## **Supporting Information**

Luque et al. 10.1073/pnas.0808498106

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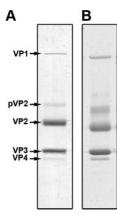


Fig. S1. Analysis by SDS/PAGE and autoradiography (A) or Coomassie blue stain (B) of E5 population virions. Protein profiles for E2, E3, E4, and E6 populations are similar. Bands corresponding to proteins VP1, pVP2, VP3, and VP4 are indicated.

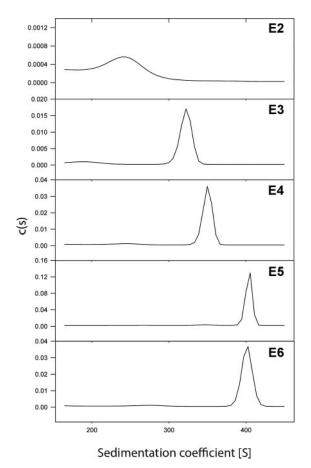
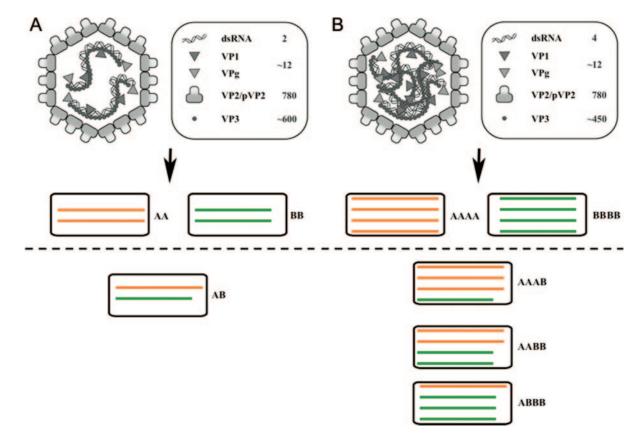


Fig. S2. Sedimentation velocity analysis of IBDV populations. Apparent sedimentation coefficient distribution, c(s), of E2-E6 IBDV particles obtained from sedimentation velocity taken at 8,000  $\times$  g and 20 °C.

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**Fig. 53.** Haploidy versus polyploidy in IBDV. (*A*) Scheme representing the standard IBDV model with its estimated stoichiometry for the structural components (*Right*). Note that the virion is haploid (2 dsRNA segments, 1 copy each of A and B, inside the capsid). If all progeny virion particles contain 2 dsRNA segments (i.e., E3 particles), a random packaging mechanism implies that 66% of the viral progeny is noninfectious (particles AA and BB, *Upper*). Only particles containing a copy of segment A and one of segment B are infectious (particles AB, *Lower*). The particle-to-infectivity ratio is relatively low (pfu/pp = 0.33). (*B*) Scheme showing the new IBDV model with its associated stoichiometry (*Right*), as deduced from this and other studies. A polyploid virion with four packaged dsRNA segments is shown as E5 and E6 IBDV particles. In this scenario, only particles AAA and BBBB (*Upper*), accounting for 40% of the viral progeny, are defective. All other combinations result in infectious particles (*Lower*), and generation of an infectious, i.e., AAA and BBB particles; the other 50% are infectious contained and particles). Note that the larger the number of segments packaged, the greater the chance of a full genome complement, hence more infectious particles.

## Table S1. Genome packaging densities in dsRNA viruses<sup>a</sup>

|                                      | dsRNA features     |                         |                          |                             |  | Inner capsid features |                      |                 |                                  | dsRNA                            |                                       |
|--------------------------------------|--------------------|-------------------------|--------------------------|-----------------------------|--|-----------------------|----------------------|-----------------|----------------------------------|----------------------------------|---------------------------------------|
| Virus family                         | No. of segments    | Size <sup>d</sup> , kbp | MW <sup>e</sup> ,<br>MDa | Length <sup>f</sup> ,<br>µm | Packed dsRNA<br>spacing, nm <sup>g</sup> | т                     | RdRPs<br>copy<br>no. | $\phi^{h}$ , nm | <sub>i</sub> r <sup>i</sup> (nm) | V <sup>j</sup> , nm <sup>3</sup> | density,<br>bp/100<br>nm <sup>3</sup> |
| HSV                                  | 1                  | ≈152                    | 103.7                    | 51.4                        | 2.6                                      | _                     |                      | ≈60             | 43.0                             | 333.000                          | 46                                    |
| Reoviridae                           |                    |                         |                          |                             |  |                       |                      |                 |                                  |                                  |                                       |
| Orthoreovirus                        | 10                 | ≈23.5                   | 16                       | 7.0                         | 2.6                                      | 2                     | 12                   | ≈60             | 24.5                             | 61,600                           | 38                                    |
| Rotavirus                            | 11                 | ≈18.5                   | 12.6                     | 5.6                         | 2.5-3.0                                  | 2                     | 12                   | ≈52             | 23.5                             | 54,400                           | 34                                    |
| Orbivirus                            | 10                 | ≈19.2                   | 13.1                     | 5.8                         | 3.0                                      | 2                     | 12                   | ≈52             | 22.0                             | 44,600                           | 43                                    |
| Aquareovirus                         | 11                 | ≈23.6                   | 16.0                     | 7.1                         |  | 2                     | 12                   | ≈60             | 23.0                             | 51,000                           | 46                                    |
| Phytoreovirus                        | 12                 | ≈25.7                   | 17.5                     | 7.7                         |  | 2                     | 12                   | ≈57             | 26.0                             | 73,600                           | 35                                    |
| Cypovirus                            | 10                 | ≈31.4                   | 21.4                     | 9.5                         | 2.5                                      | 2                     | 12                   | ≈58             | 24.0                             | 57,900                           | 54                                    |
| Cystoviridae, phage $\phi$ 6         | 3                  | ≈13.4                   | 9.1                      | 3.4                         |  | 2                     | 12                   | $\approx$ 50    | 20.0                             | 33,500                           | 40                                    |
| Totiviridae, L-A                     | 1                  | ≈4.6                    | 3.1                      | 1.3                         | 3.6-4.0                                  | 2                     | 1                    | ≈43             | 17.0                             | 20,600                           | 22                                    |
| Chrysoviridae, PcV                   | 1 (4) <sup>c</sup> | ≈3.2 (12.6)             | 2.2 (8.6)                | 0.9 (3.5)                   | 4.0*                                     | 1                     | 1/2                  | ≈40             | 16.0                             | 17,150                           | 19                                    |
| Patitiviridae, PsV-S<br>Birnaviridae | 1 (2)              | ≈1.7 (3.3)              | 1.2 (2.2)                | 0.5 (0.9)                   |  | 2                     | 1                    | ≈35             | 12.0                             | 7,240                            | 23                                    |
| IBDV, $T = 13$ capsid                |                    |                         |                          |                             |  | NPb                   | 12                   | ≈65             | 26.5                             | 77,900                           |                                       |
| 1 genome                             | 2                  | ≈6.0                    | 4.1                      | 1.8                         | 6.1*                                     |                       |                      |                 |                                  |                                  | 10                                    |
| 2 genomes                            | 4                  | ≈12.0                   | 8.2                      | 3.6                         | 4.3*                                     |                       |                      |                 |                                  |                                  | 20                                    |
| IBDV, $T = 7$ capsid                 | 2                  | ≈6.0                    | 4.1                      | 1.8                         | 2.9*                                     |                       |                      | ≈55             | 18.1                             | 24,400                           | 31                                    |

<sup>a</sup>Data reviewed in Castón *et al.* [Castón JR, *et al.* (2003) Three-dimensional structure of *Penicillium chrysogenum* virus: A double-stranded RNA virus with a genuine T=1 capsid. *J Mol Biol* 331:417–431].

<sup>b</sup>NP, not present.

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<sup>c</sup>PcV dsRNA features: the genome is formed by 4 dsRNA molecules, but an average value was calculated for each column since there is a dsRNA molecule/particle. <sup>d</sup>Data taken from Virus taxonomy: classification and nomenclature of viruses. Seventh Report of the International Committee on Taxonomy of Viruses. <sup>e</sup>MW were calculated assuming a mass of 682 Da/bp.

<sup>f</sup>Length were calculated assuming 3 Å axial rise per bp for members of the family *Reoviridae*, 2.56 Å for phage  $\phi$ 6, and 2.81 Å for L-A and PcV dsRNAs. HSV dsDNA is assumed to have a B-form structure (3.38 Å/bp).

<sup>9</sup>Measurements were mostly obtained from cryo-EM and 3DR studies and represent average Bragg spacings. An \* denotes estimated values. The corresponding interstrand spacings are calculated by introducing the factor (2/ $\sqrt{3}$ ), if strands exhibit a quasi-hexagonal packing.

<sup>h</sup>Outer diameter.

<sup>i</sup>Inner radius.

<sup>j</sup>Volume when a perfect sphere is assumed and any other internal components are ignored.

Table S2. Infectivity of E1-E6 IBDV populations

| IBDV<br>population | %, wt/wtª | [pp/pfu] <sup>b</sup> | [pfu/ <sub>total</sub> pp] <sup>c</sup> | [pfu/ <sub>total</sub> pfu] <sup>d</sup> |
|--------------------|-----------|-----------------------|---|--|
| E1                 | 3         | 7000                  | 0,0004                                  | 0,004                                    |
| E2                 | 5         | 7000                  | 0,0007                                  | 0,006                                    |
| E3                 | 12        | 100                   | 0,1200                                  | 1,080                                    |
| E4                 | 16        | 50                    | 0,3200                                  | 2,881                                    |
| E5                 | 48        | 6                     | 8,0000                                  | 72,021                                   |
| E6                 | 16        | 6                     | 2,6667                                  | 24,007                                   |

 $^aFraction$  (wt/wt) of each population: [physical particle/total physical particle] ([pp/\_{total}pp]).

<sup>b</sup>[pp/pfu] ratios deducted by titration of IBDV populations.

 $[pfu/_{total}pp] = [pp/_{total}pp]/[pp/pfu]; \Sigma(E1-E6)[pfu/_{total}pp] = [_{total}pfu/_{total}pp] = 11,1078.$ 

 $\label{eq:constraint} {}^d[pfu/_{total}pfu] = [pfu/_{total}pp]/ \left[ {}_{total}pfu/_{total}pp]; \ \Sigma(\text{E1-E6}) \left[ pfu/_{total}pfu \right] = \ 100.$ 

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