1 # Advances in Plant Virus Evolution: Translating Evolutionary
2 Insights into Better Disease Management

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34

35 Virus Acronyms: *African cassava mosaic virus* (ACMV), *Beet necrotic yellow vein virus*
36 (BNYVV), *Brome Mosaic virus* (BMV), *Cabbage leaf curl virus* (CaLCuV), C*auliflower mosaic*
37 *virus* (CaMV), *Chrysanthemum chlorotic mottle viroid* (CChMVd), *Citrus leaf blotch virus*
38 (CLBV), *Citrus tristeza virus* (CTV), *Cucumber mosaic virus* (CMV), *Cucumber vein yellowing*
39 *virus* (CVYV), *East African cassava mosaic virus* (EACMV), *Mung bean yellow mosaic virus*
40 (MYMV), *Oilseed rape mosaic virus* (ORMV), *Plum pox virus* (PPV), *Potato virus X* (PVX),
41 *Potato virus Y* (PVY), *Rice yellow mottle virus* (RYMV), *Soil-borne wheat mosaic virus*
42 (SBWMV), *Tobacco etch virus* (TEV), *Tobacco mild green mosaic virus* (TMGMV), *Tobacco*
43 *mosaic virus* (TMV), Turnip *mosaic virus* (TuMV), *Turnip vein clearing virus* (TVCV), *Wheat*
44 *streak mosaic virus* (WSMV).

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49 ## ABSTRACT

50 Recent works in plant virus evolution are revealing that genetic structure and behavior of
51 virus and viroid populations can explain important pathogenic properties of these agents, such
52 as host resistance breakdown, disease severity, and host shifting among others. Genetic
53 variation is essential for the survival of organisms. The exploration of how these subcellular
54 parasites generate and maintain a certain frequency of mutations at the intra- and inter-host
55 levels is revealing novel molecular virus-plant interactions. They emphasize the role of host
56 environment in the dynamic genetic composition of virus populations. Functional genomics has
57 identified host factors that are transcriptionally altered after virus infections. The analyses of
58 these data by means of systems biology approaches are uncovering critical plant genes
59 specifically targeted by viruses during host adaptation. Also, a next-generation re-sequencing
60 approach of a whole virus genome is opening new avenues to study virus recombination and
61 the relationships between intra-host virus composition and pathogenesis. Altogether, the
62 analyzed data indicate that systematic disruption of some specific parameters of evolving virus
63 populations could lead to more efficient ways of disease prevention, eradication, or tolerable
64 virus-plant coexistence.

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71 Viruses and viroids appear to be the fastest-evolving plant pathogens (39), and cause
72 tremendous economical crop losses annually. Some, such as the single-stranded DNA
73 begomoviruses, are emergent problems worldwide (117, 126). These subcellular pathogens
74 have higher mutation rates than, and distinct evolutionary dynamics from, bacterial and fungal
75 phytopathogens. Understanding their reproductive and transmission strategies – their biology,
76 ecology, and evolution – can lead to insights and interventions for effective crop disease
77 management. This review highlights how viruses and viroids achieve and maintain their unique
78 parasitic lifestyles and how evolutionary virology and systems biology approaches to virus-plant
79 interactions have implications for pathogen control.

80 The ability of viruses and viroids to change, and to change rapidly, underlies many
81 disease management concerns. Excepting migration from distant locations and other countries,
82 variability in plant pathogen populations is the necessary initial step in adaptation to new plants
83 (host shifting), resistance breaking (RB), and changes in symptoms and virulence. Many times
84 the rise and fall of different genotypes in a population is due to the effects of natural selection:
85 variant genomes that generate more viable descendants become more frequent over time. This
86 process can be sped up or inhibited by **bottlenecks** (see Textbox 1 for definitions of terms in
87 bold), that plant pathogens experience as they move from cell to cell and from plant to plant (57,
88 69, 88). To expand its host range, a virus population must already have a variant (perhaps at a
89 very low level in the population) that can infect that potential host. In the novel host, those
90 mutants will be fitter and will rise in frequency. However, in absence of selection on that novel
91 host, the only chance that neutral **host range mutations** have to be fixed in the population is by
92 **genetic drift**. Otherwise, neutral or even deleterious mutations will be sweep away by **purifying**
93 **selection**. Understanding the processes that generate viral diversity and the ecological
94 processes that determine selective pressures and bottlenecks can illuminate potential
95 interventions or determine where and when control measures might be most effective.

96 ***Mechanisms of virus and viroid genetic variation***. Viruses have several ways
97 to achieve variation within a plant and within a field. First and foremost is mutation: the imperfect
98 copying of genomic material from parent to offspring and subsequent chemical and enzymatic
99 changes to nucleotide bases. RNA viruses are notorious for having high **mutation rates**, due to
100 replication with RNA-dependent polymerases lacking proofreading activity. The polymerases of
101 large nidoviruses are an exception (100), but none have yet been shown to infect plants. Most
102 studies of plant virus variation measure **mutation frequencies** over a range of time, such as
103 viral mutations arising within a month after plant infection. These studies are popular in plant
104 virology because of the difficulty in relating mutation frequency to how often the viruses have
105 replicated their genome within a whole plant. Despite this limitation, mutation frequencies can
106 be used to estimate upper boundaries on plant viral mutation rates (reviewed in 120). Mutation
107 rate studies tally the mutations produced prior to the action of selection, either per round of
108 genomic replication or per cell infected. Two exceptional studies have calculated mutation rates:
109 one for TMV (94) and the other for TEV (135). In both cases, the mutation rates trend towards
110 the lower end of measured animal and bacterial RNA virus rates (Fig. 1).

111 Plant RNA viruses may indeed have lower mutation rates than animal RNA viruses, but
112 the existing data show that they are not substantially lower, and some of the differences could
113 result from methodological dissimilarities. In fact, some plant pathogens do mutate faster than
114 animal RNA viruses: plant viroids, for instance, have the highest per-base mutation rate yet
115 measured for any disease-causing agent at $2.5x10^{-3}$ per base per round of replication (61) (Fig.
116 1). Despite this highest per base mutation rate, viroids seem to obey the constant of around one
117 mutation per replicated genome reported by Drake et al., (36) for most RNA viruses. This fairly
118 constant per-genome mutation rate suggests that RNA genome sizes are limited by their
119 individual per-base mutation rates. Otherwise, a larger genome replicated with a given per-base
120 mutation rate, for example, a closterovirus with a per-base mutation rate of a viroid, would be

121 unable to maintain functional elements because of the accumulation of too many mutations in its
122 genes. Thus, a population of such larger genomes would collapse due to **lethal mutagenesis**
123 (17). Recombination may play a significant role in the virus survival by reducing the amount of
124 deleterious mutations incorporated in the same virus genome.

125 Viroids are the smallest known pathogenic agents of plants and cause diseases of
126 considerable economic importance (33, 34). Viroid genomes are composed of a single-
127 stranded, self-complementary RNA molecule of 246 to 475 nucleotides. Viroids lack the
128 capacity to code for proteins, are not encapsidated, and are replicated by host-encoded
129 polymerases (32). They are classified into two families: those that replicate in the nucleus
130 (*Pospiviroidae*) and those that replicate in the chloroplast (*Avsunviroidae*) (29). The most
131 abundant Pospiviroids are characterized by the presence of a central conserved region,
132 absence of hammerhead ribozymes, and nuclear replication via an asymmetric rolling circle by
133 a nuclear DNA-dependent RNA polymerase II. Avsunviroids are characterized by the absence
134 of a central conserved region, the presence of a hammerhead ribozyme, and replication in the
135 chloroplast via a symmetric rolling circle by another nuclear-encoded chloroplast DNA-
136 dependent RNA polymerase. Whereas pospiviroids are predicted to be primarily rod-shaped,
137 avsunviroids are predicted to be more highly branched structures. Thus, viroid molecules are a
138 collection of structural-sequence motifs that interact with host components for viroid replication,
139 processing, transport, and pathogenesis that may all influence viroid evolution (153).
140 Avsunviroids populations appear to have more haplotypes than pospiviroids (26). It has been
141 estimated that the mutation rate of avsunviroids is 10-fold larger than for pospiviroids (41, 46).
142 Apparently, most mutations in the rod-like structure of pospiviroids are deleterious. In fact, many
143 stable mutations of viroid genomes map in loops or as compensatory mutations in hairpins and
144 stems (8, 30).

145 Therefore, how viroids achieve the highest mutation rates among the known infectious
146 RNAs observed in nature is still a matter of speculation. Viroids are replicated by DNA-
147 dependent RNA polymerases with variable proofreading efficiencies. Even more, when these
148 enzymes use RNA instead of native DNA as template, their replicative fidelity could be further
149 reduced. Another putative mechanism, by which their mutation rates could be elevated above
150 that of the polymerase error, and it could also happen to viral genomes, is through enzymatic
151 changes to nucleotide bases. Cytidine deaminases are enzymes that turn cytidine (C) into
152 uridine (U), and mammals use a family of them (APOBEC) as anti-viral defense against some
153 animal viruses (73). Plants have orthologous proteins that have known roles in post-
154 transcriptional modification, and they are most active in mitochondria and chloroplasts (24). It
155 may be the case that these plant enzymes can be active on virus and viroid RNA as well, which
156 would increase C→U substitution rates. Other deaminases increase substitutions such as A→I
157 (adenosine to inosine). Patterns of frequent cytosine deamination have also been detected
158 during ssDNA geminivirus evolution (37). This could be due to these enzymes reacting with
159 single-stranded DNA viral genomes, but C→U is also the most common kind of spontaneous
160 chemical degradation that can occur on unpaired nucleotides so cytosine transitions could be
161 increased solely because geminiviral DNA is frequently single-stranded.

162 Substantial virus and viroid diversity is generated in plants also by homologous and
163 heterologous recombination. Pathogens often co-infect the same plant, allowing co-infection of
164 single cells (102), and some viruses frequently take the opportunity to unequally exchange
165 genes (23, 66, 87). While recombinants mostly appear to be tolerated between conspecific
166 viruses, intergenus recombination is also possible, and recombination can even lead to
167 incorporation of host genetic material. Recombination rates are difficult to measure, but could be
168 as high as mutation rates: BMV could exhibit an homologous crossover event per RNA
169 molecule per replication cycle (139), CaMV has an estimated recombination rate of around

170 $2x10^{-5}$ per round of replication (59). It has been proposed that recombination may have led to
171 the emergence of mosaic sequences from viroids co-infecting the same host (74, 106).
172 Recombinants have been associated with altered host range and virulence (107). One clear
173 example of a virulent recombinant was a hybrid of EACMV and ACMV that overcame crop
174 resistance to ACMV and decimated cassava production in Uganda in 1997 (113).

175 ***Plant viruses evolve quickly***. The speed of evolution can be estimated by the rate
176 at which the genetic makeup of a population changes in time by selection and/or genetic drift.
177 Representatives of the RNA families *Potyviridae* (67, 127), *Tobamoviridae* (108), and a
178 sobemovirus (52), and the ssDNA families *Geminiviridae* (37, 38, 75) and *Nanoviridae* (70)
179 evolve faster than 10^{-5} fixed nucleotide substitutions/site/year (s/s/y), and as high as 10^{-3} s/s/y.
180 There does not appear to be a distinction between the evolution rate of plant versus animal
181 viruses (39, 65), and between RNA and ssDNA viral substitution rates in plants: all plant viruses
182 appear to be fast-evolving.

183 These results were initially at odds with the plant virus evolution literature, which
184 stressed overall genetic stability over time, especially in comparison to viruses of animals with
185 adaptive immune systems (63, 66, 149). Indeed, substantial purifying selection exists for plant
186 viruses to maintain nucleotide or amino acid sequence that reduce nucleotide substitution rates.
187 One well-established source of purifying selection is the alternation in selective pressures that
188 vectored viruses experience when cycling between animal and plant cells. The capsid proteins
189 of these viruses, which interact with both host and vector, are under more pressure to be
190 unchanging, as evidenced by very low ratios of the rate of nonsynonymous changes to the rate
191 of synonymous changes (d_N/d_S) (22). This purifying selection also leads to lower average rates
192 of capsid gene evolution in vectored animal viruses than in directly transmitted viruses (79). Still,
193 the measured and estimated high nucleotide substitution rates of plant viruses occurs in
194 presence of purifying selection, and the evolutionary rate of vectored plant viruses is within the

195 same order of magnitude to some directly transmitted viruses. But an important point to stress is
196 that these rates of evolution are usually calculated over many years, if not decades, and reflect
197 an average nucleotide substitution rate. As will be shown in a subsequent section, rates of
198 mutation fixation can be higher when viruses are under **positive selection**, such as when they
199 are adapting to a novel host plant and several beneficial mutations may become ascendant in a
200 short period of time.

201 ***Variability sometimes leads to adaptability***. The average mutation and the
202 average recombination event are deleterious, and many are lethal. Around 70% of mutations
203 are deleterious (20, 35, 122). Consequently, variants that are produced are not always
204 maintained in viral populations. However, some of those changes that are deleterious in the
205 current host and environment may be adaptive under different conditions (40, 53). Mutations
206 that are deleterious in the current host will be maintained in a population if they are either mildly
207 deleterious (nearly-neutral), if they are continually created by new mutational events, or at a
208 ratio of these two factors called the **mutation-selection balance**.

209 The mutation-selection balance is one, but not the only factor determining whether a
210 mutation will be part of a virus population. **Complementation** between co-infecting viruses can
211 result in viral genomes carrying deleterious, even lethal, mutations to be maintained in
212 populations (60). Plant viruses can even develop defective interfering genomes that require at
213 least one essential component or function be supplied in *trans* by another virus genome (90).
214 Viral proteins that generally are shared among particles are involved in coating, cell-to-cell
215 movement, and suppression of gene silencing, but many other proteins may operate in *trans*
216 exclusively in some virus species. Defective interfering genomes are distinct from satellite
217 viruses, which rely on complementation but do not descend from the helper virus. Many
218 satellites attenuate the effects of the primary virus infection (128), but a minority can increase
219 virulence, such as the beta satellites of ssDNA begomoviruses (16). Similarly, defective

220 interfering genomes modulate infection severity, often competing with the complementing virus
221 and reducing virulence (111, 128).

222 Maintaining population variability is what allows for the chance encounter between a
223 novel environment and a mutation that is beneficial in that environment, such as a host range
224 mutation. However, high variability does not necessarily indicate great adaptive potential. For
225 instance, genetic **robustness** is based on a number of buffering mechanisms (including genetic
226 redundancy and cellular chaperones) that minimize mutational effects (44). Since plant viruses
227 and viroids mutate so frequently, and the vast majority of mutations are deleterious, robustness
228 is often a successful evolutionary strategy. The empirical demonstration of robustness operating
229 in an infectious RNA was provided by Codoñer et al. (26). They designed a co-infecting
230 competition experiment for this purpose. In this experiment, slow replicating and highly
231 heterogeneous populations of an avsunviroid outcompeted fast replicating and relatively
232 homogeneous pospiviroid populations only when the mutation rates of both viroids were
233 artificially increased by UV irradiation. Thus, under such conditions, the highly heterogeneous
234 viroid populations were more adapted. Whether the increased robustness in the avsunviroid is
235 due to its more relaxed RNA secondary structure or its higher genetic heterogeneity is still
236 unknown.

237 Eventually, even robust genomes will show effects due to the numerous mutations
238 accumulated in their genomes, but it is in unpredictable ways. The accumulated mutations may
239 together cause large drops in fitness, even though some individual genotypes may be adaptive
240 in the current host (18). Additionally, robustness is often specific to a virus in a particular
241 environment, shifting to another host can cause plant viruses to be less robust, and show the
242 effects of their accumulated genetic diversity and subsequent mutations. The relationship
243 between robustness and the ability to evolve and adapt, is complex and murky (99). Further
244 complicating the practical interpretation of viral and viroid genetic diversity, robustness does not

245 explain all examples where greater variability fails to lead to greater adaptability. Despite the
246 greater robustness of an avsunviroid mentioned above, members of this family generally infect
247 fewer hosts and appear to host-shift less often and have more restricted host ranges than
248 pospiviroids (41). Therefore, more research is required into the evolution of a wide variety of
249 plant subcellular pathogens to understand the interplay between genetic heterogeneity of the
250 population and adaptation.

251

252 **EPIDEMIOLOGICAL DYNAMICS OF VIRUS POPULATIONS**

253 A premise in virus evolution is that the dynamic genetic structure of virus populations
254 has a significant role in virulence, epidemiological progression of the disease, and host shifting
255 among other biological properties of viruses (76). The mechanisms that regulate the genetic
256 structure (i.e., number and frequency of haplotypes, and genetic distances among them) of virus
257 populations and their biological relevance are presented in this section.

258 ***Dynamics of the INTRA-host genetic structure of plant virus***
259 ***populations***. Theoretically, the structure of an active virus population changes within a host
260 individual during the course of a systemic infection. Despite the random emergence of
261 spontaneous mutations, these structural changes could exhibit deterministic or stochastic
262 behavior (118). The former has been observed primarily in compatible virus-plant interactions
263 (i.e., between virulent pathogen and susceptible host), whereas stochastic population structures
264 are more frequently generated in incompatible interactions (i.e., avirulent pathogen and resistant
265 host) or during viral host adaptation. Intra-host populations of TMGMV, despite their
266 heterogeneous composition, exhibit high genetic stability in field infections of *Nicotiana glauca*
267 (55, 103, 115). High genetic stability also appears to be the norm in several other plant viruses
268 infecting their compatible hosts (reviewed in by 63). This relative genetic stability suggests that

269 virus populations might not undergo substantial changes while they are interacting with their
270 natural host genotype. Purifying selection affecting the d_N/d_S ratio in the order of 0.01 to 0.31
271 has been found to operate in highly adapted virus populations, maintaining them in equilibrium
272 (62). The mutation frequency of plant viruses are influenced by host species (124). Recently, a
273 comparative bioassay of BNYVV infecting compatible versus incompatible host genotypes
274 demonstrated that more variability exists in small BNYVV populations from partially resistant
275 than in large populations produced in susceptible hosts (1). These data agree with the high
276 genetic stability of BNYVV prevailing worldwide during long periods in susceptible sugar beet
277 cultivars and the sudden stochastic diversification of BNYVV observed after the deployment of
278 resistant genotypes in the field (2, 85). Similarly, for other plant viruses, higher diversity has
279 been recorded at their centers of origin, where the plant virus presumably initiated its adaptation
280 to a new host (51, 104, 133).

281 Without external input variation by superinfections, the increase in the intra-host genetic
282 diversity that some plant virus populations experience under restrictive host conditions is most
283 likely the result from deviations of the mutation-selection balance. Accumulating evidence
284 indicates that mutation rate, rather than an invariable property of the virus, may fluctuate in
285 response to changes in viral replicase and specific cellular conditions (39, 47). For instance, the
286 terms mutators and antimutators have been coined for individuals within a species that have an
287 inheritable higher or lower mutation rate than the wild type, respectively (95). They have been
288 discovered in bacteria, bacteriophages, and human viruses (reviewed in 129). Individuals
289 encoding each one of these mutational phenotypes might coexist in the same intra-host
290 population and, under some conditions, one will eventually predominate. Although neither a
291 plant virus nor a viroid isolate has exhibited mutator or antimutator phenotypes, greater than
292 usual mutation frequencies have been detected for PVY and BNYVV only under restrictive host
293 environments (1, 4).

294 Once mutations have been introduced in a virus population, their frequency is regulated
295 by their interaction with other existing mutations. Generally speaking, the interaction between
296 genetic loci is known as **epistasis**. Epistasis can be antagonistic or synergistic depending on its
297 effect on fitness (76). Antagonistic epistasis means that two deleterious or beneficial mutations
298 carried in a given genome can be, respectively, not as bad or as good in combination as
299 expected by combining their individual fitness effects. Synergistic epistasis, on the other hand,
300 enlarges the total fitness effect of the interacting mutations. Given the extreme genome
301 compactness, gene overlapping, and lack of genetic redundancy, most mutations in virus
302 genomes show antagonistic epistasis (20). Coincidently viral genome architectures that favor
303 antagonistic epistasis are less robust to spontaneous mutations than those with greater
304 synergistic epistasis (44). *Trans* complementation and **interference** also regulate the frequency
305 and prevalence of mutants into the infecting virus population (60). The genetic structure that
306 results from the combined action of all these interactions, rather than specific genotypes
307 composing the population, could be the target of selection.

SANTIAGO ELENA F…, 4 18, 2011 12:33
Comentario [1]: Creo que así queda un poquito más claro.

308 ***Dynamics of the INTER-host diversity of virus populations***. The largest
309 amount of genetic data available on plant virus populations is for the inter-host level. The data
310 could be from individual or pooled plant samples and, typically, it is represented by consensus
311 sequences of a specific region of the virus genome. One of the most striking observations
312 derived from these data is the apparent differentiation between fully host-adapted and host-
313 adapting virus populations. In both of these types of compatible virus-plant interactions, viruses
314 reach high titers in infected plants, but in host-adapted populations the genetic diversity among
315 isolates is several orders of magnitude lower than in host-adapting populations. The genetic
316 diversification of WSMV can be used as a reference point for the rate of plant virus evolution.
317 Phylogenetic analysis of 54 WSMV field isolates from North America suggests that they arose
318 from a common ancestor introduced nearly a hundred years ago. The number of segregating

319 polymorphic sites in this representative population was 0.047 and the mean pairwise
320 **nucleotide diversity** (**π**) 0.020 (130). Phylogeographic studies of RYMV infecting rice crops in
321 Africa pinpointed the center of origin of RYMV to eastern Tanzania from where it migrated
322 northwest up the west coast of Africa (51). The largest RYMV inter-host π per Km^2 occurs in
323 Tanzania (i.e., around 200 times greater than in any other region of the continent) without a
324 correlation with the diversity of its potential host species (114). Therefore, it is likely that the high
325 RYMV diversification in Tanzania may have resulted from disruptions of the mutation selection-
326 balance that initially took place at the intra-host level and then among adapting isolates.
327 Similarly, around 240 times greater BNYVV nucleotide diversity was detected between RB
328 variants recently emerging in the Imperial Valley of California (π = 0.0024) than wild type
329 isolates collected nationwide (π = 0.00001) (3). Other factors that could be correlated with the
330 magnitude of inter-host virus diversity are the genetic diversity of the host, frequency of
331 superinfections, transmission mechanisms, etc. However, other than greater virus diversity
332 detected in sexual than asexual host genotypes (105) the effects that those variables may have
333 on plant virus populations apparently have not been explored.

334 ***Relationships between INTRA- and INTER-host virus diversity***. Except for
335 those viruses that mechanical inoculation is part of their mode of transmission in the field (11,
336 116), limited data exists concerning the intra- and inter-host genetic structure of vector-
337 transmitted virus populations. These are some of the few available examples where the
338 population was not mechanically inoculated prior to the analysis, which may disturb the natural
339 structure. The π value of whitefly-transmitted isolates of CVYV was around 0.0005 among
340 clones from two single-plant populations and 3.4 times greater among 56 consensus sequences
341 (78). Similarly, Vives et al. (142) found that the intra-host π of CLBV in 37 citrus trees naturally
342 infected (unknown vector) in a region of Spain, was three to four times lower than the inter-host
343 π. Theoretical analyses indicate that greater genetic diversities between (i.e., π_B) rather than

344 within (i.e., π_W) single-plant populations is favored by random genetic drift during virus
345 propagation (62). If genetic drift occurs more frequently at suboptimal virus fitness because π_W
346 is higher than at optimal fitness, then, it is expected that the π_W/π_B ratio of virus populations will
347 be related to virus fitness as described in Figure 2. In this way, the ratio π_W/π_B could reflect the
348 level of viral host adaptation. Initial support for this model has been obtained by comparing the
349 nucleotide diversities of BNYVV populations from composite samples of resistant *Rz1* sugar
350 beets (3). Pooled samples apparently are more reliable for this type of analysis because they
351 minimize the intrinsic plant-to-plant variation. This analysis revealed that, while BNYVV titers are
352 10^2 to 10^4 times higher in symptomatic plants infected by emerging RB variants than
353 asymptomatic plants infected by avirulent virus populations, the opposite occurs in relation with
354 the π_W/π_B ratio. It was 7.8 to 12.2 times larger in asymptomatic than symptomatic resistant
355 plants (i.e., π_W/π_B = 6.1 to 16.7 versus 0.5 to 2.1, respectively).

356 ***Population bottleneck as an additional modulator of genetic diversity***.
357 Sustaining variation is a particular challenge for plant viruses because severe genetic
358 bottlenecks exist as vectored viruses move from plant to plant and even within plants.
359 Population bottlenecks can occur during vector inoculation, cell-to-cell movement, vascular
360 access, vascular transport, vascular exit, specific tissue entry (i.e., lateral roots, endosperm,
361 meristems, etc.), vector acquisition, viruliferous vector migration, and alternate host infections
362 (57, 88). These conditions create spatial structure in how the virus diversity is distributed
363 throughout the plant and the field. Within each new plant or portion of a plant, a viral population
364 regains diversity as it multiplies from a small initial number of infecting genomes. After repeated
365 host-to-host transfers of WSMV, similar numbers of haplotypes were found whether one or two
366 strains initially infected the plant (58). Thus, bottleneck size during virus transmission is not
367 always correlated with the extent of regained variability in the derived population.

368 Throughout the life cycle of a plant virus, at any bottleneck event, the virus survival and
369 its population structure are especially vulnerable. The **multiplicity of cellular infection** (**MOI**)
370 has been developed as a way of estimating the number of virus genomes that invade a plant
371 cell during the course of local and systemic virus infections. The first reported MOI of a plant
372 virus was provided by González-Jara et al. (69). They estimated that approximately six TMV
373 genomes initially infect *Nicotiana benthamiana* cells. Then, the MOI decreases to one to two
374 genomes during the systemic infection process suggesting the involvement of mechanisms
375 inhibiting superinfection at the advanced stages of the disease. Similarly, MOI of five to six
376 genomes were estimated for SBWMV causing localized leaf infections in *Chenopodium quinoa*
377 (101). MOI values around four, with a maximum of 13 during the acute phase of systemic
378 infection, were calculated for CaMV infecting its natural host, *Brassica rapa* (72). In general, the
379 estimation of cellular MOI requires viral genomes carrying specific neutral mutations or reporter
380 genes to monitor the frequency and location of single and mixed infected cells. Newly generated
381 mutant genomes that may have derived from the inoculated transcripts are not considered in
382 these calculations, neither are virus genomes that may have been silenced or partially
383 expressed. Therefore, MOI values, rather than being an absolute number, may represent a
384 fraction of a larger and still unknown number of viral genomes per cell.

385 Theoretical analysis of the data presented above indicates that bottleneck size is critical
386 in preserving the parental population structure including both adaptive and defective mutants.
387 For instance, in virus populations where lethal mutants can only be maintained by *trans*-
388 complementation by functional virus genes, the chances that these selfish mutants will
389 predominate in the following generation are greater with broader bottlenecks because it
390 increases the probabilities of co-infecting with fitted virus genomes (101). With narrow
391 bottlenecks, on the other hand, only fit genomes will infect most of the cells and consequently
392 the genetic diversity is expected to decrease by leaving behind defective mutants. For mutants

393 that can replicate by themselves, however, the opposite outcome is more likely, i.e., the genetic
394 diversity is expected to increase at smaller bottleneck sizes because low frequency neutral or
395 near neutral mutants have better chances of moving forward by random drift and be able to
396 infect neighboring cells by themselves. With broader bottlenecks, by contrast, the parental
397 population structure has more probabilities of being reproduced in most of the infected cells.

398 The impact that different levels of intercellular bottleneck may have on the genetic
399 structure of ensuing virus populations has not been empirically determined, but coincidental
400 data suggest that it could be extremely important for disease management. For instance, two
401 resistant tobacco genotypes with the same barrier to PVY cell-to-cell movement but different
402 levels of cellular virus accumulation exhibit drastic differences in the incidence of spontaneous
403 RB infections (4). The tobacco with the weak resistance durability, NC745, has larger PVY
404 populations in the initially infected cells than the near immune tobacco VAM. Consequently, the
405 proportion of particles moving from one cell to the next is smaller in NC745. Thus, although the
406 absolute number of moving viral particles might be the same in both tobacco genotypes the
407 proportions are not. This observation suggests that relative rather than absolute MOI values
408 could be a more reliable estimation of the effect of bottlenecking on plant resistance durability.

409

410 **THE SYSTEM BIOLOGY APPROACH**

411 Systems Biology (SB) allows assessing gene expression at a genome-wide scale,
412 providing unprecedented views of the virus-host interaction. SB deals with the study of
413 interactions between components of biological systems and how these interactions give rise to
414 the function and behavior of the system (14, 84).

415 To complete their infectious cycle, the few viral components must establish multiple and
416 complex interactions among them (54, 71, 89, 138) as well as with a large number of

417 components from the host (13, 132, 146). These interactions result either in the plant controlling
418 the infection or in the virus overcoming defenses and establishing a systemic infection. Indeed,
419 the recent application of the SB approach to the analysis of virus-host interactions has revealed
420 a more complete picture of the sets of host factors required for virus infection (86, 146).
421 Moreover, SB has uncovered highly connected host genes that operate as central elements in
422 the plant regulatory network and are specifically targeted by viruses to control the host
423 metabolism (19, 28, 42). Additionally, SB has evidenced topological changes of the intra-viral
424 interaction network that are caused by its integration within the host network (93, 137).

425 While the SB approach has been increasingly used in the analysis of animal virus-host
426 interactions (e.g. hepatitis C, human immunodeficiency, yellow fever, influenza A, or
427 herpesviruses), plant virology has not yet benefitted to the same extent, and the most relevant
428 studies in the field generally apply transcriptomic techniques to generate lists of genes with
429 altered mRNA abundance in infected plants. However, the proper network analysis of virus-
430 plant interaction is still a pending task (45).

431 ***Different viruses, common targets.*** Although some studies have analyzed
432 changes in mRNA profiles resulting from viruses infecting their natural hosts, such as ACMV
433 infecting cassava (56) or RYMV infecting rice (141), *Arabidopsis thaliana* has been extensively
434 used as a model host in combination with viruses belonging to different taxonomic families
435 (Table 1). However, even using the same host species, direct comparison across experiments is
436 not straightforward because differences in profiling techniques and platforms, plant ecotypes,
437 sampling schemes, inoculation conditions and dosages, and environmental variables may all
438 exert some unpredictable effects on the expression pattern of multiple genes.

439 Whitham *et al*. (145) carried out the most comprehensive of such studies, including five
440 viruses mentioned in Table 1 (CMV, ORMV, PVX, TVCV, and TuMV) while keeping all other

441 experimental variables and techniques constant. Some generalities were drawn from this study
442 that are extensible to most of the other studies listed in Table 1. First, approximately one-third of
443 over-expressed plant genes are associated with cell responses to situations of stress, defense
444 against infection, apoptosis, programed cell death, and ageing. Second, defense-like responses
445 of *A. thaliana* to viruses are dependent on salicylic acid (SA) and require upstream signaling
446 components (148). Third, a spectrum of heat-shock proteins (HSP) is also induced after
447 infection with all viruses by a yet unknown SA-independent mechanism. HSP over-expression
448 may be a generic unspecific response of the plant to stress or, alternatively, directly triggered
449 and controlled by viruses to assist the right folding of their own proteins, many of which may be
450 misfolded (and thus aggregating) as a consequence of mutations produced during replication
451 (81). Fourth, cell wall modification genes are preferentially down-regulated. Because the
452 expression of these genes is correlated with plant cell growth and expansion, their reduced
453 expression may well result in the stunting syndrome associated with some infections. Fifth,
454 similarly, plastid genes and genes involved in chloroplast functioning are also preferentially
455 down-regulated, resulting in chlorotic symptoms. Sixth, ribosomal proteins and protein turnover
456 genes are up-regulated. This may either reflect an increased demand on the cells for protein
457 synthesis or a response triggered by viruses to enhance its own replication.

458 ***Host-adaptation and changes in gene expression profiles.*** The actual
459 interactions between viruses, natural hosts, and vectors are the results of natural selection
460 operating during many generations. Hence, to have a precise description of the interactions
461 established between viral and cellular components, it is necessary to take into account the
462 evolutionary perspective of the process: the degree of adaptation of the virus to its host.
463 Unfortunately, this evolutionary perspective has not been taken in most studies listed in Table 1:
464 only TuMV, CMV and CaMV are prevalent in wild *A. thaliana* populations (109).

465 To test whether adaptation to a host may result in changes in mRNA profile, Agudelo-
466 Romero *et al*. (6) performed an evolution experiment adapting TEV to the susceptible ecotype
467 L*er*-0 of *A. thaliana* (25). The TEV clone used as the ancestral virus was able of systemically
468 infect L*er*-0 plants although the infection was asymptomatic. After 17 serial passages, the
469 resulting virus (labeled as TEV-*At*17) accumulated three orders of magnitude more than the
470 ancestral one per gram of infected tissue, its infectivity was 100% (compared with the low 10%
471 of the ancestral genotype) and induced severe symptoms including stunting, etching, and leaf
472 malformation. A single amino acid substitution in the VPg was enough to trigger these
473 symptoms. TEV-*At*17 infection caused the differential expression of a total of 505 up-regulated
474 and 1335 down-regulated plant genes relative to its ancestor virus (Figure 3 in 6). Both viruses
475 also differentially affected the expression of transcription factors, with 51 up-regulated and 84
476 down-regulated only by the evolved virus. Interestingly, only genes up-regulated by the
477 ancestral virus and unaffected by the evolved virus were significantly enriched in categories
478 related to plant responses to different abiotic and biotic stresses, including systemic acquired
479 resistance and activation of the innate immune resistance.

480 At face value, the above results support the hypothesis that by adapting to a host,
481 viruses should change and improve the way they interact with the components of the host cell
482 transcriptional network. Therefore, studies of virus-plant interactions should concentrate on
483 naturally coevolved pairs rather than *ad hoc* pairs. While keeping in mind this concern, global
484 profiling experiments will allow identifying sets of genes that are essential for the replication of a
485 given virus, but also other sets that may be required for closely relative viruses and even for
486 unrelated viruses. Furthermore, if all these genes are placed into the context a host regulatory
487 networks, we may identify pathways, rather than individual genes, that may be targets of
488 intervention for therapeutics without undesired side effects.

489

490 **WHOLE VIRUS GENOME ANALYSIS: CASE STUDY OF CTV**

491 Repeated infections of perennial hosts often result in mixed infections by multiple strains
492 of the same virus or related viruses (7, 92, 110, 131, 143, 144). This occurs, to a lesser extent,
493 in annual crops as well (31, 98, 112). Natural viral complexes create an environment that is
494 conducive to high frequencies of recombination, and consequently appearance of an enormous
495 collection of genetic variants that have potentials to evolve into novel genotypes or strains. In
496 addition, functional complementation of viral proteins in such an environment may nurture
497 mutations that could have been negatively selected in single infections. Studying such complex
498 systems may require novel experimental approaches. An example of these approaches is the
499 analyses of whole genome CTV complexes by high throughput genomic sequencing of viral
500 populations (144, 150). These analyses have revealed detailed, genome-wide information on
501 virus recombination, mutation, and evolution.

502 CTV, one of the largest plant viruses, is encoded by a positive sense single-stranded
503 RNA molecule of 19.2 to 19.3 kb. Its transmission is through vegetative grafting and by aphids
504 in a semi-persistent manner. Its natural host, citrus, is propagated by budwood grafting and has
505 longevity of more than one hundred years. Repeated transmissions by aphids and vegetative
506 propagation have resulted in an increase in the complexity of CTV populations over hundreds of
507 years. The initial infection may have originated from a single strain, but subsequent infections by
508 different strains occurred during the long history of human cultivation of citrus species, resulting
509 in co-existence of multiple strains (genotypes) in a single host. Similar scenarios of viral
510 accumulation and consequently the existence of viral complexes with multiple strains are
511 common in other viruses that persistently infect perennial, long living trees (80). Within CTV
512 complexes, promiscuous recombination between genomes occurred at remarkably high rates
513 (143, 144). Recent data also suggests extraordinary stability and low mutation rates of CTV
514 (Weng, Z., Dawson, W. O., and Xiong, Z., unpublished). These lead to hypothesis that the high

515 level of promiscuous RNA recombination compensates for the extreme genome stability and low
516 mutation rate of CTV, and functions as a major force driving the production of genetic variants
517 important for adaptation and evolution. These variants can be selected upon in a new
518 environment and can potentially evolve to become an emerging viral strain. This hypothesis
519 perhaps explains the origin of the sequenced CTV SY568 genome (152), which consists of
520 mosaic sequences from a severe and mild strains (143).

521 To study these complexes, a high density CTV re-sequencing microarray was designed
522 to simultaneously re-sequence multiple genotypes in CTV populations with high accuracy (Fig.
523 3) (144, 150). A large number of natural isolates and single-aphid transmitted isolates have
524 been analyzed using this array. Nearly all of the isolates, even some single-aphid-transmitted
525 isolates, were found to contain more than one strain (144). The re-sequencing microarray
526 provided direct visual identification of multiple components in a mixed infection and at the same
527 time re-sequenced the predominant viral sequences in the complex.

528 This whole genome strategy further showed that CTV complexes comprised one or more
529 predominant genotypes, with one or more genotypes as minor components. For example, a
530 severe stem-pitting isolate from Florida, FL278, contains a predominant T30-like strain. T30 is a
531 mild strain that causes little or no symptoms and does not cause significant economic damage
532 (9), which does not agree with the severity of the disease observed in the source plant. Further
533 analysis using the CTV re-sequencing microarray and real-time PCR revealed a minor genotype
534 (<1% of the population) that resembles a type T36 strain (Table 2), a quick-decline strain that is
535 commonly associated with rapid death of trees on sour orange rootstock (82). The presence of
536 this unusual CTV strain raises a possibility that the T36-like strain may in fact be a contributor to
537 the observed stem-pitting symptoms. This example illustrates that a minor component in a CTV
538 complex can play a significant role in pathogenesis. A thorough knowledge of the genetic

539 composition within a CTV isolate is therefore critical to understand the interaction among
540 different genotypes in a disease complex and their roles in disease development.

541 Evidence for CTV recombination has been documented before (83, 97, 119, 143). As
542 high as 4% of cloned viral genome fragments have been found to be recombinants between two
543 co-infecting strains (143). However, these studies examined only select regions of the large
544 CTV genome. When the recombination analysis is extended to the entire CTV genome by whole
545 genome sequencing analysis, the scale and the degree of promiscuous recombination between
546 co-infecting strains are even more astonishing. For instance, a natural field isolate, FS2-2,
547 contains a CTV complex harboring three distinct strains that are visually identifiable in the
548 hybridized re-sequencing microarray (144) (Fig. 3). A genome-wide 454 sequencing analysis of
549 FS2-2 revealed that a large number of 454 sequencing reads (5%) were recombinants, despite
550 the fact that these sequences were relatively short, with an average length of 256 nucleotides.
551 The recombination events were throughout the entire genome, with the most active
552 recombination occurring toward the 3' half of the CTV genome. FS2-2 contains at least three co-
553 infecting strains: T30, T36, and VT. Promiscuous recombination occurred among all the
554 identified strains. A deeper analysis of 1 kb genomic fragments, sequenced by the traditional
555 Sanger method, corroborated the high level of recombination activity in the FS2-2 complex. A
556 surprisingly large percentage (17.9%) of the cloned molecules was found to be recombinants
557 between the three constituent strains. Additionally, four recombinants possessed two crossover
558 sites, resulting from either a double-crossover or two independent recombination events (144).
559 Further divergence of some recombinants after recombination was also evident in this study.
560 Thus, this data suggests that promiscuous, intergenic recombination can generate a large
561 amount of genetic variants, which could subsequently diverge and evolve to distinct CTV
562 genotypes.

563 An interesting phenomenon in mixed infections of CTV is the lack of apparent cross
564 protection and interference between multiple strains. Even though the 3' halves of multiple CTV
565 genomes within an infected plant share 90% or higher sequence identity, they replicate
566 independently and do not seem to interfere with each other. The inability of the predominant
567 mild T30-like genotype in the above FL278 example to mitigate the effect of the severe T36-like
568 genotype illustrates this failed cross-protection. Nevertheless, successful cross-protection using
569 mild isolates can be achieved (140). Therefore, it is plausible that only strains with highly similar
570 sequences across the entire genome protect against each other, or that in some cases the
571 powerful, three-component RNA silencing suppression system in CTV (50, 91) somehow
572 circumvents the effect of cross protection. Further genome-wide characterization of CTV
573 complexes and other plant viruses will likely shed light on this important yet puzzling biological
574 phenomenon, and provide guidance on effective implementation of cross protection and gene
575 silencing strategies.

576

577 **INTEGRATION INTO DISEASE MANAGEMENT STRATEGIES**

578 Theoretically, an assembly of measures that altogether reduce the **effective population**
579 **size** (N_e), increase the genetic diversity, and maximize the bottleneck effect could gradually
580 exclude a virus from its host species (i.e., virus exclusion = small N_e + large π + low MOI).
581 Unfortunately, the quantification of each one of these parameters is labor intensive, and the
582 magnitude required to obtain the expected effect have not been empirically determined. N_e is
583 generally lower than the census of the population, *N*, and requires co-inoculation experiments to
584 be estimated. On the other hand, *N* can be directly estimated by ELISA or real-time PCR and,
585 for practical purposes, it may follow the same trend than N_e in some virus-plant interactions. A
586 greater technological challenge is to develop a more feasible approach of estimating π than the

587 traditional cloning and sequencing. Technological advances such as next generation
588 sequencing systems (15) may provide part of the solution to this task. Significant advancement
589 has been made estimating bottleneck sizes at both ecological and intra-host levels, but these
590 calculations may still need to include the population proportions that represent the estimated
591 MOI. Plant resistance genes may affect N_e, π, or MOI in the desired way. However, few *R*
592 genes, other than *Rz1* and *Rz2* affecting π in sugar beet, have been quantitatively
593 characterized in relation with some of these parameters to predict resistance durability (21, 49).
594 Antivirals based on nucleotide analogs increase π enough to drive the population into lethal
595 mutagenesis (27), but their economic cost and environmental safety in agriculture still need to
596 be evaluated. More viable antiviral strategies may be those based on natural plant resistance
597 mechanisms such as gene silencing or those involving posttranscriptional enzymatic
598 modifications of virus nucleotides.

599 In addition to the genetic plant restriction of virus cell-to-cell and vascular movements,
600 bottleneck narrowing could be achieved by reducing the number of initial infection events to
601 which a plant or plant population is exposed. Traditionally, it has been assumed that the amount
602 of virus particles inoculated in a susceptible plant is epidemiologically irrelevant (57), but it might
603 not be true for some incompatible virus-plant interactions, mainly those where the frequency of
604 virulent genomes is constrained by intra-population interactions.

605 The host environment influences viral population variability by controlling co-infection
606 dynamics, which determine the potential for complementation and recombination (147).
607 Therefore, the role that alternate host reservoirs may have in the generation of virus variation
608 should be taken in consideration during disease risk assessments.

609 *Trans*-complementation among different versions of the same viral element expressed in
610 a common cellular compartment may improve virus robustness through direct reciprocity (e.g., a

611 defective transporter could benefit from an efficient replicator and vice versa) (136). A practical
612 consequence of modeling virus robustness is that, under mutagenic conditions imposed by the
613 host or external mutagens, some virus populations could be eradicated through lethal
614 mutagenesis whereas others would recover following the generation-selection of RB mutations.
615 Serial passage of wild type BNYVV through strongly resistant *Rz2* sugar beets caused an
616 increase of its genetic heterogeneity to such levels that, in some lineages, the virus infection
617 was gradually eliminated while in others its apparent robustness was improved (1).

618 The relationship between virus fitness and the π_W/π_B ratio describe in Figure 2 suggests
619 that, if a virus have reach the adapted stage without causing significant damage to the crop, it
620 most likely will persist in that condition for a long time. Therefore, engaging in a two-arm race
621 with the virus to eradicate the disease may not be a profitable strategy. In conclusion, plant
622 viruses and viroids have become important experimental systems for studying pathogen
623 evolution, and an increasing amount is known about their mutation, recombination, and
624 evolutionary dynamics. The ways in which populations of viruses interact with one or a number
625 of hosts has begun to be probed using high-throughput techniques, and is revealing how small
626 fractions of an infecting population might be driving symptom severity, how rapid viral adaptation
627 to a novel host leads to massive changes in host response to the virus, and how sometimes the
628 hosts that permit the lowest viral titers harbor the greatest viral population diversity. These
629 insights are helping to explain the field data, which often show very little change in viral
630 sequences despite large changes in symptoms, host range, and disease severity. Future work
631 will continue to expand our basic knowledge of phytopathology and direct applied research into
632 how best to control viral and viroid population diversity, effective population size and limit
633 pathogen spread.

634

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644

645 **LITERATURE CITED**

646

647 1. Acosta-Leal, R., Bryan, K. B., and Rush, C. M. 2010a. Host effect on the genetic
648 diversification of *Beet necrotic yellow vein virus* single-plant populations. Phytopathology
649 100: 1204-1212.
650 2. Acosta-Leal, R., Bryan, K. B., Smith, J. T., and Rush, C. M. 2010b. Breakdown of host
651 resistance by independent evolutionary lineages of *Beet necrotic yellow vein virus* involves a
652 parallel C/U mutation in its *p25* gene. Phytopathology 100: 127-133.
653 3. Acosta-Leal, R., Fawley, M. W., and Rush, C. M. 2008. Changes in the intra-isolate genetic
654 structure of *Beet necrotic yellow vein virus* populations associated with plant resistance
655 breakdown. Virology 376: 60-68.
656 4. Acosta-Leal, R. and Xiong, Z. 2008. Complementary functions of two recessive *R*-genes
657 determine resistance durability of tobacco 'Virgin A Mutant' (VAM) to *Potato virus Y*. Virology
658 379: 275-283.
659 5. Agudelo-Romero, P, Carbonell, P., De la Iglesia, F., Carrera ,J., Rodrigo, G., Jaramillo, A.,
660 Pérez-Amado, M. A., and Elena, S. F. 2008a. Changes in the gene expression profile of
661 *Arabidopsis thaliana* after infection with *Tobacco etch virus*. Virol. J. 5: 92.
662 6. Agudelo-Romero, P., Carbonell, P., Pérez-Amador, M. A., and Elena, S. F. 2008b. Virus
663 adaptation by manipulation of host’s gene expression. PLoS ONE 3: e2397.
664 7. Al Rwahnih, M., Daubert, S., Golino, D., and Rowhani, A. 2009. Deep sequencing analysis
665 of RNAs from a grapevine showing Syrah decline symptoms reveals a multiple virus
666 infection that includes a novel virus. Virology 387: 395-401.
667 8. Ambrós, S., Hernández, C., Desvignes, J. C., and Flores, R. 1998. Genomic structure of
668 three phenotypically different isolates of peach latent mosaic viroid: implication of the
669 existence of constraints limiting the heterogeneity of viroid quasispecies. J. Virol. 72: 7397-
670 7406.

671 9. Albiach-Martí, M. R., Mawassi, M., Gowda, S., Satyanarayana, T., Hilf, M. E., Shanker, S.,
672 Almira, E. C., Vives, M. C., Lopez, C., Guerri, J., Flores, R., Moreno, P., Garnsey, S. M., and
673 Dawson, W. O. 2000. Sequences of *Citrus tristeza virus* separated in time and space are
674 essentially identical. J. Virol. 74: 6856-6865.
675 10. Ascencio-Ibáñez, J. T., Sozzani, R., Lee, T. J., Chu, T. M., Wolfinger, R. D., Cella, R., and
676 Hanley-Bowdoin, L. 2008. Global analysis of *Arabidopsis* gene expression uncovers a
677 complex array of changes impacting pathogen response and cell cycle during geminivirus
678 infection. Plant Physiol. 148: 436-454.
679 11. Ayllón, M. A., Rubio, L., Moya, A., Guerri, J., and Moreno, P. 1999. The haplotype
680 distribution of two genes of *Citrus tristeza virus* is altered after host change or aphid
681 transmission. Virology 255: 32-39.
682 12. Babu, M., Griffiths, J. S., Huang, T. S., and Wang, A. 2008. Altered gene expression
683 changes in *Arabidopsis* leaf tissues and protoplats in response to *Plum pox virus* infection.
684 BMC Genomics 9: 325.
685 13. Bailer, S. M., and Haas, J. 2009. Connecting viral with cellular interactomes. Curr. Opin.ion
686 Microbiol. 12: 453-459.
687 14. Barábasi, A. L., and Oltvai, Z. N. 2004. Network biology: understanding the cell's functional
688 organization. Nat. Rev. Genet. 5: 101-113.
689 15. Bentley, D. R. 2006. Whole-genome re-sequencing. Curr. Opin. Gen. Develop. 16: 545-552.
690 16. Briddon, R. W., Bull, S. E., Amin, I., Idris, A. M., Mansoor, S., Bedford, I. D., Dhawan, P.,
691 Rishi, N., Siwatch, S. S., Abdel-Salam, A. M., Brown, J. K., Zafar, Y., and Markham, P. G.
692 2003. Diversity of DNA beta, a satellite molecule associated with some monopartite
693 begomoviruses. Virology 312: 106-121.
694 17. Bull, J. J., R. Sanjuán, R., and Wilkie, C. O. 2007. Theory of lethal mutagenesis for viruses.
695 J. Virol. 81: 2930-2939.

696 18. Burch, C. L. and Chao, L. 2000. Evolvability of an RNA virus is determined by its mutational
697 neighbourhood. Nature 406: 625-628.
698 19. Calderwood, M. A., Venkatesan, K., Xing, L., Chase, M. R., Vazquez, A., Holthaus, A. M.,
699 Ewence, A. E., Li, N., Hirozane-Kishikawa, T., Hill, D. E., Vidal, M., Kieff, E., and Johannsen,
700 E. 2007. Epstein-Barr virus and virus human protein interaction maps. Proc. Natl. Acad. Sci.
701 USA 104: 7606-7611.
702 20. Carrasco, P., de la Iglesia, F., and Elena, S. F. 2007. Distribution of fitness and virulence
703 effects caused by single-nucleotide substitutions in tobacco etch virus. J. Virol. 81: 12979-
704 12984.
705 21. Chain, F., Riault, G., Trottet, M., and Jacquot, E. 2007. Evaluation of the durability of the
706 Barley yellow dwarf virus-resistant Zhong ZH and TC14 wheat lines. Eur. J. Plant Pathol.
707 117: 35-43.
708 22. Chare, E. R. and Holmes, E. C. 2004. Selection pressures in the capsid genes of plant RNA
709 viruses reflect mode of transmission. J. Gen. Virol. 85: 3149-3157.
710 23. Chare, E. R. and Holmes, E. C. 2006. A phylogenetic survey of recombination frequency in
711 plant RNA viruses. Arch. Virol. 151: 933-946.
712 24. Chateigner-Boutin, A. L. and Small, I. 2010. Plant RNA editing. RNA Biol. 7: 213-219.
713 25. Chisholm, S. T., Mahajan, S. K., Whitham, S. A., Yamamoto, M. L., and Carrington, J. C.
714 2000. Cloning of *Arabidopsis* RTM1 gene, which controls restriction of long-distance
715 movement of *Tobacco etch virus*. Proc. Natl. Acad. Sci. USA 97: 489-494.
716 26. Codoñer, F. M., Daròs, J.-A., Sole, R. V., and Elena, S. F. 2006. The fittest versus the
717 flattest: Experimental confirmation of the quasispecies effect with subviral pathogens. PLoS
718 Pathog. 2: 1187-1193.
719 27. Crotty, S., Cameron, C. E., and Andino, R. 2001. RNA virus error catastrophe: Direct
720 molecular test by using ribavirin. Proc. Natl. Acad. Sci. USA 98: 6895-6900.

721 28. De Chassey, B., Navratil, V., Tafforeau, L., Hiet, M. S., Aublin-Gex, A., Agaugué, S.,
722 Meiffren, G., Pradezynski, F., Faria, B. F., Chantier, T., Le Breton, M., Pellet, J., Davoust,
723 N., Mangeot, P. E., Chaboud, A., Penin, F., Jacob, Y., Vidalain, P. O., Vidal, M., André, P.,
724 Rabourdin-Combe, C., and Lotteau, V. 2008. Hepatitis C virus infection protein network.
725 Mol. Syst. Biol. 4: 230.

726 29. Daròs, J.-A., Elena, S. F., and Flores, R. 2006. Viroids: and Ariadne's thread into the RNA
727 labyrinth. EMBO reports 7: 593-598.

728 30. De la Peña, M. and Flores, R. 2002. *Chrysanthemum chlorotic mottle viroid* RNA: Dissection
729 of the pathogenicity determinant and comparative fitness of symptomatic and non-
730 symptomatic variants. J. Mol. Biol. 321: 411-421.

731 31. Díaz-Pendón, J. A., Canizares, M. C., Moriones, E., Bejarano, E. R., Czosnek, H., and
732 Navas-Castillo, J. 2010. Tomato yellow leaf curl viruses: menage a trois between the virus
733 complex, the plant, and the whitefly vector. Mol. Plant Pathol. 11: 441-450.

734 32. Diener, T. O. 1971. Potato spindle tuber "virus". IV. A replicating, low molecular weight RNA.
735 Virology 45: 411-428.

736 33. Diener, T. O. 1989. Circular RNAs: relics of precellular evolution? Proc. Natl. Acad. Sci. USA
737 86: 9370-9374.

738 34. Diener, T. O. 2001. The viroid: biological oddity or evolutionary fossil? Adv. Virus Res. 57:
739 137-184.

740 35. Domingo-Calap, P., Cuevas, J. M., and Sanjuán, R. 2009. The fitness effects of random
741 mutations in single-stranded DNA and RNA bacteriophages. PLoS Genet. 5: e1000742.

742 36. Drake, J. W., Charlesworth, B., Charlesworth, D., and Crow, J. F. 1998. Rates of
743 spontaneous mutations. Genetics 148: 1667-1686.

744 37. Duffy, S. and Holmes, E. C. 2008. Phylogenetic evidence for rapid rates of molecular
745 evolution in the single-stranded DNA begomovirus *Tomato yellow leaf curl virus* (TYLCV). J.
746 Virol. 82: 957-965.

747 38. Duffy, S. and Holmes, E. C. 2009. Validation of high rates of nucleotide substitution in
748 geminiviruses: Phylogenetic evidence from East African cassava mosaic viruses. J. Gen.
749 Virol. 90: 1539-1547.
750 39. Duffy, S., Shackelton, L. A., and Holmes, E. C. 2008. Rates of evolutionary change in
751 viruses: Patterns and determinants. Nat. Rev. Genet. 9: 267-276.
752 40. Duffy, S., Turner, P. E., and Burch, C. L. 2006. Pleiotropic costs of niche expansion in the
753 RNA bacteriophage Phi 6. Genetics 172: 751-757.
754 41. Durán-Vila, N., Elena, S. F., Darós, J. A., and Flores, R. 2008. Structure and evolution of
755 viroids, p. 43-64. *In* E. Domingo, C. R. Parrish, and J. J. Holland (eds.), Origin and Evolution
756 of Viruses. Academic Press, London.
757 42. Dyer, M. D., Murali, T. M., and Sobral, B. W. 2008. The landscape of human proteins
758 interacting with viruses and other pathogens. PLoS Pathog. 4: e32.
759 43. Elena, S. F., Agudelo-Romero, P., Carrasco, P., Codoner, F. M., Martin, S., Torres-Barcelo,
760 C., and Sanjuán, R. 2008. Experimental evolution of plant RNA viruses. Heredity 100: 478-
761 483.
762 44. Elena, S. F., Carrasco, P., Darós, J. A., and Sanjuán, R. 2006. Mechanisms of genetic
763 robustness in RNA viruses. Embo Reports 7: 168-173.
764 45. Elena, S. F., Carrera, J., and Rodrigo, G. 2011. A systems biology approach to the
765 evolution of plant-virus interactions. Curr. Opin. Plant Biol. doi:10.1016/j.pbi.2011.03.013.
766 46. Elena, S. F., Gómez, G., and Daròs, J-A. 2009. Evolutionary constraints to viroid evolution.
767 Viruses 1: 241-254.
768 47. Elena, S. F. and Sanjuán, R. 2005. Adaptive value of high mutation rates of RNA viruses:
769 Separating causes from consequences. J. Virol. 79: 11555-11558.
770 48. Espinoza, C., Medina, C., Somerville, S., and Arce-Jonhson, P. 2007. Senescence-
771 associated genes induced during compatible viral interactions with grapevine and
772 *Arabidopsis*. J. Exp. Bot. 58: 3197-3212.

773 49. Fabre, F., Brouchou, C., Palloix, A., and Moury, B. 2009. Key determinants of resistance
774 durability to plant viruses: Inshights from a model linking within- and between-host
775 dynamics. Virus Res. 141: 140-149.
776 50. Fagoaga, C., Lopez, C., de Mendoza, A. H., Moreno, P., Navarro, L., Flores, R., and Pena,
777 L. 2006. Post-transcriptional gene silencing of the p23 silencing suppressor of *Citrus tristeza*
778 *virus* confers resistance to the virus in transgenic Mexican lime. Plant Mol. Biol. 60:153-165.
779 51. Fargette, D., Pinel, A., Abubakar, Z., Traoré, O., Brugidou, C., Fatogoma, S., Hébrard, E.,
780 Choisy, M., Séré, Y., Fauquet, C., and Konaté, G. 2004. Inferring the evolutionary history of
781 *Rice yellow mottle virus* from genomic, phylogenetic, and phylogeographic studies. J. Virol.
782 78: 3252-3261.
783 52. Fargette, D., Pinel, A., Rakotomalala, M., Sangu, E., Traoré, O., Sérémé, D., Sorho, F.,
784 Issaka, S., Hébrard, E., Séré, Y., Kanyeka, Z., and Konaté, G. 2008. *Rice yellow mottle*
785 *virus*, an RNA plant virus, evolves as rapidly as most RNA animal viruses. J. Virol. 82: 3584-
786 3589.
787 53. Ferris, M. T., Joyce, P., and Burch, C. L. 2007. High frequency of mutations that expand the
788 host range of an RNA virus. Genetics 176: 1013-1022.
789 54. Fossum, E., Friedel, C. C., Rajagopala, S. V., Titz, B., Baiker, A., Schmidt, T., Kraus, T.,
790 Stellberger, T., Rutenberg, C., Suthram, S., Bandyopadhyay, S., Rose, D., von Brunn, A.,
791 Uhlmann, M., Zeretzke, C., Dong, Y. A., Boulet, H., Koegl, M., Bailer, S. M., Koszinowski,
792 U., Ideker, T., Uetz, P., Zimmer, R., and Haas, J. 2009. Evolutionarily conserved herpesviral
793 protein interaction networks. PLoS Pathog. 5: e1000570.
794 55. Fraile, A., Malpica, J. M., Aranda, M. A., Rodríguez-Cerezo, E., and García-Arenal, F. 1996.
795 Genetic diversity in tobacco mild green mosaic tobamovirus infecting the wild plant
796 *Nicotiana glauca*. Virology 223: 148-155.

797 56. Fregene, M., Matsumura, H., Akano, A., Dixon, A., and Terauchi, R. 2004. Serial analysis of
798 gene expression (SAGE) of host-plant resistance to the cassava mosaic disease (CMD).
799 Plant Mol. Biol. 56: 563-571.
800 57. French, R. and Stenger, D. C. 2003. Evolution of Wheat streak mosaic virus: Dynamics of
801 population growth within plants may explain limited variation. Annu. Rev. Phytopathol. 41:
802 199-214.
803 58. French, R. and Stenger, D. C. 2005. Population structure within lineages of *Wheat streak*
804 *mosaic virus* derived from a common founding event exhibits stochastic variation
805 inconsistent with the deterministic quasi-species model. Virology 343: 179-189.
806 59. Froissart, R., Roze, D., Uzest, M., Galibert, L., Blanc, S., and Michalakis, Y. 2005.
807 Recombination every day: Abundant recombination in a virus during a single multi-cellular
808 host infection. PLoS Biol. 3: e89.
809 60. Froissart, R., Wilke, C. O., Montville, R., Remold, S. K., Chao, L., and Turner, P. E. 2004.
810 Co-infection weakens selection against epistatic mutations in RNA viruses. Genetics 168: 9-
811 19.
812 61. Gago, S., Elena, S. E., Flores, R., and Sanjuán, R. 2009. Extremely high mutation rate of a
813 hammerhead viroid. Science 323: 1308.
814 62. García-Arenal, F., Fraile, A., and Malpica, J. M. 2001. Variability and genetic structure of
815 plant virus populations. Annu. Rev. Phytopathol. 39: 157-186.
816 63. García-Arenal, F., Fraile, A., and Malpica, J. M. 2003. Variation and evolution of plant virus
817 populations. International Microbiology 6: 225-232.
818 64. Geri, C., Cecchini, E., Giannakou, M. E., Covey, S. N., and Milner, J. J. 1999. Altered
819 patterns of gene expression in *Arabidopsis* elicited by *Cauliflower mosaic virus* (CaMV)
820 infection and by CaMV gene VI transgene. Mol. Plant-Microbe Interact. 12: 377-384.
821 65. Gibbs, A. J., Fargette, D., García-Arenal, F., and Gibbs, M. J. 2010. Time -The emerging
822 dimension of plant virus studies. J. Gen. Virol. 91: 13-22.

823 66. Gibbs, A., Gibbs, M., Ohshima, K., and García-Arenal, F. 2008a. More about plant virus
824 evolution: past, present and future, p. 229-250. *In* E. Domingo, C. R. Parrish, and J. J.
825 Holland (eds.), Origin and Evolution of Viruses Academic Press, London.
826 67. Gibbs, A. J., Ohshima, K., Phillips, M. J., and Gibbs, M. J. 2008b. The prehistory of
827 potyviruses: Their initial radiation was during the dawn of agriculture. PLoS ONE 3: e2523.
828 68. Golem, S., Culver, J. N. 2003. *Tobacco mosaic virus* induced alterations in the gene
829 expression profile of *Arabidopsis thaliana*. Mol. Plant-Microbe Interact. 16: 681-688.
830 69. González-Jara, P., Fraile, A., Canto, T., and García-Arenal, F. 2009. The Multiplicity of
831 infection of a plant virus varies during colonization of its eukaryotic host. J. Virol. 83: 7487-
832 7494.
833 70. Grigoras, I., Timchenko, T., Grande-Perez, A., Katul, L., Vetten, H. J., and Gronenborn, B.
834 2010. High variability and rapid evolution of a nanovirus. J. Virol. 84: 9105-17.
835 71. Guo, D., Rajamäki, M. L., Saarma, M., and Valkonen, J. P. T. 2001. Towards a protein
836 interaction map of potyviruses: Protein interaction matrixes of two potyviruses based on the
837 yeast two-hybrid system. J. Gen. Virol. 82: 935-939.
838 72. Gutierrez, S., Yvon, M., Thebaud, G., Monsion, B., Michalakis, Y., and Blanc, S. 2010.
839 Dynamics of the multiplicity of cellular infection in a plant virus. PLoS Pathog. 6(9):
840 e1001113.
841 73. Hamilton, C. E., Papavasiliou, F. N., and Rosenberg, B. R. 2010. Diverse functions for DNA
842 and RNA editing in the immune system. RNA Biol. 7: 220-228.
843 74. Hammond, R., Smith, D. R., and Diener, T. O. 1989. Nucleotide sequence and proposed
844 secondary structure of *Columnea latent viroid*: A natural mosaic of viroid sequences. Nucleic
845 Acids Res. 17: 10083-10094.
846 75. Harkins, G., Delport, W., Duffy, S., Wood, N., Monjane, A. L., Owor, B. E., Donaldson, L.,
847 Saumtally, S., Verabudren, S., Triton, G., Markham, P. G., Briddon, R. W., Shepherd, D. N.,

848 Rybicki, E. P., Martin, D. P., and Varsani, A. 2009. Experimental evidence indicating that
849 mastreviruses probably did not co-diverge with their hosts. Virology J. 6: 104.
850 76. Holmes, E. C. 2009. The evolutionary genetics of emerging viruses. Annu. Rev. Ecol. Evol.
851 Syst. 40: 353-372.
852 77. Ishihara, T., Sakurai, N., Sekine, K. T., Hase, S., Ikegami, M., Shibata, D., and Takahashi,
853 H. 2004. Comparative analysis of expressed sequence tags in resistant and susceptible
854 ecotypes of *Arabidopsis thaliana* infected with *Cucumber mosaic virus*. Plant Cell Physiol.
855 45: 470-480.
856 78. Janssen, D., L. Velasco, G. Martin, E. Segundo, and Cuadrado. I. 2006. Low genetic
857 diversity among *Cucumber vein yellowing virus* isolates from Spain. Virus Genes 34: 367-
858 371.
859 79. Jenkins, G. M., Rambaut, A., Pybus, O. G., and Holmes, E. C. 2002. Rates of molecular
860 evolution in RNA viruses: A quantitative phylogenetic analysis. J. Mol. Evol. 54:156-165.
861 80. Jridi, C., Martin, J. F., Marie-Jeanne, V., Labonne, G., and Blanc, S. 2006. Distinct viral
862 populations differentiate and evolve independently in a single perennial host plant. J. Virol.
863 80: 2349-2357.
864 81. Jockusch, H., Wiegand, C., Mersch, B., and Rajes, D. 2001. Mutants of *Tobacco mosaic*
865 *virus* with temperature-sensitive coat proteins induce heat shock response in tobacco
866 leaves. Mol. Plant-Microbe Interact. 14: 914-917.
867 82. Karasev, A. V., Boyko, V. P., Gowda, S., Nikolaeva, O. V., Hilf, M. E., Koonin, E. V., Niblett,
868 C. L., Cline, K., Gumpf, D. J., Lee, R. F., Garnsey, S. M., Lewandowski, D. J., and Dawson,
869 W. O. 1995. Complete sequence of the *Citrus tristeza virus* RNA genome. Virology 208:
870 511-520.
871 83. Kim, D. H., Shim, H. K., Hyeon, J. W., Kwon, H. M., Kim, K. S., Choi, M. S., Lee, J. K., Kim,
872 D. G., Yang, J. S., and Lee, S. C. 2006. SSCP analysis of variations in haplotypes of *Citrus*

873 *tristeza virus* isolated from yuzu (*Citrus junos*) in geographically separate regions of Korea.
874 J. Plant Biol. 49: 88-96.
875 84. Kitano, H. 2002. Systems Biology: A brief overview. Science 295: 1662-1664.
876 85. Koenig, R. and Lennefors, B. L. 2000. Molecular analyses of European A, B and P type
877 sources of *Beet necrotic yellow vein virus* and detection of the rare P type in Kazakhstan.
878 Arch. Virol. 145: 1561-1570.
879 86. Krishnan, M. N., Ng, A., Sukumaran, B., Gilfoy, F. D., Uchil, P. D., Sultana, H., Brass, A. L.,
880 Adametz, R., Tsui, M., Qian, F., Montgomery, R. R., Lev, S., Mason, P. W., Koski, R. A.,
881 Elledge, S. J., Xavier, R. J., Agaisse, H., and Fikrig, E. 2008. RNA interference screen for
882 human genes associated with West Nile virus infection. Nature 455: 242-245.
883 87. Lefeuvre, P., Lett, J. M., Reynaud, B., and Martin, D. P. 2007. Avoidance of protein fold
884 disruption in natural virus recombinants. PLoS Pathog. 3: e181.
885 88. Li, H. Y. and Roossinck, M. J. 2004. Genetic bottlenecks reduce population variation in an
886 experimental RNA virus population. J. Virology 78: 10582-10587.
887 89. Lin, L., Shi, Y., Luo, Z., Lu, Y., Zheng, H., Yan, F., Chen, J., Chen, J., Adams, M. J., and
888 Wu, Y. 2009. Protein-protein interactions in two potyviruses using the yeast two-hybrid
889 system. Virus Res. 142: 36-40.
890 90. Llamas, S., Sandoval, C., Babin, M., Pogany, J., Bujarski, J. J., and Romero, J. 2004. Effect
891 of the host and temperature on the formation of defective RNAs associated with *Broad bean*
892 *mottle virus* Infection. Phytopathology 94: 69-75.
893 91. Lu, R., Folimonov, A., Shintaku, M., Li, W. X., Falk, B. W., Dawson, W. O., and Ding, S. W.
894 2004. Three distinct suppressors of RNA silencing encoded by a 20-kb viral RNA genome.
895 Proc. Natl. Acad. Sci. USA 101: 15742-15747.
896 92. Lunden, S., Meng, B. Z., Avery, J., and Qiu, W. P. 2010. Association of *Grapevine fanleaf*
897 *virus*, *Tomato ringspot virus* and *Grapevine rupestris stem pitting-associated virus* with a
898 grapevine vein-clearing complex on var. Chardonnay. Eur. J. Plant Pathol. 126: 135-144.

899 93. MacPherson, J. I., Dikerson, J. E., Pinney, J. W., Robertson, D. L. 2010. Patterns of HIV-1
900 protein interaction identify perturbed host-cellular subsystems. PLoS Comp. Biol. 6:
901 e1000863.
902 94. Malpica, J. Fraile, A., Moreno, I., Obies, C. I., Drake, J. W., and García-Arenal, F. 2002. The
903 rate and character of spontaneous mutation in an RNA virus. Genetics 162: 1505-1511.
904 95. Mansky, L. M. and Cunninham, K. S. 2000. Virus mutators and antimutators: Roles in
905 evolution, pathogenesis, and emergence. Trends Genet. 16: 512-517.
906 96. Marathe, R., Guan, Z., Anandalakshmi, R., Zhao, H., and Dinesh-Kumar, S. P. 2004. Study
907 of *Arabidopsis thaliana* resistome in response to *Cucumber mosaic virus* infection using
908 whole genome microarray. Plant Mol. Biol. 55: 501-520.
909 97. Martín, S., Sambade, A., Rubio, L., Vives, M. C., Moya, P., Guerri, J., Elena, S. F. and
910 Moreno, P. 2009. Contribution of recombination and selection to molecular evolution of
911 *Citrus tristeza virus*. J. Gen. Virol. 90: 1527-1538.
912 98. Mascia, T., Cillo, F., Fanelli, V., Finetti-Sialer, M. M., De Stradis, A., Palukaitis, P., and
913 Gallitelli, D. 2010. Characterization of the interactions between *Cucumber mosaic virus* and
914 *Potato virus Y* in mixed infections in tomato. Mol. Plant-Microbe Interact. 23: 1514-1524.
915 99. McBride, R. C., Ogbunugafor, C. B., and Turner, P. E. 2008. Robustness promotes
916 evolvability of thermotolerance in an RNA virus. BMC Evol. Biol. 8: 231.
917 100. Minskaia, E., Hertzig, T., Gorbalenya, A. E., Campanacci, V., Cambillau, C., Canard, B.,
918 and Ziebuhr, J. 2006. Discovery of an RNA virus 3'->5' exoribonuclease that is critically
919 involved in coronavirus RNA synthesis. Proc. Natl. Acad. Sci. U S A 103: 5108-5113.
920 101. Miyashita, S. and Kishino, H. 2010. Estimation of the size of genetic bottlenecks in cell-
921 to-cell movement of Soil-borne wheat mosaic virus and the possible role of the bottlenecks
922 in speeding up selection of variations in trans-acting genes or elements. J. Virol. 84: 1828-
923 1837.

924 102. Morilla, G., Krenz, B., Jeske, H., Bejarano, E. R., and Wege, C. 2004. Tete a tete of
925 *Tomato yellow leaf curl virus* and *Tomato yellow leaf curl Sardinia virus* in single nuclei. J.
926 Virology 78: 10715-10723.
927 103. Moya, A., Rodríguez-Cerezo, E., and García-Arenal, F. 1993. Genetic-structure of
928 natural-populations of the plant RNA virus *Tobacco mild green mosaic virus*. Mol. Biol. Evol.
929 10: 449-456.
930 104. Ohshima, K., Akaishi, S., Kajiyama, H., Koga, R., and Gibbs, A. J. 2010. Evolutionary
931 trajectory of *Turnip mosaic virus* populations adapting to a new host. J. Gen. Virol. 91: 788-
932 801.
933 105. Ooi, K. and Yahara, T. 1999. Genetic variation of geminiviruses: Comparison between
934 sexual and asexual host plant populations. Mol. Ecol. 8: 89-97.
935 106. Owens, R. A., Yang, G., Gundersen-Rindal, D., Hammond, R. W., Candresse, T., and
936 Bar-Joseph, M. 2000. Both point mutation and recombination contribute to the sequence
937 diversity of *Citrus viroid III*. Virus Genes 20: 243-252.
938 107. Padidam, M., Sawyer, S., and Fauquet, C. M. 1999. Possible emergence of new
939 geminiviruses by frequent recombination. Virology 265: 218-225.
940 108. Pagán, I., Firth, C., and Holmes, E. C. 2010a. Phylogenetic analysis reveals rapid
941 evolutionary dynamics in the plant RNA virus genus tobamovirus. J. Mol. Evol. 71: 298-307.
942 109. Pagán, I., Fraile, A., Fernández-Fueyo, E., Montes, N., Alonso-Blanco, C., and García-
943 Arenal, F. 2010b. *Arabidopsis thaliana* as a model for the study of plant-virus coevolution.
944 Phil Tras R Soc B 365: 1983-1995.
945 110. Pantaleo, V., Saldarelli, P., Miozzi, L., Giampetruzzi, A., Gisel, A., Moxon, S., Dalmay,
946 T., Bisztray, G., and Burgyan, J. 2010. Deep sequencing analysis of viral short RNAs from
947 an infected Pinot Noir grapevine. Virology 408: 49-56.
948 111. Patil, B. L. and Dasgupta, I. 2006. Defective interfering DNAs of plant viruses. Crit. Rev.
949 Plant Sci. 25: 47-64.

950 112. Perotto, M. C., Cafrune, E. E., and Conci, V. C. 2010. The effect of additional viral
951 infections on garlic plants initially infected with Allexiviruses. Eur. J. Plant Pathol. 126: 489-
952 495.
953 113. Pita, J. S., Fondong, V. N., Sangare, A., Otim-Nape, G. W., Ogwal, S., and Fauquet, C.
954 M. 2001. Recombination, pseudorecombination and synergism of geminiviruses are
955 determinant keys to the epidemic of severe cassava mosaic disease in Uganda. J. Gen.
956 Virol. 82: 655-665.
957 114. Poulicard, N., Pinel-Galzi, A., Hebrard, E., and Fargette, D. 2009. Why *Rice yellow*
958 *mottle virus*, a rapidly evolving RNA plant virus, is not efficient at breaking *rymv1-2*
959 resistance. Mol. Pl. Pathol. 11: 145-154.
960 115. Rodríguez-Cerezo, E., Elena, S. F., Moya, A., and García-Arenal, F. 1991. High genetic
961 stability in natural populations of the plant RNA virus *Tobacco mild Green mosaic virus*. J.
962 Mol. Evol. 32: 328-332.
963 116. Rodríguez-Cerezo, E. and García-Arenal, F. 1989. Genetic heterogeneity of the RNA
964 genome population of the plant virus U5-TMV. Virology 170: 418-423.
965 117. Rojas, M. R. and Gilbertson, R. L. 2008. Emerging plant viruses: A diversity of
966 mechanisms and opportunities, p. 27-51. *In* M. J. Roossinck (ed.), Plant Virus Evolution.
967 Springer-Verlag, Berlin.
968 118. Rouzine, I. M., Rodrigo, A., and Coffin, J. M. 2001. Transition between stochastic
969 evolution and deterministic evolution in the presence of selection: General theory and
970 application to virology. Microb. Mol. Biol. Rev. 65: 151-185.
971 119. Rubio, L., Ayllón, M. A., Kong, P., Fernandez, A., Polek, M., Guerri, J., Moreno, P., and
972 Falk, B. W. 2001. Genetic variation of *Citrus tristeza virus* isolates from California and Spain:
973 Evidence for mixed infections and recombination. J. Virol. 75: 8054-8062.
974 120. Sanjuán, R., Agudelo-Romero, P., and Elena, S. F. 2009. Upper-limit mutation rate
975 estimation for a plant RNA virus. Biol. Lett. 5: 394-6.

976 121. Sanjuán, R., Cuevas, J. M., Moya, A., and Elena, S. F. 2005. Epistasis and the
977 adaptability of an RNA virus. Genetics 170: 1001-1008.
978 122. Sanjuán, R., Moya, A., and Elena, S. F. 2004. The distribution of fitness effects caused
979 by single-nucleotide substitutions in an RNA virus. Proc. Natl. Acad. Sci. USA 101: 8396-
980 8401.
981 123. Sanjuán, R., Nebot, M. R., Chirico, N., Mansky, L. M., and Belshaw, R. 2010. Viral
982 mutation rates. J. Virol. 84: 9733-48.
983 124. Schneider, W. L. and Roossinck, M. J. 2001. Genetic diversity in RNA virus
984 quasispecies is controlled by host-virus interactions. J. Virol. 75: 6566-6571.
985 125. Schrag, S. J., Rota, P. A., and Bellini, W. J. 1999. Spontaneous mutation rate of
986 measles virus: Direct estimation based on mutations conferring monoclonal antibody
987 resistance. J. Virol. 73: 51-54.
988 126. Seal, S. E., VandenBosch, F., and Jeger, M. J. 2006. Factors influencing begomovirus
989 evolution and their increasing global significance: Implications for sustainable control. Crit.
990 Rev. Plant Sci. 25: 23-46.
991 127. Simmons, H. E., Holmes, E. C., and Stephenson, A. G. 2008. Rapid evolutionary
992 dynamics of *Zucchini yellow mosaic virus*. J. Gen. Virol. 89: 1081-1085.
993 128. Simon, A. E., Roossinck, M. J., and Havelda, Z. 2004. Plant virus satellite and defective
994 interfering RNAs: New paradigms for a new century. Annu. Rev. Phytopathol. 42: 415-437.
995 129. Sniegowski, P. D., Gerrish, P. J., Johnson, T., and Shaver, A. 2000. The evolution of
996 mutation rates: Separating causes from consequences. BioEssays 22: 1057-1066.
997 130. Stenger, D. C., Seifers, D. L., and French, R. 2002. Patterns of polymorphism in *Wheat*
998 *streak mosaic virus*: Sequence space explored by a clade of closely related viral genotypes
999 rivals that between the most divergent strains. Virology 302: 58-70.
1000 131. Susaimuthu, J., Tzanetakis, I. E., Gergerich, R. C., Kim, K. S., and Martin, R. R. 2008.
1001 Viral interactions lead to decline of blackberry plants. Plant Dis. 92: 1288-1292.

1002 132. Tan, S. L., Ganji, G., Paeper, B., Proll, S., and Katze, M. G. 2007. Systems biology and
1003 the host response to viral infection. Nat. Biotech. 25: 1383-1389.
1004 133. Tomimura, K., Spak, J., Katis, N., Jenner, C. E., Walsh, J. A., Gibbs, A. J., and
1005 Ohshima, K. 2004. Comparisons of the genetic structure of populations of *Turnip mosaic*
1006 *virus* in West and East Eurasia. Virology 330: 408-423.
1007 134. Trinks, D., Rajeswaran, R., Shivaprasad, P. V., Akbergenov, R., Oakeley, E. J.,
1008 Veluthambi, K., Hohn, T., and Pooggin, M. M. 2005. Suppression of RNA silencing by a
1009 geminivirus nuclear protein, AC2, correlates with transactivation of host genes. J. Virol. 79:
1010 2517-2527.
1011 135. Tromas, N. and Elena, S. F. 2010. The rate and spectrum of spontaneous mutations in a
1012 plant RNA virus. Genetics 185: 983-989.
1013 136. Turner, P. E. and Chao, L. 1999. Prisoner's dilemma in an RNA virus. Nature 398: 441-
1014 443.
1015 137. Uetz, P., Dong, Y. A., Zeretzke, C., Atzler, C., Baiker, A., Berger, B., Rajagopala, S. V.,
1016 Roupelieva, M., Rose, D., Fossum, E., and Haas, J. 2006. Herpesviral protein networks and
1017 their interaction with the human proteome. Science 311: 239-242.
1018 138. Uetz, P., Rajagopala, S. V., Dong, Y. A., and Haas, J. 2004. From ORFeomes to protein
1019 interaction maps in viruses. Genome Res. 14: 2029-2033.
1020 139. Urbanowicz, A., Alejska, M., Formanowicz, P., Blazewicz, J., Fligerowicz, M., and
1021 Bujarski, J. J. 2005. Homologous crossover among molecules of *Brome mosaic virus* RNA1
1022 and RNA2 segments *in vivo*. J. Virol. 79: 5732-5742.
1023 140. Vanvuuren, S. P., Collins, R. P., and Dagraca, J. V. 1993. Evaluation of *Citrus tristeza*
1024 *virus* isolates for cross protection of grapefruit in South-Africa. Plant Dis. 77: 24-28.
1025 141. Ventelon-Debout, M., Delalande, F., Brizard, J. P., Diemer, H., Van Dorsselaer, A., and
1026 Brugidou, C. 2004. Proteome analysis of cultivar-specific deregulations of *Oryza sativa*

1027 *indica* and *O. sativa japonica* cellular suspensions undergoing *Rice yellow mottle virus*
1028 *infection*. Proteomics 4: 216-225.
1029 142. Vives, M. C., Rubio, L., Galipienso, L., Navarro, L., Moreno, P., and Guerri, J. 2002. Low
1030 genetic variation between isolates of *Citrus leaf blotch virus* from different host species and
1031 of different geographical origins. J. Gen. Virol. 83: 2587-2591.
1032 143. Vives, M. C., Rubio, L., Sambade, A., Mirkov, T. E., Moreno, P., and Guerri, J. 2005.
1033 Evidence of multiple recombination events between two RNA sequence variants within a
1034 *Citrus tristeza virus* isolate. Virology 331: 232-237.
1035 144. Weng, Z., Barthelson, R., Gowda, S., Hilf, M. E., Dawson, W. O., Galbraith, D. W., and
1036 Xiong, Z. 2007. Persistent infection and promiscuous recombination of multiple genotypes of
1037 an RNA virus within a single host generate extensive diversity. PLoS ONE 2: e917.
1038 145. Whitham, S. A., Quan, S., Chang, H. S., Cooper, B., Estes, B., Zhu, T., Wang, X., and
1039 Hou, Y. M. 2003. Diverse RNA viruses elicit the expression of common sets of genes in
1040 susceptible *Arabidopsis thaliana* plants. Plant J. 33: 271-283.
1041 146. Whitham, S. A. and Wang, Y. 2004. Roles for host factors in plant viral pathogenicity.
1042 Curr. Opin. Plant. Biol. 7: 365-371.
1043 147. Wintermantel, W. M., Cortez, A. A., Anchieta, A. G., Gulati-Sakhuja, A., and Hladky, L. L.
1044 2008. Co-infection by two criniviruses alters accumulation of each virus in a host-specific
1045 manner and influences efficiency of virus transmission. Phytopathology 98: 1340-5.
1046 148. Wise, R. P., Moscou, M. J., Bogdanove, A. J., Whitham, S. A. 2007. Transcript profiling
1047 in host-pathogen interactions. Annu. Rev. Phytopathol. 45: 329-369.
1048 149. Wu, B., Melcher, U., Guo, X., Wang, X., Fan, L., and Zhou, G. 2008. Assessment of
1049 codivergence of mastreviruses with their plant hosts. BMC Evol. Biol. 8: 335.
1050 150. Xiong, Z., Barthelson, R., Weng, Z., and Galbraith, D. W. 2006. Designing and testing of
1051 a *Citrus tristeza virus* re-sequencing microarray. Proc. Int. Org. Citrus Virolog. 16: 11-22.

1052 151. Yang, C., Guo, R., Jie, F., Nettleton, D., Peng, J., Carr, T., Yeakely, J. M., Fan, J. B.,
1053 and Whitham, S. A. 2007. Spatial analysis of *Arabidopsis thaliana* gene expression in
1054 response to *Turnip mosaic virus* infection. Mol. Plant-Microbe Interact. 20: 358-370.
1055 152. Yang, Z. N., Mathews, D. M., Dodds, J. A., and Mirkov, T. E. 1999. Molecular
1056 characterization of an isolate of *Citrus tristeza virus* that causes severe symptoms in sweet
1057 orange. Virus Genes 19: 131-142.
1058 153. Zhong, X., Archuall, A. J., Amin, A. A. and Ding, B. 2008. A genomic map of viroid RNA
1059 motifs critical for replication and systemic trafficking. Plant Cell 20: 35-47.

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1061 Textbox 1. Definitions of important terms

1062 **Complementation**: The rescue of loss-of-function mutants by functional alleles contained in the
1063 population.

1064 **Effective population size (N_e)**: Number of individuals capable of producing viable progeny.

1065 **Epistasis**: The effect of a mutation in one gene over the expression of another gene or mutation
1066 of the same genome.

1067 **Genetic Bottleneck**: A severe reduction in population size.

1068 **Genetic drift**: Changes in the genetic structure of a population caused by random sampling of
1069 haplotypes moving from generation to generation.

1070 **Host range mutation**: A mutation that affects the host range of a parasite.

1071 **Interference**: Competition between beneficial mutations to become fixed in the population.

1072 **Lethal mutagenesis**: Excessive accumulation of mutations in a population that causes
1073 population extinction.

1074 **Multiplicity of cellular infection (MOI)**: Relative number of infectious particles that penetrate a
1075 cell.

1076 **Mutation frequency**: Proportion of mutations in a population remaining after the action of
1077 selection.

1078 **Mutation rate**: Proportion of mutations in a population that accumulate prior to the action of
1079 host selection.

1080 **Mutation-selection balance**: The coupling between mutation rate and selection pressures that
1081 define the frequency of mutations in a population.

1082 **Nucleotide diversity (π)**: Average number of nucleotide differences per site between any two
1083 randomly chosen haplotypes from a population.

1084 **Positive selection**: Selection of adaptive mutations.

1085 **Purifying (negative) selection**: Conditions that favor the removal of deleterious mutations from
1086 the population.

1087 **Robustness**: Molecular mechanisms that allow for the accumulation of mutations without
1088 concomitant phenotypic change.

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1091 Table 1. Studies of gene expression global profiling for *A. thaliana* in response to viral infection

Virus genus	Virus species	Reference
Caulimovirus	*Cauliflower mosaic virus* (CaMV)	(64)
Cucumovirus	*Cucumber mosaic virus* (CMV)	(77, 96, 145)
Geminivirus	*Mung bean yellow mosaic virus* (MYMV)	(134)
	Cabbage leaf curl virus (CaLCuV)	(10)
Potexvirus	*Potato virus X* (PVX)	(145)
Potyvirus	*Turnip mosaic virus* (TuMV)	(145, 151)
	Plum pox virus (PPV)	(12)
	Tobacco etch virus (TEV)	(5)
Tobamovirus	*Turnip vein clearing virus* (TVCV)	(145)
	Oilseed rape mosaic virus (ORMV)	(145)
	Tobacco mosaic virus (TMV)	(48, 68)

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1097 Table 2. Amounts (fg)[1] of CTV strains in CTV complexes

Strains	CTV complex	
	FS2-2	FL278
VT-like	155.9 ± 9.9	N/A
T30-like	37.1 ± 4.4	86.28 ± 7.7
T36-like	62.0 ± 12.4	0.43 ± 0.2

[1] The amount in fg of CTV cDNA in 1 µg of tissues calculated using standard curves generated for each genotype (Xiong, Z. and Weng, Z., unpublished data).

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1100 **FIGURE CAPTIONS**

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1102 **Fig. 1**. Average mutation rates (mutations per base per infected cell, except as noted) of viruses
1103 and viroids with RNA and ssDNA genomic architectures. The rates of two plant RNA viruses are
1104 shown as green squares: TMV (94) and TEV (135). Mutation rates of one RNA bacteriophage
1105 and eight RNA animal viruses are shown as orange squares (Measles virus' mutation rate is
1106 given as mutations per base per genomic replication event, and cannot be extrapolated to cell
1107 infected (125). Similarly, the per base per genomic replication event mutation rate of CChMVd is
1108 shown as the green triangle. Two measured mutation rates of single-stranded DNA
1109 bacteriophages are shown as blue squares. Rates are from Sanjuán et al. (123) and references
1110 therein.

1111 **Fig. 2**. Model of the relationships between virus fitness and nucleotide diversity of virus
1112 populations at the intra- (π_W) and inter- (π_B) isolate levels along virus host adaptation. The arrow
1113 represents the direction of the evolutionary steps followed by a virus adapting to a new host
1114 genotype (time scales could be significantly different in each one of the four represented
1115 evolutionary phases). The intermediate adapting phase is subdivided into an initial genetically
1116 incompatible virus-plant interaction and a subsequent compatible interaction at which host
1117 resistance has been genetically defeated. Virus adaptations in the old and new hosts are
1118 characterized by the lowest π_B and highest virus fitness.

1119 **Fig. 3**. Images of hybridized re-sequencing microarrays showing multiple strains in isolate FS2-
1120 2. The CTV Affymetrix microarray chips were hybridized with target DNA prepared from full
1121 length clones of T30 and T36 strains and from full genomic DNA of FS2-2 amplified by RT-PCR.
1122 Warm colors represent higher hybridization intensities and cool colors represent lower
1123 hybridization intensities. Locations of CTV genomes tiled on the microarray are indicated to the
1124 left.