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Bromoviruses (Bromoviridae)

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Introduction

The family Bromoviridae contains important genera of plant viruses, with host ranges varying from narrow to very wide, and infecting herbaceous plants, shrubs and even trees. Several of them are responsible for major epidemics in fodder crops such as tomato, cucurbits, bananas, or alfalfa. The members of the family Bromoviridae have spherical or bacilliform particles with a trisegmented, positive-sense, single-stranded RNA (ssRNA) genome, packaged in separate virions. Bromovirids can be transmitted mechanically, via the pollen, seeds or by insect vectors.

As shown in Tables 1 and 2, the Bromoviridae family includes six genera: Alfamovirus (one member, type species: Alfa mosaic virus, AMV), Anulavirus (two members, type species: Pelargonium zonate spot virus, PZSV), Bromovirus (six members, type species: Brome mosaic virus, BMV), Cucumovirus (four members, type species: Cucumber mosaic virus, CMV), Ilarvirus (22 members, type species: Tobacco streak virus, TSV), and Oleavirus (one member, type species: Olive latent virus 2, OLV-2). Genus demarcation criteria are based on natural host range, method of transmission, detailed morphology and properties of particles, organization of RNA genome, replication schemes and producing defective RNAs and satellite RNAs.

The two prototype genera, Bromovirus and Cucumovirus, are the genera mostly related, with the latter being agriculturally important. Both bromo- and cucumoviruses share such properties like the molecular and genetic features of their tripartite RNA genome, the number of encoded proteins and similar virion structure. The computer-assisted comparisons of aa sequences reveal significant similarity among their RNA replication proteins, much beyond the presence of characteristic GDD motif for 2a or for helicase/transferase domains in 1a. More broadly, the replication proteins share aa sequence similarity within the alphavirus-like super-family of positive-strand RNA viruses, which includes numerous plant and important animal/human viruses. The type members of different genera, such as CMV, BMV and...
Table 1  Main characteristics of the RNA genome in six genera of the family Bromoviridae

| Genus            | Acronym     | RNA1  | RNA2  | RNA3  | 3 UTR | sgRNA/diRNA |
|------------------|-------------|-------|-------|-------|-------|-------------|
| Alfamovirus      | (AMV)       | 3644  | 2593  | 2037  | Complex| +/-         |
| Anulaviruses     | (PZSV)      | 3383  | 2435  | 2639  | Complex| +/-         |
| Bromovirus       | (BMV)       | 3234  | 2865  | 2117  | tRNA-like| +/-         |
| Cucumovirus      | (CMV)       | 3357  | 3050  | 2216  | tRNA-like| +/-        |
| Ilarvirus        | (TSV)       | 3491  | 2926  | 2205  | Complex| +/-         |
| Oleavirus        | (OLV-2)     | 3126  | 2734  | 2438  | Complex| +/-         |

*Partial sequence.

AMV, have and continue to constitute excellent models for molecular research on viral gene expression, RNA replication, virion assembly, RNA recombination, epidemiology or the role of cellular genes in basic virology.

**Phylogeny and Biodiversity of the Family Bromoviridae**

Although RNA1, 2 and 3 overall keep a great similitude in their sequences, clearly rearrangements of RNAs has been done for members of the Bromoviridae. Both RNA recombination but also segment reassortment played a major role as the sources of variation in shaping the bromovirids member groups, being important contributors to the evolutionary history of the family, especially for the genera Bromovirus, Cucumovirus and Ilarvirus (Figs. 1 and 2). However, doubts have been shed on the biological significance of the official taxonomy of the family Bromoviridae. To better understand the taxonomy, attempts have been made to reconcile the incongruences observed in the viruses’ evolutionary radiation caused by recombination and reassortment. These two processes could create new genetic variability and then these primary variants would undergo further selection for functional genomes of individual viruses. Consequently, the variants generated by reassortment and recombination events must have been initially viable which represents the first selective filter while further directional selection fine tunes the newly created RNAs. RNA segment reassortment was probably common at the origin of the bromoviruses and cucumoviruses as well as at the origin of Alfalfa mosaic virus, American plum line pattern virus and Citrus leaf rugose virus. Furthermore, recombination analyzes done for each of the three genomic RNAs revealed very common crossovers within the members of the genera Bromovirus, Cucumovirus and Ilarvirus, but also mixed recombination involving species from different genera. It seems that bromoviruses and cucumoviruses did split from a common ancestor forming distinct clades due to crossover events in RNA3, whereas protein 2b promoted the selection of a CMV-TAV RNA1/2-RNA3 recombinant. In the 5′ untranslated regions (UTR) of CMV RNA3 the sequence rearrangements have likely been the precursors of the radiation of three cucumovirus subgroups. The results illustrated in Fig. 2 confirm a clear separation between the genera Bromovirus and Cucumovirus, while the ilarviruses constitute their own cluster; two other genera (*Anulavirus* and *Oleavirus*) are more unique within the family. Although these results suggest that AMV should be included in the ilarviruses, its unequivocal assignment has yet to be resolved, especially because AMV differs from other ilarviruses with the mode of transmission, by aphids versus by pollen and thrips. Bromovirids are members of a larger alpha-like supergroup based upon both 1a and 2a proteins whereas 3a proteins cluster the bromovirids together with other viral groups into a separate pool of movement-associated proteins. In general, the constructed phylogenetic network not only reflects the initial genetic exchanges but also confirms the taxonomic status of the different genera within the family Bromoviridae, notwithstanding the phylogenetic disturbances caused by genetic exchange.

**Virion Properties and Structure**

Virions of bromovirids are non-enveloped, being either spherical or pseudo-spherical, with $T = 3$ icosahedral symmetries, and a diameter of 26–35 nm (genera *Anulavirus, Bromovirus, Cucumovirus* and *Ilarvirus*), whereas genera *Alfamovirus* and some *ilarviruses* have bacilliform virions, of diameters 18–26 nm and lengths of 30–85 nm. In *Oleavirus* virions have different shape.

In genus Bromovirus three types of viral particles are composed of 180 molecules of a 20 kDa CP, and encapsidate different RNA components: RNA1 (Mr c. 1.1 × 10⁶), RNA2 (Mr c. 1.0 × 10⁶) and RNA3 plus sgRNA4 (Mr c. 0.75 × 10⁶ and 0.3 × 10⁶). In addition to viral RNAs, BMV virions have been recently reported to package small amounts of host RNAs, with their
functions yet to be determined. The members of the genus *Cucumovirus*, in addition to three genomic RNAs, encapsidate two subgenomic RNAs (sgRNAs) and a satellite RNA.

The crystal structures of both BMV and CCMV have been resolved showing very similar organization (Fig. 3), with the CP subunits folded into a beta-barrel core organized within the protruding both pentameric and hexameric capsomers. The interactions among hydrophobic aa residues stabilize the capsomers, and the hexameric subunits are further stabilized via interactions between N-terminal portions, where six short beta-strands form a tubule called beta-hexamer. Mutational analysis demonstrated that beta-hexamer was not required for virion formation but rather modulated virus spread *in planta*. In addition, the capsomers

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### Table 2  
List of genera and species in the family Bromoviridae. Type species are written in bold.

| Genus          | Species                     | Acronym  | GenBank accession no.  |
|----------------|-----------------------------|----------|------------------------|
|                |                             | RNA 1 (P1) | RNA 2 (P2) | RNA 3 (MP and CP)     |
| Alfamovirus    | Alfalfa mosaic virus        | (AMV)    | NC_001495 | NC_002024 | NC_002024     |
| Anulavirus     | Amazon lily mild mottle virus | (ALMMoV) | NC_018402 | NC_018403 | NC_018404     |
| **Pelargonium zonate spot virus** | (PZSV)    | NC_003649 | NC_003650 | NC_003651     |
| **Tentative species** | Cassava Ivorian bacilliform virus | (CiIBV) | NC_025482 | NC_025483 | NC_025484     |
| **Bromovirus** | Broad bean mottle virus     | (BBMV)   | NC_004006 | NC_004007 | NC_004008     |
|                | *Brome mosaic virus*        | (BMV)    | NC_002026 | NC_002027 | NC_002028     |
|                | *Cassia yellow blotch virus* | (CyBV)   | NC_006999 | NC_007000 | NC_007001     |
|                | *Cowpea chlorotic mottle virus* | (CCMV)   | NC_003543 | NC_003541 | NC_003542     |
|                | *Melandrium yellow fleck virus* | (MeYFV) | NC_013266 | NC_013267 | NC_013268     |
|                | *Spring beauty latent virus* | (SBLV)   | NC_004120 | NC_004121 | NC_004122     |
| **Cucumovirus** | *Cucumber mosaic virus*     | (CMV)    | NC_002034 | NC_002035 | NC_001440     |
|                | Gayfeather mild mottle virus | (GMMoV)  | NC_012134 | NC_012135 | NC_012136     |
|                | *Peanut stunt virus*       | (PSV)    | NC_002038 | NC_002039 | NC_002040     |
|                | *Tomato aspermy virus*     | (TAV)    | NC_003837 | NC_003838 | NC_003836     |
| **Ilarvirus**  | **Subgroup 1**  |          |          |          |               |
|                | *Ageratum latent virus*    | (ALV)    | NC_022127 | NC_022128 | NC_022129     |
|                | *Blackberry chlorotic ringspot virus* | (BCChRSV) | NC_011553 | NC_011554 | NC_011555     |
|                | *Parietaria mottle virus*  | (PaMoV)  | NC_005848 | NC_005849 | NC_005854     |
|                | *Privet ringspot virus*    | (PrRSV)  | NC_027928 | NC_027929 | NC_027930     |
|                | *Strawberry necrotic shock virus* | (SNSV) | NC_008706 | NC_008707 | NC_008708     |
|                | *Tobacco streak virus*     | (TSV)    | NC_003844 | NC_003842 | NC_003845     |
| **Subgroup 2** | *Asparagus virus 2*        | (AsV2)   | NC_011808 | NC_011809 | NC_011807     |
|                | *Citrus leaf rugose virus* | (CLRv)   | NC_003548 | NC_003547 | NC_003546     |
|                | *Citrus variegation virus* | (CVV)    | NC_009537 | NC_009538 | NC_009536     |
|                | *Elm mottle virus*         | (EMoV)   | NC_003569 | NC_003568 | NC_003570     |
|                | *Lilac ring mottle virus*  | (LrMoV)  | EU919668* | NC_038777 | NC_038776     |
|                | *Spinach latent virus*     | (SpLV)   | NC_003808 | NC_003809 | NC_003810     |
|                | *Tomato necrotic streak virus* | (ToNSV) | NC_039075 | NC_039074 | NC_039076     |
|                | *Tulare apple mosaic virus* | (TAMV)  | NC_003833 | NC_003834 | NC_003835     |
| **Subgroup 3** | *Apple mosaic virus*       | (ApMV)   | NC_003464 | NC_003465 | NC_003480     |
|                | *Blueberry shock virus*    | (BSV)    | NC_022250 | NC_022251 | NC_022252     |
|                | *Lilac leaf chlorosis virus* | (LiLChV) | NC_025477 | NC_025478 | NC_025481     |
|                | *Prunus necrotic ringspot virus* | (PNRSV) | NC_004362 | NC_004363 | NC_004364     |
| **Subgroup 4** | *Fragaria chiloensis latent virus* | (FLCV) | NC_006566 | NC_006567 | NC_006568     |
|                | *Prune dwarf virus*        | (PDV)    | NC_008039 | NC_008037 | NC_008038     |
| **Unassigned species** | *American plum line pattern virus* | (APLPV) | NC_003451 | NC_003452 | NC_003453     |
|                | *Apple necrotic mosaic virus* | (ANMV) | NC_040469 | NC_040471 | NC_040470     |
|                | *Cape gooseberry virus 1*  | (CGV1)   | NC_040393 | NC_040392 | NC_040394     |
|                | *Humulus japonicus latent virus* | (HuJLV) | NC_006064 | NC_006065 | NC_006066     |
|                | *Tea plant line pattern virus* | (TPLLr) | NC_040345 | NC_040346 | NC_040347     |
| **Oleavirus**  | *Olive latent virus 2*     | (OLV)    | NC_003673 | NC_003674 | NC_003671     |

*aPartial sequence.

The crystal structures of both BMV and CCMV have been resolved showing very similar organization (Fig. 3), with the CP subunits folded into a beta-barrel core organized within the protruding both pentameric and hexameric capsomers. The interactions among hydrophobic aa residues stabilize the capsomers, and the hexameric subunits are further stabilized via interactions between N-terminal portions, where six short beta-strands form a tubule called beta-hexamer. Mutational analysis demonstrated that beta-hexamer was not required for virion formation but rather modulated virus spread *in planta*. In addition, the capsomers
are hold together by interactions through C-terminal portions that extend radially from the capsid. The C-termini are anchored between the beta-barrel core and the N-proximal loop, and this interaction might be responsible for initiation of assembly of CCMV capsids. The structure of BMV is organized similarly to that of CCMV such that both capsids undergo well-studied reversible structural transitions where shifting pH from 5.0 to 7.0 causes capsid expansion. However, some CP mutations can further stabilize the capsids. Capsids are also stabilized by metals at multiple binding sites that coordinate the amino acids from adjacent CP subunits.

The packaged RNAs interact with the basic N-terminal aa of the CP in the torus-shaped sub-shell inside the BMV capsid so to neutralize the phosphate groups. Other sites of RNA interaction localize to the internally-proximal basic aa of the CP subunits. The RNA encapsidation signals have been mapped on BMV RNAs, especially to both the 3' UTR and a central sequence in RNA3. The co-packaging of sgRNA4 is contingent upon both RNA replication and translation of CP.

The detailed knowledge of CCMV capsids provided opportunities in nanotechnology, e.g., for the reversible pH-dependent gating, useful during the regulation of size-constrained biomimetic mineralization. The interior surface of CCMV capsids can be engineered of as differentially functionalized CP subunits, to act e.g., as a ferritin surrogate that spatially constrains the formation of mineral nuclei.
of iron oxide nanoparticles. Also, the electrostatically driven adsorption on Si and amine-functionalized Si as well as the fabrication of multilayer CCMV films have been reported. The electrostatically patchy protein cages of CCMV can be used to direct the assembly of super lattices for RNA encapsulation.
The total length of the Bromoviridae RNA genome is approximately 8 kb, with three RNA segments capped at the 5' terminus. Whereas the highly conserved within members, the not polyadenylated 3' termini fold into strong secondary structures. These structures are either aminoacylable tRNA-like (Bromovirus and Cucumovirus) or forming other not aminoacylated arrangements (Alfamovirus, Anulavirus, Ilarvirus and Oleavirus) (Table 1). RNAs 1 and 2 are monocistronic and they code, respectively, for viral replicase proteins 1a and 2a (Fig. 4). Protein 1a has two distinct domains of guanylyl transferase and helicase, and it is involved in anchoring the replicase complex to the endoplasmic reticulum membrane, and induces the formation of membranous vesicular mini-organelles called spherules where RNA replication occurs. In AMV the replicase proteins interact with the tonoplast. Protein 2a is the actual RNA-dependent RNA polymerase enzyme that interacts with protein 1a and synthesizes the vRNAs. Mutations and deletions in 1a/2a ORFs helped to identify the regions active in BMV RNA replication as well as regions responsible for interaction with the cellular membrane or for interactions between 1a/2a polypeptides. An active BMV replicase preparation has been extracted. The anulavirus encodes the smallest RdRp (2a) protein within the family. For cucumoviruses and in some ilarviruses RNA2 is dicistronic encoding a protein 2b, that is part of the C-terminal region of the protein 2a. Protein 2b was found to act as suppressor of RNA interference, being involved in the inhibition of viral gene silencing but also in systemic movement and affecting the symptoms.
RNA3 encodes the movement (MP) and coat (CP) proteins, the latter being translated from the subgenomic (sg)RNA4. BMV CP is a multi-functional protein. In addition to its structural/encapsidation role, it also coordinates the viral infection processes including (1) participation in the formation of replication factories, (2) repression of RNA replication but also translation, and (3) stimulation of BMV RNA accumulation at lower CP levels. Moreover, the BMV CP participates in RNA recombination events; an analogous function to that of BMV CP was assigned to nucleocapsid proteins in retroviruses and coronaviruses. The multiple functions of CP are exercised by effective binding to several distinct sites in RNA3, including the 3' non-coding hairpin, two central regions, and possibly at the 5' end. The contact BMV CP amino acids have been mapped at distinct parts of the CP monomers. Since BMV replicase complex also binds to most of these sites it has been postulated that the CP is involved in the regulation of BMV RNA replication such that the repression of RNA accumulation and translation occurs at higher levels of BMV CP, while stimulation of BMV RNA accumulation at the lower CP levels. While CP is usually translated from the encapsidated 3' sgRNA4, Olive latent virus 2 encapsidates a sgRNA with no apparent messenger activity whereas CP is translated from another non-encapsidated sgRNA4. The purified genomic RNAs are directly infectious but for some bromovirids the presence of CP is necessary (e.g., for AMV or ilarviruses).

The 32 kDa MP of bromovirids has a conserved RNA binding domain that binds to vRNA and assists with viral transport. There are two groups among the members of the family Bromoviridae regarding the cell-to-cell transport; those that do not require participation of viropes and those that transport whole virions. In the first group only the MP-RNA entity is transported (non-virion transport) in a form of either the complex of MP and vRNA, or a triple complex of vRNA, MP, and CP. For the first group, the prototype example is TMV whereas members of the genus Bromovirus have a similar mechanism, which has been demonstrated for CCMV. The MP of these viruses belongs to the 30K superfamily of MPs that appear to interact with cellular microtubules. In the second subtype, where the transported complex is vRNA-MP-CP, the typical example is CMV, a comovirus. In this case, the CMV MP exhibits the binding affinity to the actine microfilaments. The second group transports whole virions intercellularly through plasmodesmata inside the microtubules (virion transport). BMV and AMV are the members of two genera that are transported this way. Also for ilarviruses such as PDV or PNRSV, their MPs likely assist during translocation of the entire viral particles alongside the tubular structures. It appears that MPs of these viruses interact with virion CP subunits via a 44C-terminal key aa domain.

For long distance transport, most viruses require the CP which suggests that they are transported in the form of viral particles (virions). This has been demonstrated by showing that bromovirids require unmodified CP and the wild type C-terminus of MP for long distance spread, such as AMV, BMV and CMV. Very likely the CP-MP interactions enhance the systemic transport, independently of the mechanism of short distance (cell-to-cell) transport.

Genomic RNA Replication and Recombination

Replication of bromovirus RNAs are the best studied among the members of the family Bromoviridae. Only viral proteins 1a (helicase and methyl-transferase domains) and 2a (RdRp), but not the proteins 3a or CP, are required for RNA synthesis (Fig. 4), first demonstrated for BMV, both in plant and in yeast cells. The cytoplasmic RNA replicase complex localizes to the endoplasmic reticulum membranes called spherules. The extracted bromoviral RdRp preparations have allowed for mapping in vitro on three genomic BMV RNAs the promoter of (-) strand synthesis within the 3' UTR. The promoters of (+) strand synthesis have also been mapped to the 5' proximal non-coding region in BMV RNAs. Likewise, the sub-genomic promoter (sgp) has been localized as a 100 nt subset of the 250 nt intercistronic region in (-) strand in BMV RNA3, being responsible for synthesis (transcription) of sgRNA4. In addition, in the plus strand the 250 nt intercistronic sequence supports other functions including the efficient RNA3 replication, the maintenance of a proper ratio of (+) to (-) strands of RNA3, stabilization of RNA3 via interaction with protein 1a, synthesis (transcription) of sgRNA4, and the assembly of the active RNA replication complex. It also serves as an efficient RNA recombination hot spot. Some bromoviruses as well as togaviruses carry the internal poly(A) tract, as part of the intercistronic region of the RNA3 segment. Besides viral RNA sequences and viral proteins, a variety of essential host genes affecting BMV RNA replication have been identified, by using yeast knockout libraries, as apparently the yeast cells can support a complete replication cycle of this virus Fig. 5.

Both homologous and non-homologous RNA-RNA crossovers has been demonstrated between bromoviral RNAs during infection. Homologous recombination has also been shown during co-infection between two strains of BMV, with some distinct hot spots localized within both the coding and non-coding regions. The role in recombination of proteins 1a and 2a as well as of the CP have been demonstrated in BMV, suggesting the template switching as a likely mechanism for RNA recombination. For the cucumoviruses, the control of recombination frequency resides mainly in the 2a gene.

Members of the genera Bromovirus and Cucumovirus are capable of producing the defective (d)RNAs and defective-interfering (di)RNAs during infection (Table 1). In particular, strains of Broad bean mottle virus (BBMV) do accumulate RNA2-derived deletion variants after serial passages through broad bean. In BMV, both replicating and non-replicating truncated RNA2-derived artificial diRNAs have been shown to interfere with BMV RNAs in protoplasts. For CMV, several types of RNA3-derived diRNAs have been described.

Biology

The family Bromoviridae is one of the most important families of plant RNA viruses, with some members widely distributed in the world. In its entirety, the family has a wide host range (more than 10,000 species) and some members are causing agronomically
important diseases. However, the host range of members of individual genera ranges from significantly narrow (genera Bromovirus, Oleavirus) to extremely broad (genus Cucumovirus). CMV can infect one of the largest number of plant species among plant viruses. Some of these viruses cause major disease epidemics in vegetables, fodder and fruit crops, e.g., in tomato, cucurbits, bananas, or alfalfa, and in fruit trees (ilarviruses). Different virus species are transmitted mechanically, via pollen/thrips, through seeds or by insect vectors like aphids or beetles. It has been speculated that lack of efficient vectors evolved some bromovirids toward producing larger concentration of viral particles. For CMV, although the virus is prone to recombination, the recombinants are rare during infection, suggesting the presence of strong selection bottlenecks. No direct correlation between virus yield and symptom severity have been observed, but rather the symptoms seem to be associated with changes in specific regions in the RNA genome, as it has been mapped by using the natural strains of BMV, BBMV or CCMV.

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