HAL
open science

The Curious Strategy of Multipartite Viruses

Yannis Michalakis, Stéphane Blanc

To cite this version:

Yannis Michalakis, Stéphane Blanc. The Curious Strategy of Multipartite Viruses. Annual Review of Virology, 2020, 7 (1), pp.203-218. 10.1146/annurev-virology-010220-063346 . hal-02981664

HAL Id: hal-02981664

https://hal.inrae.fr/hal-02981664v1

Submitted on 25 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Title: The Curious Strategy of Multipartite Viruses

Authors: Yannis Michalakis[1] and Stéphane Blanc[2]

Affiliations:

[1] *MIVEGEC, CNRS, IRD, Univ Montpellier, Montpellier, France*

[2] *UMR BGPI, INRA, CIRAD, Montpellier SupAgro, Univ. Montpellier, Montpellier, France*

ORCID IDs:

YM: 0000-0003-1929-0848
SB: 0000-0002-3412-0989

Correspondence should be sent to both YM and SB:

Yannis.Michalakis@ird.fr

Stephane.blanc@inra.fr

Keywords : multipartite virus, genome architecture, virus evolution, multicomponent virus, genome formula, multicellular functioning.

Abstract : Multipartite virus genomes are composed of several segments, each packaged in a distinct viral particle. While this puzzling genome architecture is found in ~17% of known viral species, its distribution among hosts or among distinct types of genome composing nucleic acid remain poorly understood. No convincing advantage of multipartitism has been identified, yet the maintenance of genomic integrity appears problematic. We here review recent studies shedding light on these issues. Multipartite viruses rapidly modify the copy number of each segment/gene from one host species to another, a putative benefit if host switches are common. One multipartite virus functions in a multicellular way: the segments do not need to all be present in the same cell and can functionally complement across cells maintaining genome integrity within hosts. The genomic integrity maintenance during host-to-host transmission needs further elucidation. These features challenge several Virology foundations and could apply to other multicomponent viral systems.

34 **1. WHAT ARE MULTIPARTITE VIRUSES – DISTINCTION WITH MONOPARTITE/SEGMENTED**

35 Viruses show exceptional variation in how they package their genetic information for
36 transmission to future generations. Most viruses have their genetic information carried by a
37 single molecule of DNA or RNA packaged in a transmission vehicle, the viral particle. These are
38 the monopartite viruses such as the dsDNA herpesviruses, the ssDNA geminiviruses (with the
39 exception of some begomoviruses) or parvoviruses, the dsRNA totiviruses, the (+)ssRNA
40 polioviruses and the (-)ssRNA filoviruses, and they represent ~75% of all viral annotated species
41 (1). Another strategy occurs only among RNA viruses and consists in having the genetic
42 information carried by several molecules which are all packaged together in a single viral
43 particle. These are the segmented viruses, which despite their relative scarcity, representing
44 only ~9% of current viral species (1), are well known probably because they comprise some
45 famous human parasites such as the Influenza A Virus. A third, puzzling, strategy goes one step
46 further: the genetic information is carried by several molecules, but instead of packaging all
47 these molecules together, these viruses package them in different viral particles. These are the
48 multipartite viruses. Despite the fact that they represent ~17% of all viral species, their study
49 has been widely neglected and their Biology remains largely mysterious. They recently
50 attracted renewed interest, as two recent reviews (1, 2) illustrate. In this paper we rapidly recall
51 some important points and review important recent discoveries (Figure 1). We then provide
52 arguments on how these recent findings on multipartite viruses challenge some Virology
53 foundations, call for a reconsideration of some important notions in Biology, and invite us to
54 reconsider a number of features of other viral multicomponent systems.

55 **2. DISTRIBUTION OF MULTIPARTITISM IN THE VIRAL WORLD:**

56 Multipartite viruses are very common parasites of plants and fungi. Indeed, about a
57 third of viral genera parasitizing plants have adopted this peculiar genomic organization.
58 Because they cause disease in many agriculturally important plants, multipartite viruses have
59 important financial and well-being consequences (2).

60 The host distribution of multipartite viruses is intriguing (Figure 2). Indeed, none has
61 ever been reported to infect bacteria, while the reports in animals can be counted with the
62 fingers of just one hand and are of two sorts. The first sort corresponds to plant viruses, e.g. the
63 genus *Tenuivirus* (3), which also replicate in their arthropod vectors. The genus *Dichorhavirus*
64 (Family *Rhabdoviridae*) may also be included in this category (4), although both the replication
65 within the mite vector (5) and the actual separate encapsidation of the two genome segments
66 await definitive confirmation. The second sort regroups viruses exclusively infecting animal
67 hosts. The separate encapsidation of the genome segments has been definitely confirmed
68 solely for bidensoviruses of silkworms (6). One additional case, a Jingmenvirus infecting
69 mosquitoes (Guaico culex virus, GCXV), has recently been proposed (7). To infer that GCXV is
70 multipartite, this study used dilution of virus particles-containing solutions and titer monitoring,
71 as well as infection of cell cultures at low MOI demonstrating that the distinct particles infecting
72 different cells do not contain the full set of segments. However, a similar low MOI-cell culture
73 infection has been used in a segmented bunyavirus to demonstrate that most viral particles do
74 not contain all the genomic segments due to their non-specific encapsidation (8). Therefore
75 further studies in Jingmenviruses are needed to conclusively discard alternative genomic
76 characterizations.

77 In fact, the formal qualification of a viral species as “multipartite” cannot derive from
78 sequence comparisons and homology because there are numerous examples of monopartite
79 species highly homologous to multipartite ones, belonging to the same viral families or even
80 genera (e.g. the family *Potyviridae* or the genus *Begomovirus*). It can also not be derived solely
81 from the dilution techniques mentioned above. Despite their usefulness to originally conceive
82 the existence of multipartite viruses of plants (9), it is now clear that serial dilutions of virus
83 suspensions could similarly affect the titration (concentration of infectious units) for both
84 multipartite and segmented viruses producing a large amount of semi-infectious particles (8,
85 10). Consequently, approaches directly assessing the number and nature of segments
86 encapsidated in distinct particles within a virus population are required. This can be achieved by
87 the physical separation of distinct classes of virus particles from a population and analysis of
88 their respective genome content (11, 12). But techniques of structural biochemistry (13, 14) or
89 fluorescent high-resolution microscopy (8, 15) are also efficient.

90 Current data indicate that multipartite viruses appear common in plants and fungi,
91 extremely rare in animals and inexistent in bacteria (Figure 2A). The reason for this distribution
92 is at present unclear. Although it is difficult to formally exclude a sampling bias without detailed
93 quantifications of relative sampling effort, this explanation is not very plausible: for example,
94 there is no reason to expect that the viromes of animal farms have been investigated in a more
95 biased way than those of crops, nor is there a reason to see why phage investigations would
96 have missed all multipartite species if they existed. The distribution is so skewed, that a few
97 hypothetical missed cases would not balance it, and thus it likely represents a biological reality.
98 What may be the reasons underlying it?

99 Multipartite viruses attracted some theoretical interest in the early '90s (16–20). These
100 studies were based, understandably, on the then knowledge, or beliefs. It was then believed
101 that plant viruses had high multiplicities of infection, which could avoid or at least limit the risk
102 of losing genomic integrity upon cell-to-cell or host-to-host transmission. Zwart and Elena (21)
103 reviewed empirical estimations of the multiplicities of infection at the individual cell, within-
104 host across organ, and the between host levels and the existing evidence does not indicate that
105 plant viruses, independently of their genomic architecture, stand out in any way (see later
106 section for more discussion on this issue). A recent explanation put forward by Zhang et al. (22)
107 is that, under some assumptions, multipartite viruses require lower endemic thresholds to be
108 maintained in hosts connected by dense static networks, i.e. networks with numerous neighbor
109 contacts which do not change over time, than in annealed networks where neighbor contacts
110 are reshuffled continuously. Zhang et al. concluded that this result explains why multipartite
111 viruses occur mostly in plants and almost never in animals, because static networks would
112 better represent plant contact networks while annealed networks would better represent
113 animal contact networks. There are several issues with this proposed explanation but the most
114 important is that horizontally transmitted plant multipartite viruses are vectored by animals,
115 their vast majority aphids of whiteflies, and thus the contact network of the plant viruses
116 depends mostly on animal movements and whatever motivates them. The reason underlying
117 the host distribution of multipartite viruses thus still eludes us, and it is not even clear whether
118 we should expect an ecological or a cellular/physiological type of explanation.

119 Another intriguing aspect of the distribution of multipartite viruses concerns the nucleic
120 acid supporting their genomic information (Figure 2B and (1, 2, 23)). One can note that:

121 - dsDNA viruses are exclusively monopartite; their genome is never segmented nor multipartite
122 (we exclude polydnaviruses for reasons discussed in (2)

123 - ssDNA viruses are either mono- or multipartite; there are no segmented ssDNA viruses

124 - (+)ssRNA viruses are mostly mono- or multipartite, only nodaviruses and omegatetravirus
125 being segmented.

126 - (-)ssRNA viruses are mostly monopartite or segmented, with the exception of the genera
127 *Ophiovirus*, *Varicosavirus* et *Dichorhavirus* which are multipartite

128 - dsRNA viruses are the only case where monopartite are a minority; most of them are
129 segmented, with roughly equal numbers of genera among multipartite and monopartite.

130 This distribution has never received an explanation. Why are ssDNA and (+)ssRNA
131 viruses more prone to be multipartite than segmented, why are (-)ssRNA viruses more prone to
132 be segmented than multipartite, why are dsRNA viruses so labile and why are dsDNA viruses so
133 intolerant of any sort of genome segmentation? To complicate matters, the reasons underlying
134 host distributions and those underlying nucleic acid distributions could be confounded. For
135 example, only few dsDNA viruses parasitize plants or fungi (Figure 2C) (24). Of the 27
136 bacteriophage families currently recognized by the ICTV all have dsDNA genomes but four
137 ssDNA and two RNA families (23, Figure 2C). Thus for this group of viruses it is difficult to tell
138 whether they do not become multipartite because they rarely infect plants or fungi, or whether
139 they do not become multipartite because there is something in dsDNA that makes it less
140 amenable to multipartitism and segmentation. Each of the remaining nucleic acid genomic
141 constitutions are capable of infecting both multipartite-prone hosts (i.e. plants and fungi) and

142 multipartite-excluding hosts (i.e. animals, bacteria and Archaea) such that within each the
143 relative representation of genomic architectures may at least partially reflect that of the
144 respective hosts.

145 Probably because their existence is puzzling, it is often assumed, e.g. in theoretical
146 considerations, that multipartite viruses are derived from ancestral monopartite viruses.
147 Plausible as this may be, it has actually never been investigated through e.g. phylogenetic
148 reconstructions. The relationship between multipartite and segmented viruses has also eluded
149 phylogenetic analyses so far: as the previous paragraph discusses these two genomic
150 architectures do not co-occur in some nucleic acid configurations, but they do in other. When
151 they do co-occur, do they co-occur in the same branches, and if yes, which is ancestral? These
152 important questions still await for a rigorous analysis.

153 **3. PROPOSED ADVANTAGES OF MULTIPARTITE VIRUSES**

154 The proposed advantages have been already reviewed elsewhere (1, 25). We thus only
155 briefly mention them here.

156 The factors initially put forward, and developed with theoretical models, all derive from
157 the fact that each segment of a multipartite virus is smaller than the entire genome of a
158 monopartite, for a given total genome length. Thus multipartite viruses would have evolved
159 because (i) smaller segments would suffer less from high mutational loads, due to high
160 mutation rates (16, 17); (ii) would replicate faster (17, 19, 26); (iii) because of their segmented
161 nature they would benefit from genetic exchanges (26–28). It is worth noting that none of
162 these hypotheses is specific to multipartite viruses: while the theoretical models developed to

163 back them explicitly considered multipartite genomic architectures, the above features are
164 shared with segmented viruses. It is thus not obvious why a multipartite rather than a
165 segmented genomic architecture should be adopted; the latter would not incur the costs
166 specific to multipartitism pertaining to the preservation of viral genomic integrity (see next
167 section). Further, while these putative benefits have unfortunately rarely been tested, the only
168 published test failed to find any evidence in their favor (29): after passaging the monopartite
169 foot and mouth disease virus (FMDV) at high MOI, Garcia-Arriaza and colleagues observed the
170 spontaneous evolution to a bipartite variant (30). Ojosnegros and colleagues subsequently
171 showed that the bipartite outcompeted the monopartite variant, but none of the above cited
172 processes was responsible for this outcome; instead, the viral particles of the bipartite variant
173 were more stable allowing for a longer infectious period (29). The lack of any other cases
174 renders it impossible to know how general this explanation could be, both as to its occurrence
175 and as to the magnitude of the potential benefit for multipartite forms. Finally it was recently
176 shown (31) that even though the evolution of multipartitism is improbable in the absence of an
177 intrinsic advantage, it may rarely occur following the stochastic extinction of monopartite forms
178 under specific parameters allowing for the maintenance of multipartite variants. This is unlikely
179 as a general explanation of the evolution of multipartitism, but should not be ignored for
180 explaining specific cases.

181 **4. POTENTIAL COSTS AND HOW MULTIPARTITE VIRUSES SOLVE THEM:**

182 The existence of multipartite viruses appeared problematic since their discovery
183 because of the potential issue of the maintenance of their genomic integrity: if all segments
184 need to be present in the same host individual and, presumably, individual host cell for the

185 infection to function, by packaging the segments separately multipartite viruses potentially
186 incur the risk of producing many incomplete non-functional inoculations.

187 It is *a priori* possible to imagine two potential ways through which this transmission cost
188 could be resolved (Figure 1): (i) through independent transmission of a sufficiently large
189 number of viral particles to ensure that at least one copy of each is transmitted at the relevant
190 level (individual host for between-host transmission, Figure 1.5, or individual cell for within-
191 host transmission, Figure 1.2); or (ii) through some sorting mechanism that manages to bring
192 together at least one copy of each segment and achieve their sorted transmission even if the
193 total number of transmitted segments is small. Either of these mechanisms could apply at
194 either level. The potential of the first mechanism to resolve this cost has been evaluated
195 theoretically by Iranzo and Manrubia (32), who calculated the number of viral particles that
196 would need to be transmitted, designated by the authors as MOI for multiplicity of infection, in
197 order for a multipartite variant to outcompete a monopartite ancestor. These calculations
198 showed that while for multipartite viruses with few segments the threshold MOIs are not too
199 high, for viruses with more than four segments they are orders of magnitudes larger than the
200 currently available empirical MOI estimates for any kind of virus.

201 ***4.1. Within-host cost***

202 *4.1.1. Genome formulas*

203 A first study formally questioned whether the eight segments of the octopartite faba
204 bean necrotic stunt virus (FBSNV, genus *Nanovirus*, family *Nanoviridae*), which code for one
205 gene each and are all approximately 1 kb long, accumulate within individual plants at equal

206 frequencies. The answer was that they do not: some segments accumulate at low frequencies
207 while others are much more frequent (33). Infections converge to a sort of 'equilibrium'
208 frequency distribution, termed the 'genome formula', as the disease progresses within plants.
209 When the virus is transmitted from one host plant species to another, the genome formula
210 changes within a single passage. Similar observations have since been reported in another plant
211 multipartite virus, the (+)ssRNA tripartite alfalfa mosaic virus (AMV (34)). The existence of an
212 uneven genome formula has also been concluded for one of the rare multipartite viruses
213 infecting an animal, the ssDNA bipartite bombyx mori bidensovirus (BmBDV (35)), although a
214 single host was tested in this latter case. It is also possible to reinterpret the results of previous
215 studies as indicative of the existence of a genome formula in other viruses (see the end of the
216 Discussion of (33) for references), suggesting that the unequal accumulation of the distinct
217 genome segments is a general feature during infection of hosts by multipartite viruses.

218 The existence of the formula adds to the cost: rare segments would require an even
219 larger MOI to be transmitted than that corresponding to all segments having equal frequencies.
220 Indeed, Sofonea and colleagues (appendix of (36)) extended the calculations of Iranzo and
221 Manrubia (32) and showed that when accounting for the existence of the genome formula the
222 threshold MOIs beyond which multipartitism may evolve become even larger.

223 On the other side, the fact that the genome formula readily changes when the viruses
224 are passaged from one host species to another suggests that the multipartite nature of the
225 genome of these viruses allows them to rapidly modify the relative expression of their genes
226 (Figure 1.4). The fact that variation in segment copy numbers has functional consequences was
227 recently investigated by Gallet and colleagues (R. Gallet, J. Di Mattia, S. Ravel, R. Vitalis, Y.

228 Michalakis and S. Blanc in preparation): they found that the amount of DNA of each segment is
229 positively correlated to the amount of its corresponding mRNA in two host plant species. This
230 result shows that the genome formula variation indeed impacts gene expression. Whether this
231 variation is adaptive remains to be formally demonstrated empirically; the possibility that it
232 may represent an adaptive strategy in a rapidly changing environment, such as an environment
233 where a virus changes host species frequently, has been demonstrated by a recent theoretical
234 study (M. Zwart & S.F. Elena in review).

235 *4.1.2. A multicellular way of life*

236 The fact that in order to avoid the cost of multipartitism viruses would need to have
237 unrealistically high MOIs, at least under the hypothesis that the different segments do not
238 'travel' in a sorted way, led us to question the premise of the cost at least at the within-host
239 level: is it true that all genomic segments need to be concomitantly present within each host
240 cell for the infection to function? Using fluorescent-labeling techniques we found that the
241 distinct segments of FBNSV do not co-occur in most cells of an infected host (37). This per se
242 could be interpreted as a manifestation of the cost of multipartitism. However, even though the
243 segments often do not co-occur, a given segment often co-occurs in a cell with the protein
244 encoded by a segment which is locally absent. Thus, the function of a segment is present in cells
245 where the genetic information is absent, suggesting that at least FBNSV infections operate at a
246 supra-cellular level through trafficking of gene products; whether the function circulates under
247 the form of mRNA, protein or both, remains to be investigated. The main point, however, is
248 that the genomic segments do not need to be concomitantly present in the same cell for the
249 infection to progress; therefore the putative cost of multipartitism should be much smaller than

250 initially anticipated at least at the within-host level (Figure 1.1). A more precise quantification of
251 this putative cost would require identifying the spatial scale at which the function trafficking
252 occurs. If this is the entire plant, then the putative cost at this level could potentially be nil.

253 *4.1.3. Sorted transmission*

254 Although not experimentally demonstrated, the possibility that sorted transmission of
255 the distinct genome segments could occur in multipartite viruses has been suggested for some
256 viral species. We use the term 'sorted transmission' here to distinguish it from "collective
257 transmission" as commonly used in the literature. Collective transmission connotes the co-
258 transmission of several virus particles to a cell or a host, and because it is most commonly
259 conceived for monopartite viruses, it usually designates the joint delivery of multiple copies of a
260 viral genome and thus increased MOI (for review see (38)). The phenomenon we discuss here is
261 that multipartite viruses could sort their distinct genome segments, somehow assembling them
262 to constitute a transmitted group containing the integral genome without necessarily increasing
263 the MOI.

264 While the ssDNA octopartite FBNSV has obviously not adopted this strategy at the
265 within host level (see the multicellular lifestyle described above), a possible sorted transmission
266 of distinct genome segments is suggested by Gilmer and colleagues (39, 40) for multipartite
267 (+)ssRNA viruses. When reviewing the literature on the cell-to-cell and long distance within host
268 movement of diantho-, bromo-, virga and bennyviruses the authors convincingly argue that
269 these RNA genomes travel within the vascular system of their plant hosts under the form of
270 nucleo-protein complexes (RNPs) and not mature assembled viral particles. The authors

271 postulate that this matter of fact makes the RNA accessible within the moving RNPs and
272 consider the possibility that specific RNA-RNA interactions between segments can elaborate a
273 moving complex containing one copy of each. The movement of RNPs and the intersegment
274 RNA-RNA interactions are well supported by empirical results. RNA-RNA intersegment
275 interactions could have diverse roles in the viral cycle, however, and the direct demonstration
276 that they tie the segments together during long distance within-host movement (Figure 1.3) is
277 still lacking.

278 ***4.2. Between-host cost***

279 As we just saw, the within-host cost can be at least partially alleviated either through a
280 still hypothetical sorted transmission or because some viruses, such as the FBNSV, may adopt a
281 multicellular way of life which dispenses them from the obligation of maintaining their genomic
282 integrity at the single cell level. There is nevertheless an obligation to maintain genomic
283 integrity at the individual host level: viruses need all genome segments (indispensable
284 segments) in the same host individual in order to be able to successfully complete their life-
285 cycle: replicate, colonize the host and successfully transmit the integral genome to another
286 host. The between-host genomic integrity maintenance cost is obviously related to the
287 transmission mechanisms. In plants, >90% of the multipartite viral species are transmitted
288 from-host-to-host by animal vectors (1) and must thus find a way to transfer all their genetic
289 information.

290 An obvious solution could be through the massive inoculation of viral particles during
291 vector transmission (Figure 1.5). This possibility is strongly contradicted by the currently

292 available evidence: independently of their genomic architecture, nearly all viruses for which an
293 empirical estimate exists undergo severe bottlenecks during host-to-host transmission (21).
294 Only two multipartite viruses have been investigated in this respect: the tripartite (+)ssRNA
295 cucumber mosaic virus (CMV; family *Bromoviridae*) and the octopartite ssDNA FBNSV. In both
296 cases, the estimated effective bottleneck sizes were very low, ranging from one to 6 copies per
297 segment ((41) for CMV; (36) for FBNSV), one to three orders of magnitude lower than the
298 threshold beyond which mutlipartitism is theoretically favored (32, 36). It is true that effective
299 sizes may differ from the number of particles actually transmitted. However, the FBNSV study
300 used two methods: one based on genetic variant frequencies and estimating the genetic
301 bottleneck, the other based on the success of infection and estimating the population
302 bottleneck; both methods yielded very similar numbers.

303 Our admittedly very limited current knowledge thus leaves us with a still unresolved
304 question as to how multipartite viruses manage to maintain their genomic integrity at this level.
305 A potential critique of the laboratory-based estimations of the transmission bottlenecks is that
306 they perhaps use unrealistically low numbers of vector individuals (aphids in these cases) per
307 host plant, one to ten. Many aspects of multipartite virus ecology are currently unknown or at
308 best understudied, and 'details' on aphid transmission are part of these aspects. The limited
309 available evidence, however, does not suggest that a high density of vectors is the solution to
310 this paradox: Schinghamer and colleagues found that only 10 individual aphids out of 447
311 investigated, belonging to three out of fourteen species, were able to transmit at least one out
312 of nine virus diseases in a field population of faba bean (42).

313 A bolder possibility is that perhaps all the segments do not need to colonize individual
314 plants concomitantly (Figure 1.7). We know for example that when the ssDNA nanoviruses
315 successfully infect a host cell, their genetic information exits the capsid, moves to the host cell
316 nucleus where the cell machinery polymerizes the second strand to produce dsDNA, which then
317 associates with histone proteins to form a 'minichromosome' supporting both transcription and
318 rolling circle replication (43). However, we do not know what happens if particles containing
319 only some of the segments enter a host cell (Figure 1.8). The genetic information is likely
320 similarly decapsidated, and if so, what is its fate in the absence of some of the segments? Does
321 it wait for some signal under the form of a minichromosome? For how long can it stay in this
322 state? Similar or at least analogous questions apply for genome segments of RNA multipartite
323 viruses. It is likely that the answer depends on the nature of the nucleic acid, on the subcellular
324 compartment or organelle with which they associate, on whether they stay in the host
325 cytoplasm or move to the nucleus, etc. In this context it is interesting to mention observations
326 on the stability of the RNAs of viral satellites of CMV and TMV (44, 45). These authors showed
327 that satellite RNAs were able to survive, both in vivo and in vitro, in the absence of their helper
328 virus for at least ten days, while at the same time the stability of the RNAs of their helper was
329 much lower (<48h). In any case, if incomplete inoculations of at least some viruses may survive
330 for some time and be 'rescued' by subsequent inoculations the between-host transmission cost
331 would be greatly reduced if not entirely alleviated. The operation of such a mechanism would
332 also imply that such viruses have an unappreciated capacity to reassort.

333 The existence of this infection rescue mechanism is entirely hypothetical at present, but
334 preliminary results in our laboratory suggest that it may exist. Indeed, even though all eight

335 genome segments of FBNSV have always been found to co-occur in field samples (46, 47), three
336 segments, N, C and U4, are dispensable for infection to occur, at least under laboratory
337 conditions (46, 47). It is however worth mentioning that the lack of at least some of these
338 segments may have important phenotypic effects, e.g. in the absence of segment N successful
339 infections are produced but aphid transmission becomes impossible (47). We inoculated one
340 set of plants without segment C and another set of plants without segment U4. We then either
341 (i) allowed different groups of aphids to feed on these two sets of plants and subsequently
342 transferred both groups of aphids on naïve plants; or (ii) allowed the same aphids to acquire
343 viral infections sequentially on the two sets of plants and then transferred them on naïve
344 plants. In both cases we obtained successful infections of recipient plants containing all FBNSV
345 segments. In an additional experiment aphids acquired first all segments but U4 from an
346 incompletely infected plant, and then, three days later, U4 from an infected plant lacking N.
347 Using segment specific in situ hybridization, we could show that despite their sequential
348 acquisition, N and U4 reunite and accumulate in the exact same intracellular membranous
349 vesicles of the cells of the anterior midgut of the aphid vectors (Di Mattia J, Yvon M, Zeddam JL,
350 Vernerey MS, Michalakis Y and Blanc S. In preparation). These experiments demonstrate that
351 reassortment of incomplete infections may restore complete infections both very early within
352 the gut of insect vectors upon cumulative storage of the segments sequentially acquired, or
353 later within the plant after merging of incomplete infectious subsets of segments to restore the
354 integral genome. It remains to be seen whether a similar outcome can be reached from
355 incomplete inoculations which on their own are unable to establish successful infections, and to

356 characterize the time-interval between inoculations allowing for the rescue of such incomplete
357 infections (Figure 1.8).

358 **5. MULTICOMPONENT VIRAL SYSTEMS**

359 The previous sections outlined a number of issues imposed by their multipartite nature
360 on these viruses, and some potential ways they found to accommodate them. Even though the
361 situation is not identical, it is worth noting the analogies that exist between multipartite viruses
362 and other multicomponent viral systems, such as segmented viruses, viral satellites or defective
363 particles (48): the constraint to maintain genomic integrity and the possibility to regulate gene
364 expression by differentially modifying the gene (segment) copy number. The solutions adopted
365 by multipartite viruses could inspire research on these other viral entities, and reciprocally.

366 Segmented viruses do not face in principle the genomic integrity cost once viral particles
367 have been formed. However, they must find a way to ensure that this indeed happens within
368 individual host cells. This could happen either (i) through some specific sorting mechanism
369 when packaging the distinct segments of the genome; or (ii) non-specifically packaging within
370 each viral particle more segments than their genome consists of, analogous to a high MOI at
371 the viral particle level.

372 Specific sorting mechanisms governing the packaging of the different genome segments
373 have been described in several viruses. For example, Borodavka et al. (49) review the processes
374 through which several dsRNA segmented viruses package their genomic segments in a specific
375 sequence, which apparently ensures the incorporation of one copy of each segment in the viral
376 particle. However, evidence accumulates that the (-)ssRNA Influenza A Virus (IAV) which also

377 possesses specific packaging mechanisms (50), produces a large number of particles which fail
378 to express all IAV genes, termed semi-infectious particles (or SIP (10, 51)). They actually
379 constitute the majority of IAV viral particles: a recent study reported that individual viral
380 particle infections lead to the successful replication of all eight IAV segments in only 1.22 % of
381 all cases (52), and there is even variation among isolates in their propensity to produce such
382 particles (see (53) for a review). As this review argues, SIPs may be generated by several
383 mechanisms. Failure to package all eight segments has been reported to occur in up to 20% of
384 viral particles (54). Failure to express the proteins of all eight segments could also be explained
385 by post-inoculation segment loss during, e.g. segment trafficking within the cytoplasm or from
386 the cytoplasm to the nucleus, or replication failure during the early stages of infection.
387 Whatever the mechanism responsible for SIP production, however, it is believed that the IAV
388 manages to successfully infect its hosts through complementation resulting from multiple
389 infections of single individual host cells (51–53), complementation being also the mechanism
390 allowing the persistence of defective particles (55) and viral satellites (56). Observations on the
391 (-)ssRNA Bunyavirales, e.g. Rift Valley fever virus (RVFV), reviewed by Wichgers Schreur et al.
392 (57), also show that the majority of mature virions lack one or more genome segments, and
393 that the genome segment ratio in mature virions departs from 1:1:1. Thus the existence of very
394 specific packaging mechanisms does not per se alleviate the genomic integrity problem for
395 viruses with genomes carried by more than one molecule. At least some of these viruses must
396 resolve the issue through higher MOI.

397 It has been reported that at least one virus uses a “within-particles high MOI strategy”
398 by packaging more segments in its viral particles than its genome consists of, the bi-segmented

399 dsRNA infectious bursal disease virus (IBDV) of the family *Birnaviridae* (58): IBDV packages up to
400 four segments in each particle. This results in an increased probability that at least one copy of
401 each of its two segments will be carried by each particle, while at the same time some particles
402 contain several copies of a given segment and other segments are missing: viral particles can be
403 aneuploid, polyploid, and potentially both. None of the known multipartite viruses has adopted
404 this packaging strategy: cases where more than one segments are packaged in the same
405 particle exist, i.e. the RNA3 and RNA4 segments of bromoviruses (59) or RNA1 and RNA2 of
406 dianthoviruses (60, 61), but it is always the same segments which are packaged together; the
407 process is not random (62).

408 Finally, the possibility that the genomic integrity issue, at least at the within-host level,
409 can be resolved by a multicellular way of life, as observed in the multipartite FBNSV (37),
410 deserves further investigation in segmented viruses and also for accumulation/maintenance of
411 satellites and defective particles. Host cells continuously traffic host functions, under the form
412 of mRNA, proteins or even organelles (63–65). While it has long been evident that viruses
413 exploit intra-host cell communication for cell-to-cell transmission, we do not know how
414 frequently and how intensively they might use these avenues to circulate gene functions, thus
415 adopting a multicellular way of life. This was shown to be the case in the multipartite FBNSV,
416 but there is no a priori reason that such a functioning could be restricted to multipartite viruses;
417 it could well occur e.g. in segmented viruses and even in monopartite.

418 Another feature put forward recently in multipartite viruses is the between segment
419 variation in gene copy number, which further depends for a given virus on the host species (33,
420 34). It was suggested that this variation may lead to their ability to rapidly adapt their gene

421 expression to the challenges imposed by differing host physiologies ((33, 34); M. Zwart and S.F.
422 Elena in review), and it was recently confirmed in the FBNSV that indeed DNA segment copy
423 number variation is correlated with RNA expression: the quantitative variation at the gene level
424 has a functional role in terms of gene expression (R. Gallet, J. Di Mattia, S. Ravel, R. Vitalis, Y.
425 Michalakis and S. Blanc in preparation). The existence of SIPs and DIPs in many viruses result in
426 within and between host variation of gene copy numbers and protein expression (8, 53, 55, 56),
427 and it has been recently argued that this variation may at least in some cases provide
428 advantages to the viruses ((53) for SIPs; (55, 66) for DIPs). It is thus possible to imagine that
429 segmented viruses (and why not even some monopartite viruses) could function through SIP (or
430 DIP for monopartite) production and a multicellular way of life as multipartite viruses within
431 hosts, while resolving the between-host genomic integrity cost through the production of some
432 viral particles containing all genomic segments (non-defective particles) and a relatively high
433 MOI.

434

435 **6. RECAP ON CHALLENGES TO VIROLOGY PRINCIPLES POSED BY MULTIPARTITES AND OTHER**
436 **MULTICOMPONENT SYSTEMS**

437 The existence of specific packaging mechanisms were at the basis of several
438 foundational principles of virology: that one virus particle may be able to successfully infect one
439 cell; that the viral genome travels between cells and hosts in a single transmission vehicle; that
440 the viral replication cycle is “cell-autonomous”, i.e. is completed within a cell and then
441 reiterated in next contaminated cells. Multipartite viruses violate these principles.

442 That they violate the first two derives directly from the multipartite nature of these
443 viruses: because they are multipartite the genetic information does not circulate in a single
444 transmission vehicle. Instead, it is packaged in as many as eight different particles. The
445 successful infection of a single cell cannot be achieved by a single viral particle, and the
446 genomic integrity cost derives from these violations.

447 That the replication cycle is not cell-autonomous is a much more surprising violation,
448 and one that allows to alleviate the genomic integrity cost, at least at the within-host level. It
449 has been shown to occur in the octopartite nanovirus FBNSV (37). It is presently unclear
450 whether this is a general feature of multipartite viruses, though there is no a priori reason to
451 believe it should be restricted to just FBNSV, nanoviruses, or ssDNA viruses. It directly implies
452 that the spatial unit of infection is not the individual host cell, but some larger level whose scale
453 awaits further characterization.

454 We earlier mentioned the possibility that the between-host genomic integrity cost may
455 be resolved if inoculation of a given host by all indispensable genomic segments does not need
456 to be concomitant: if incomplete inoculations, where only some of the segments are passed to
457 a host individual, may remain latent and be rescued by subsequent inoculations which
458 complement them. We emphasize that this is still a hypothesis. But would it be proven to occur
459 it would not only imply that the genomic integrity cost imposed by multipartitism is much
460 weaker than initially anticipated. It would also imply that multipartite viruses can shuffle their
461 genome through segment reassortments at a much larger scale than previously appreciated,
462 even among individual viruses which do not co-occur on the same host individual, or even host
463 population or perhaps host species – depending on vector behavior. It is unlikely that this

464 potential, should its existence be proved, may have led to the evolution of multipartitism:
465 considering a scenario where a rare multipartite mutant invades a monopartite population,
466 because of its initial rareness the multipartite mutant could only benefit from its increased
467 reassorting capacity too rarely: its genome segments would have a low probability to encounter
468 other segments, and the very rare reassortments would need to be beneficial to constitute an
469 advantage. This mechanism is thus unlikely to be at the origin of multipartitism. It can
470 nevertheless provide the opportunity to multipartite viruses, once multipartitism established,
471 to reshuffle their genome at a very high rate and e.g. generate host range variants (67) with
472 obvious agricultural and economic consequences.

473 Multipartite viruses have been shown to be able to modify their gene copy number
474 depending on the host species they infect through their genome formula modulation. In at least
475 one of the viruses in which this phenomenon was observed, it was further shown that it has
476 functional consequences since gene copy number variation is correlated with gene expression
477 variation. These modifications occur 'immediately' upon a single passage from one host species
478 to another, and they are not correlated with any mutations at the DNA sequence level (R.
479 Gallet, J. Di Mattia, S. Ravel, R. Vitalis, Y. Michalakis and S. Blanc. in preparation). These
480 observations lead to a number of important considerations:

481 First, they suggest that multipartite viruses may possess a mechanism allowing them to
482 adjust their gene expression to their host species "instantly", upon a single transmission event.
483 Because their aphid vectors are most often polyphagous, it may well be the case that they are
484 often submitted to host species changes. This DNA mutation free gene expression adjustment
485 mechanism could thus constitute an important advantage deriving directly from the

486 multipartite nature of this viruses, a consideration supported by recent modeling results (M.
487 Zwart and S.F. Elena in review). Even though monopartite viruses can also adapt to a
488 challenging environment through copy number variation (68, 69), such adaptations require
489 sequence mutations through gene duplication and, though relatively fast, are thus not
490 immediate. This capacity to instantly modulate gene expression upon host species change,
491 derived directly from multipartitism, may thus constitute an evolutionary advantage of this
492 genomic architecture which under some circumstances may be able to overcome the genomic
493 integrity cost – or whatever remains of it.

494 Second, the existence of the genome formula and its DNA-mutation free modification
495 pose some formidable conceptual issues. What is the genome of a multipartite virus? Is it the
496 concatenation of the DNA sequences of its genomic segments? Or is it instead the genome
497 formula, i.e. the collection of all the copies of the different segments/genes? The latter would
498 imply that the genome of a virus depends on its host species… Is gene copy number variation a
499 genetic mutation or a manifestation of phenotypic plasticity in multipartite viruses? Nobody
500 disputes that the adaptation of the monopartite poxviruses through gene copy number
501 variation, termed 'genetic accordions' (68, 69) results from genetic mutations. Multipartite
502 viruses react to host species switching through essentially the same mechanism, copy number
503 variation; yet, if their genome is defined as the concatenation of the DNA sequences of their
504 genomic segments, this adjustment should be considered as phenotypic plasticity since it does
505 not result from a DNA sequence modification. To the extent that SIPs, and even DIPs, may not
506 always represent junk for segmented viruses but functional explorations in gene copy number

507 variation at least under some circumstances, as discussed earlier, these considerations may
508 apply much more generally than one could think.

509

510 **DISCLOSURE STATEMENT**

511 The authors are not aware of any affiliations, memberships, funding, or financial holdings that
512 might be perceived as affecting the objectivity of this review.

513

514 **ACKNOWLEDGMENTS**

515 We thank Rosemary Dorrington for clarifications on packaging of RNA segments of
516 omegatetraviruses. This project was funded by the French national research funding agency
517 (grants ANR-14-CE02-0014-01 and ANR-18-CE92-0028-01), the SPE department of INRA, and
518 the IRD and CNRS research institutes.

519

520 **LITERATURE CITED**

521 1. Lucía-Sanz A, Manrubia S. 2017. Multipartite viruses: adaptive trick or evolutionary treat? *npj*
522 *Systems Biology and Applications*. 3(1):
523 2. Sicard A, Michalakis Y, Gutiérrez S, Blanc S. 2016. The strange lifestyle of multipartite viruses. *PLOS*
524 *Pathogens*. 12(11):e1005819
525 3. Liu W, Hajano J-U-D, Wang X. 2018. New insights on the transmission mechanism of tenuiviruses
526 by their vector insects. *Current Opinion in Virology*. 33:13–17

527 4. Dietzgen RG, Freitas-Astúa J, Chabi-Jesus C, Ramos-González PL, Goodin MM, et al. 2018. Chapter
528 Five - Dichorhaviruses in their host plants and mite vectors. In *Advances in Virus Research*, ed P
529 Palukaitis, MJ Roossinck. 102:119–48. Academic Press
530 5. Leastro MO, Kitajima EW, Silva MS, Resende RO, Freitas-Astúa J. 2018. Dissecting the subcellular
531 localization, intracellular trafficking, interactions, membrane association, and topology of Citrus
532 Leprosis Virus C proteins. *Front. Plant Sci.* 9:
533 6. Hu Z, Li G, Li G, Yao Q, Chen K. 2013. Bombyx mori bidensovirus: The type species of the new genus
534 Bidensovirus in the new family Bidnaviridae. *Chin. Sci. Bull.* 58(36):4528–32
535 7. Ladner JT, Wiley MR, Beitzel B, Auguste AJ, Dupuis AP, et al. 2016. A multicomponent animal virus
536 isolated from mosquitoes. *Cell Host & Microbe*. 20(3):357–67
537 8. Wichgers Schreur PJ, Kortekaas J. 2016. Single-molecule FISH reveals non-selective packaging of
538 Rift Valley Fever Virus genome segments. *PLOS Pathogens*. 12(8):e1005800
539 9. Gokhale DV, Bald JG. 1987. Relationship between plant virus concentration and infectivity: a
540 'growth curve' model. *Journal of Virological Methods*. 18(4):225–32
541 10. Brooke CB, Ince WL, Wrammert J, Ahmed R, Wilson PC, et al. 2013. Most Influenza A virions fail to
542 express at least one essential viral protein. *Journal of Virology*. 87(6):3155–62
543 11. Lister RM. 1966. Possible relationships of virus-specific products of tobacco rattle virus infections.
544 *Virology*. 28(2):350–53
545 12. Van Kammen A. 1967. Purification and properties of the components of cowpea mosaic virus.
546 *Virology*. 31(4):633–42
547 13. Chu PWG, Helms K. 1988. Novel virus-like particles containing circular single-stranded DNAs
548 associated with subterranean clover stunt disease. *Virology*. 167(1):38–49
549 14. Hesketh EL, Saunders K, Fisher C, Potze J, Stanley J, et al. 2018. The 3.3 Å structure of a plant
550 geminivirus using cryo-EM. *Nature Communications*. 9(1):2369

551 15. Chou Y, Vafabakhsh R, Doğanay S, Gao Q, Ha T, Palese P. 2012. One influenza virus particle
552 packages eight unique viral RNAs as shown by FISH analysis. *Proceedings of the National Academy*
553 *of Sciences*. 109(23):9101–9106
554 16. Pressing J, Reanney DC. 1984. Divided genomes and intrinsic noise. *J Mol Evol*. 20(2):135–46
555 17. Nee S. 1987. The evolution of multicompartmental genomes in viruses. *J Mol Evol*. 25(4):277–81
556 18. Nee S, Maynard Smith J. 1990. The evolutionary biology of molecular parasites. *Parasitology*.
557 100(S1):S5–18
558 19. Szathmáry E. 1992. Natural selection and dynamical coexistence of defective and complementing
559 virus segments. *Journal of Theoretical Biology*. 157(3):383–406
560 20. Szathmáry E. 1992. Viral sex, levels of selection, and the origin of life. *Journal of Theoretical*
561 *Biology*. 159(1):99–109
562 21. Zwart MP, Elena SF. 2015. Matters of size: genetic bottlenecks in virus infection and their potential
563 impact on evolution. *Annual Review of Virology*. 2(1):161–79
564 22. Zhang Y-J, Wu Z-X, Holme P, Yang K-C. 2019. Advantage of being multicomponent and spatial:
565 multipartite viruses colonize structured populations with lower thresholds. *Physical Review Letters*.
566 123(13):138101
567 23. Varsani A, Lefeuvre P, Roumagnac P, Martin D. 2018. Notes on recombination and reassortment in
568 multipartite/segmented viruses. *Current Opinion in Virology*. 33:156–66
569 24. Lefkowitz EJ, Adams, MJ, Davison, AJ, Siddell, SG, Simmonds, P, eds. 2015. *Virus Taxonomy: The*
570 *classification and nomenclature of viruses. The online 10th Report of the ICTV (EC 47, London,*
571 *2015). URL: https://talk.ictvonline.org/ictv-reports/ictv_online_report/*
572 25. Lucía-Sanz A, Aguirre J, Manrubia S. 2018. Theoretical approaches to disclosing the emergence and
573 adaptive advantages of multipartite viruses. *Current Opinion in Virology*. 33:89–95

574 26. Chao L. 1991. Levels of selection, evolution of sex in RNA viruses, and the origin of life. *Journal of*
575 *Theoretical Biology*. 153(2):229–46
576 27. Chao L. 1988. Evolution of sex in RNA viruses. *Journal of Theoretical Biology*. 133(1):99–112
577 28. Nee S. 1989. On the evolution of sex in RNA viruses. *Journal of Theoretical Biology*. 138(3):407–12
578 29. Ojosnegros S, García-Arriaza J, Escarmís C, Manrubia SC, Perales C, et al. 2011. Viral genome
579 segmentation can result from a trade-off between genetic content and particle stability. *PLOS*
580 *Genetics*. 7(3):e1001344
581 30. Garcia-Arriaza J, Manrubia SC, Toja M, Domingo E, Escarmis C. 2004. Evolutionary transition
582 toward defective RNAs that are infectious by complementation. *J. Virol.* 78:11678–85
583 31. Valdano E, Manrubia S, Gómez S, Arenas A. 2019. Endemicity and prevalence of multipartite
584 viruses under heterogeneous between-host transmission. *PLOS Computational Biology*.
585 15(3):e1006876
586 32. Iranzo J, Manrubia SC. 2012. Evolutionary dynamics of genome segmentation in multipartite
587 viruses. *Proc Biol Sci*. 279(1743):3812–19
588 33. Sicard A, Yvon M, Timchenko T, Gronenborn B, Michalakis Y, et al. 2013. Gene copy number is
589 differentially regulated in a multipartite virus. *Nature Communications*. 4:DOI:
590 10.1038/ncomms3248
591 34. Wu B, Zwart MP, Sánchez-Navarro JA, Elena SF. 2017. Within-host evolution of segments ratio for
592 the tripartite genome of Alfalfa Mosaic Virus. *Scientific Reports*. 7(1):5004
593 35. Hu Z, Zhang X, Liu W, Zhou Q, Zhang Q, et al. 2016. Genome segments accumulate with different
594 frequencies in Bombyx mori bidensovirus. *Journal of Basic Microbiology*. 56(12):1338–43
595 36. Gallet R, Fabre F, Thébaud G, Sofonea MT, Sicard A, et al. 2018. Small bottleneck size in a highly
596 multipartite virus during a complete infection cycle. *Journal of Virology*. 92(14):e00139-18

597 37. Sicard A, Pirolles E, Gallet R, Vernerey M-S, Yvon M, et al. 2019. A multicellular way of life for a
598 multipartite virus. *eLife*. 8:e43599
599 38. Sanjuán R, Thoulouze M-I. 2019. Why viruses sometimes disperse in groups. *Virus Evol*. 5(1):
600 39. Dall'Ara M, Ratti C, Bouzoubaa SE, Gilmer D. 2016. Ins and outs of multipartite positive-strand RNA
601 plant viruses: packaging versus systemic spread. *Viruses*. 8(8):228
602 40. Gilmer D, Ratti C, Michel F. 2018. Long-distance movement of helical multipartite phytoviruses:
603 keep connected or die? *Current Opinion in Virology*. 33:120–28
604 41. Betancourt M, Fereres A, Fraile A, García-Arenal F. 2008. Estimation of the effective number of
605 founders that initiate an infection after aphid transmission of a multipartite plant virus. *Journal of*
606 *Virology*. 82(24):12416–21
607 42. Schwinghamer MW, Nicholas AH, Schilg MA. 2009. Three aphid vectors of faba bean (Vicia faba)
608 viruses in northern New South Wales and occurrence of Acyrthosiphon pisum-transmitted isolates
609 of Soybean dwarf virus. *Australasian Plant Pathology*. 38(3):262–69
610 43. Gronenborn B. 2004. Nanoviruses: genome organisation and protein function. *Veterinary*
611 *Microbiology*. 98(2):103–9
612 44. Mossop DW, Francki RIB. 1978. Survival of a satellite RNA in vivo and its dependence on cucumber
613 mosaic virus for replication. *Virology*. 86(2):562–66
614 45. Mossop DW, Francki RIB. 1979. The stability of satellite viral RNAs in vivo and in vitro. *Virology*.
615 94(2):243–53
616 46. Timchenko T. 2006. Infectivity of nanovirus DNAs: induction of disease by cloned genome
617 components of Faba bean necrotic yellows virus. *Journal of General Virology*. 87(6):1735–43
618 47. Grigoras I, Vetten H-J, Commandeur U, Ziebell H, Gronenborn B, Timchenko T. 2018. Nanovirus
619 DNA-N encodes a protein mandatory for aphid transmission. *Virology*. 522:281–91

620 48. Michalakis Y, Blanc S. 2018. Editorial overview: Multicomponent viral systems. *Current Opinion in*
621 *Virology*. 33:vi–ix
622 49. Borodavka A, Desselberger U, Patton JT. 2018. Genome packaging in multi-segmented dsRNA
623 viruses: distinct mechanisms with similar outcomes. *Current Opinion in Virology*. 33:106–12
624 50. Hutchinson EC, von Kirchbach JC, Gog JR, Digard P. 2010. Genome packaging in influenza A virus.
625 *Journal of General Virology,*. 91(2):313–28
626 51. Brooke CB. 2017. Population diversity and collective interactions during Influenza Virus infection.
627 *Journal of Virology*. 91(22):e01164-17
628 52. Jacobs NT, Onuoha NO, Antia A, Steel J, Antia R, Lowen AC. 2019. Incomplete influenza A virus
629 genomes occur frequently but are readily complemented during localized viral spread. *Nat*
630 *Commun*. 10(1):1–17
631 53. Diefenbacher M, Sun J, Brooke CB. 2018. The parts are greater than the whole: the role of semi-
632 infectious particles in influenza A virus biology. *Current Opinion in Virology*. 33:42–46
633 54. Nakatsu S, Sagara H, Sakai-Tagawa Y, Sugaya N, Noda T, Kawaoka Y. 2016. Complete and
634 incomplete genome packaging of influenza A and B viruses. *mBio*. 7(5):
635 55. Vignuzzi M, López CB. 2019. Defective viral genomes are key drivers of the virus–host interaction.
636 *Nature Microbiology*. 4(7):1075
637 56. Gnanasekaran P, Chakraborty S. 2018. Biology of viral satellites and their role in pathogenesis.
638 *Current Opinion in Virology*. 33:96–105
639 57. Wichgers Schreur PJ, Kormelink R, Kortekaas J. 2018. Genome packaging of the Bunyavirales.
640 *Current Opinion in Virology*. 33:151–55
641 58. Luque D, Rivas G, Alfonso C, Carrascosa JL, Rodriguez JF, Caston JR. 2009. Infectious bursal disease
642 virus is an icosahedral polyploid dsRNA virus. *Proceedings of the National Academy of Sciences*.
643 106(7):2148–52

644 59. Chaturvedi S, Rao A. 2018. Molecular and biological factors regulating the genome packaging in
645 single-strand positive-sense tripartite RNA plant viruses. *Current Opinion in Virology*. 33:113–19
646 60. Basnayake VR, Sit TL, Lommel SA. 2006. The genomic RNA packaging scheme of Red clover necrotic
647 mosaic virus. *Virology*. 345(2):532–39
648 61. Basnayake VR, Sit TL, Lommel SA. 2009. The Red clover necrotic mosaic virus origin of assembly is
649 delimited to the RNA-2 trans-activator. *Virology*. 384(1):169–78
650 62. Newburn LR, White KA. 2019. Trans-acting RNA–RNA interactions in segmented RNA viruses.
651 *Viruses*. 11(8):751
652 63. Woith E, Fuhrmann G, Melzig MF. 2019. Extracellular vesicles—connecting kingdoms. *International*
653 *Journal of Molecular Sciences*. 20(22):5695
654 64. Drab M, Stopar D, Kralj-Iglič V, Iglič A. 2019. Inception mechanisms of tunneling nanotubes. *Cells*.
655 8(6):626
656 65. Gill S, Catchpole R, Forterre P. 2019. Extracellular membrane vesicles in the three domains of life
657 and beyond. *FEMS Microbiol Rev*. 43(3):273–303
658 66. Rezelj VV, Levi LI, Vignuzzi M. 2018. The defective component of viral populations. *Current Opinion*
659 *in Virology*. 33:74–80
660 67. Pavithra BS, Govin K, Renuka HM, Krishnareddy M, Jalali S, et al. 2019. Characterization of
661 cucumber mosaic virus infecting coleus (Plectranthus barbatus) in Karnataka. *VirusDis.* 30(3):403–
662 12
663 68. Elde NC, Child SJ, Eickbush MT, Kitzman JO, Rogers KS, et al. 2012. Poxviruses deploy genomic
664 accordions to adapt rapidly against host antiviral defenses. *Cell*. 150(4):831–41
665 69. Bayer A, Brennan G, Geballe AP. 2018. Adaptation by copy number variation in monopartite
666 viruses. *Current Opinion in Virology*. 33:7–12
667

668 **REFERENCE ANNOTATION LIST**

669 (reference number as in Literature Cited, annotation text):

670 1: important review on the evolution of multipartite viruses

671 2: important review on multipartite viruses with focus on different steps of their life cycle

672 10: Founding study revealing the problem of genome integrity in segmented viruses

673 21: reviews concepts and results on virus bottleneck sizes and MOI

674 23: reviews observations in recombination and reassortment in segmented and multipartite
675 viruses

676 29: only empirical paper exploring the potential advantages of multipartite variants

677 32: theoretical investigation of the parameter range favoring multipartitism depending on the
678 number of segments

679 33: first paper demonstrating the existence of the genome formula in multipartite viruses

680 37: first paper demonstrating a supra-cellular way of life for a multipartite virus

681 41: first paper estimating the bottleneck size in a multipartite virus

Caption figure 1

A multipartite virus with 8 genome segments is used as an example for the illustration

1-When particles randomly enter cells at low MOI (thin arrows), each infected cell rarely receives the full set of segments. Complementation of distinct segments across cells allows the viral system to infect and maintain its genome integrity at a supra cellular level. This possibility has been experimentally demonstrated for FBNSV and is visible in the inset micrograph where two segments are FISH-labeled with distinct colours (37).

2-Each cell could randomly receive the full set of segments if entering at high MOI (thick arrows). This possibility has thus far not received empirical support (see text)

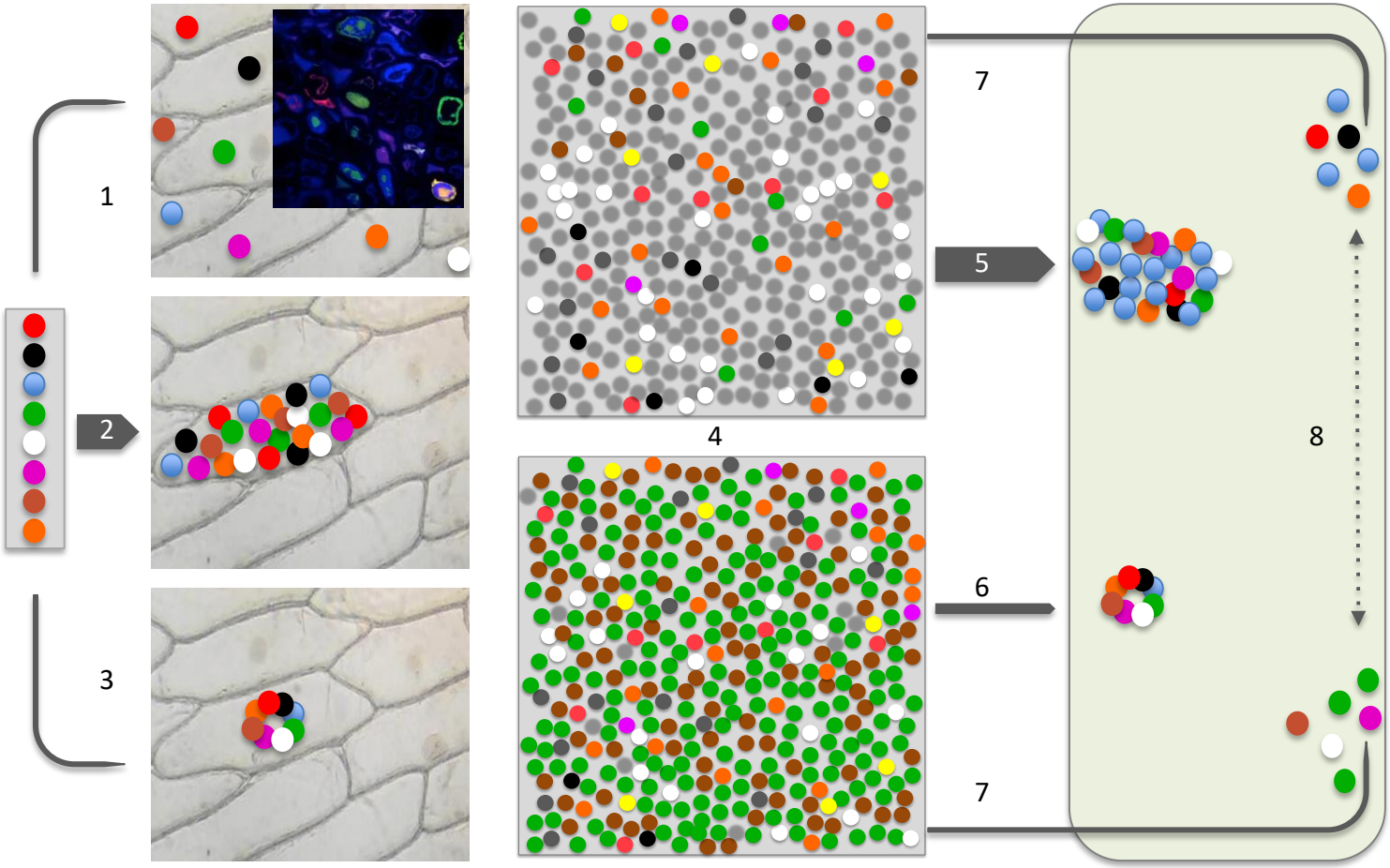
3-The full set of segments could be introduced into individual cells at low MOI if sorted cell-to-cell transfer can operate. Such a specific sorting of the segments during cell-to-cell transfer has been discussed (40) but never fully demonstrated experimentally.

4-Once the host is infected, populations of multipartite viruses accumulate at specific frequencies for each segment (genome formula) that are host dependent. Here two distinct host species are represented in the upper and lower panels. It has been demonstrated in FBNSV that these distinct formulas correspond to different gene expression in the two hosts (R. Gallet, J. Di Mattia, S. Ravel, R. Vitalis, Y. Michalakis and S. Blanc in preparation). This ability to rapidly modify gene expression could represent an evolutionary advantage if host switches are frequent.

5-Multipartite viruses could be transmitted between hosts at high MOI, but no experimental data support this possibility

6-Sorted transmission of the segments could also occur at this level but this has not been shown in any case and it is not considered probable.

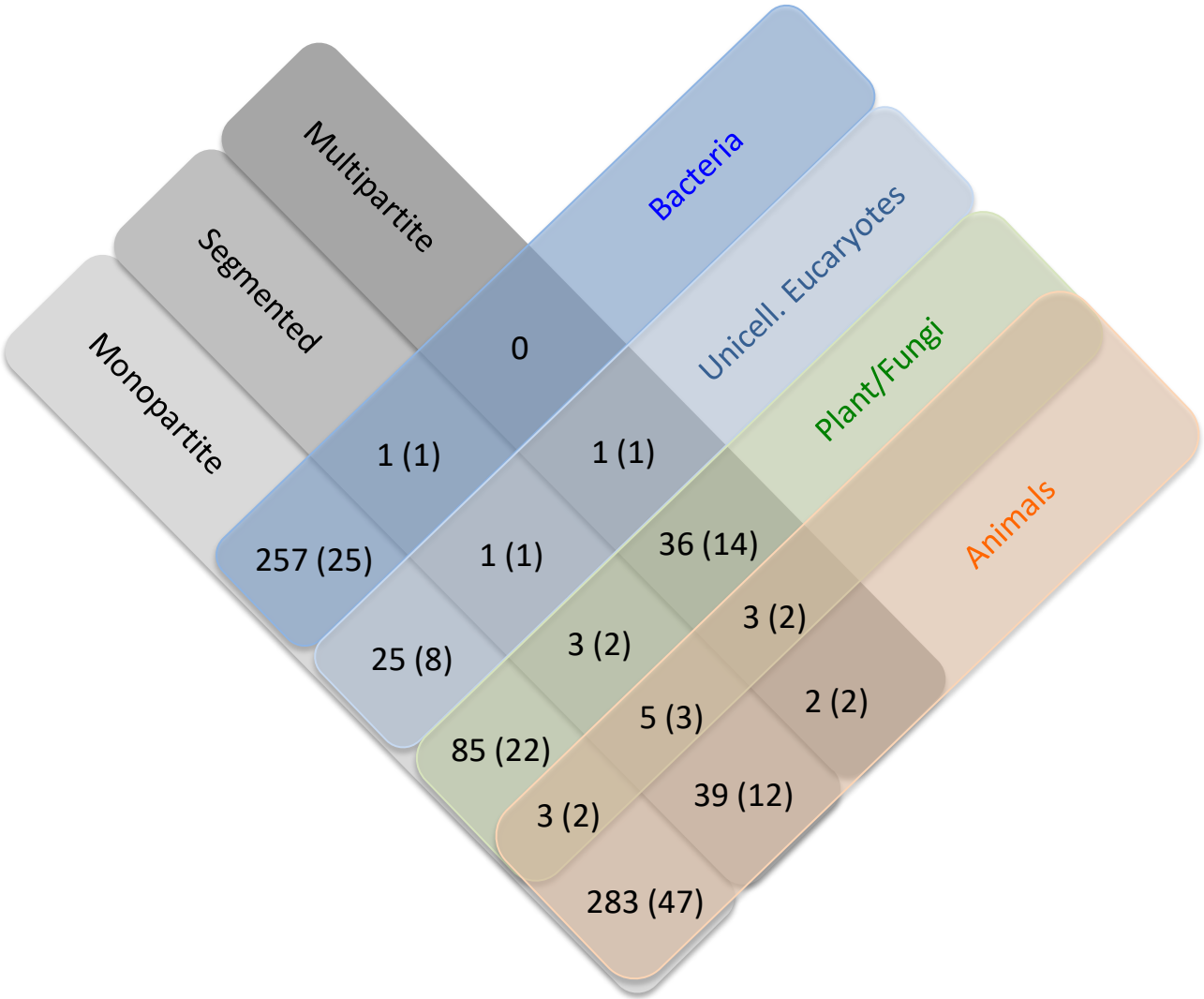
7-Current data indicate that transmission by insect vectors is at low MOI and so it is imaginable that distinct vector individuals could transmit incomplete sets of segments that would complement within the host (8) and initiate infection.


1
2
3
4
5
6
7
7
8

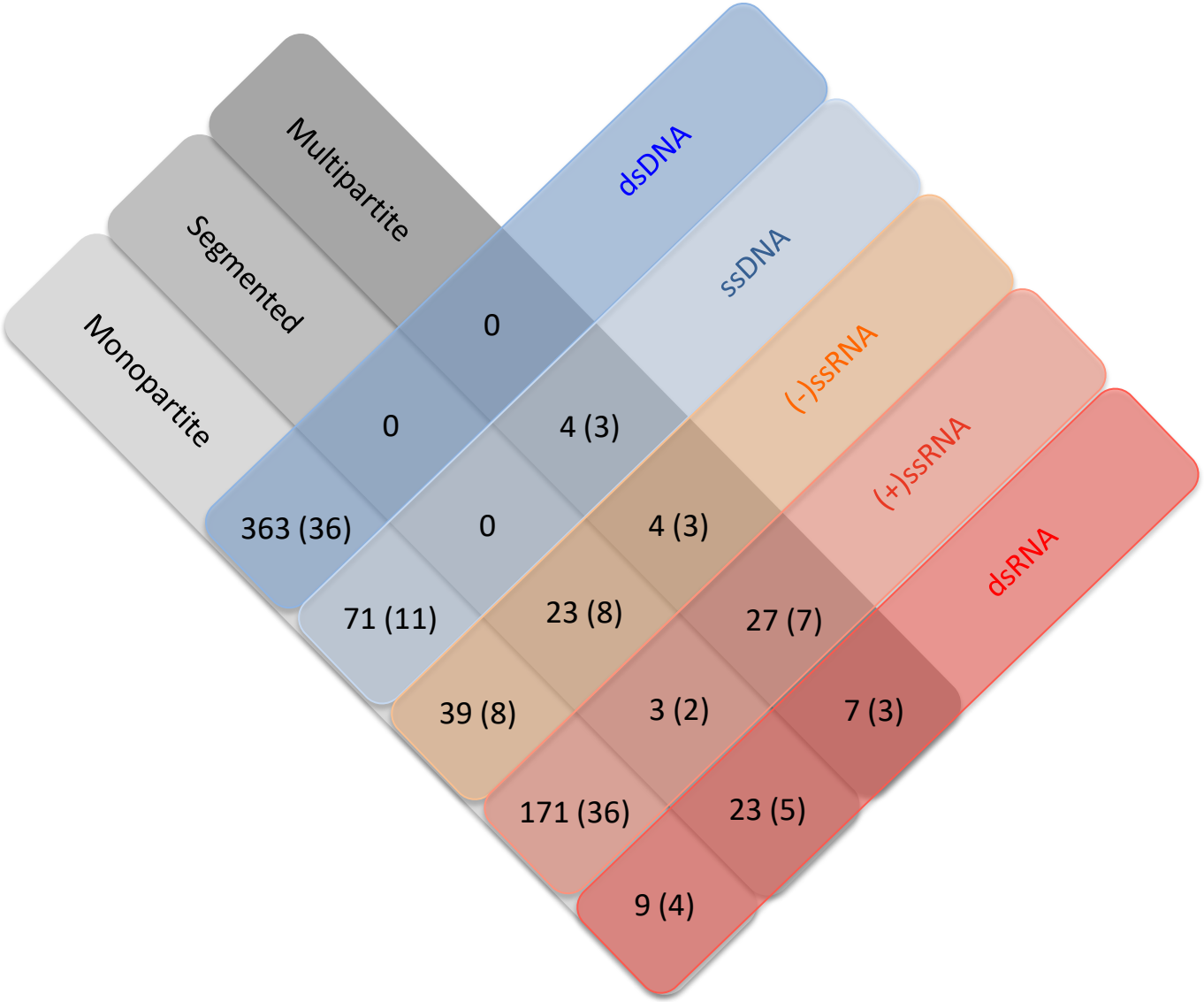
Caption figure 2

This figure is intended to use the data available at the time of preparation of this review to reveal the major trends in the distribution of the multipartite viral genome organization among types of host organisms and among the nature of the various nucleic acids composing the viral genomes. The numbers may not be exact for several reasons: i) packaging mechanisms have not been experimentally validated in many cases and may thus be deduced from relatedness with viral species where they were exprerimentally established, ii) some genera are unassigned to families, iii) when a family contains species in distinct categories, it is counted in each case, iv) the taxonomy is not fixed and so the number of genera and families is continuously changing over the years. This figure represents our interpretation of information from the literature and from ViralZone (https://viralzone.expasy.org/). In each diagram the numbers represent numbers of genera and the numbers in parentheses numbers of families. **A**: variation in genomic architecture across different types of hosts. The cells in Animals overlapping with those in plants/fungi correspond to plant viruses replicating in their animal vectors. When the hosts are not identified, the corresponding genera and families are simply not counted. **B**: variation in genomic architecture across different types of nucleic acid; **C**: variation in nucleic acid across different types of hosts. The cells in Animals overlapping with those in plants/fungi correspond to plant viruses replicating in their animal vectors. When the hosts are not identified, the corresponding genera and families are simply not counted.

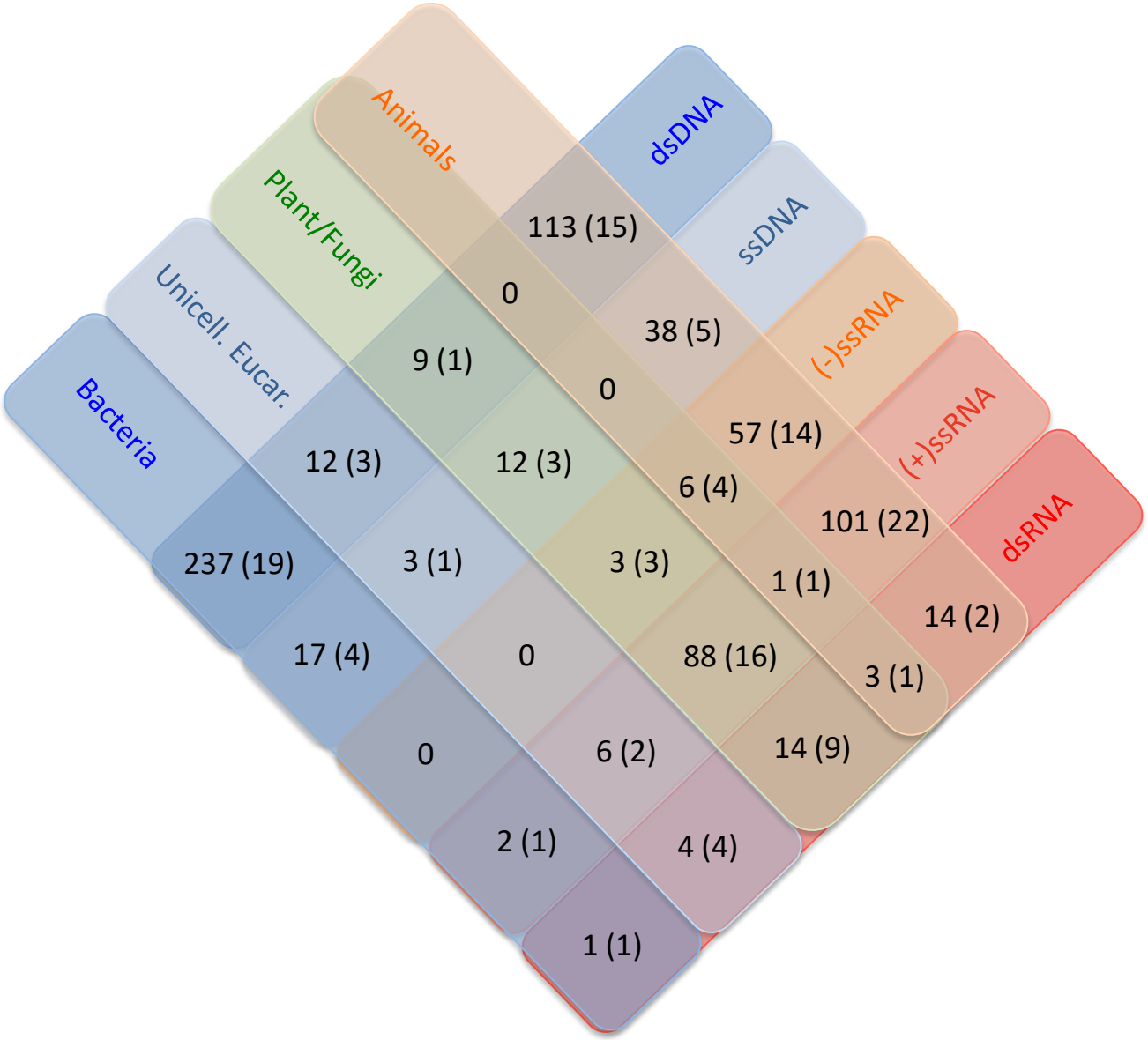
A

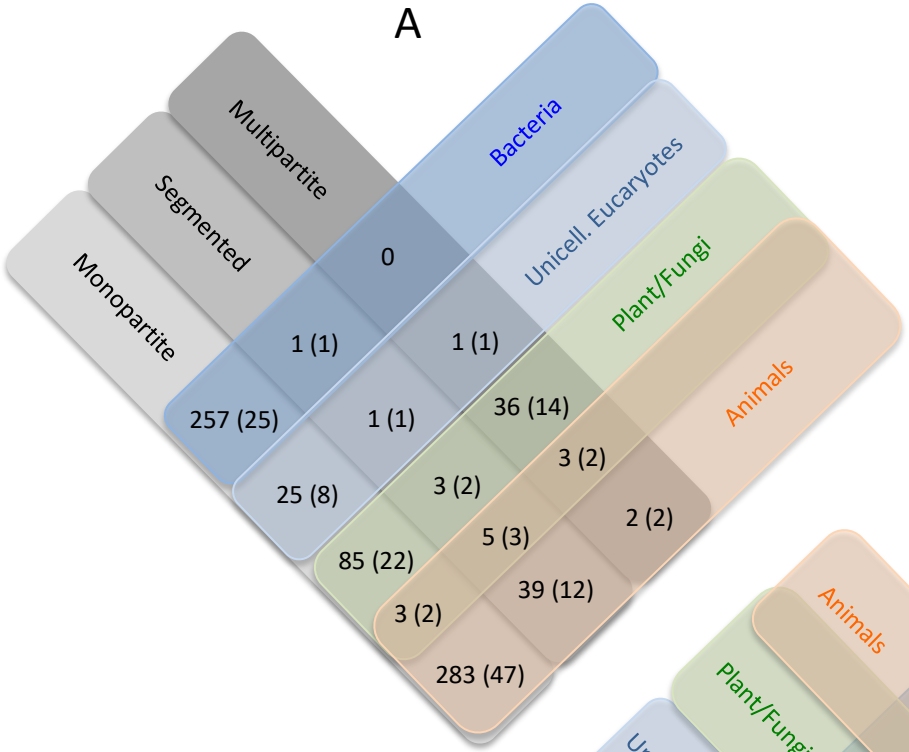

Multipartite
Segmented
Monopartite
Bacteria
Unicell. Eucaryotes
Plant/Fungi
Animals
0
1 (1)
1 (1)
257 (25)
1 (1)
36 (14)
25 (8)
3 (2)
3 (2)
85 (22)
5 (3)
2 (2)
3 (2)
39 (12)
283 (47)

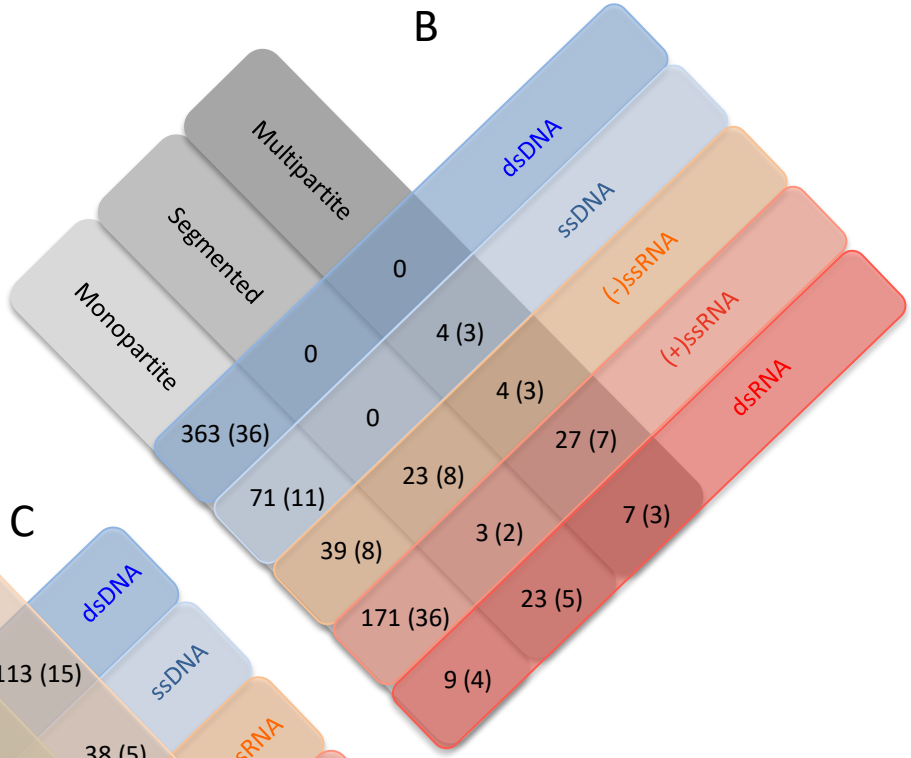
B

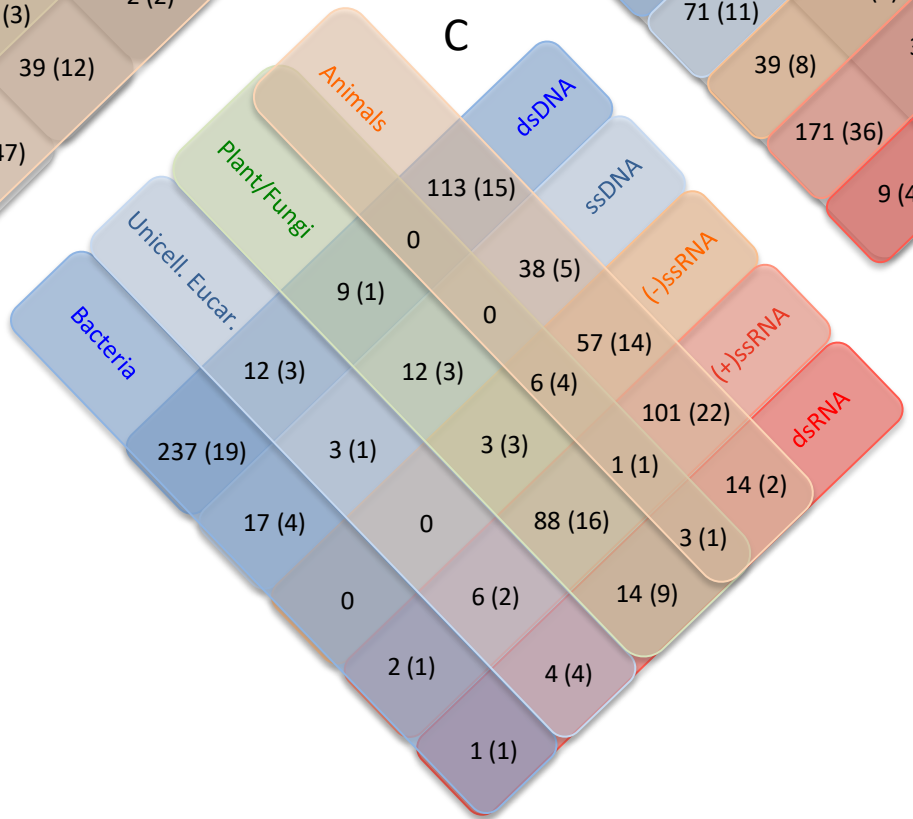

Multipartite
Segmented
Monopartite
dsDNA
ssDNA
(-)ssRNA
(+)ssRNA
dsRNA
0
0
4 (3)
363 (36)
0
4 (3)
71 (11)
23 (8)
27 (7)
39 (8)
3 (2)
7 (3)
171 (36)
23 (5)
9 (4)

C


Bacteria
Unicell. Eucar.
Plant/Fungi
Animals
dsDNA
ssDNA
(-)ssRNA
(+)ssRNA
dsRNA
237 (19)
12 (3)
9 (1)
0
113 (15)
38 (5)
57 (14)
101 (22)
14 (2)
17 (4)
3 (1)
12 (3)
0
3 (3)
6 (4)
1 (1)
3 (1)
0
0
6 (2)
88 (16)
14 (9)
2 (1)
4 (4)
1 (1)


A
Multipartite
Segmented
Monopartite
Bacteria
Unicell. Eucaryotes
Plant/Fungi
Animals
0
1 (1)
1 (1)
257 (25)
1 (1)
36 (14)
25 (8)
3 (2)
3 (2)
85 (22)
5 (3)
2 (2)
3 (2)
39 (12)
283 (47)


B
Multipartite
Segmented
Monopartite
dsDNA
ssDNA
(-)ssRNA
(+)ssRNA
dsRNA
0
4 (3)
0
4 (3)
363 (36)
0
27 (7)
23 (8)
71 (11)
7 (3)
3 (2)
39 (8)
23 (5)
171 (36)
9 (4)


C
Animals
Plant/Fungi
Unicell. Eucar.
Bacteria
dsDNA
ssDNA
(-)ssRNA
(+)ssRNA
dsRNA
113 (15)
0
38 (5)
9 (1)
0
57 (14)
12 (3)
12 (3)
6 (4)
101 (22)
237 (19)
3 (1)
3 (3)
1 (1)
14 (2)
17 (4)
0
88 (16)
3 (1)
0
6 (2)
14 (9)
2 (1)
4 (4)
1 (1)