Questions and Concepts in Plant Virus Evolution: a Historical Perspective

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Abstract The interest in plant virus evolution can be dated to the late 1920s, when it was shown that plant virus populations were genetically heterogeneous, and that their composition changed according to the experimental conditions. Many important ideas were generated prior to the era of molecular virology, such as the role of host- and vector-associated selection in virus evolution, and also that small populations, gene coadaptation and evolutionary trade-offs could limit the efficiency of selection. The analysis of viral genomes in the 1980s and 1990s established the quasispecies-like structure of their populations and allowed extensive analyses of the relationships among virus strains and species. The concept that virus populations had huge sizes and high rates of adaptive mutations became prevalent in this period, with selection mostly invoked as explaining observed patterns of population structure and evolution. In recent times virus evolution has been coming into line with evolutionary biology, and a more complex scenario has emerged. Population bottlenecks during host colonization, during host-to-host transmission or during host population fluctuations may result in smaller population sizes, and genetic drift has been recognized as an important evolutionary factor. Also, particularities of viral genomes such as low levels of neutrality, multifunctionality of coding and encoded sequences or strong epistasis could constrain the plasticity of viral genomes and hinder their response to selection. Exploring the complexities of plant virus evolution will continue to be a challenge for the future, particularly as it affects host, vector and ecosystem dynamics.

1.1 Introduction

As is the case with all living entities, reproduction of plant viruses may result in the generation of individuals that differ genetically from their parents, which are called mutants or, more vaguely, variants. Hence, populations of plant viruses are genetically heterogeneous, and the frequency distribution of genetic variants in the population (i.e., the genetic structure of the population) may change with time. This process is called evolution. A major area in the study of evolution aims at understanding the mechanisms of evolution and how they shape the genetic structure of populations. Another area aims at understanding the evolutionary history of organisms and the resulting taxonomic relationships among them. Both aspects of evolutionary studies have a long history in plant virology and have attracted much interest in the last few decades, particularly since the availability of molecular analytical techniques, such as those allowing the rapid determination of nucleotide sequences.

In this chapter we will review how the analysis of plant virus evolution has itself evolved. We do not pretend to make an exhaustive review, but we hope rather to put emphasis on the concepts that have driven the development of the field, illustrated with references to the publications that introduced those concepts or that, in our opinion, best developed them.

1.2 The Early Period

By this, we refer to the period from the origins of plant virology until the widespread use of molecular techniques for nucleic acid analyses. The heterogeneous nature of plant virus populations was evident as early as 1926, by the isolation of symptom variants from areas with atypical symptoms in systemically infected plants (Kunkel 1947) or after biological cloning through single-lesion passage, once necrotic local lesion hosts (i.e., hypersensitive hosts) had been discovered (Holmes 1929). It was also soon perceived that the major components of virus preparations could vary according to the conditions in which the virus was multiplied and passaged. Numerous reports of serial passage experiments including host shifts showed host-associated changes in viral properties, what led to the concept of host adaptation (Yarwood 1979). These observations were interpreted as due to selection in the new conditions. A major concern was whether selection acted on variants present in the original population, or on variants generated under the new conditions. This conceptual dispute was related to a second one about the possibility of obtaining genetically homogeneous virus preparations by single-lesion cloning. Some virologists, particularly Milton Zaitlin, claimed that the frequent appearance of mutants in virus stocks, known from earlier research with *Tobacco mosaic virus* (TMV; Gierer and Mundry 1958), prevented population homogeneity. The reversibility of host adaptation and the first molecular characterization of the phenomenon (Dennis Keller et al. 1981) supported the hypothesis of host-associated selection of pre-existing variants. Early molecular analyses also showed that continuous generation of mutants prevented genetic homogeneity in single-lesion-derived stocks (García-Arenal et al. 1984). Hence, the confrontation of the two hypotheses was irrelevant, but it promoted research that showed the relevance of selection as an evolutionary process in plant viruses and the intrinsic heterogeneity of plant virus populations.

Evidence that selection could operate rapidly in viral populations also came from natural populations, particularly in relation to the overcoming of resistance factors in crops. The analysis of the selection of pathotype P1 of *Tomato mosaic virus*, which overcomes *Tm-1* gene resistance in tomato, continues to be a classic (Pelham et al. 1970). However, it was also noticed that selection would not always be so effective, as evidenced by the durability of some resistance factors to viruses in crops. Bryan D. Harrison was responsible for three seminal concepts in this respect. He proposed that the evolutionary relevant size of virus populations could approach the number of infected plants or of viruliferous vectors, being thus much smaller than suggested by the high number of virus particles accumulating in the infected plant. Relatively small population sizes could hinder the efficiency of selection in virus populations (Harrison 1981). In addition, his work on *Raspberry ringspot virus* showed two phenomena also limiting the efficiency of selection: selection for mutual compatibility between RNAs 1 and 2 of the virus, and the existence of evolutionary trade-offs, two concepts that became very important in pathogen evolution theory (Hanada and Harrison 1977).

Interest in the evolution of viruses as taxonomic entities (the concept of virus species was slow to be accepted by plant virologists) also originated in this period. Analyses of relatedness among viruses or strains were initially based on biological assays, such as the extent of cross-protection. Later, serological differentiation indices or the amino acid composition of the coat proteins allowed development of quantitative analyses (Van Regenmortel 1975). The work of Adrian Gibbs pioneered the establishment of phylogenetic relationships among plant viruses, and he was also a pioneer in the development of analytical tools, as exemplified by his work on the relationships among the species of tobamoviruses (Gibbs 1986).

Thus, many of the ideas and conceptual approaches relevant to understanding virus evolution, to be developed later on, were generated in this early period on the bases of sound biological experiments or observations, in spite of limited experimental tools.

1.3 The Analysis of Viral Genomes and Its Impact on Virus Evolution Research: Quasispecies and Phylogenetics

The development in the 1970s of methods for the analyses of nucleic acids had a deep impact on the study of virus evolution. These methods allowed the comparison of virus isolates on the basis of genomic regions or viral proteins other than the structural ones, and eventually allowed the comparison of complete genomes.

Comparison of viral variants made much use of ribonuclease T1 fingerprinting, restriction fragment length polymorphisms (RFLPs), ribonuclease protection assay (RPA) of a labeled complementary RNA probe or single-stranded conformation polymorphisms (SSCPs), in addition to nucleotide sequence determination of genomes or parts of genomes. Data from fingerprints, RFLPs and, of course, nucleotide sequences can be used to directly estimate genetic distances between genotypes, while data from RPA and SSCPs cannot, as they depend on sequence context. These methodological limitations often were overlooked because initial analyses of virus variability focused just on the detection of variants, but later handicapped the development of quantitative analyses of population structure.

The availability of methods allowing the differentiation of closely related genotypes, and the availability of biologically active complementary DNA (cDNA) clones of RNA genomes, definitively determined that virus populations are intrinsically heterogeneous owing to errors during replication. Following the trend with animal- and bacteria-infecting viruses, research focused on RNA viruses, and heterogeneity of cDNA-derived populations was initially shown for *Cucumber mosaic virus* (CMV) satellite RNA and for TMV (Aldahoud et al. 1989; Kurath and Palukaitis 1989). It was shown also, initially for *Tobacco mild green mosaic virus* (TMGMV; Rodríguez-Cerezo and García-Arenal 1989), that the frequency distribution of genotypes in virus populations was gamma, with a major genotype plus a set of minor variants newly generated by mutation or kept at a low level by selection. It was shown later on that the shape of this distribution depended on both the virus and the host plant (Schneider and Roossinck 2000, 2001). This genetic structure had been previously reported for RNA viruses infecting bacteria or animals and had been named a quasispecies (Domingo and Holland 1997), as it corresponded to that predicted by Eigen's quasispecies theory, proposed to describe the evolution of an infinite population of asexual replicators at high mutation rate (Eigen and Schuster 1977). The quasispecies concept has been used often in virology as a mere description for genetically heterogeneous virus populations ("swarms" of mutants), with no concern or awareness for further implications, or for the specific conditions required for the quasispecies concept to materialize, as pointed out by Eigen (1996) himself and developed in the next section. Regardless of the limited appreciation of its implications, the quasispecies concept was crucial in making virologists in the 1980s aware of the intrinsic heterogeneity of virus populations, an early discovery that had been overlooked in an era focused on the molecular analyses of viral genomes.

The quasispecies concept assumed high mutation rates for RNA viruses. It was indeed shown with bacteriophages and with lytic viruses infecting mammalian cells that RNA-dependent RNA polymerases lacked a proofreading activity, and had error rates several orders of magnitude higher than DNA-dependent DNA polymerases of large DNA phages or of cellular organisms (on the order of 10^{-4}–10^{-6} per position and replication round; Drake et al. 1998). Because of high mutation rates of RNA viruses and high accumulation levels in host cells, it was concluded that RNA viruses had large and highly diverse populations. As a consequence, viral populations would easily respond to changing selection pressure, and the evolution of high mutation rates would have an adaptive value, allowing the virus to survive in changing environments. This concept became the "dogma" that has presided over analyses of RNA virus evolution for more than two decades since the early 1980s. Challenges to this dogma, coming initially from the plant virus field, will be described in the next section.

Nucleotide sequence determination, and the development of methods for the comparison of distantly related sequences, led to phylogenetic analyses of proteins with a similar function in viruses belonging to different genera. These analyses, first done with RNA-dependent RNA polymerases (Kamer and Argos 1984), allowed the classification of viruses in large groups or "superfamilies" (Koonin and Dolja 1993; Goldbach and de Haan 1994) although the validity of the higher-order comparisons was later seriously questioned (Zanotto et al. 1996). Availability of nucleotide sequences of complete viral genomes showed that phylogenies of different gene families were not congruent and that gene organization within the genomes could vary between viral taxonomic groups that were otherwise related. This could be explained by "reassortment of functional modules of coding and regulatory sequences" (Haseloff et al. 1984) according to the concept of "modular evolution," first proposed for bacteriophages (Botstein 1980). Also, availability of whole genome sequences showed that virus genes were often contained totally or partially within another gene, in a different reading frame. This observation led Adrian Gibbs to propose the very novel concept of de novo generation of genes by "overprinting," and methods to analyze which of the two overlapping genes was the novel one (Keese and Gibbs 1992).

The ease of comparing viral genomes also prompted analyses of the genetic structure of natural populations of plant viruses. Phylogenetic approaches were generally preferred to population genetics ones. Both approaches showed from the early 1990s that virus populations could be structured according to different factors, such as geographic or host origin, and different selection pressures were invoked to explain the observed population structures. Again, Gibbs's work on tymoviruses infecting wild plants (Skotnicki et al. 1993, 1996) was pioneering in this field. Major selection pressures acting on virus genomes were identified in this period. Selection was associated with the need to maintain a functional structure, for instance, in the capsid protein of tobamoviruses (Altschuh et al. 1987) or in noncoding subviral pathogenic nucleic acids such as satellites or viroids (Fraile and García-Arenal 1991; Elena et al. 1991). Host-associated selection, already known from passage experiments, was also invoked to explain population structure, for instance, in *Kennedya yellow mosaic virus* (KYMV; Skotnicki et al. 1996), *Hop stunt viroid* (Kofalvi et al. 1997) or *Barley yellow dwarf virus* (BYDV; Mastari et al. 1998). Evidence of vector-associated selection initially derived from loss of transmissibility upon mechanical passage or vegetative propagation of the virus host (Reddy and Black 1977). Population structure in relation to vector transmission has been analyzed in few instances, mostly with begomoviruses (Harrison and Robinson 1999; Simón et al. 2003) supporting vector-associated selection.

Because most analyses of virus population structure followed a phylogenetic approach and because analytical methods were able to differentiate between closely

related variants, the resulting data were interpreted mostly as conforming to the dogma of high genetic diversity of RNA virus populations. However, the genetic diversity of a population does not depend only on the number of genotypes present in the population, but also on the frequency of each genotype and on the genetic distances among them. The few reports that considered these three factors for the analysis of population structure, such as those for solanaceae-infecting tobamoviruses (Rodríguez-Cerezo et al. 1991) showed low population diversities. Later on, analyses of populations of other viruses also provided evidence of low population diversity and, importantly, showed that population diversity did not depend on the nature, RNA or DNA, of the virus genome (García-Arenal et al. 2001). Also, nucleotide diversity in virus genes was not higher for RNA than for DNA plant viruses and, interestingly, diversity values were in the range of those of the genes of their eukaryotic hosts and vectors. Data showed that negative selection was important in keeping low nucleotide diversities and, more important, that the degree of negative selection was not related to the function of the virus protein, at odds with observations on cellular organisms, in which certain classes of proteins are more conserved than other. Multifunctionality of viral proteins could explain these observations (García-Arenal et al. 2001). Hence, evidence showed high genetic conservation, rather than high diversity, for most plant virus populations analyzed.

1.4 The Challenge to the Dogma: Viruses Might Be Not So Variable nor Might Their Populations Be So Big

The fact that plant viral populations did not show the big diversity assumed by the dogma led to the questioning of the two premises on which that dogma rested: high population sizes and high rates of adaptive mutations. As stated, it was proposed as early as 1981 that in spite of high levels of virus accumulation in the infected hosts, population size perhaps would not be so high (Harrison 1981). In fact, the relevant evolutionary parameter is not the census size of the population, but the effective population size, which could be grossly defined as the fraction of the population that passes its genes to the new generation. Expansions and contractions of population size during the virus life cycle, i.e., the occurrence of population bottlenecks, would affect the effective population size. It was first shown for TMV that virus population indeed passed through severe bottlenecks during plant colonization, and that effective sizes of the population that initiates colonization of a new leaf could be as low as units or tens of individuals (Sacristán et al. 2003). Detailed analyses of within-host population structure of *Wheat soil borne mosaic virus* led to similar numbers, derived from a different approach (French and Stenger 2003). It was also shown that severe population bottlenecks occurred both during CMV colonization of new leaves within a plant and during horizontal transmission by aphids to new plants (Li and Roossinck 2004; Ali et al. 2006). Genetic drift can result in the elimination from the population of the fittest genotypes and the accumulation of deleterious mutations, eventually leading to population extinction (i.e., mutational *etch virus* (de la Iglesia and Elena 2007). Mutation accumulation and population extinction was also shown to occur in nature in the TMV population infecting *Nicotiana glauca*, owing to a reduction in the TMV population size caused by coinfection with TMGMV, to our knowledge the only report of mutational meltdown occurring in viral populations in nature (Fraile et al. 1997). Hence, random genetic drift, as opposed to selection, can be an important evolutionary factor for plant viruses, a possibility not contemplated by the quasispecies theory, which is a deterministic model of evolution.

The first years of this century also brought evidence that the high potential to vary of RNA viruses need not result in high adaptability. The spontaneous mutation rate of TMV was determined using a large (804-nt) mutational target in conditions of minimal selection against deleterious mutants (Malpica et al. 2002). Mutation rates were high but slightly less than those previously reported for lytic RNA viruses (0.05–0.1 compared with approximately one mutation per genome and replication round, but see a new estimation of *Vesicular stomatitis virus* (VSV) mutation rate in line with that of TMV in Furió et al. 2005). More importantly, the mutational spectrum for an RNA genome was reported for the first time in this work. A large percentage of mutants were multiple mutants, and about one third of mutations were insertions and deletions, so a large fraction of mutations will be highly deleterious if not immediately lethal. An analysis of the mutational spectrum of VSV also showed that most point mutations were deleterious (Sanjuán et al. 2004a). Thus, the high mutation rate of RNA viruses seems not to have evolved as an adaptive trait facilitating adaptation to new environments (Elena and Sanjuán 2005). Also, epistatic interactions between different site mutations were shown to be strong for VSV (Sanjuán et al. 2004b). Genetic exchange by recombination or reassortment of genomic segments (i.e., sexuality) is another important source of genetic variation in viruses, often with large phenotypic effects such as host switches, host range expansion and is often at the root of the emergency of new viral diseases. A typical example is the role of genetic exchange in the origin of the pandemic of cassava mosaic disease in East Africa (Fargette et al 2006). Genetic exchange also has been shown to be important in the evolution of taxonomic entities (White et al. 1995). Genotypes generated by recombination can be frequent in virus populations, as shown particularly for begomoviruses (Sanz et al. 2000), but also for RNA virus such as *Turnip mosaic virus* (TuMV) (Tan et al. 2004). Last, a recent report has shown the importance of recombination in *Cauliflower mosaic virus* that can make up to 50% of experimental populations. This report also provides the only estimate of recombination rates, 2×10^{-5}–5×10^{-5} per base and replication cycle, i.e., on the order of mutation rates in RNA viruses (Froissart et al. 2005). However, genetic structure of field RNA virus populations often indicates constraints to genetic exchange (Bonnet et al. 2005), and experiments with both DNA and RNA viruses (*Maize streak virus* and CMV, respectively) have shown that heterologous gene combinations are selected against, supporting the coadaptation of gene complexes in viral genomes (Martin et al. 2005; Escriu et al. 2007). Thus, this is also evidence that epistatic interactions would constrain the plasticity of the small genomes of RNA viruses, further limiting their possibility to respond to selection

the common occurrence of resistance-breaking isolates (Harrison 2002; García-Arenal and McDonald 2003), is in agreement with these observations.

Thus, the general view on the evolution of RNA viruses that dominated the scene in the 1980s and 1990s and that we have called the dogma is unsupported by a large body of evidence. Theoretical and experimental challenges of the quasispecies theory have also multiplied (see the exchange in Holmes and Moya 2002 and Domingo 2002). Conditions for application of the quasispecies theory, such as equilibrium conditions, single-peak (or master sequence) fitness landscapes, large values for the product of effective population size and mutation rate, lack of lethal mutants, or asexuality may often not apply to viral populations (Eigen 1996; Wilke 2005), as shown in this section. In addition, the quasispecies theory may be in fact in perfect agreement with standard population genetics (Wilke 2005). Hence, the view is prevailing that virus evolution is not intrinsically different in its processes from that of other living entities, in spite of particularities. One such particularity would be a not well-defined ploidy, derived from the possibility of coinfection of the same cell by different virus particles. A direct consequence of coinfection is that deleterious or lethal mutants may be efficiently complemented by functional genotypes sharing the same cell, which will provide the affected function *in trans*. Complementation may counter the effect of selection (Moreno et al. 1997) and, thus, may have important consequences on virus evolution, for instance, the maintenance in viral populations of more virulent but less fit variants.

1.5 Recent Times: New Concepts and New Challenges

If virus evolution is not intrinsically different in its processes from that of other living entities, viruses may be good experimental systems to test general evolutionary hypotheses (Elena and Lenski 2003). The use of plant viruses to this aim has only started recently, but probably will be a major component of research on plant virus evolution in the near future. Another important recent trend likely to explode in the near future is the consideration of plant virus evolution on a broader context than the virus population itself, incorporating the interaction of viral and host and/or vector populations.

Analyses of the genetic structure of viral populations and viral phylogenies have benefited in recent times from the availability of new computational tools that allow detailed and more informative analyses of sequence data. Examples are tools that implement different methods for the detection of recombination (Posada and Crandall 2001) or of positive selection acting on particular codons of protein-encoding genes (Yang et al. 2000). These tools have recently been applied to different plant virus systems (Moury 2004; Codoñer and Elena 2006). Progress in methods to obtain and analyze phylogenetic or population structure data have resulted also in an increased awareness that their interpretation is often hindered by limited information on the factors that act on virus populations and determine their evolution. Particularly, host- and vector-associated factors act on virus populations in ways probably dependent on properties of the virus itself, such as host and vector

nge. Thus, there is a need to analyze virus evolution within broader epidemiologi-al and ecological frames. Good examples of integration of ecological and epide-iological data in virus evolution studies are in a series of reports on the volutionary biology of *Rice yellow mottle virus* (Fargette et al. 2006) and TuMV Tomitaka and Ohshima 2006). An important motor of research on virus evolution om an ecological perspective is related to the development of transgenic plants ith pathogen-derived resistance, and the need to evaluate the risks that their wide-pread use could have for agricultural and wild ecosystems (Tepfer 2002).

Another important field just starting to be developed is the role in virus evolution f the effects that viruses have on their host and vector populations. Viruses often can e virulent pathogens that harm their hosts. In the context of evolutionary biology, irulence is often defined as the deleterious effects of parasite infection on the host's itness. As such, virulence affects the population genetics and dynamics of hosts and s, thus, the major factor determining host–pathogen coevolution (Frank 1996). Because virulence is the key property of pathogens, much theoretical work has been lone in the last two decades aimed at modeling virulence evolution. Plant viruses are nost adequate systems to test the assumptions and predictions of these models, but he study of virulence evolution in plant viruses is largely an unexplored field. Recent nterest in this subject is shown by reports that have addressed questions such as the elationship between virulence and virus multiplication, mode of transmission, host daptation or within-host competition in mixed infections (Escriu et al. 2003; Sacristán et al. 2005; Stewart et al. 2005). Also, viruses may affect the population lynamics of their insect vector. For instance, it has been shown that virus infection nas an influence on both the attraction of insect vectors by host plants and on their reproductive potential (Fereres et al. 1999; Jiu et al. 2007). Interestingly, the effect of virus infection on aphid biology may depend on the transmission manner, nonpersist-ent or persistent (Eigenbrode et al. 2002). Research on virus–vector interactions pres-ently lags behind that on virus–host interactions.

In the past, most analyses of virus evolution focused on agricultural systems, and little work has been done in natural ecosystems. An analysis of the prevalence of five generalist viruses on 21 species of wild plants has shown a selective interaction between viruses and hosts and, more important, that host selectivity is a successful strategy for generalist viruses (Malpica et al. 2006). This result is relevant, as mod-els of virulence evolution predict that pathogens will evolve to specialism, against the evidence that most plant and animal pathogens are generalists (Woolhouse et al. 2001). The observed tendency to specialize could reconcile both views. This report also showed that viruses tended to associate positively in mixed infected hosts (Malpica et al. 2006), which again is relevant, as coinfection of different pathogens may have important consequences for the pathogen, the infected host and for host–pathogen coevolution.

The role of plant viruses in ecosystem dynamics, as it relates to virus evolution, is also an emergent area of research. Virus infection of wild plants often goes unnoticed because it is asymptomatic, and it generally has been considered not to harm the host. However, several reports have shown that virus infection can decrease the fitness of wild plants (Kelly 1994; Friess and Maillet 1996; Maskell et al. 1999). Perhaps the best

studied case is the effect of BYDV and *Cereal yellow dwarf virus* infection in wild grasses in California. Prevalence, accumulation and virulence vary according to the host plant species, and have a complex influence on grassland dynamics, mediated by phenomena such as pathogen spillover (an epidemic in one host is affected by transmission from another host population) and pathogen-mediated apparent competition (Power and Mitchell 2004). Virus infection also influences the effects of herbivory, showing the importance of multitrophic interactions in virus ecology (Malmstrom et al. 2006). The relevance of multitrophic interactions had been shown long ago in wild legumes in Australia infected by KYMV, which were less affected by herbivory than noninfected plants (Gibbs 1980). Another outstanding example of complex interactions is shown by an analysis demonstrating that the increased stress tolerance of grasses associated with colonization by endophytic fungi is due to virus infection of the fungus (Márquez et al. 2007). The analysis of virus populations in ecosystems is, thus, uncovering highly complex networks of interactions. How these interactions affect virus evolution should be an important area of research in the upcoming years.

1.6 Final Comments

In the earlier period of plant virology, prior to the advent of molecular techniques for the analysis of viral genomes, research often had a population-oriented perspective, and reflected the very broad biological understanding that characterized the earlier generations of plant virologists. Many important ideas, often to be fully developed in later years, were generated at this time. The impact of molecular virology in the 1980s somewhat detracted from the interest in plant virus population research, and when this interest was renewed later on most plant virologists, even those interested in evolution, had a molecular rather than a populational formation, which conditioned the perception of evolutionary processes. In recent times, plant virus evolution has again attracted population biologists and the study of plant virus evolution is, thus, losing its peculiarities and is coming in line with evolutionary biology at large. As a consequence, new questions are being asked from new perspectives and broader contexts, including the reciprocal complex interactions of virus populations with those of their hosts and vectors. Exploring the complexities of plant virus evolution will certainly keep scientists busy for a long time.

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References

Aldahoud R, Dawson WO, Jones GE (1989) Rapid, random evolution of the genetic structure of replicating tobacco mosaic virus populations. Intervirology 30:227–233

Ali A, Li H, Schneider WL, Sherman DJ, Gray S, Smith D, Roossinck MJ (2006) Analysis of genetic bottlenecks during horizontal transmission of Cucumber mosaic virus. J Virol 80:8345–8350

Altschuh D, Lesk AM, Bloomer AC, Klug A (1987) Correlation of co-ordinated amino acid substitutions with function in viruses related to tobacco mosaic virus. J Mol Biol 193:693–707

Bonnet J, Fraile A, Sacristán S, Malpica JM, García-Arenal F (2005) Role of recombination in the evolution of natural populations of Cucumber mosaic virus, a tripartite RNA plant virus. Virology 332:359–368

Botstein D (1980) A theory of modular evolution for bacteriophages. Ann N Y Acad Sci 354:484–490

Codoñer FM, Elena SF (2006) Evolutionary relationships among members of the Bromoviridae deduced from whole proteome analysis. Arch Virol 151:299–307

de la Iglesia F, Elena SF (2007) Fitness declines in *Tobacco etch virus* upon serial bottlenecks transfers. J Virol 81:4941–4947

Domingo E (2002) Quasispecies theory in virology. J Virol 76:463–465

Domingo E, Holland JJ (1997) RNA virus mutations and fitness for survival. Annu Rev Microbiol 51:151–178

Donis-Keller H, Browning KS, Clarck JM (1981) Sequence heterogeneity in satellite tobacco necrosis virus RNA. Virology 110:43–54

Drake JW, Charlesworth B, Charlesworth D, Crow JF (1998) Rates of spontaneous mutation. Genetics 148:1667–1686

Eigen M (1996) On the nature of virus quasispecies. Trends Microbiol 4:216–218

Eigen M, Schuster P (1977) The hypercycle. A principle of natural self-organization. Pt.A.: emergence of the hypercycle. Naturwissenchaften 64:541–565

Eigenbrode SD, Ding H, Shiel P, Berger PH (2002) Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). Proc R Soc Lond Ser B 269:455–460

Elena SF, Lenski RE (2003) Evolution experiments with microorganisms: The dynamic and genetic bases of adaptation. Nat Rev Genet 4:457–469

Elena SF, Sanjuán R (2005) Adaptive value of high mutation rates of RNA viruses: separating causes from consequences. J Virol 79:11555–11558

Elena SF, Dopazo J, Flores R, Diener TO, Moya A (1991) Phylogeny of viroids, viroid-like satellite RNAs, and the viroid-like domain of hepatitis δ virus RNA. Proc Natl Acad Sci USA 88:5631–5634

Escriu F, Fraile A, García-Arenal F (2003) The evolution of virulence in a plant virus. Evolution 57:755–765

Escriu F, Fraile A, García-Arenal F (2007) Constraints to genetic exchange support gene coadaptation in a tripartite RNA virus. PLoS Pathog 3:e8

Fargette D, Konate G, Fauquet C, Muller E, Peterschmitt M, Thresh JM (2006) Molecular ecology and emergence of tropical plant viruses. Annu Rev Phytopathol 44:235–260

Fereres A, Kampmeier GE, Irwin ME (1999) Aphid attraction and preference for soybean and pepper plants infected with Potyviridae. Ann Entomol Soc Am 92:542–548

Fraile A, García-Arenal F (1991) Secondary structure as a constraint on the evolution of a plant viral satellite RNA. J Mol Biol 221:1065–1069

Fraile A, Escriu F, Aranda MA, Malpica JM, Gibbs AJ, García-Arenal F (1997) A century of tobamovirus evolution in an Australian population of *Nicotiana glauca*. J Virol 71:8316–8320

Frank SA (1996) Models of parasite virulence. Q Rev Biol 71:37–78

French R, Stenger DC (2003) Evolution of wheat streak mosaic virus: dynamics of population growth within plants may explain limited variation. Annu Rev Phytopathol 41:199–214

Friess N, Maillet J (1996) Influence of cucumber mosaic virus infection on the intraspecific competitive ability and fitness of purslane (*Portulaca oleraceae*) New Phytol 132:103–111

Froissart R, Roze D, Uzest M, Galibert L, Blanc S, Michalakis Y (2005) Recombination every day: abundant recombination in a virus during a single multi-cellular host infection. PLoS Biol 3:389–395

Furió V, Moya A, Sanjuán R (2005) The cost of replication fidelity in an RNA virus. Proc Natl Acad Sci USA 102:10233–10237

García-Arenal F, McDonald B (2003) An analysis of the durability of resistance of plant to viruses. Phytopathology 93:941–952
García-Arenal F, Palukaitis P, Zaitlin M (1984) Strains and mutants of tobacco mosaic virus are both found in virus derived from single-lesion-passaged inoculum. Virology 132:131–137
García-Arenal F, Fraile A, Malpica JM (2001) Variability and genetic structure of plant virus populations. Annu Rev Phytopathol 39:157–186
Gibbs AJ (1980) A plant virus that partially protects its wild legume host against hebivores. Intervirology 13:42–47
Gibbs AJ (1986) Tobamovirus classification. In: Van Regenmortel MHV, Fraenkel-Conrat H (eds) The plant viruses, vol 2. The rod-shaped plant viruses. Plenum, New York, pp 168–180
Gierer A, Mundry KW (1958) Productions of mutants of tobacco mosaic virus by chemical alteration of its ribonucleic acid *in vitro*. Nature 182:1457–1458
Goldbach R, de Haan P (1994) RNA viral supergroups and the evolution of RNA viruses. In: Morse SS (ed) The evolutionary biology of viruses. Raven, New York, pp 105–119
Hanada K, Harrison BD (1977) Affects of virus genotype and temperature on seed transmission of nepoviruses. Ann Appl Biol 85:79–92
Harrison BD (1981) Plant virus ecology: ingredients, interactions and environment influences. Ann Appl Biol 99:195–209
Harrison BD (2002). Virus variation in relation to resistance-breaking in plants. Euphytica 124:181–192
Harrison BD, Robinson DJ (1999) Natural genomic and antigenic variation in whitefly-transmitted geminiviruses (begomoviruses). Annu Rev Phytopathol 37:369–398
Haseloff J, Goelet P, Zimmern D, Ahlquist P, Dasgupta R, Kaesberg P (1984) Striking similarities in amino acid sequence among nonstructural proteins encoded by RNA viruses that have dissimilar genomic organization. Proc Natl Acad Sci USA 81:4358–4362
Holmes FO (1929) Local lessions in tobacco mosaic. Bot Gaz 87:39–55
Holmes EC, Moya A (2002) Is the quasispecies concept relevant to RNA viruses? J Virol 76:460–462
Jiu M, Zhou XP, Tong L, Xu J, Yang X, Wan FH, Liu SS (2007). Vector-virus mutualism accelerates population increase of an invasive whitefly. PLoS ONE 2:e182
Kamer G, Argos P (1984) Primary structural comparisons of RNA-dependent polymerases from plant, animal and bacterial viruses. Nucleic Acids Res 12:7269–7282
Keese P, Gibbs A (1992) Origins of genes: big bang or continuous creation? Proc Natl Acad Sci USA 89:9889–9893
Kelly SE (1994) Viral pathogens and the advantage of sex in the perennial grass *Anthoxantum odoratum*: a review. Philos Trans R Soc Lond Ser B 346:295–302
Kofalvi SA, Marcos JF, Cañizares MC, Pallás V, Candresse T (1997) Hop stunt viroid (HSVd) sequence variants from *Prunus* species: evidence for recombination between HSVd isolates. J Gen Virol 78:3177–3186
Koonin EV, Dolja VV (1993) Evolution and taxonomy of positive-strand RNA viruses: implications of comparative analysis of amino acid sequences. Crit Rev Biochem Mol Biol 28:375–430
Kunkel LO (1947) Variation in phytopathogenic viruses. Annu Rev Microbiol 1:85–100
Kurath G, Palukaitis P (1989) RNA sequence heterogeneity in natural populations of three satellite RNAs of cucumber mosaic virus. Virology 173:231–240
Li H, Roossinck MJ (2004) Genetic bottlenecks reduce population variation in an experimental RNA virus population. J Virol 78:10582–10587
Malmstrom CM, Stoner CJ, Brandenburg S, Newton LA (2006) Virus infection and grazing exert counteracting influences on survivorship of native bunchgrass seedling competing with invasive exotics. J Ecol 94:264–275
Malpica JM, Fraile A, Moreno I, Obiés CI, Drake JW, García-Arenal F (2002) The rate and character of spontaneous mutation in an RNA virus. Genetics 162:1505–1511
Malpica JM, Sacristán S, Fraile A, García-Arenal F (2006) Association and host selectivity in
Márquez LM, Redman RS, Rodríguez RJ, Roossinck MJ (2007) A virus in a fungus in a plant-three way symbiosis required for thermal tolerance. Science (in press)
Martin DP, van der Walt E, Posada D, Rybicki EP (2005) The evolutionary value of recombination is constrained by genome modularity. PLoS Genet 1:e51
Maskell LC, Raybould AF, Cooper JI, Edwards M-L, Gray AJ (1999) Effects of turnip mosaic and turnip yellow mosaic virus on the survival, growth and reproduction of wild cabbage (*Brassica oleracea*). Ann Appl Biol 135:401–407
Mastari J, Lapierre H, Dessens JT (1998) Asymmetrical distribution of barley yellow dwarf virus PAV variants between host plant species. Phytopathology 88:818–821
Moreno I, Malpica JM, Rodríguez-Cerezo E, García-Arenal F (1997) A mutation in tomato aspermy cucumovirus that abolishes cell-to-cell movement is maintained to high levels in the viral RNA population by complementation. J Virol 71:9157–9162
Moury B (2004) Differential selection of genes of cucumber mosaic virus subgroups. Mol Biol Evol 21:1602–1611
Pelham J, Fletcher JT, Hawkins JH (1970) The establishment of a new strain of tobacco mosaic virus resulting from the use of resistant varieties of tomato. Ann Appl Biol 65:293–297
Posada D, Crandall KA (2001) Evaluation of methods for detecting recombination from DNA sequences: computer simulations. Proc Natl Acad Sci USA 98:13757–13762
Power AG, Mitchell CE (2004) Pathogen spillover in disease epidemics. Am Nat 164:S79–S89
Reddy DVR, Black LM (1977) Isolation and replication of mutant populations of wound tumor virions lacking certain genome segments. Virology 80:336–346
Rodríguez-Cerezo E, García-Arenal F (1989) Genetic heterogeneity of the RNA genome population of the plant virus U5-TMV. Virology 170:418–423
Rodríguez-Cerezo E, Elena SF, Moya A, García-Arenal F (1991) High genetic stability in natural populations of the plant RNA virus tobacco mild green mosaic virus. J Mol Evol 32:328–332
Sacristán S, Malpica JM, Fraile A, García-Arenal F (2003) Estimation of population bottlenecks during systemic movement of *Tobacco mosaic virus* in tobacco plants. J Virol 77:9906–9911
Sacristán S, Fraile A, Malpica JM, García-Arenal F (2005) An analysis of host adaptation and its relationship with virulence in *Cucumber mosaic virus*. Phytopathology 95:827–833
Sanjuán R, Moya A, Elena SF (2004a) The distribution of fitness effects caused by single-nucleotide substitutions in an RNA virus. Proc Natl Acad Sci USA 101:8396–8401
Sanjuán R, Moya A, Elena SF (2004b) The contribution of epistasis to the architecture of fitness in an RNA virus. Proc Natl Acad Sci USA 101:15376–15379
Sanz AI, Fraile A, García-Arenal F, Zhou X, Robinson DJ, Khalid S, Butt T, Harrison BD (2000) Multiple infection, recombination and genome relationships among begomovirus isolates found in cotton and other plants in Pakistan. J Gen Virol 81:1839–1849
Schneider WL, Roossinck MJ (2000) Evolutionary related Sindbis-like plant viruses maintain different levels of population diversity in a common host. J Virol 74:3130–3134
Schneider WL, Roossinck MJ (2001) Genetic diversity in RNA virus quasispecies is controlled by host-virus interactions. J Virol 75:6566–6571
Simón B, Cenis JL, Beitia F, Khalid S, Moreno IM, Fraile A, García-Arenal F (2003) Genetic structure of field populations of Begomoviruses and of their vector *Bemisia tabaci* in Pakistan. Phytopathology 93:1422–1429
Skotnicki ML, Mackenzie AM, Ding SW, Mo JQ, Gibbs AJ (1993) RNA hybrid mismatch polymorphisms in Australian populations of turnip yellow mosaic tymovirus. Arch Virol 132:83–99
Skotnicki ML, Mackenzie AM, Gibbs AJ (1996) Genetic variation in populations of kennedya yellow mosaic tymovirus. Arch Virol 141:99–110
Stewart AD, Logson JM Jr, Kelley SE (2005) An empirical study of the evolution of virulence under both horizontal and vertical transmission. Evolution 59:730–739
Tan Z, Wada Y, Chen J, Ohshima K (2004) Inter- and intralineage recombinants are common in natural populations of *Turnip mosaic virus*. J Gen Virol 85:2683–2696
Tepfer M (2002) Risk assessment of virus-resistant transgenic plants. Annu Rev Phytopathol 40:467–91

Tomitaka Y, Ohshima K (2006) A phylogeographical study of the *Turnip mosaic virus* population in East Asia reveals an "emergent" lineage in Japan. Mol Ecol 15:4437–4457
Van Regenmortel MHV (1975) Antigenic relationships between strains of tobacco mosaic virus. Virology 64:415–420
White PS, Morales FJ, Roossinck MJ (1995) Interspecific reassortment in the evolution of a cucumovirus. Virology 207:334–337
Wilke CO (2005) Quasispecies theory in the context of population genetics. BMC Evol Biol 5:44
Woolhouse MEJ, Taylor LH, Haydon DT (2001) Population biology of multihost pathogens. Science 292:1109–1112
Yang Z, Nielsen R, Goldman N, Krabbe Pedersen A-M (2000) Codon-substitution models for heterogeneous selection pressure at amino acid sites. Genetics 155:431–449
Yarwood CE (1979) Host passage effects with plant viruses. Adv Virus Res 24:169–190
Zanotto PM, Gibbs MJ, Gould EA, Holmes EC (1996) A reevaluation of the higher taxonomy of viruses based on RNA polymerases. J Virol 70:6083–6096