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Molecular Variation in Vector-Borne Plant Viruses: Epidemiological [and Discussion] Significance

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Molecular variation in vector-borne plant viruses: epidemiological significance

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Patterns of variation are examined in four groups of plant viruses, with special reference to their particle proteins and to changes in vector transmissibility and specificity. In the nepoviruses and potyviruses, non-circulative transmission, by nematodes and aphids respectively, seems dependent on structural features on the surface of the virus particles. The N-terminal part of the particle protein may play the key role in potyviruses. Similarly in the luteoviruses, and possibly in the geminiviruses, specificity of circulative transmission by aphids, whiteflies and leafhoppers is linked to the antigenic specificity of the virus particles. Among naturally occurring isolates of the same virus, variation seems often to be discontinuous, and is predominantly of two sorts. Minor variations, characterized by loss of an epitope or substitutions of a few amino acids, can be associated with loss of transmissibility in luteoviruses and potyviruses, or have no effect. Major variations are associated with differences in vector specificity and seem likely to involve radical genetic changes that have evolved over long periods. The adaptation of virus particle proteins for transmission by vectors probably results in conservation of the genes that encode them, and in greater conservation of some parts of these genes than of others.

Introduction

Plant viruses differ greatly in their survival strategies. At one extreme some, such as prunus necrotic ringspot ilarvirus, are spread relatively slowly from plant to plant of the same species in pollen, usually over short distances, and are maintained in a few long-lived perennial species and by vegetative propagation of infected mother plants. At another extreme others, such as beet curly top geminivirus, can be spread rapidly and over long distances by active flying vectors migrating from overwintering virus reservoirs that may include a wide range of plant species. The occurrence and course of epidemics of plant virus disease are therefore the resultant of the interplay of many factors and processes (Harrison 1981), which impose a variety of selection pressures on the viruses, and conversely are affected by modifications in their genomes. Virus isolates are selected for ability to spread from plant to plant, usually with the aid of invertebrate or fungal vectors; for ability to survive in different and fluctuating environmental conditions, especially temperature; and for ability to infect, replicate to a high concentration in and spread throughout plants of different species and of different genotypes of the same species, without damaging them so severely that they will not act as inoculum sources for further spread. In some instances, where host species have been exposed to infection continuously for many years, there is evidence of co-evolution of virus and host to a situation in which the viruses survive in but do not greatly damage their plant hosts (Jones 1981).

The amount of information about the molecular basis of changes in epidemiologically important characteristics of plant viruses is still small, much smaller than that available for some vertebrate-infecting viruses, such as the influenza viruses (Webster *et al.* 1982; McCauley

1987). However, vertebrate virus systems differ from plant virus systems in important ways. First, the vertebrate viruses mostly spread without a vector or are spread by arthropod vectors that they infect, whereas most plant viruses rely on transmission by vectors that, in most instances, are not themselves infected. Secondly, vertebrate viruses, but not plant viruses, are selected for ability to overcome antibody-mediated defence reactions. Thirdly, whereas viruses infecting warm-blooded animals replicate at relatively constant temperatures, plant virus infections must proceed at a range of environmental temperatures that fluctuate daily and seasonally. There are therefore several reasons for examining the limited but now rapidly increasing amount of information on molecular variation of epidemiological importance in plant viruses. In this paper special emphasis will be given to variation that relates to transmissibility by different types of vectors that the viruses seem not to infect.

Genetic systems of plant viruses

Different groups of plant viruses have a variety of types of genome: RNA or DNA, single-stranded or double-stranded, monopartite or multipartite. However, the great majority of plant virus genomes contain from four to eight genes, and about 80% consist of single-stranded RNA of positive-sense polarity. Information on the four virus groups dealt with here is summarized in table 1.

Table 1. Genome and vector types of four groups of plant viruses

	genome properties					
virus group	type	no. species	approx. size (nucleotides)	no. genes	vectors	transmission type
geminivirus						
sub-group A	ssDNA	2	2800 and 2750	6	whiteflies	circulative
sub-group B	ssDNA	1	2750	?4	leafhoppers	circulative
luteovirus	ssRNA	1	6500	?5	aphids	circulative
nepovirus	ssRNA	2	8500 and 4000–7000	?6	nematodes	non-circulative
potyvirus	ssRNA	1	9500	7–8	aphids	non-circulative, dependent on helper component

Three main kinds of genetic variants of plant viruses are known. One type includes mutants in which nucleotide substitutions, deletions or duplications occur. A second comprises pseudo-recombinants, which are variants of viruses with multipartite genomes that are produced by reassorting the genome parts of different virus strains so that the new isolate has one or more parts derived from each parental strain. The biological fitness of such pseudo-recombinants depends in part on the mutual compatibility of their genome segments. This need results in near identity of the non-coding nucleotide sequences in different genome segments, at both their 5′ and 3′-ends. Among plant viruses, pseudo-recombination may be important in nature for increasing the amount of variability that is derived from a set number of mutations. Among vertebrate viruses it is almost certainly responsible for causing the large genetic shifts in influenza virus A seen in the strains that cause major epidemics.

The third type of genetic variant consists of recombinants, which possess genome nucleic acid molecules made up of sequences derived from different sources. Recombination is well known among viruses with double-stranded DNA genomes but has only recently been reported in

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plant viruses with single-stranded RNA genomes. For example, when the 5′-terminal sequence of one of the three genome RNA species of brome mosaic bromovirus was deliberately altered, it reverted to the original form when the virus was propagated in plants, presumably as a result of recombination between a mutant molecule and a wild-type molecule of one of the other genome RNA species (Bujarski & Kaesberg 1986). Evidence has also been obtained that recombination occurs in nature in tobraviruses. Two isolates of tobacco rattle virus were found in which the smaller genome segment (RNA-2) contained the particle protein gene of pea early-browning virus instead of that of tobacco rattle virus but in which the 5′ and 3′-terminal non-coding sequences of tobacco rattle virus were retained (Robinson *et al.* 1987).

Plant viruses therefore produce variants of several types, with the absolute and relative frequencies of the different types depending on the type of genome.

Variation in geminiviruses

The geminivirus group comprises viruses that produce characteristic geminate particles and have genomes consisting of one or two species of circular single-stranded DNA (table 1). Both genome DNA and its complement contain open reading frames. The viruses, which mostly occur in tropical or sub-tropical regions, fall into two main sub-groups. Viruses in one sub-group have one species of genomic DNA and are transmitted by leafhoppers, whereas those in the other sub-group have two species of genomic DNA and whitefly vectors.

A larger number of complete viral genomes have been sequenced among geminiviruses than among other groups of plant viruses and this allows the differences between genomes, and the relative variability of different genes, to be assessed. When the mean similarities of all the gene products in different pairs among four whitefly-transmitted geminiviruses were compared, genome similarity was not correlated with similarity in host range. Indeed, the greatest similarity (79%) was between bean golden mosaic and tomato golden mosaic viruses, which have non-overlapping host ranges, and the least similarity (45%) was between bean golden mosaic and mungbean yellow mosaic viruses, which both infect leguminous species. When the general variability of different gene products was compared, that of gene V was the most variable and that of gene I the least (table 2). The function of the gene V product is not known, and this gene may not have a counterpart among leafhopper-transmitted geminiviruses, but gene I encodes the virus particle protein, which is similar in size to, and can be compared

Table 2. Similarities among amino acid sequences of proteins encoded by genes of four whitefly-transmitted geminiviruses

(Comparisons are based on nucleotide sequences of African cassava mosaic virus (Stanley & Gay 1983), bean golden mosaic virus (Howarth *et al.* 1985), mungbean yellow mosaic virus (M. Ikegami, personal communication) and tomato golden mosaic virus (Hamilton *et al.* 1984).)

gene	location of gene	direct sequence similarity (%)
I	DNA-1	74 (67–90)
II	DNA-1 (complementary strand)	65 (60–71)
III	DNA-1 (complementary strand)	56 (49–68)
IV	DNA-1 (complementary strand)	49 (36–76)
V	DNA-2	34 (19–70)
VI	DNA-2 (complementary strand)	56 (37–90)

[a] Gene III was not detected in mungbean yellow mosaic virus. Figures are for the other three viruses.

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directly with, that of the leafhopper-transmitted viruses. The results of such a comparison show that the particle proteins of the three leafhopper-transmitted viruses have little amino acid sequence similarity to those of the four whitefly-transmitted ones (mean 12%), somewhat more similarity among themselves (mean 27%), but much less than that found among the whitefly-transmitted viruses (mean 74%) (table 3). Furthermore, similar comparisons of all the putative gene products of four leafhopper-transmitted geminiviruses show that, in contrast to the whitefly-transmitted viruses, the particle proteins have no greater sequence similarity than other proteins (Donson *et al.* 1987).

The pattern of variation in geminivirus particle proteins mirrors the relationships previously established among geminiviruses by tests with homologous and heterologous polyclonal antisera and by nucleic acid hybridization tests with cloned DNA probes (Roberts *et al.* 1984). All the whitefly-transmitted viruses were found to be strongly related, whereas the leafhopper-transmitted ones were not related either to the whitefly-transmitted viruses or, with one exception, to one another. The serological relationships among whitefly-transmitted geminiviruses can be analysed further by using monoclonal antibodies. When seven whitefly-transmitted geminiviruses were tested against a panel of monoclonal antibodies to African cassava mosaic virus, each virus gave a distinctive pattern of reactions, but one epitope was specific for the homologous virus and another occurred in all the other viruses apart from the leafhopper-transmitted beet curly top virus (table 4).

TABLE 3. SIMILARITIES AMONG AMINO ACID SEQUENCES OF PARTICLE PROTEINS OF SEVEN GEMINIVIRUSES

		direct sequence similarity (%)[a]					
virus	vector[b]	BGMV	MYMV	TGMV	BCTV	MSV	WDV
African cassava mosaic (ACMV)	WF	68	67	71	15	10	12
bean golden mosaic (BGMV)	WF	100	72	90	15	9	11
mungbean yellow mosaic (MYMV)	WF	–	100	75	15	9	10
tomato golden mosaic (TGMV)	WF	–	–	100	14	9	11
beet curly top (BCTV)	LH	–	–	–	100	25	22
maize streak (MSV)	LH	–	–	–	–	100	35
wheat dwarf (WDV)	LH	–	–	–	–	–	100

[a] Based on nucleotide sequences from sources cited in table 2, and from Stanley *et al.* (1986) (BCTV), Mullineaux *et al.* (1984) (MSV) and MacDowell *et al.* (1985) (WDV).
[b] WF, whitefly; LH, leafhopper.

TABLE 4. REACTIONS OF SIX MONOCLONAL ANTIBODIES TO AFRICAN CASSAVA MOSAIC VIRUS WITH PARTICLES OF OTHER GEMINIVIRUSES

(Data from Thomas *et al.* (1986) and Harrison *et al.* (1987), obtained by indirect enzyme-linked immunosorbent assay (ELISA).)

	monoclonal antibody					
virus	SCR11	SCR13	SCR15	SCR17	SCR18	SCR20
African cassava mosaic	+	+	+	+	+	+
Australian tomato leaf curl	0	0	0	(+)[a]	0	+
bean golden mosaic	0	0	0	+	0	+
euphorbia mosaic	0	0	(+)	+	0	+
Indian cassava mosaic	0	0	+	0	+	+
mungbean yellow mosaic	0	0	0	+	(+)	+
tomato golden mosaic	0	(+)	(+)	+	0	+
beet curly top	0	0	0	0	0	0

[a] (+) indicates a weak reaction

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It is not clear how variation in geminivirus genes II to VI affects ability of the viruses to survive and spread in nature. However, circumstantial evidence suggests that the geminivirus particle protein plays a key role not only in protecting genomic DNA from inactivation but also in mediating virus transmission by the specific insect vectors. Geminiviruses can be acquired from infected plants in minimum feeding periods of 5 min (leafhoppers) or 15 min (whiteflies), cannot be inoculated to healthy plants until at least 6 h (leafhoppers) or 4 h (whiteflies) after the beginning of acquisition and remain transmissible for several or many days after acquisition but not necessarily for the life of the vector (Harrison 1985). The leafhopper-transmitted maize streak virus does not seem to multiply in its vector but appears to pass through the gut wall into the haemolymph and from there into salivary gland cells and saliva, with which it is introduced to virus-free plants to cause new infections (Storey 1933). Comparably detailed work has not been done with whitefly-transmitted geminiviruses but there is no reason to suppose that they behave differently. Clearly the vector-species specificity of such a method of transmission, involving transport of intact virus particles through the gut wall and salivary gland cells, must reflect properties of the surface of the virus particles, focusing attention on the virus particle protein. Indeed, in the luteoviruses, which have vector relations resembling those of geminiviruses but are transmitted by aphids, there is strong evidence that the virus particle protein determines vector specificity (see next section). Such a role could therefore explain the remarkable evolutionary conservation of the particle protein of whitefly-borne geminiviruses, all of which are transmitted by the same whitefly species, *Bemisia tabaci*, and the lack of its conservation among the leafhopper-borne members of the group, each of which has a different species as its principal vector (Roberts *et al.* 1984; Harrison 1985). If this hypothesis is correct, the epitope detected by monoclonal antibody SCR20 (table 4) would be a candidate for the structure that determines whitefly-transmissibility. This epitope is not detected in denatured particle protein after electrophoresis in polyacrylamide gel, indicating that it is sensitive to the way in which the polypeptide chain is folded (M. M. Aiton & B. D. Harrison, unpublished results).

Further information has been obtained by determining the distribution of 17 epitopes of a west Kenyan isolate of African cassava mosaic virus among geminivirus isolates from mosaic-affected cassava plants from different regions. Figure 1 summarizes the results of testing 87 virus isolates from 10 countries in Africa or the Indian subcontinent. The isolates fall into three groups. Group A isolates came from Ivory Coast, Nigeria, Angola, South Africa and western Kenya; group B isolates came from coastal Kenya, Malagasy, Malawi and Tanzania; and group C isolates were from India and Sri Lanka. Within each group there was some variation: group A isolates shared at least 14 epitopes with the west Kenyan isolate, group B isolates shared 4 to 9 epitopes with it and group C isolates shared only 2 or 3. Moreover, isolates in the same group tended to share the same epitopes.

This pattern of variation resembles that resulting from genetic shift and drift in influenza A virus. In influenza, shift results from pseudo-recombination. However, the variation between groups of isolates from cassava does not seem to have arisen in this way because nucleic acid hybridization tests indicate that the DNA-2 species of group B and C isolates differ in sequence from the DNA-2 of group A isolates considerably more than do the respective DNA-1 species (which contain gene I) (Harrison *et al.* 1987). Geminiviruses have not been found in cassava in South America, which is the centre of origin of cassava, and the variation now observed among isolates from the Old World could reflect the three main paths of introduction of cassava

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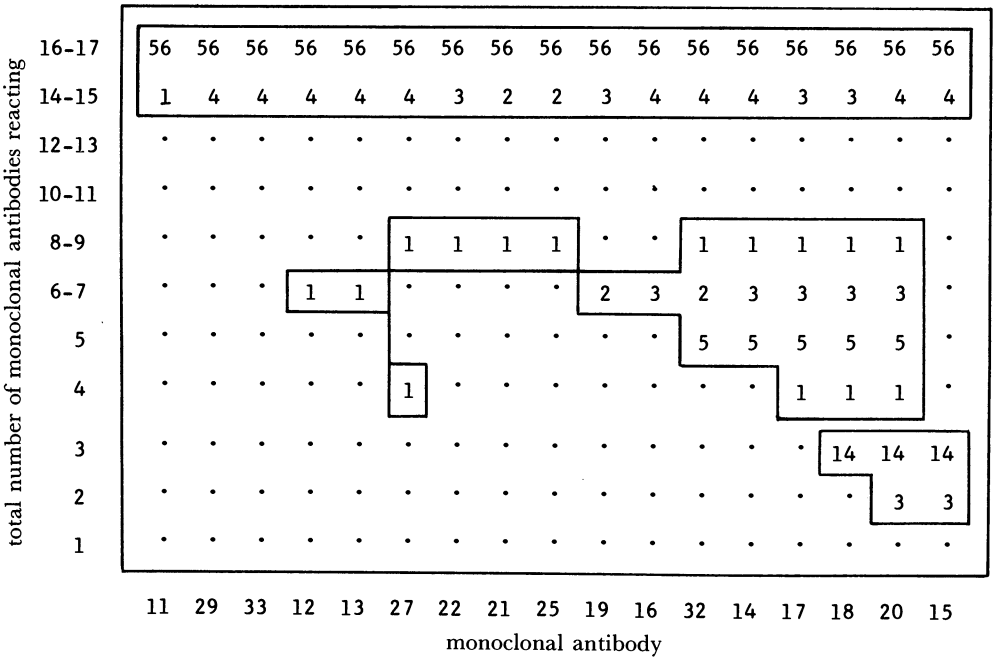

FIGURE 1. Distribution of particle protein epitopes among 87 geminivirus isolates from cassava obtained from ten countries in Africa and the Indian subcontinent. Each isolate was tested against 17 monoclonal antibodies (codes given on horizontal axis), each of which detects a different epitope in the particle protein of the type isolate of African cassava mosaic virus from western Kenya. The number of isolates reacting with each monoclonal antibody is subdivided according to the total number of antibodies reacting with each of these isolates (vertical axis). Group A isolates (from western Kenya, Angola, Ivory Coast, Nigeria and South Africa) reacted with 14–17 monoclonal antibodies, group B isolates (from coastal Kenya, Malagasy, Malawi and Tanzania) reacted with 4–9, and group C isolates (from India and Sri Lanka) reacted with 2 or 3. Data from Harrison *et al.* (1987) and Aiton & Harrison (1988).

from South America to Africa and India by Portuguese colonists and traders in the 16th to 18th centuries: (*a*) in the 16th and 17th centuries to the Congo region, from which it was distributed to other parts of West Africa and eastwards across the continent; (*b*) in the 18th century around the Cape of Good Hope to settlements along the East African coast and from there inland to the west; and (*c*) in the 18th century via Reunion and/or Mauritius to Sri Lanka and India. The variation now observed between isolates could be explained if the cassava was virus-free when introduced to each of these regions but became infected by three different kinds of indigenous virus that were established respectively in the three areas in other species (Harrison *et al.* 1987). If this hypothesis is correct, the differences between group A, B and C isolates from cassava may represent evolutionary divergence that occurred before the 18th century. Further work is needed to ascertain whether this divergence has been enforced by the need to adapt to divergent forms of *Bemisia* that may differ in distribution, but it is worth noting that all the isolates tested have the particle protein epitope detected by monoclonal antibody SCR20.

The small-scale variation analogous to genetic drift cannot be explained historically. Thus although variants of group A occur in different countries, the most prevalent forms in all countries were indistinguishable antigenically from the isolate used to prepare the monoclonal antibodies. Perhaps the minor variants result from mutations that do not have much effect on biological fitness. If so, they might be expected to be clustered in particular districts within a country because much of the dissemination of virus in vegetative planting material is likely to

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be local, and *B. tabaci* probably seldom moves over large distances. To ascertain whether regional variation occurs, 60 isolates collected throughout an area measuring 400 km × 400 km within the Ivory Coast were tested against the panel of monoclonal antibodies. Four minor variants were identified, three of which came from within 60 km of each other and had related but not identical changes. This indicates that some geographical variation does occur (Aiton & Harrison 1988) but whether it has epidemiological significance is unclear.

Variation in luteoviruses

In contrast with the geminiviruses, only a little sequence information is available for the single-stranded RNA genomes of luteoviruses, which seem to contain about 5 genes (Miller *et al.* 1987). Serological tests provide a reliable method of identifying different members of the group, but a network of serological relationships exists among the particle proteins of the different viruses. Some cereal hosts, such as barley, are susceptible to infection with several serologically distinguishable luteovirus variants, known collectively as barley yellow dwarf virus. These each have a different aphid species or combination of species as their main vector(s) (Rochow 1969) and are probably best assigned to two distinct viruses, known as RPV (transmitted by *Rhopalosiphon padi*) and MAV (transmitted by *Sitobion* (formerly *Macrosiphum*) *avenae*). *R. padi* seldom transmits MAV unless the source plants contain RPV as well, when virus isolates indistinguishable from MAV form a substantial proportion of those transmitted. This happens because some of the MAV-RNA in the doubly infected plants becomes packaged in particles that have the RPV coat protein, which confers transmissibility by *R. padi* (Rochow 1970).

Like geminiviruses, luteoviruses circulate through the bodies of their vectors, passing from the gut to the haemocoele and then into cells of the accessory salivary glands and saliva. Few, if any, cells become infected during this sequence of events. Vector specificity is mainly determined by the ability of virus particles to pass from the haemocoele into 'coated pits' within salivary gland cells. Thus particles of non-transmitted isolates are found in the basal lamina of the salivary gland cells but apparently do not enter them (Gildow & Rochow 1980). The luteovirus particle protein therefore seems to determine ability to enter salivary gland cells, and the requirements for entry evidently differ in different aphid species. However, transmissibilities by different aphid species are not mutually exclusive: for example, the PAV strain of barley yellow dwarf virus is transmitted by *R. padi* as well as *S. avenae*, and its particles possess one or more epitopes not found in either MAV or RPV, as well as those shared with MAV (Torrance *et al.* 1986). Taken together, these findings indicate that the emergence of barley yellow dwarf virus variants with altered particle proteins is likely to affect their degree of transmissibility by different members of the complex of aphid species that infest barley and other cereals, with consequent effects on virus epidemiology.

The antigenic differences found between these variants of the barley yellow dwarf viruses are relatively large and seem likely to have arisen from more than one or a few mutational events. To look for smaller differences that might affect aphid transmissibility, tests were made with another luteovirus, potato leafroll, all British isolates of which had proved to be closely related when tested with polyclonal antisera (Tamada & Harrison 1980). When 30 isolates were screened against a panel of 10 monoclonal antibodies, all but two reacted with each antibody and were readily transmitted by the main aphid vector, *Myzus persicae*. The remaining two

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isolates did not react with two of the monoclonal antibodies and were transmitted seldom or not at all by *M. persicae* (Massalski & Harrison 1987) (table 5). They had presumably been maintained by vegetative propagation of infected plants. One of these poorly transmitted isolates had previously been examined in some detail and was known to be acquired by, and to persist in, *M. persicae* in substantial amounts. Also, whereas aphids fed through membranes on purified particles of a transmissible isolate could inoculate the virus to plants, those fed on the poorly transmissible isolate could not (Tamada *et al.* 1984). These results again point to the importance of the luteovirus particle constituents in determining aphid transmissibility and suggest that one or more epitopes in the particle protein play a key role. By analogy with barley yellow dwarf virus, this role may concern the passage of virus from the haemocoele into salivary gland cells. Further tests have failed to distinguish the epitopes detected by the two monoclonal antibodies that do not react with the poorly transmissible isolates, and they may be identical. They are not detected in immunoblots of denatured virus particle protein and hence are conformation-sensitive (table 5).

TABLE 5. REACTIONS OF MONOCLONAL ANTIBODIES PREPARED TO POTATO LEAFROLL LUTEOVIRUS PARTICLES

(Data from Massalski & Harrison (1987) and Mayo & Massalski (1987).)

monoclonal antibody	particles of aphid non-transmissible isolate[a]	aphid-transmissible isolate		
		particles[a]	disrupted particles[a]	denatured protein[b]
SCR1	+	+	+	+
SCR2	+	+	+	+
SCR6	+	+	(+)	0
SCR7	+	+	0	0
SCR8	0	+	0	0

[a] Reaction in ELISA of antigen trapped on plates at pH 7.4 (particles) or pH 9.6 (disrupted particles). (+) indicates weak reaction.

[b] Reaction of particle protein in immunoblotting tests after electrophoresis in polyacrylamide gel.

These results show that luteovirus particle proteins are highly adapted for transmissibility by particular aphid species and that a small change in the protein can virtually abolish aphid transmissibility. The selection pressure which aphid transmissibility exerts on the luteovirus particle protein gene must be a strongly conservative influence on it.

VARIATION IN NEPOVIRUSES

The genome structure and biological properties of nepoviruses differ greatly from those of geminiviruses and luteoviruses (table 1). The two segments of the nepovirus single-stranded RNA genome are each translated into one large polyprotein that is cleaved into functional gene products, only two of which, the virus particle and genome-linked proteins, are reasonably well characterized. Nepoviruses depend for their survival and spread on their wide host ranges, transmissibility by nematodes and passage through seed to a proportion of progeny plants. Their survival in dormant seeds, especially weed seeds, helps them to persist at sites of disease outbreaks during crop-free periods and to be disseminated to other sites (Murant & Lister 1967). In some nepoviruses, notably raspberry ringspot virus, variant strains differ in

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transmissibility by nematodes or through seed, or in plant host range or invasiveness in selected species. By ascertaining the properties of pseudo-recombinant isolates produced by reassortment of the genome segments of the variants, most of the genetic determinants of these biological characteristics could be assigned to either RNA-1 or RNA-2 (table 6). A few characteristics were found to be determined by both RNA-1 and RNA-2, and in some instances there was evidence of the need for mutual adaptation of the two genomic RNA species for maximum expression of a characteristic (Harrison *et al.* 1974*a*; Harrison & Hanada 1976).

TABLE 6. LOCATION OF GENETIC DETERMINANTS FOR BIOLOGICAL PROPERTIES IN THE GENOME OF RASPBERRY RINGSPOT NEPOVIRUS

(Data from Harrison *et al.* (1972, 1974*a*) and Hanada & Harrison (1977).)

biological property	genome segment	
	RNA-1	RNA-2
particle antigenic specificity	0	+
nematode vector transmissibility	0	+
seed transmissibility	+	(+)
infectivity for raspberry cultivar Lloyd George	+	0
systemic invasiveness in French bean	+	0

These results indicate that the two genome segments are subject to different selection pressures. For example, RNA-1 variants will be at a selective disadvantage if they do not maintain ready seed transmissibility, as will RNA-2 variants if they do not maintain ease of nematode transmission. Moreover, different selection pressures may act on the same genome segment. This was seen when plantings of susceptible raspberry cultivars with a high incidence of infection with raspberry ringspot virus were replaced by the cultivar Lloyd George, which is immune to the common Scottish strain of the virus. In some of these replanted fields, Lloyd George plants became infected with a resistance-breaking strain (strain LG) that is readily nematode-transmitted, but is less readily seed-transmitted in weed hosts than the common strain (Murant *et al.* 1968; Hanada & Harrison 1977). Indeed strain LG was not found in raspberry cultivars susceptible to the common strain, suggesting that it is normally not prevalent among the population of virus isolates infecting weed reservoir-hosts. However, degree of seed-transmissibility and ability to infect Lloyd George raspberry are both determined by RNA-1 (table 6). This genome segment is therefore exposed to two selection pressures in raspberry fields and, because no strain is known that infects Lloyd George and is readily seed-transmitted, these pressures are opposite and their relative strengths appear to determine the incidence of strain LG. It seems either that the two viral genes are closely linked or that both characteristics are controlled by the same gene and that a mutation to a form that is more favourable for one characteristic is necessarily deleterious for the other. Indeed, it is probably commonly true, for the many viruses that have only a few genes, that individual genes and gene products have more than one function, that most mutations that are advantageous for one function may be disadvantageous for another, and that virus gene evolution is subject to compromises, which are needed to maintain or improve biological fitness under a variety of selection pressures.

Nepoviruses do not infect or circulate within their nematode vectors but rely for transmission on attachment and detachment of virus particles on specific surfaces in the nematodes'

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mouthparts or oesophagus (Taylor & Robertson 1969). The viruses may be retained by vector nematodes for weeks or months and presumably are transmitted when their particles are egested along with saliva. Each virus is transmitted by only one or a few nematode species, which usually differ for different viruses and sometimes for different strains of the same virus. However, in general, ability of a nematode species to transmit is correlated with ability of virus particles to bind to its mouthparts or foregut (Harrison *et al.* 1974*b*). The transmissibility of a particular virus isolate is also correlated with the serological specificity of the virus particle protein, and experiments with pseudo-recombinants show that both these characteristics are controlled by RNA-2 (table 6). Presumably, structures on the virus particle surface determine the ability of the particles to attach to surfaces in the nematode.

As with luteoviruses, differences in vector specificity between nepovirus strains are accompanied by antigenic differences that are too large to be caused by a few mutations. Indeed, in tomato black ring virus, the RNA-2 molecules (which contain the particle protein gene) of strains differing in vector specificity have only about 12% similarity in nucleotide sequence, whereas strains with similar vector specificity have at least 88% sequence similarity (Dodd & Robinson 1984). However, small changes in the antigenic specificity of virus particles can be tolerated without loss of nematode transmissibility, as exemplified by the LG and common Scottish strains of raspberry ringspot virus (Murant *et al.* 1968). The picture that emerges is that some variants have become adapted for transmission by different specific vectors, probably over a long period, and have many genome differences, whereas other have smaller genome differences that may increase host range and alter seed transmissibility. For a virus strain to survive in natural conditions it must have these qualities in combinations that differ according to the circumstances at the site.

Variation in potyviruses

The potyvirus group includes over 100 viruses, which can be acquired and inoculated by aphids within a few minutes and usually are not retained by vector insects for more than an hour or two. Although some of these viruses are transmitted through seed, usually to only a small proportion of progeny seedlings, all rely for their survival and spread mainly on transmission by aphids. Moreover, vector specificity is not highly developed and most individual potyviruses can be transmitted by several species of aphid. The single-stranded RNA genome of potyviruses is probably translated into a single large polypeptide that is cleaved, with the aid of a virus-coded protease (Carrington & Dougherty 1987), into functional gene products. These products include two that play key roles in aphid transmission: the virus particle protein and a non-structural protein known as the helper component. The helper component consists of a polypeptide of *ca.* 55 kDa (Thornbury & Pirone 1983) that is responsible for binding the virus particles to surfaces in the aphid's maxillary stylets (Berger & Pirone 1986). The helper component of one potyvirus typically can assist the transmission of other, but not necessarily all, members of the group.

Only a limited amount of nucleotide sequence information is available for potyvirus genomes, but enough to indicate the extent to which the encoded proteins are conserved in a few members of the group. For example, the amino acid sequences of the putative helper components and the particle proteins of tobacco vein mottling and tobacco etch viruses have 54% and 61% similarity, respectively. This compares with figures of 31–67% for four other

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virus-coded proteins (Domier *et al.* 1987), suggesting that the particle protein and helper component are relatively well conserved but somewhat less so than the putative viral polymerase (67%). However, within the particle proteins of five potyviruses, some amino acid sequences are conserved strongly and others hardly at all (figure 2). For example, the N-terminal sequences (up to residue 66 in johnson grass mosaic virus and up to residues 26–29

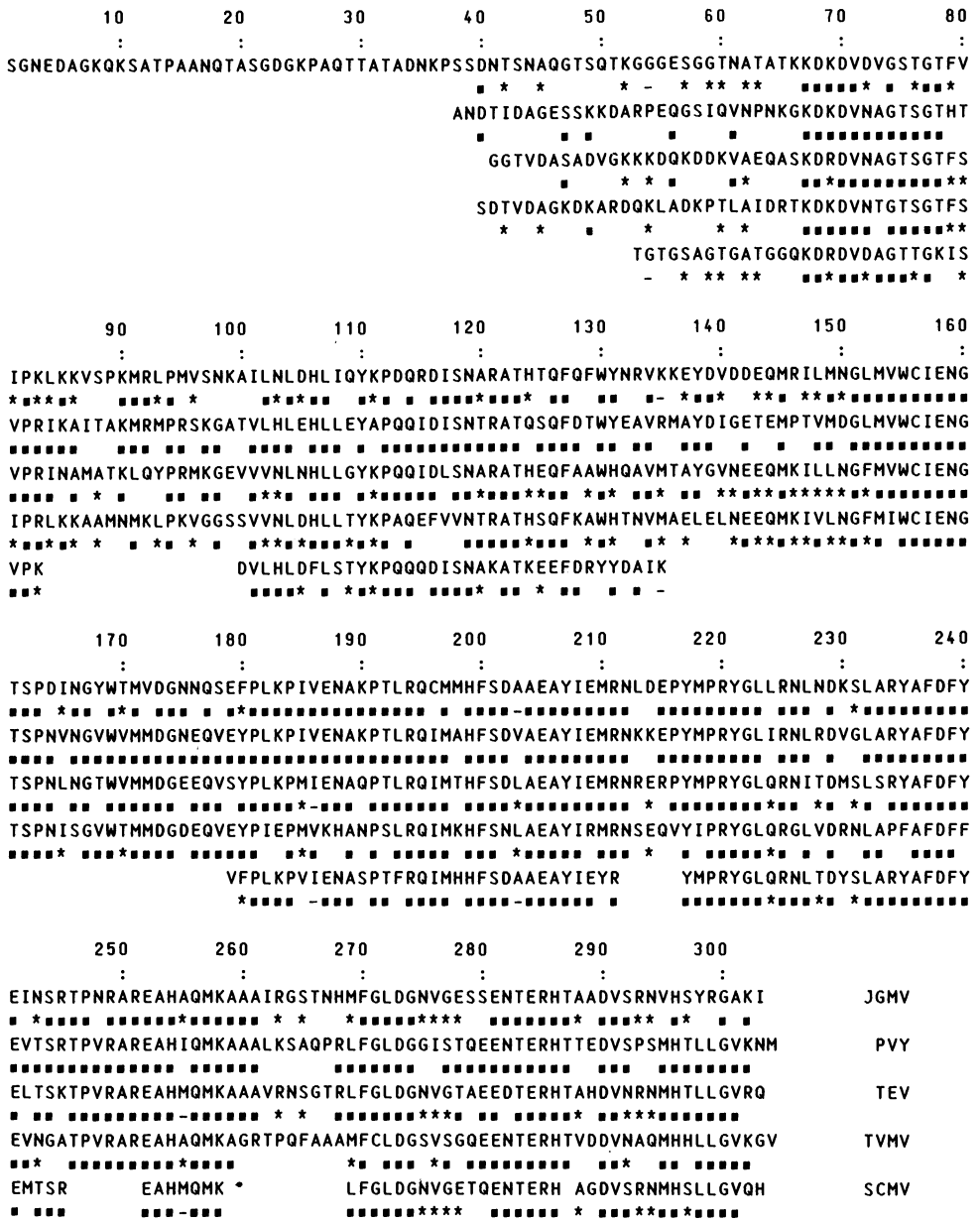

FIGURE 2. Amino acid sequences of the particle proteins of five potyviruses, aligned to show direct similarities. Residues that occur at the same position in the proteins of potato virus Y (PVY) and one or more other viruses are marked with a square below them; other similarities are indicated by other symbols. Sequences are from the following sources: johnson grass mosaic (JGMV) and sugar cane mosaic (SCMV) viruses, Shukla *et al.* (1987); potato virus Y (PVY), Shukla *et al.* (1986); tobacco etch virus, aphid non-transmissible strain (TEV), Allison *et al.* (1985); tobacco vein mottling virus (TVMV), Domier *et al.* (1986). Gaps in the sequence for SCMV represent undetermined portions.

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in the smaller proteins of the other viruses) are in general not conserved, whereas others including residues 67–86 and 148–261 (numbered as for johnson grass mosaic virus: figure 2) are almost all conserved in two or more viruses. Partial digestion with trypsin indicates that the N-terminal and C-terminal regions of the proteins are exposed at the surface of the virus particles and suggests that much of the remainder of the sequence is not exposed (Hiebert *et al.* 1984; Shukla *et al.* 1988). Furthermore, results of tests with monoclonal antibodies to tobacco etch virus indicate that virus-specific epitopes consist largely of terminal regions of the protein, whereas epitopes shared between potyviruses are in other regions (Dougherty *et al.* 1985). This picture is consistent with that derived from experiments with monoclonal antibodies to potato virus V. The epitopes specific to this virus were found by immuno-electron microscopy to be on the surface of virus particles and they are conformation-sensitive, whereas the epitopes shared with other potyviruses are conformation-insensitive (sequence-dependent) and not located on the particle surface, but can be revealed by disrupting the virus particles (Farmer & Harrison 1988).

These results do little to identify the features of the virus particle protein that interact with the helper component and play a key role in transmission by aphids. However, comparison of the proteins of aphid-transmissible and aphid non-transmissible isolates of tobacco etch virus provides a clue. The helper component of the non-transmissible isolate is known to be functional in transmission of another isolate, implying that it does not recognize the particle protein of the non-transmitted isolate. Conversely the helper component of potato virus Y can assist the transmission of particles of the normally non-transmissible isolate of tobacco etch virus (Pirone & Thornbury 1983), suggesting that in this isolate it is the adaptation of particle protein to its own helper component that has been lost. The nucleotide sequence of the particle protein-encoding region of the genome of the non-transmissible isolate shows six amino acid differences from the transmissible isolate (positions 1, 7, 10, 57, 136 and 146 from the N′-terminus (Allison *et al.* 1985)). Of these, residues 57, 136 and 146 are probably not on the surface of the virus particles, whereas residues 1, 7 and 10 are (Hiebert *et al.* 1984; Allison *et al.* 1985). The replacements at positions 7 and 10 destroy two DAG amino acid triplets, one copy of which also occurs in the first eight residues in the sequences of the proteins of three other aphid-transmissible potyviruses (figure 2), and it seems possible that this triplet is involved in binding to the helper component. Transmission would also require the release of virus particles from their sites of attachment on the maxillary stylets but there is no experimental evidence on how this occurs. However, further inspection of figure 2 shows that all the proteins contain duplicated potential cleavage sites for trypsin at positions 67–70, and several other similar sites occur between these and the DAG sequence. Cleavage at these sites by a trypsin-like enzyme might therefore release the particles from their retention sites in the aphid feeding apparatus so that they could be egested and act as inoculum during subsequent exploratory probes by the aphids. Such cleavages are known not to destroy infectivity (Hiebert *et al.* 1984). Further work is needed to test this hypothesis.

These comparisons suggest that a few sequences near the N-terminus of the potyvirus particle protein, and most sequences in the middle and at the C-terminus, are strongly conserved, presumably because many of the mutations that occur in these regions are disadvantageous. Moreover, with potyviruses as with nepoviruses, there is evidence that different selection pressures favour variants of different kinds. For example, when potyviruses are subcultured several times without the use of aphid vectors, they tend to lose their aphid transmissibility

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(Simons 1976), presumably because aphid non-transmissible variants outgrow their transmissible progenitors. In some examples, as already discussed, the key change is in the virus particle protein, but in others it is in the helper component (Kassanis & Govier 1971). Both these proteins must therefore be conserved and retain their mutual adaptation if they are to function in transmission, and the virus is to maintain it biological fitness. Although firm information is lacking, quite small amino acid changes in the helper protein seem likely to be as significant epidemiologically as are those in the particle protein.

Discussion

The examples considered in this paper make it abundantly clear that the particle proteins in several groups of viruses are highly adapted for their function in transmission by vectors. In the nepoviruses and potyviruses this function involves attachment and detachment of virus particles at surfaces in the feeding apparatus or oesophagus of the vector. By contrast, in the luteoviruses, and possibly the geminiviruses, it involves the passage of virus particles through the gut wall and salivary gland cells. Among vertebrate viruses these types of transmission seem uncommon and may not be strictly analogous. Perhaps this is because the close structural adaptation of virus particle proteins that is needed for transmission by such mechanisms is incompatible with the need of vertebrate viruses to generate antigenic variants that can evade the protection provided by antibodies. These mechanisms of transmission could, therefore, have been able to evolve in viruses infecting plants but not in those with vertebrate hosts.

Considering the patterns of variation found among particle proteins of plant viruses, two sorts predominate. First, there are small variations of the kind that might be produced by point mutation and which apparently do not impair either transmission by vectors or other characteristics that contribute to biological fitness. However, other variations of this type result in loss of transmissibility, as seen with the variants of potato leafroll luteovirus and tobacco etch potyvirus that can only be maintained by non-natural methods. There are therefore strict limits to the variation that can be tolerated in field isolates of a virus.

Secondly, there are much larger variations that must involve either many point mutations or more radical alterations in the particle protein gene and are often associated with differences in vector specificity. These are found, for example, in individual nepoviruses and in barley yellow dwarf luteoviruses. It is not clear how variants of this kind have arisen from a common stock, but in general they seem stable genetically and do not give rise to alternative form(s) when they are subcultured many times. Perhaps the most detailed information available for variants of this type is that relating to the geminiviruses infecting cassava, summarized in figure 1. However, evidence is lacking on whether the separation of these isolates into groups A, B and C, which corresponds to their geographical distribution, also corresponds with differences in vector specificity. It is also not clear whether these viruses that possess single-stranded DNA genomes are more prone to produce major variants, possibly by recombination, than are viruses with single-stranded RNA genomes.

Much less information exists about variation in helper components than that in virus particle proteins. However, helper components too seem likely to be closely adapted for their function in transmission. The relative frequency with which aphid non-transmissible isolates of potyviruses are obtained by subculturing successively without the aid of aphid vectors may be partly because point mutations in helper components can cause loss of function. Work with

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caulimoviruses, a group of viruses with monopartite double-stranded DNA genomes, leads to a similar conclusion. Thus the helper component of cauliflower mosaic virus is inactivated by a small change in the helper component-encoding gene II (Woolston *et al.* 1987).

These considerations lead to the conclusion that small variations in the particle proteins or helper components of individual viruses frequently lead to loss of vector transmissibility but that acquisition of ability to be transmitted by additional or alternative vectors is a more complex matter and in no example are the underlying genetic changes known.

This paper has dealt mainly with the molecular variation that affects vector transmissibility and has only mentioned in passing other epidemiologically important characteristics such as host range, seed transmission, ability to reach a high concentration in tissues, ability to cause diseases of differing severity and adaptation to environmental temperature. The basis of host range is poorly understood and likely to be complicated, and is an important subject for further research. However, variants differing in pathogenicity arise frequently, probably by point mutations, suggesting that genetic drift plays an important role in adaptive variation in this property. Similarly, variants with different temperature tolerances and optima arise readily (Holmes 1934; Kassanis 1957), probably by point mutations. The processes affected have not been determined in most instances but, in principle, the production or function of any viral nucleic acid or virus-coded protein could be affected.

A small beginning has been made towards gaining an understanding of the molecular basis of biological properties of plant viruses, and hence of the characteristics that affect their survival and spread. Continuation and intensification of this effort is a major task for molecular geneticists, plant virologists and epidemiologists.

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Discussion

D. A. J. Tyrrell, F.R.S. (*MRC Common Cold Unit, Salisbury, U.K.*). What is the mechanism of the different types of carriage: do the viruses multiply in the vector?

B. D. Harrison. With only one of the four groups of viruses I have discussed is there any evidence for viral replication in the vector. With this group, the luteoviruses, the evidence is equivocal. Weidemann (1982) reported that potato leafroll virus antigen, detected by fluorescent antibody staining, accumulates in some cells in the midgut and principal salivary gland of vector aphids. However, the virus content of aphids, measured by ELISA, does not increase after aphids have left virus source plants (Tamada & Harrison 1981). Extensive multiplication of potato leafroll virus in vector aphids therefore seems to be ruled out. The possible occurrence of limited multiplication is not excluded but has not been proved.

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