic reo-like viruses seem to represent only the tip of the iceberg, and very many more must exist. So far there is no apparent relationship of these viruses to plant reoviruses; some of them might however be related to vertebrate reoviruses transmitted by insects.

Particle morphology

Particle morphology is an important means of distinguishing between the genera of plant reoviruses. Phytoreoviruses are angular and without spikes, whereas Fijiviruses have more rounded particles with external spikes (A spikes) and also prominent spikes (B spikes) on the easily formed subviral particle.^{7,9,11,59} For oryzaviruses, particle structure differs between RRSV and ERSV; ERSV appears to resemble Fijiviruses in structure.²⁶ RRSV, however, possesses distinct morphology with broad-based spikes entirely covering the core; there does not seem to be a complete outer shell, though the basic construction appears similar to the B-spiked core of Fijiviruses.^{21-23,59} This particle morphology is one of the main features separating RRSV from members of the genera *Phytoreovirus* and *Fijivirus*, within the third genus of plant reoviruses.^{11,75} Despite apparently different morphology, RRSV and ERSV would appear to be correctly placed in the same genus as they share symptomatology, host range, vector relationships,^{9,11} and terminal nucleotide sequences.⁷⁶

Hatta and Francki⁵³ have pointed out that the Fijivirus B-spiked subviral particle (core) somewhat resembles the similarly spiked particles of cypoviruses. Cypoviruses lack an outer shell but are normally embedded in a virus-encoded protein matrix (the polyhedron) which may serve the same purpose as an outer capsid.

The three non-pathogenic reoviruses all resemble the Fijiviruses in particle structure. Thus LAV has a double-shelled particle with A spikes and resembles FDV, with which it was compared.⁵³ PgMV also has a complete outer shell and a typically B-spiked subviral particle.⁴³ NLRV has double-shelled particles about 65 nm in diameter, with B spikes on the inner capsid and A spikes on the outer shell; however, the cypovirus polyhedrin protein is much smaller⁷⁷ than the outer capsid protein of NLRV, and significant sequence homology was not detected between the two (N. Nakashima and H. Noda, unpublished results).

Among the plant reoviruses, Fijivirus-type particle morphology is linked with possession of 10 genome segments and multiplication in planthoppers. Among NLRV, LAV and PgMV, all with Fijivirus-type particles, only the first conforms to this rule, since LAV has 10 segments but a leafhopper host, and PgMV has a planthopper host but 12 segments.

Vertical transmission

Vertical transmission may occur in two ways: directly from the female to internal tissues of the egg (transovarial transmission) or by contamination of the egg surface, so that progeny become infected upon emergence (transovum transmission).⁷⁸ Transovum transmission can be eliminated by sterilizing the egg surface. In earlier experiments on vertical transmission of plant reoviruses, the distinction between transovarial and transovum transmission was not made.

The phyto-reoviruses are vertically transmitted in their hopper hosts. For WTV the rate is reported as 14 to 70 %⁷⁹ RDV is vertically transmitted in infected females of *N. cincticeps* at a rate of 60–85 %, ¹⁴ and in *R. dorsalis*, 0–64 %.⁸⁰ For RGDV in *N. nigropictus*, the figures are 66.7–92.8 %¹⁷ Fijiviruses and oryzaviruses show no vertical transmission in their insect hosts, or at most, extremely low rates.^{7,80–84} In all these studies, transmission rates were measured by observing virus transmission to plants, whereas more recent studies have used immunological methods.

About 20 % vertical transmission (from females but not males) is reported for LAV.⁴¹ In these experiments, eggs were surface-sterilized with 1 % hyamine, so transmission must have been ovarial. When the case of NLRV was examined by ELISA, the rate of transovum transmission was 14 %, but transovarial transmission was only 2 %.⁴⁵

We cannot at present account for the above large differences in rates of vertical transmission, except that high rates are associated with leafhoppers and

low rates with planthoppers. (Nevertheless, planthoppers can vertically transmit tenuiviruses at high rates.⁵⁵) WTV invades various vector organs⁸⁵ including the ovary,⁸⁶ but the case is similar with RBSDV Fijivirus.⁸³ With hopper ovarian structures⁸⁷ in mind, Nasu⁸⁸ has suggested ways in which viruses may invade oocytes, and observed such invasion by RDV in a leafhopper,⁸⁹ but further investigation is needed to resolve the problem.

Horizontal transmission via plants

To maintain a virus in a hopper colony by female vertical transmission alone, a rate of 100 % is apparently necessary;⁷⁸ this means that horizontal transmission must occur. NLRV is in fact transmitted from hopper to hopper through contaminated rice plants, and the virus probably moves through the phloem and/or xylem, as it can be acquired at points distant from the inoculation site.⁴⁵ Failure of PCR to amplify genomic material from an inoculated plant suggests that NLRV does not multiply in the plant. Moreover, as with LAV,⁴¹ NLRV concentration in inoculated rice plants gradually falls. Could NLRV be multiplying in other plants? Probably not, as rice is the only natural host of *N. lugens*. The hopper has been reared on some other *Oryza* species,⁹⁰ but does not seem to live actively on these in nature (R. Kisimoto, personal communication). There remains the slight possibility that NLRV may multiply in some wild ancestor of the rice plant.

In contrast, PgMV reportedly cannot be acquired from previously inoculated maize plants.⁴² This result needs to be verified because acquisition tests were not made immediately following inoculation feedings. As with LAV and NLRV, there is no evidence of multiplication in the plant; on the contrary, inoculated virus soon disappears.

NLRV can be propagated in a second planthopper, *Laodelphax striatellus*, but not in some hoppers tested; virus concentrations were not as stable in *L. striatellus* as in *N. lugens*.⁴⁵ *L. striatellus* is a cosmopolitan species⁹¹ that may not be an optimal host but may participate in the infection cycle in the field.

Proteins and genome

Five to seven structural proteins are usually resolved by electrophoresis of purified preparations of plant reoviruses.⁹ PgMV has six major proteins⁴³ but those of LAV have not been reported. NLRV has seven proteins, three major (140 kDa, 135 kDa, 65 kDa),

Table 2. Conserved 5'- and 3'-terminal nucleotide sequences of the plus strands in Reoviridae⁹²

Virus	Terminal sequences	Reference
<i>Orthoreovirus</i> SEROTYPE 3 <i>Aquareovirus</i>	5' GCUA -----UCAUC 3'	105, 106
<i>Coltivirus</i>		
<i>Orbivirus</i> BTV	5' GUUAAA -----ACUUAC 3'	107
<i>Rotavirus</i>	5' GGCAUU -----UGUGACC 3' U G	108–111
<i>Cypovirus</i> CPV	5' AGUAA -----GUUAGCC 3'	112
<i>Phytoreovirus</i> WTV, RDV, RGDV	5' GGUA -----UGAU 3' C C	101, 104
<i>Fijivirus</i> RBSDV, MRDV	5'AAGUUUUUU -----UGUC 3'	94, 113
<i>Oryzavirus</i> RRSV	5' GAUAAA -----GUGC 3'	114
Non-pathogenic NLRV	5' AGU -----GUUGUC 3'	92

Modified from Kudo *et al.*¹⁰⁴

three intermediate (160 kDa, 110 kDa, 75 kDa), and one minor (120 kDa). The 160 kDa, 140 kDa, 110 kDa and 75 kDa proteins are from the core and the others from the outer shell.⁴⁴

S10 and S8 of NLRV have been sequenced.^{92,93} S10 codes for a non-structural protein of unknown function that appears to be homologous⁹² with the protein encoded by MRDV S6 ORF 1,⁹⁴ in turn homologous with one of the two ORFs in RBSDV S7.⁹⁵ S8 codes for the 62 kDa major outer shell protein,⁴⁴ which has a predicted amino acid sequence identity of 18.6 % with the protein encoded by RBSDV S10. This value is not as high as shown among the major capsid proteins of phytoreoviruses (48–56 %⁹⁶) but hydrophobicity plots of the predicted proteins indicate close similarity.^{93,97}

Almost all the other genome segments of NLRV have now been sequenced (N. Nakashima and H. Noda, unpublished results); there appear to be at least 11 ORFs, with a genome size of about 28,700 bp, larger than RDV, with about 25,750,⁹⁸ and in fact the largest among reoviruses known to date. As with RDV⁹⁹ and other reoviruses,¹⁰⁰ S1 is assumed to encode the RNA polymerase, which appears to be the 160 kDa protein.

As in other reoviruses,¹⁰¹ segment-specific inverted repeats are found near each end of each segment.

Classification and origins

Analysis of nucleotide sequences may throw light on the taxonomic position and evolution of NLRV and eventually that of other similar viruses,^{102,103} especially in regard to the conserved 5' and 3' terminal sequences. These sequences for NLRV are closely similar to those of the Fijiviruses (Table 2), as one would expect from other similarities already established.

It has been suggested^{3-6,115} for the following reasons that plant reoviruses have greater affinity with their insect than their plant hosts. (1) All presently known plant reoviruses multiply in and are vectored by hoppers. (2) The viruses are not, or are only mildly pathogenic in their insect hosts, but cause disease in their plant hosts. (3) Plant species infected by reoviruses are usually the prime food and breeding hosts of their hopper vectors (though a clear exception is MRDV in maize, which is only an occasional and unpalatable host of the main vector, *L. striatellus*). (4) Virus concentration is generally higher in hoppers than in plants, and more tissues are invaded. (5) Six out of nine genera in the Reoviridae are composed of viruses that multiply in insects.

Two hypotheses have been suggested for the origin and evolution of plant reoviruses. One involves co-

evolution with the vector.⁵ For example, differences between phyto-reoviruses and Fijiviruses may have evolved during divergence of the Cicadelloidea (leafhoppers) and Fulgoroidea (planthoppers).^{5,115} Nault and Ammar¹¹⁵ have also speculated that ancestral Reoviridae probably had 10 segments, and particles without spikes.

The second hypothesis is that plant reoviruses originated in insects and later became adapted to plants.^{3,6,115} LAV and NLRV use the host plant as a passive and transitory reservoir and this seems a likely beginning for the change from passive to active.¹¹⁵ It would seem more adaptive for insect viruses to acquire the ability to multiply in plants, and so widen their habitat and enlarge the virus reservoir. The step from passive circulation in plants to active multiplication may have been taken independently by phyto-reoviruses and Fijiviruses, and LAV, PGMV and NLRV may be poised to take such a step, although it is of course not excluded that they would once have been plant viruses, and have lost this characteristic.

Prospect

The genome organization and encoded proteins will soon be known for NLRV. The sequences of the terminal and internal nucleotides will tell us more about phylogenetic relationships within the Reoviridae, and an understanding of the gene functions will shed light on host specificity and pathogenicity.

Very many insect viruses undoubtedly await discovery and study, since the known numbers and varieties of insect are vast, and even so may represent only a modest fraction of the total. Moreover the viruses we are discussing here are non-pathogenic, and were discovered by accident. For example, LAV was studied because of its supposed pathogenicity,⁷ which turned out to be an effect of the host hopper's salivary toxins;^{40,53} during the study of LAV itself, two other viruses of the hopper (small polyhedral, and rhabdovirus-like) came to light.⁴⁰ We have observed other viruses in *N. lugens* through our study of NLRV (unpublished results). How many more non-pathogenic insect viruses of great interest are waiting out there? How many are reoviruses with unforeseen new combinations of properties?

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