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VIRAL EVOLUTION AND INSECTS AS A POSSIBLE VIROLOGIC TURNING TABLE

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SUMMARY

Three lines of observation demonstrate the role of arthropods in transmission and evolution of viruses. a) Recent outbreaks of viruses from their niches took place and insects have played a major role in propagating the viruses. b) Examination of the list of viral families and their hosts shows that many infect invertebrates (I) and vertebrates (V) or (I) and plants (P) or all kingdoms (VIPs). This notion holds true irrespective of the genome type. At first glance the argument seems to be weak in the case of enveloped and non-enveloped RNA viruses with single-stranded (ss) segmented or non-segmented genomes of positive (+) or negative polarity. Here, there are several families infecting V or P only; no systematic relation to arthropods is found. c) In the non-enveloped plant viruses with ss RNA genomes there is a strong tendency for segmentation and individual packaging of the genome pieces. This is in contrast to ss+ RNA animal viruses and can only be explained by massive transmission by seed or insects or both, because individual packaging necessitates a multihit infection. Comparisons demonstrate relationships in the nonstructural proteins of double-stranded and ss+ RNA viruses irrespective of host range, segmentation, and envelope. Similar conclusions apply for the negative-stranded RNA viruses. Thus, viral supergroups can be created that infect V or P and exploit arthropods for infection or transmission or both. Examples of such relationships and explanations for viral evolution are reviewed and the arthropod orders important for cell culture are given.

Key words: arthropods; superfamily and host range; viral evolution; reassortment; recombination; substitution.

CLASSIFICATION OF VIRUSES AND THEIR HOSTS

There are several reasons why virology is one of the hot beds of contemporary research. First, the introduction of cell culture enabled researchers to perform meaningful work on these obligatory cell parasites. Second, the importance of viruses as pathogens has increased since the introduction of antibiotics to fight bacteria. Worldwide traveling, ecologic changes, and altered human behavior have led to the evasion of viruses from their original ecologic niches and sometimes to spectacular pandemics, such as AIDS. In several cases evolution of viruses and traveling acted in concert (66,67). Third, viruses are relatively simple organisms consisting in principle of an encoated genome without any translation machinery, so that modern techniques of molecular biology and genetic engineering can be applied successfully. Finally, a renewed interest in taxonomy and evolution has stimulated the latest developments in, for example, sequence comparisons. The taxonomy of viruses is mainly still based on classical criteria, such as the symmetry (as visualized in the electron microscope; icosahedral, bacilliform, rod-like, helical, complex), the lipid bilayer envelope surrounding the inner parts of the virion (the genome containing nucleocapsid), and the type of the nucleic acid [double-stranded (ds) DNA, single-stranded (ss) DNA, ds RNA and ss RNA]. In addition, viruses can be grouped according to the host kingdoms they parasitize.

Table 1 has been composed from the most recent classification scheme (27) of viruses including some 2430 viruses belonging to 73 families or groups. The taxonomic hierarchy uses the terms of supergroup or superfamily, family, genus, species and types, variants, and strains. The term "species" is not very precise (114); the reason for this will be given below. Some interesting facts can be gleaned from the table in which viral families are listed according to the criteria of nucleic acid type and envelope (present or not) versus host. There are no ss DNA enveloped viruses; ds RNA viruses with an envelope occur in bacteria only. All other combinations can be found in vertebrates (V) and in invertebrates (I); some families are also represented in plants (P) and can be coined as VIP-families such as the Reo-, the Bunya- and the Rhabdoviridae. This is probably the first piece of evidence that insects (arthropods) have played the role of a virologic turning table between the mobile vertebrates and the nonmobile plants. Taking into account that genomic ss RNA can either be positive-stranded (+) (acting as a messenger RNA) or negative-stranded (−) (no messenger function), the family and group frequency is approximately as follows (without bacterial and fungal viruses): ds DNA, envelope, 5; ds DNA, no envelope, 4; ss DNA, no envelope, 1 (2); ds RNA, no envelope, 3; ss RNA, +, envelope, 4; ss RNA, −, envelope, 6; ss RNA, +, no envelope, 27 (tentative); ss RNA, −, no envelope, (?) 1. Thus, the + stranded virions without an envelope represent the overwhelming majority of all viral families, reflecting the many families or groups that infect plants.

In addition to the criteria mentioned above, there exist virions with segmented genomes. The family and group distribution is shown in Table 2 (without bacterial and fungal viruses). In virions

TABLE 1

IMPORTANT VIRAL FAMILIES GROUPED ACCORDING TO TYPE OF GENOME, ENVELOPE AND HOST[a]

Host	ds DNA Envelope	ds DNA No Envelope	ss DNA No Envelope	ds RNA No Envelope		ss RNA Envelope		ss RNA No Envelope	
Bacteria	yes	yes	yes	no		no		yes	
Protozoa, algae, fungi	no	yes	no	yes		no		yes	
Invertebrates	Pox Baculo Polydna	Irido	Parvo	Reo Birna	10–12 2	Flavi Toga Rhabdo Bunya MyxoD	+1 +1 −1 −3 −6–7	Picorna Nudaurelia (Tetra) Noda	1+ 1+ 2+
Vertebrates	Pox Herpes Hepadna (Pararetro)	Irido Adeno Papova	Parvo	Reo Birna	10–12 2	Corona Flavi Toga Retro Filo Paramyxo Rhabdo Arena Bunya Orthomyxo	+1 +1 +1 (+1) −1 −1 −1 −2 −3 −7–8	Picorna Calici	1+ 1+
Plants		Caulimo (Pararetro)	Parvo (Gemini) 1–2	Reo Crypto	10–12 2	Rhabdo Bunya	−1 −3	Carmo (Calici) Sobemo (Picorna) + ca. 20 groups Tenui	(1, 2, 3+) (4−)

[a] The terms "viridae" for families and "virus" for groups or genera are not given for the sake of brevity. Numbers indicate numbers of genomic segments; +, positive polarity; −, negative polarity.

with partite genomes there is again a certain correlation between insects and the other kingdoms, with the exception of the extreme Polydna family which is restricted only to insects. The ss DNA Geminiviruses contain either one (subgroups I and II) or two (subgroup III) DNA segments. Members of subgroup I infect gramineae, whereas members of subgroup II infect dicotyledonous plants and both are transmitted by leafhoppers; and subgroup III infects dicots and is transmitted in a persistent manner by the whitefly (38). The ds RNA Birnaviridae have a host range encompassing fish, molluscs, birds, and Drosophila and are transmitted horizontally and vertically by as yet unknown direct vectors (24). The Reoviridae have 10 to 12 segments of linear ds RNA and a host range including vertebrates (Orthoreo), mammals, insects (Orbi), and dicots and gramineae, transmitted by leafhoppers during their entire life (Phytoreo) (58). The Bunyaviruses are enveloped viruses with three molecules of negative or ambisense ss RNA. They are mostly transmitted by insects to vertebrates, with the exception of the genus Hantavirus (101). The members of the genus Tospovirus infect plants, being transmitted by thrips. The prototype Tomato spotted wilt virus infects more than 360 plant species belonging to 50 families (22). The Orthomyxoviruses, carrying 8 (A and B) or 7 (C) segments include also members (Dhori and Thogoto viruses) which are tick borne, occasionally infecting man. These contain 6 or 7 ss minus RNA segments; the sequenced segments show relatedness to the A, B, and C types (28). Note that all the animal viruses contain the full complement of genomic segments in each virion (single component viruses); there is no separate encapsidation. In plant viruses the situation is different (57). As shown in Table 2, the segments of plant viruses are mostly separately encapsidated (multicomponent viruses). This is possible due to the cell-to-cell and seed transmission and massive mechanical inoculation (by the vectors). As mentioned above, these viruses do not usually carry an envelope, probably reflecting the fact that they do not bud through a cellular membrane as enveloped animal viruses do to reach the outside world. Rather, the plant viruses are transported from cell to cell (21,55,81,115). The only exception among the partite + stranded RNA viruses without envelope is the Noda group (94). Here, common encapsidation seems to be a prerequisite for infection of mosquitoes and transfer to vertebrates. The families with partite genomes infecting invertebrates, vertebrates, and plants—the VIP's—form very large families. These viruses replicate in their insect hosts. The monopartite families of the Rhabdo-, the Picorna-, and the Flaviviruses are also large and possess representatives replicating in insects. In contrast, many plant viruses are transmitted by insects in a nonpersistent or semipersistent manner, forming larger and smaller groups. It should be added here that according to classical taxonomy there is no systematic correlation between virion structure and genome type.

Viral Evolution

Although the classical viral families are not clearly based on evolutionary considerations, the organization of a given genome must have something to do with its origin and evolution. Indeed, supergroups can be defined as a collection of families with a nucleic acid type, a genome organization, a replication strategy, and a few

TABLE 2

VIRAL FAMILIES WITH SEGMENTED GENOMES AND THEIR HOSTS[a]

Genome Type	Number of Segments	Family or Group	Hosts	Packaging
ds DNA envelope	multiple	Polydna	I	1 particle
ds DNA no envelope	—	—	—	—
ss DNA no envelope	2	Gemini	P, I	separate
ds RNA	2	Crypto	P	intracellular
no envelope	2	Birna	V, I	1 particle
	10–12	Reo	V, I, P	1 particle
ss RNA, + envelope	dimer	Retro	V	1 particle
ss RNA, −	2	Arena	V	1 particle
envelope	3	Bunya	V, I, P	1 particle
	(6)7–8(9)	Orthomyxo	V, I	1 particle
ss RNA, + no envelope	2	Como, Nepo, Bymo, Diantho, Faba, Tobra, Furo	P, (I)	separate
		Noda	V, I, P	1 particle
	3	Hordei, Cucumo, Bromo, Ilar, Alfalfa	P, (I)	separate
ss RNA, − no envelope	4	Tenui	P, I	separate

[a] The terms "viridae" and "virus" are not given. I, invertebrates; V, vertebrates; P, plants; +, positive polarity; −, negative polarity; ds, double-stranded; ss, single-stranded. The names of plant virus groups are usually from prototype virus names.

protein sequence motifs in common; members of a family exhibit a genome with a similar gene order and stronger homologies; genera within families may possess dissimilar host ranges; species, types, and variants distinguish themselves from a prototype or consensus genome by a few nucleotides only. However, such small differences at the genomic level may have drastic consequences when it concerns biological activity. Therefore, the question arises as to the role of insects in the divergence of viral families.

Short-term evolution by substitutions at the genomic level. Parental viral populations become heterogeneous, but apparently remain stable due to a dynamic equilibrium between original types and viable mutants, which arise rapidly and disappear again due to selection (quasispecies nature of viral genomes). Many papers have covered this topic (19,25,31,52,62,66–68,72,78,97,106,109–111,116,120). The primary source of viral change is obviously "mutation." It seems appropriate to clarify the term because base substitution at the genomic level, mutation at the protein level, and evolution at the functional or structural (phenotypical) level of the mature virion involve different niveaux. Due to silent substitutions and constraints such as protein folding or selection of non-viable or non-infective mutants, the three corresponding rates are proportional but the proportion factor may be different between viral families. The substitution rate of a virus can be defined as the probability that in the course of a single replication of its genome a given nucleotide position is altered. The mutation rate corresponds to the probability that in a viral protein set an amino acid at a given position is exchanged. The evolution rate reflects the probability that an infectious virion competent for progeny formation exhibits altered functions. Substitution rates (error rates) and evolution rates can be measured; as a rule amino acid sequence changes are deduced (direct and indirect methods) (106). The reason for error is the intrinsic noisiness of RNA-dependent RNA and DNA polymerases which do not possess an editing system (25,52,97,106,109). Despite all the difficulties in exactly measuring the rates mentioned above, it seems that RNA viruses have mutation rates much higher than those of their hosts. The question is not settled in the case of DNA viruses (78,106). The rates in vitro do not necessarily correlate with the rates of field variation (106), which may rather reflect constraints and selection. Dependent upon selective pressures, for example during epidemics in which ecologic niches are expanded or changed or both, the dynamic equilibrium of the viruses and the viral population may also change (66). Paramount examples are Picorna- and influenza viruses. In a recent epidemic of acute hemorrhagic conjunctivitis (Enterovirus 70: Picornaviridae, 1 ss+ RNA, no envelope), a range of variants quickly arose and persisted in the viral and human population so that the RNAs differed between every sampling place and time over the whole world. A nucleotide substitution rate of 1.8×10^{-3} per base position per year was calculated (85). A rate of nucleotide change of about 2×10^{-3} per site per year has been reported for influenza A (Orthomyxoviridae) in human populations (16). The surface proteins of the virions, exposed to newly formed antibodies (change of niche conditions!) are under strong selective pressure (3,4). Unchanged virions will be neutralized and eliminated. Thus, the RNA quasispecies equilibrium is shifted in the next generation. This in turn permits the accumulation of amino acid changes (protein level) in the antigenic sites of the hemagglutinin, which corresponds to about 20% on the genome level (11). This type of change is called the antigenic "drift." Comparative sequence analysis has allowed the creation of a phylogenetic tree of the human influenza A viruses and the pinpointing of their origin in the middle of the last century (29). Thus, by selection and counterselection of countless virions during consecutive generations, the sites of changes in the genes will reflect sites and regions of freedom of change in the proteins and stretches where stability is needed. However, the nucleotide changes in all the influenza genes occur in a clocklike manner, the rate of "silent" substitutions being similar in all the genes. The host type plays a major role in selection. In human infections a larger proportion of substitutions in the hemagglutinin gene is fixed as mutations than, for example, in birds. This again favors the conclusion that substitutions are random but that there is selection for or against amino acid changes (3,31).

Many other examples have been described. The "same" viruses (human immunodeficiency virus: Retroviridae) from different patients within a clustered outbreak due to a contaminated transfusion batch were distinguishable (80); the same applied to individual human beings (45). Poliovirus (Picornaviridae) changed during epidemics in an unvaccinated community (89) and in a vaccinated child (84) or during chronic infection of individuals (64). Nonetheless, most viruses have quite stable populations in the wild. Thus, vaccines produced from old isolates such as polio and yellow fever vaccines still work. On the other hand, the unpredictable often small changes at the molecular level may have drastic changes at the disease level, e.g., with respect to virulence (104,117), to host range (105), or to organ tropism (2). Steinhauer and Holland (109) review many other examples of changes of cell, tissue, and species specificity and of virulence due to one or a few nucleotide changes.

The important lessons to be learned are: a) The permanently occurring substitutions may lead to mutations which are selected for

or against according to the reigning selective forces, the structural and survival needs of the virions, and the host type. This creates diversification, new strains, and eventually new species. b) The term species is unclear; it is a collection of strains with similar (known) properties. Van Regenmortel (114) has coined the term "polythetic species." c) Mutations of single amino acids can have drastic consequences allowing, for example, a virus to jump the species barriers. This is a very important aspect in viral evolution.

Long-term evolution by reassortment. Several groups of viruses possess segmented genomes as indicated in Table 2. A segment corresponds to a viral gene or genes. Structure genesis and infectivity usually depend on the full segment complement. As stated above, segmentation is not typical in the case of the ss + RNA viruses infecting animals, with the exception of the Nodaviruses of insects. In + RNA viruses of plants the opposite trend is found (57,71). Segmented multicomponent viruses exist only in plants and fungi. This has probably to do with selection phenomena occurring during transmission. However, segmented + RNA viruses as derivatives from nonsegmented RNA can be constructed artificially (30). This finding shows that during evolution the transition between monopartite and multipartite genomes is possible, a conclusion that is also drawn from the analysis of superfamilies. In case of double stranded (Reo-, Birnaviridae) and negative-stranded (Orthomyxo-, Bunya- and Arenaviridae) animal viruses, the RNA is copackaged in the form of a single component virus. If one host cell suffers a mixed infection by different strains of a partite virus there is some probability that the progeny virions contain a mixed set of genome segments. This important parasexual mechanism (62), called reassortment, creates a significant pathogenic potential. Influenza pandemics (1957, strain Singapore; 1968, strain Hong Kong) can be explained on this basis. Interspecies transfer of viruses may occur from time to time (62,103). Reassortment is the reason for the so-called antigenic "shift." It has been described in Reoviridae in vitro and in vivo (23,96,100). Bunyavirus genome reassortment has been successfully demonstrated in mosquitoes (8,76). In Arenaviruses (bipartite, −, animal) certain reassortants of Lymphocytic choriomeningitis virus lead to a drastic increase of virulence in mice (98).

Long-term evolution by RNA-RNA recombination and formation of defective (interfering) RNA. Another important mechanism for RNA virus diversification is RNA-RNA recombination. It corresponds to a covalent linkage between two RNA stretches, and most probably is the result of a double infection of a cell with two related (nonsegmented) RNA genomes. Homologous recombination refers to a intratypic site-correct recombination, with parental genomes being nearly identical. Intertypic recombination between RNAs of viruses with different serotypes (10 to 15% sequence differences) is sometimes called nonhomologous recombination. It has been described in the Picornaviruses Poliovirus (20) and foot and mouth disease virus, in the coronavirus mouse hepatitis virus in vitro and in vivo (61,79), and in the Bromoviruses (plants) brome mosaic (15) and cowpea chlorotic mottle viruses (5). In cell culture, recombination between RNAs of the Alphatogavirus Sindbis has been demonstrated (118). The related Western equine encephalitis virus is a natural recombinant virus (46). The only representative of the Rubivirus genus of the Togaviruses, rubella, may be a nonhomologous recombinant with loss of an envelope protein and rearrangements of protein motifs (26). It is not known whether recombination can occur in all RNA viruses. The frequency has not been determined for all cases. It seems rarely to be site specific. It is probably very rare in negative-strand RNA viruses. It is assumed that copy-choice by template switching of the viral RNA polymerase during negative-strand synthesis (65) is the underlying mechanism (56). Such a phenomenon would explain homologous and nonhomologous recombinations and even large deletions in an RNA, as, for example, in the case of defective (interfering) RNA. Nonhomologous recombination might even explain integration of cellular sequences such as $tRNA^{Asp}$ sequences at the 5′-end of Sindbis virus defective interfering RNA (86) or the uptake of parts of the ubiquitin gene by Pestiviruses (a genus of the Flaviviridae, animal). Irrespective of whether RNA-RNA recombination is a rare event or more frequent than anticipated, it certainly has had its role in viral evolution and may have led to the creation of "new" genera or, together with mutation, to "new" families.

RNAs of the defective (interfering) (DI) type have been demonstrated for nearly every animal virus. They are more or less extensively deleted RNAs which can be packaged and externalized with the help of viral proteins encoded by intact genomes coexisting in the same cell. DI particles can infect because of their usurped surface proteins, but they are individually not viable due to lack of genetic information. They can affect viral evolution in the course of consecutive high multiplicity passages by competing with full-length RNA for polymerases encoded by coinfecting intact virions (53,54). Defective genomes may evolve as gene modules (12) which may return to autonomous particles by rare recombinations, causing large phenotypic changes. DI particles can also modulate virulence, triggering a persistent infection. Cells then survive and the viral RNA can change. In mosquito cells, DI RNAs appeared soon after infection with the Alphatogavirus Semliki Forest; this may be a reason for the chronic infection invariably established in these cells (107).

Short RNA, multipartite genomes, and translation strategy. Mutation pressure seems to favor small RNAs. Models predict that high multiplicities of infection and high mutation rates support the evolution of multicomponent viruses (19). However, multicomponent viruses which package their RNA segments separately have a severe survival problem, at least in animals, due to the mode of transmission. The infection is a multihit phenomenon (57). It is interesting to note that among DNA viruses the Gemini virus group is essentially the only one with a divided genome. In RNA, the vicinal 2′-OH group leads to bonding capacities and labilization not possible in DNA (112). The inherent noisiness of RNA polymerases (transcriptases, replicases) and the quasispecies nature of viral RNA together with the inbuilt lability may explain why no RNA genome larger than 9 to 11×10^6 Da (Corona viridae) has ever been found, whereas ds DNA genomes may be much larger than 100×10^6. The genomic Toga RNA amounts to 4×10^6; the Picorna RNA is 2.5×10^6, and similar to the molecular weight (M_r) of the monopartite plant virus genomic RNAs (2 to 3×10^6). In the case of segmented plant genomes, the M_r of individual segments is as a rule only about 1×10^6. Selective interaction of segments during maturation may result in a kind of proofreading (97), so that in co-packaged segmented genomes the number of errors in a virion is lower than the average sum of the errors in the components. In short genomes, compacting the genetic information may be important. One possibility is to overlap genes in different reading frames as in the Influenza segments 7 and 8 (77) or in phase with leaky read through of stop codons (33–37,110,111). The ss+ RNA ge-

nomes are polycistronic. In view of the difficulties of translating polycistronic messages in eukaryotic cells (73) such viruses often produce subgenomic messengers. In certain plant viruses, such as Cucumo-, Bromo-, Ilar-, and Alfalfaviruses subgenomic mRNA is (co)-packaged. Subgenomic RNAs could re-assort with genomic RNAs from different parents to form a new partite genome, especially in case of massive transfer by insects. In mixed infections, RNA 3 of cowpea chlorotic mottle virus can replace RNA 3 of brome mosaic virus (both bromoviruses, tripartite, RNA 3 coding for the coat protein) (7). Certainly, during evolution many such combinations have been tried. Eventually, copy choice or ligation may also have led to longer, continuous genomes.

Different strategies are used to overcome the problem that internal initiation by eukaryotic ribosomes is not easily feasible (59). Negative-strand viruses synthesize monocistronic messengers. Some + stranded viruses such as Coronaviruses form quasi-monocistronic messengers. Togaviruses and the Bromo-, Cucumo-, Hordei-, Sobemo-, Tobra-, Tobamo-, and other plant viruses (ss+) use subgenomic RNAs. Others such as Picorna- and Flaviviruses (ss+) translate the whole message into a covalent polyprotein which has to be posttranslationally cleaved by virus- and host-coded endoproteases into the correct proteins (protein processing). Subgenomic messenger RNA formation and processing can be combined. Separation and unification of viral genomes seem to be a reversible process occurring in evolution. The number of segments characterizes viral families in a formal way only. The feasibility of a divided Sindbis genome (30) points in the direction of genomic module formation and module shuffling.

Viral Superfamilies

Single-positive-strand RNA viruses. Comparisons of the gene order, transcription, and translation strategies and finally nucleotide sequences have shown that ss + strand RNA viruses can be divided into two supergroups (49), somewhat arbitrarily, centered around the Picornaviruses (polio, hepatitis A, foot and mouth disease) and the Togaviruses (Alphaviruses and Rubivirus), respectively. Both superfamilies possess members that replicate in insects, e.g., the cricket paralysis virus of the Picornaviridae and all Alphaviruses, mosquitoes being the natural vectors for transmission to vertebrates. In the case of the *Picorna-like* (Poty-like) supergroup, the Calici- and Picornaviruses of animals, and the Como-, Nepo-, Poty-, and Bymoviruses of plants belong together even though the Como-, Nepo-, and Bymoviruses have bipartite genomes. The genomic RNAs contain single open reading frames, encoding polyproteins which must be cut into mature proteins by virus encoded endoproteases. No subgenomic messenger RNAs are formed. All the viruses of this superfamily are nonenveloped. The RNAs are 3′ polyadenylated; the 5′-terminus is not capped but is covalently linked to the virus encoded protein Vpg. The gene order is shown in Table 3. Thus, at the 3′ ends of the genomic RNA of polio and the RNAs B or 1 of the plant viruses there is a series of 4 genes in the same order encoding analogous functions (33–37). These four nonstructural (ns) proteins seem to be part of a membrane-associated replication complex. The polymerases contain the GDD motif (6), to be found in many polymerases including those of the Togaviridae. The 3C and 24K proteinases contain conserved residues with an active-site cysteine. Vpg is present at the 5′ end of all newly synthesized RNAs. The proteins 2C (polio) and 58K (como) termed MEM are membrane associated proteins thought to fix the whole replication complex to the host membranes where replication takes place. They share homologies; one motif is the GKS/T motif, known in many nucleotide-binding proteins (42,51). In the RNA of Potyvirus and RNA 1 of Nepo- and Bymoviruses this set of replication proteins, exhibiting sequence similarities, is again found. However, in Poty- and Bymoviruses the coat protein gene is located on the 3′-terminal side of the polymerase gene; this is interesting in view of recombination events and modular evolution. More information concerning initiation of protein synthesis, shut-off of host coded protein synthesis, protein processing, and virion structure can be found in King et al. (63).

TABLE 3

GENE ORDER IN THE POTYVIRUS (PICORNA-LIKE) SUPERGROUP[a]

Picorna	5′. . . .	MEM(2C)	3A	Vpg(3B)	3C	Pol(3D)	
		*		O	+	×	
Como RNA B	5′. . . .	MEM(58K)		Vpg(4K)	24K	Pol(87K)	
		*		O	+	×	
Nepo RNA 1	5′. . . .	*		O	+	×	
Poty	5′. . . .	CI(54K)		Vpg	NIa	NIb	CP
		*		O	+	×	
Bymo RNA 1	5′. . . .	*		?	+	×	CP

[a] Strongly simplified and not to scale. Individual viral names, RNA2 and M respectively, 5′ genes such as transport proteins and helper components, 3′ Poly(A) stretch not indicated. For a review *see* (63). MEM, membrane protein, with the nucleoside triphosphate (NTP, *) binding motif GKS/T (42). O, gene for the 5′ covalently bound protein Vpg. +, contains a serine proteinase like motif with cysteine in the active site. ×, RNA polymerase with the GDD motif (6). CP, coat protein. K, kilodaltons. In Picornaviruses the coat protein genes are on the 5′ terminal side, in Como- and Nepoviruses on RNA M and 2, respectively.

The *Alpha- or Sindbis-like* (Tobamo-like) viruses represent a second large superfamily (1). Their genomic RNA is capped at the 5′ end, whereas the 3′ end shows variable structures [X_{OH}, tRNA, or Poly(A)]. They all produce one or several subgenomic RNAs. They encode ns proteins by a series of genes of similar order. Leaky termination codons and corresponding read-through proteins occur. However, the structures of the virions and their hosts are variable and the genomic RNAs may be mono-, bi-, or tripartite. Examples are given in Table 4. In Alpha-like plant viruses a proteinase motif generally is not present, whereas in Alphaviruses at least one ns proteinase activity has been described (40,43,74). All conserved proteins of the Alpha-like group are involved in RNA multiplication (47,48,75). The polymerase contains the GDD motif (6,60). Near the nucleotide binding motif is a helicase motif (the DEAD family) which may unwind replicative form RNA molecules during replication (39,41,70). The fourth conserved motif, a methyltransferase in nsP1 of Sindbis virus, may be involved in capping of the genomic RNA at the 5′ end (82). The structural proteins are variable and nonhomologous and probably reflect the different selective pressures of the plant and the animal environments. Thus, unique genes and conserved genes are recombined or reassorted, making use of a constant replication module (33–37).

Recently, the creation of a Carmo-like and a Sobemo-like supergroup of plant viruses has been proposed (37). These genomes do not exhibit genes for putative helicases nor for putative methyltransferases. Carmoviruses exhibit homologies with the polymerases of Flavi- and Pestiviruses and hepatitis C virus (44,72,83). Sobemo-

TABLE 4

EXAMPLES FOR THE GENE ORDER IN THE TOBAMOVIRUS (SINDBIS-LIKE) SUPERGROUP[a]

Alpha	5′	nsP1		nsP2		nsP3	nsP4	C	E	$(A)_n$ 3′
		O		* +			→ ×			
Tobamo	5′		126K		183K		TRA	CP		$(tRNA)_{His}$ 3′
		O		* →	×					
Bromo										
RNA1	5′	O		*						
RNA2					×					$(tRNA)_{tyr}$ 3′
RNA3							TRA	CP		

[a] Strongly simplified and not to scale. Individual viral names and 5′ cap not indicated. For a review *see* (37). nsP, nonstructural proteins; C, CP, nucleocapsid and coat proteins, respectively; E, 2-3 envelope proteins; TRA, transport proteins; $(A)_n$, Poly(A); O, methyltransferase (capping enzyme); *, nucleotide binding sequence motif; +, papain-like proteinase domain (43); ×, polymerase domain; → leaky termination codon and readthrough protein. nsP3 of Alphaviruses is unique.

viruses, on the other hand, have a 5′ Vpg and a putative serine proteinase (40). In Tables 5 and 6 some motifs encompassing these groups are presented.

Based on all these considerations and sequence comparisons, trees of the known ss+ RNA viruses have been constructed. There is, with small discrepancies, an overall consensus that all these viruses are related (13,17,37,44,72,110). Bruenn (13) compared 50 RNA-dependent viral RNA polymerases to compose a dendrogram based on every amino acid. He found a large group of vertebrate, plant, and insect viruses whose common characteristic is exploitation of insect hosts or vectors. He gives the following relationship: Picornaviruses-ds RNA viruses-Alphaviruses-Tobamovirus-like-Potyvirus-like-Flavivirus-like-Luteo (Carmo)virus-like.

Double-strand RNA viruses. ds RNA viruses provoke important diseases in vertebrates (99,100), in insects (9), and in plants (90). Apart from fungal and bacterial viruses, the Reoviridae, the Birnaviridae, and the Cryptoviruses of plants are the important families. Orbiviruses of the Reoviridae, e.g., African horse sickness, Bluetongue virus (Culicoides), and Colorado tick fever virus (ticks, mosquitoes?) multiply in their invertebrate hosts. Phytoreoviruses (90) multiply in the transmitting leafhoppers which infect dicotyledonous plants; Fijiviruses are transmitted by planthoppers and infect Gramineae. The classification and the physicochemical properties are given elsewhere (27,92). The viruses mentioned here are naked, icosahedral, the genomes are segmented and mostly monocistronic (Table 2). Deletions may alter the number and size of segments (90) and reassortment of segments is probable (119). The viruses of fungi are the only ones that are separately encapsidated. The RNA polymerases are virion-associated; these enzymes transcribe full-length positive-strands which serve for protein biosynthesis and RNA replication. Capping is known in the case of Reo- and Birnaviridae.

Sequence comparisons of the RNA-dependent RNA polymerases have demonstrated relationships among the positive-strand and double-strand RNA viruses (13,69,88), as indicated above. Similarly, Gorbalenya et al. (41,42) have described ds DNA-, ss-DNA-, ds-RNA-, and ss+ RNA-viruses with proteins containing the motifs A and B (Table 5).

Single-minus-strand RNA viruses. The order Mononegavirales encompasses the monopartite Paramyxoviridae, the Rhabdoviridae, and the Filoviridae. Representatives of the Rhabdoviridae infect insects or plants. In the order of the segmented Multinegavirales (Orthomyxoviridae, Bunyaviridae, Arenaviridae, and Tenuiviruses) there are again representatives infecting insects or plants or both (Tables 1 and 2). The Mononegavirales all have a similar gene order (93). The most extensive homologies can be found in the replicase-polymerase genes (113). Although the gene orders within the Rhabdoviridae are very similar, the plant virus genomes are larger as they encode additionally a transport protein (81). Some plant Rhabdoviruses are transmitted by planthoppers to Gramineae, others by leafhoppers. Plant Rhabdoviruses replicate in their vectors which are quite specialized to a restricted number of host species. The animal Vesicular stomatitis virus has been experimentally transmitted to animals by horseflies, sandflies, and mosquitoes, but evidence for similar phenomena in nature is lacking. It can be imagined that Rhabdoviruses evolved in insects and were maintained by vertical transmission through the egg.

The Bunyaviruses of the order Multinegavirales contain three negative sense ss RNAs per virus particle. The RNA is complexed to the nucleocapsid (N) and to the replicase (R) protein. The envelope has two membrane associated proteins, G1 and G2. Nonstructural (NS) proteins may be formed. Reassortment has been described (10). The genome organization resembles that of the Rhabdoviruses, as shown in Table 7. Bunyaviruses, except Hantaviruses, are transmitted by mosquitoes, sandflies, gnats, or ticks. Tomato spotted wilt virus is transmitted by thrips; the larvae acquire the virus and the adults transmit it.

Other distant relationships are known, for example with the Tenuiviruses transmitted by planthoppers to grasses. Within the Bunyaviridae the 3′ and 5′ terminal sequences of the Tomato spotted wilt virus are different from the other genera however, similar to the

TABLE 5

EXAMPLES FOR PARTIAL SEQUENCE HOMOLOGIES IN CONSERVED MOTIFS[a]

A. Motifs A and B of the Nucleotide Binding/Helicase Region

1.	165	FQGKSRTGKSLLMS	95	KVRDDEAFKNRR ....
2.	81	VRGAVGSGKSTGLP	73	FVIIDECHVNDA ....
3.	354	NRGKVKLGKREFAW	.....	.....
4.	830	VDGVPGCGKTKEIL	57	RLFIDEGLMLHT ....
5.	183	VIGTPGSGKSAIIK	50	VLYVDEAFACH .....
6.	183	VFGVPGSGKSAIIK	50	ILYVDEAFACH .....
		G G GKS T		DEA

[a] 1 and 2: from the Picornavirus-like supergroup C (44): 1: Cowpea mosaic virus (CPMV) of the Comoviruses, 58K protein coded for by RNA B (Table 3); 2: Tobacco etch virus (TEV) of the Potyviruses, CI = 54K protein. 3: from the Luteovirus-like supergroup B (44), Southern bean mosaic virus (SBMV) of the Sobemoviruses, 105K protein. 4-6: from the Sindbisvirus-like supergroup A (44): 4: Tobacco mosaic virus (TMV) of the Tobamoviruses, 126K protein (32) (Table 4); 5: Sindbis virus of the Alphatogaviruses, nsP2 (Table 4); 6: Semliki Forest virus of the Alphatogaviruses, nsP2 (Table 4). To the left the N-terminal A site, and to the right with a gap indicated by the number of amino acids the C-terminal B site. The A site corresponds to a putative purine triphosphate binding domain, the B site to the putative helicase domain ("DEAD-family"). More sequences and references can be found in (41, 42, 44, 102).

TABLE 6

EXAMPLES FOR PARTIAL SEQUENCE HOMOLOGIES IN CONSERVED MOTIFS[a]

B. Motifs I to IV of the RNA Polymerase

1.	1434	DYSSFDGL	53	SGFPMT	3	NS	37	GDD	50	L . .
2.	2521	VYCDADGS	59	SG 3 T	3	NT	26	GDD	46	L . .
3.	699	DISGFDWS	56	SG 3 T	3	NS	18	GDD	38	FC . .
4.	1385	DISKYDKS	51	SG 3 T	3	NT	22	GDD	35	FC . .
5.	2271	DIASFDKS	51	SG 3 T	3	NT	24	GDD	37	FC . .

[a] Same viruses as given in Table 5. SFV is omitted. 1: CPMV, 87 K protein; 2: TEV, NI b protein; 3: SBMV, ORF 2; 4: TMV, 183 K; 5: Sindbis, nsP4. The four motifs are separated by the larger gaps indicated by numbers. The GDD motif is number 3. *See* also Tables 3 and 4. Numbering of amino acids from beginning of the polyprotein. More sequences and references can be found in (17, 44, 88).

3′ terminal sequence of the third segment of Thogotovirus, a tick transmitted Orthomyxovirus (22,108).

According to Peters (93), the following evolutionary history can be envisaged: A common origin gave rise to the Protorhabdo-Paramyxoviruses on one hand and to Protobunya-Orthomyxoviruses on the other hand. Divergent evolution in different ecologic niches then led to the contemporary diversification and eventual partial gain/loss of infectivity for insects.

Insects (Arthropods) as a Virological Turning Table?

At first glance the number of viral families, genera, and species is bewildering. However, despite the many possibilities of viral evolution, the relationships between families and groups have not been completely blurred. The main lesson to be learned is that accumulation of substitutions, reassortment, and recombination act together, allowing modular evolution. Not all evolutionary pathways have been mentioned, for example, biased hypermutation (18), formation of mixed protein coats after double infections by related viruses (phenotypic mixing), induction of receptors for a virus by infection by another virus, or viral proteins expressed in the plasma membrane acting as receptors. Such possibilities can enlarge the host ranges. Certain mutations can even increase the substitution rate (95). Thus the taxonomic criteria of envelope or segmentation of genomes are relative. They are useful for diagnosis but irrelevant when it concerns relationships. Whether the ss+ RNA viruses arose from an ancestral ds RNA virus (13) or vice versa (69), the consensus is that ds and ss+ RNA viruses are related and can be classified within superfamilies, which again exhibit relationships irrespective of envelope (animal viruses), segmentation (plant viruses), or virion architecture.

TABLE 7

GENE ORDER OF SOME NEGATIVE-STRAND RNA VIRUSES[a]

Rhabdo	3′	N	P	M	G	R	5′
Bunya	3′	N	NS_S	G_2	G_1	R	
		S RNA		M RNA		L RNA	
Arena	3′	N		G_1	G_2	R	
		S RNA				L RNA	

[a] Strongly simplified and not to scale. Individual viral names, leader sequences and some genes for nonstructural proteins not indicated. For a review *see* (93, 111). N, nucleoprotein; P, phosphoprotein; M, matrix protein; G, membrane glycoproteins; R, replicases; NS, nonstructural proteins.

Negative-stranded mono- or multipartite RNA viruses seem to have an origin of their own, but they also can be taken together into a superfamily (93,110,111). It must be stated that relationships are much more difficult to evaluate within DNA viruses due to the large genomes. However, considering the contemporary facts and the known viral relationships one cannot escape the conclusion that arthropods have played a role as a "turning table" in viral evolution and still have this function. In the family of the Poxviridae (ds DNA, envelope) are the Entomopoxvirinae, infecting Coleoptera, Lepidoptera, Orthoptera, and Diptera. Some members seem to be transmitted by mosquitoes to rodents. The insect iridescent viruses are ds DNA viruses (Iridoviridae), some with an envelope. The ss DNA Geminiviruses are transmitted in a persistent manner by leafhoppers or whiteflies to plants. The transmission to vertebrates of some Reoviruses (ds RNA) by Culicoides, Phlebotomines, and ticks has been mentioned. The cytoplasmic polyhedrosis virus (Cypovirus; Reoviridae) infects Lepidoptera, Diptera, and Hymenoptera. Plant reoviruses are transmitted in a persistent manner by leafhoppers and planthoppers. The ds RNA Birnaviruses infect vertebrates and Drosophila. In the supergroup of the negative-ss RNA viruses (Mononegavirales) some Rhabdoviruses multiply in leafhoppers, planthoppers, or aphids but also in mosquitoes; in the related Polynegavirales some Influenza D viruses replicate in ticks, and many Bunyaviruses are transmitted by mosquitoes, ticks, phlebotomines, or thrips.

In the huge superfamily of the ss+ RNA viruses there are many representatives infecting arthropods or transmitted by arthropods. Some Picornaviruses infect bees, Drosophila, crickets, flies, and aphids. The Alphaviruses infect, without exception, mosquitoes. The corresponding plant viruses are mostly transmitted by insects in a nonpersistent or semipersistent manner. The Tobamo-like Closteroviruses are transmitted by aphids, mealy bugs, or whiteflies in a semipersistent manner. The Potyvirus-like viruses contain members transmitted by aphids or whiteflies. The Flaviviruses are transmitted by infected mosquitoes or ticks. In the Luteovirus-like supergroup (13), Luteovirus is transmitted to plants by aphids in a persistent manner.

This brief overview demonstrates that arthropods, mainly insects, are participating in the life of all superfamilies of viruses. Obviously, not all infected insects become transmitters. Baculovirus infection is not transmitted to the other kingdoms. There is a large range from infection, infection-transmission (e.g., Alphaviruses), persistent, se-

mipersistent, or nonpersistent transmission. The question cannot be answered whether insects transmitting in a nonpersistent manner have lost the corresponding receptors for the viruses or whether they never possessed them. However, the compilation shows that the transmitting insects are ideally suited for virus propagation. They belong either to the superorder Hemipterodea or the superorder Holometabola of the insects (14). Thrips of the order Thysanoptera have piercing-sucking mouthparts with a stylet. The Homoptera (aphids, hoppers, mealy bugs, whiteflies) with more than 33 000 species have also piercing-sucking mouthparts, a stylet, and a salivary pump. They are plant feeders. The order Diptera (true flies, phlebotomines, mosquitoes, gnats) (superorder Holometabola) with about 150 000 species encompasses blood or plant juice suckers with a stylet and an elaborate pump. Insects with biting-chewing (Orthoptera) or sucking (Lepidoptera) mouthparts seem to be less apt as true virus transmitters. Ticks belong to the Cheliceriformes of the arthropods, subclass Arachnida, order Acari; they are blood-sucking ectoparasites with piercing mouthparts. Thus, for the virologist, cell cultures of organisms of the orders Thysanoptera, Homoptera, Diptera, and Acari are the important tools. Hink and Hall (50) have published the list of the recently established invertebrate cell lines and their use in virus research.

Convergent evolution or transduction of host genes seem to be more remote answers to explain the co-linearities in the genetic maps and common motifs in plant and animal viruses. Common ancestors, interviral recombination, and divergent evolution are a more favorable hypotheses (33,35,36). The separation of plant and animal cells occurred before 10^9 yr ago, so that in view of the high substitution frequency of viral RNA, the common viral ancestor cannot be equally old. Here, arthropods come into play again. The host ranges of plant and animal viruses flow together in arthropods. Either the RNA viruses of plants and higher animals stem from arthropod viruses or arthropods were the mailing stations for the transfer to the two kingdoms, irrespective of the fact that the relevant arthropods feed either on plants or on animals, but not on both. An ancestral insect RNA virus could have spread the modules of RNA-dependent RNA polymerases by interviral recombinations in coinfected tissues (13), and RNA segments could have been distributed by reassortment in a similar way (91,97). To name a common precursor is guesswork. However, the Nodamura virus, an insect virus, has unique properties (87,94). It infects mosquitoes in an inapparent infection, but kills invertebrates (moths, bees) and vertebrates (suckling mice). The virus multiplies in BHK cells. It is unique among the ss+ RNA viruses of vertebrates and arthropods (Tables 1 and 2) because it exhibits a bipartite genome with two small RNAs which are not individually infectious and must be co-packaged. There is probably no envelope. Infected cells contain three ss RNAs. Other members of the Nodaviridae, infecting Diptera, Coleoptera, or Lepidoptera, have been described. It has been integrated between the Poty-like and the Flavi-like supergroups (13).

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