Molecular Ecology and Emergence of Tropical Plant Viruses

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Rice yellow mottle virus, Cassava mosaic geminiviruses, *Maize streak virus*, *Banana streak virus*, tropical agriculture, resistance durability

Abstract

An appreciation of the risks caused by emergent plant viruses is critical in tropical areas that rely heavily on agriculture for subsistence and rural livelihood. Molecular ecology, within 10 years, has unraveled the factors responsible for the emergence of several of the economically most important tropical plant viruses: *Rice yellow mottle virus* (RYMV), Cassava mosaic geminiviruses (CMGs), *Maize streak virus* (MSV), and *Banana streak virus* (BSV). A large range of mechanisms—most unsuspected until recently—were involved: recombination and synergism between virus species, new vector biotypes, genome integration of the virus, host adaptation, and long-distance dispersal. A complex chain of molecular and ecological events resulted in novel virus-vector-plant-environment interactions that led to virus emergence. It invariably involved a major agricultural change: crop introduction, cultural intensification, germplasm movement, and new genotypes. A current challenge is now to complement the analysis of the causes by an assessment of the risks of emergence. Recent attempts to assess the risks of emergence of virulent virus strains are described.

INTRODUCTION

Over the past two decades, molecular ecology has emerged as a new and rapidly expanding branch of the biological sciences. Molecular ecology is inherently interdisciplinary and derives from several scientific disciplines including biology, ecology, epidemiology, molecular evolution, and genetics. Molecular techniques are used to study the complex patterns and processes of biological diversity at all levels of organization, from genes to organisms, populations, species, and ecosystems, but from a gene-centric perspective (5, 39, 58, 106). Molecular ecology has been applied to a wide range of organisms, including viruses and other parasites. Not only can it answer key specific questions left unresolved in earlier ecological studies, but it also reveals a wide array of additional features of their ecology. We focus here on virus emergence, a critical aspect of plant virus ecology of great economic importance. Within little more than 10 years, molecular ecology has elucidated a wide variety of factors, most unsuspected until recently, involved in the emergence of plant viruses. Examples are selected from tropical plant viruses, especially those found in Africa, because many of the most damaging plant virus epidemics occur in the tropics and major breakthroughs in molecular ecology have been achieved with several tropical viruses. Moreover, molecular ecology will be most useful for the control of tropical plant virus diseases.

Features of tropical environments that influence the behavior of tropical plant virus diseases have been described elsewhere (122). Comprehensive and up-to-date information on the ecology of virus diseases of crops in tropical countries was published recently (80). Concepts and techniques of molecular ecology are described in recent textbooks (5, 39). Emerging infectious diseases are caused by pathogens that have increased in incidence, geographical distribution, or host range; have changed pathogenesis or newly evolved; or have been discovered or recognized recently (3). Among the economically most important tropical plant viruses, *Rice yellow mottle virus* (RYMV), cassava mosaic geminiviruses (CMGs), *Maize streak virus* (MSV), and *Banana streak virus* (BSV) have caused severe epidemics in the past decades. They are used here to compare and contrast molecular and ecological events that led to their emergence. These viruses belong, respectively, to the genus (family) *Sobemovirus*, *Begomovirus (Geminiviridae)*, *Mastrevirus (Geminiviridae)* and *Badnavirus (Caulimoviridae)* (35).

Rice yellow mottle disease is often referred to as a typical example of an emergent virus disease triggered by agricultural intensification. Molecular ecology reveals three phases of the emergence process that are often confounded: host range extension, dissemination of the pathogen, and increase in disease prevalence. Cassava mosaic disease (CMD), caused by whitefly-borne viruses, is the most important cassava disease in sub-Saharan Africa. Detailed epidemiology in East and West Africa in the 1970s and the 1980s (17, 28, 30, 34) and analysis of historical precedents (19) could not explain the onset of the subsequent very damaging pandemic in eastern and central Africa in the 1990s. The issue is now being resolved through major advances in understanding the complex etiology of the disease and the role of the whitefly vector *Bemisia tabaci*, which led to a dramatic change in pathogenicity and the rapid spread of the virulent virus species associated with the pandemic. "Streak" was the name given to virus diseases in Africa, affecting maize, sugarcane, and wild grasses. Studies revealed the complex and contrasting interplay between the wild indigenous hosts and the introduced crops of maize and sugarcane, leading to host range expansion through adaptation of the "streak" viruses. Banana streak disease was first reported from Côte d'Ivoire in 1958 and subsequently in many other countries in Africa and elsewhere. The unique etiology and mode of propagation of BSV were elucidated recently and may explain its exceptional molecular diversity. Infection arose from viral DNA integrated in the

nuclear genome in one of the wild species that has contributed to many of the banana cultivars currently grown. Consequently, international banana breeding and crop improvement programs have been hindered.

These examples illustrate the sequence of a wide range of complex and interacting molecular, ecological, and agro-ecological events that resulted in novel virus-vector-plant-environment interactions and led to the emergence of several tropical plant virus diseases. A challenge now for molecular ecology is to complement the analysis of the causes of emergence by assessing the risks involved. Recent attempts to assess the risks of virulent strains emerging, another major threat for tropical agriculture, are described.

RICE YELLOW MOTTLE VIRUS

Rice yellow mottle disease is caused by *Rice yellow mottle virus*, a member of the genus *Sobemovirus* (60), which was reported first in 1966 in Kenya (4) and subsequently in virtually all other rice-producing regions in Africa (**Figure 1**), but not elsewhere (126). Accordingly, the disease is categorized as emergent (3). The etiology and transmission of RYMV have been characterized. In recent years, studies of the molecular diversity of RYMV on a continental scale (1, 31, 100, 101, 126) have elucidated the transmission, dispersal and origin of RYMV, and hence the process of emergence of the virus.

Range of Transmission

The natural host range of RYMV is limited to wild (*Oryza longistaminata*, *Oryza barthii*) and cultivated (*Oryza sativa*, *Oryza glaberrima*) rice species. In addition, natural infection of a few wild grasses (*Echinocloa colona*, *Panicum repens*) has been reported (69), but their role as sources of inoculum is unclear. All these hosts are water-dependent species found mostly along riverbanks, lakeshores, swamps, temporary ponds, and irrigation canals. In subtropical areas, cultivated rice including floating and

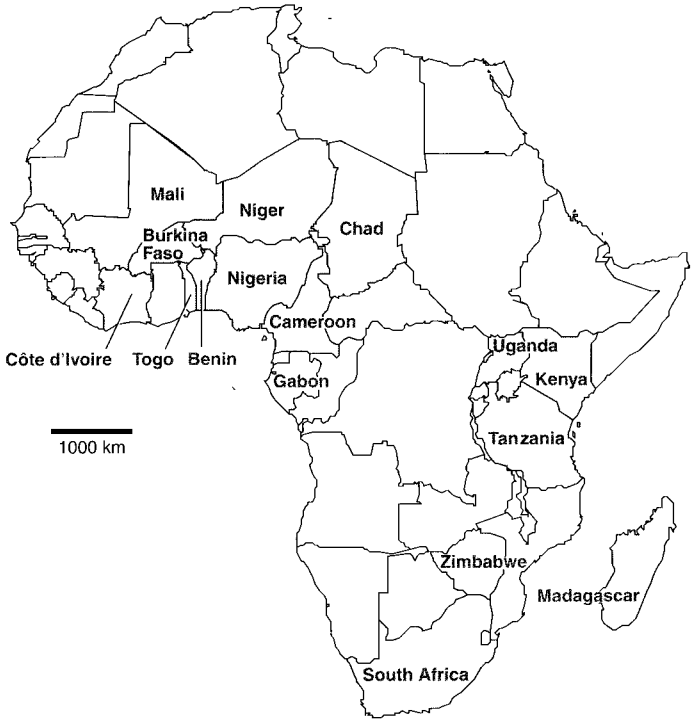


Figure 1
Map of Africa showing the countries listed in the text.

irrigated types is confined to similar environments. In tropical areas, rain-fed rice is also grown, often in mixed cropping systems. Occupying less than 1% of the agricultural land area, rice habitats in Africa are patchy, especially when compared with Asia (90% and 6% of the world areas, respectively). In the past several decades, however, rice cultivation has intensified rapidly in Africa. The area grown has increased threefold since 1966 to reach 10 million hectares in 2004 (**http://www.irri.org/science/ricestat**). Overall, RYMV habitats are heterogeneous, fragmented and expanding.

RYMV is transmitted by contact and by animal vectors. Transmission by seeds has not been detected in either cultivated or wild rice species (68). Transmission by contact is frequent between seedlings within seedbed nurseries and at transplanting (127). Seedlings are then planted into neighboring rice fields, usually located within a few hundred meters of

the nurseries. Consequently, transmission by contact allows on-site perpetuation of the inoculum. Among the various animals reported to transmit RYMV (including rats, cows, insects), several species of beetles of the family Chrysomelidae play the major role. Spread results from the interaction between vector movement and landscape structure (46, 129). Beetles have a short active flight capacity. Thus, when habitats are continuous, spread can proceed through local movement over short distances between adjoining or nearby rice fields. By contrast, when host distribution is patchy, such as for rice in much of Africa, dissemination can occur only by dispersal over greater distances, which leads to new populations which settle and spread. Long-distance wind-blown dispersion of beetles has sometimes been reported (121), although the range and frequency of these events are unknown, and cannot be extrapolated to the dispersal of RYMV, which is a non-persistent vector-borne virus with a short retention time.

Molecular characterization of RYMV isolates from two contrasting islands of Pemba and Madagascar in the Indian Ocean provided information on the distances and frequency of long-range dispersal. In Pemba, a small island circa 30 km east of the mainland coast of Tanzania where rice is heavily cultivated and RYMV is prevalent, different strains occur, but they all belong to the east Tanzania monophyletic group (126). This indicates that RYMV has been spread on several occasions over distances of several tens of kilometers. In Madagascar, a 600,000 km^2 large country and a major rice producer situated at least 400 km away from the African mainland, RYMV occurs and the isolates showed relationships with East African isolates (1). A vicariance biogeography event is unlikely as separation of Madagascar from the African mainland began circa 165 mya. More likely, the relationships reflect an influx of viruliferous insect vectors from mainland Africa (82). However, such long-distance dispersal over a few hundred kilometers is rare as serological and molecular typing of over 500 isolates from mainland Africa did not reveal any evidence of a recent isolate transfer between East, Central, and West Africa (126).

Patterns of Dispersal

The diversity of RYMV in Africa was assessed by studying isolates from 15 countries. In a few other countries in Central Africa, the disease has not been reported. According to the scenario developed here of an east to west dissemination of RYMV across Africa, the virus is likely dispersed throughout these countries. Thus, either the virus then became extinct, or, more likely, disease incidence was not sufficiently high to attract attention, or diagnostic techniques were not available. Moreover, the nonspecific yellowing symptoms could have been overlooked or confused with physiological disorders during the rare surveys conducted in these regions. Despite this apparent discontinuity, RYMV surveys are still among the most comprehensive for any plant virus.

Analyses of the spatial distribution of the genetic diversity further elucidated the dispersal process. RYMV showed a high level of spatial population structure. It was marked at the continental scale with three subdivisions: East Africa, West-Central Africa, and West Africa. At the continental scale, a close relationship was found between pairwise geographic and genetic distances, whether genetic distances were calculated on the full genome or on individual genes (1, 31). This relationship reflects the fact that dispersal and genetic differentiation of RYMV are both time-dependent processes and that they develop on a similar timescale. It suggests that the overall dissemination of RYMV in Africa was progressive. This trend, apparent at the continental scale, averages variable and sometimes contrasted relationships between genetic and geographic distances at regional or local scales. This indicates that the rapidity with which RYMV spreads over landscapes was not uniform, and that genetic differentiation does not reflect spatial separation simply measured as physical distances per se. Examples of

deviations from a close relationship between genetic and geographical distances revealed the physical, ecological, and historical factors that influenced dispersal.

Three studies conducted at regional scales illustrate the complex interplay between these factors and showed the impact of rice cultivation (cultural practices, cropping intensity, crop spacing, field spatial arrangement) on virus dispersal and the subsequent strain distribution. (*a*) Isolates from northern Tanzania are similar to those from Uganda and Kenya around Lake Victoria, but quite different from those of western Tanzania near Lake Malawi and from those of eastern Tanzania, although a similar distance of circa 700 km separates the different populations (102). Thus, geographic distances do not account for such genetic differences. Isolation from the rest of Tanzania by a 1500 to 2000 m high-altitude plateau with little rice cultivation explains the specificity of the Lake Victoria strain. Year-round growth of wild and cultivated rice around the lake ensures host continuity in time and space that facilitates virus spread, which accounts for the homogeneity of the isolates of this area. (*b*) Isolates from Niger, Benin, and Togo are more closely related to isolates of Chad and Cameroon in Central Africa 1500 kilometers eastward than to those of Burkina-Faso, Mali, and Côte d'Ivoire in West Africa 300–500 km westward. Neither distance nor physical obstacles explain the split in the distributions of the West and West-Central strains (126). Actually, the north/south limit between the two distribution areas of the strains is also the border between an area of traditional continuous rice cropping in the west ("rice belt") and discontinuous rice cropping in the east ["yam belt" and "millet belt," respectively (105)]. The subsequent isolation and differences in rate of virus spread between the two regions likely explain the West and West-Central strain differences. (*c*) There was no relationship between genetic and geographic distances in the two far opposite parts of Africa. In eastern Tanzania, a series of highly variable strains occurred within a restricted geographical range. Such pronounced genetic differentiation within a limited area reflects the older endemicity of RYMV in this region bordered by mountains and a high-altitude plateau. By contrast, in West Africa, populations have low variability with extended spatial distribution. Habitat connectivity, due to the lack of physical barriers and continuous rice cropping reinforced by the current intensification in rice cultivation, may have favored spread in the more recent phases of RYMV dispersal in West Africa.

Center of Origin

The origin and route of RYMV dissemination throughout Africa is revealed by the anisotropy of the spatial diversity of the virus. The highest diversity was observed in East Africa, with a pronounced peak in eastern Tanzania, and a decrease from the east to the west of the continent. The asymmetrical ladder-like pattern of the phylogram is characterized by a branching order of the series of internal to distal nested nodes which correlates with the geographic origin of the isolates along an east-to-west transect across Africa (31). This pattern also suggests a westward expansion with a succession of founder effects and subsequent diversification phases. Accordingly, genetic diversity would be adversely affected by recurrent bottlenecks occurring along the route of colonization, resulting in the lowest diversity in the extreme west. This, together with accumulation of de novo mutations postdating population separation, provides an explanation for the genetic differences among strains across Africa. Within Tanzania, most of the strains, including the most divergent ones, were found in the east, suggesting that this region is the center of origin of RYMV. Accordingly, the RYMV ancestor is likely to have diversified in this region before spreading to other parts of Africa following the easiest routes of transmission, in particular along rivers and lakes harboring wild and cultivated rice. The continental

westward orientation of the RYMV expansion was an inevitable consequence of the eastern position of the center of origin. At regional and local scales, the direction and rate of dissemination resulted from landscape patterns.

Interestingly, an analogous scenario has been suggested recently for cassava mosaic geminiviruses in Africa after an in-depth study of their distribution and variability across the continent (92; see below). The highest diversity, at the species level, was in Tanzania with both localized and divergent species. This pattern of variability also suggested an East African origin for CMGs, and a subsequent spread by the whitefly vector *Bemisia tabaci* from East to West Africa with corridors of propagation favoring spread and also barriers curtailing it. Eastern Tanzania is a biodiversity "hotspot" for a wide range of plant and animal species and is referred to as the Eastern Arc Mountains and coastal forest region (89). It may have harbored the primary indigenous hosts of RYMV and also those of cassava mosaic geminiviruses. The natural and experimental host range of RYMV includes several Gramineae species of the tribe Oryzeae (4, 69). Its discontinuous host range is consistent with the view that wild and cultivated rice species were not the original hosts of RYMV. More probably, these were montane grasses, endemic to the Eastern Arc Mountains region.

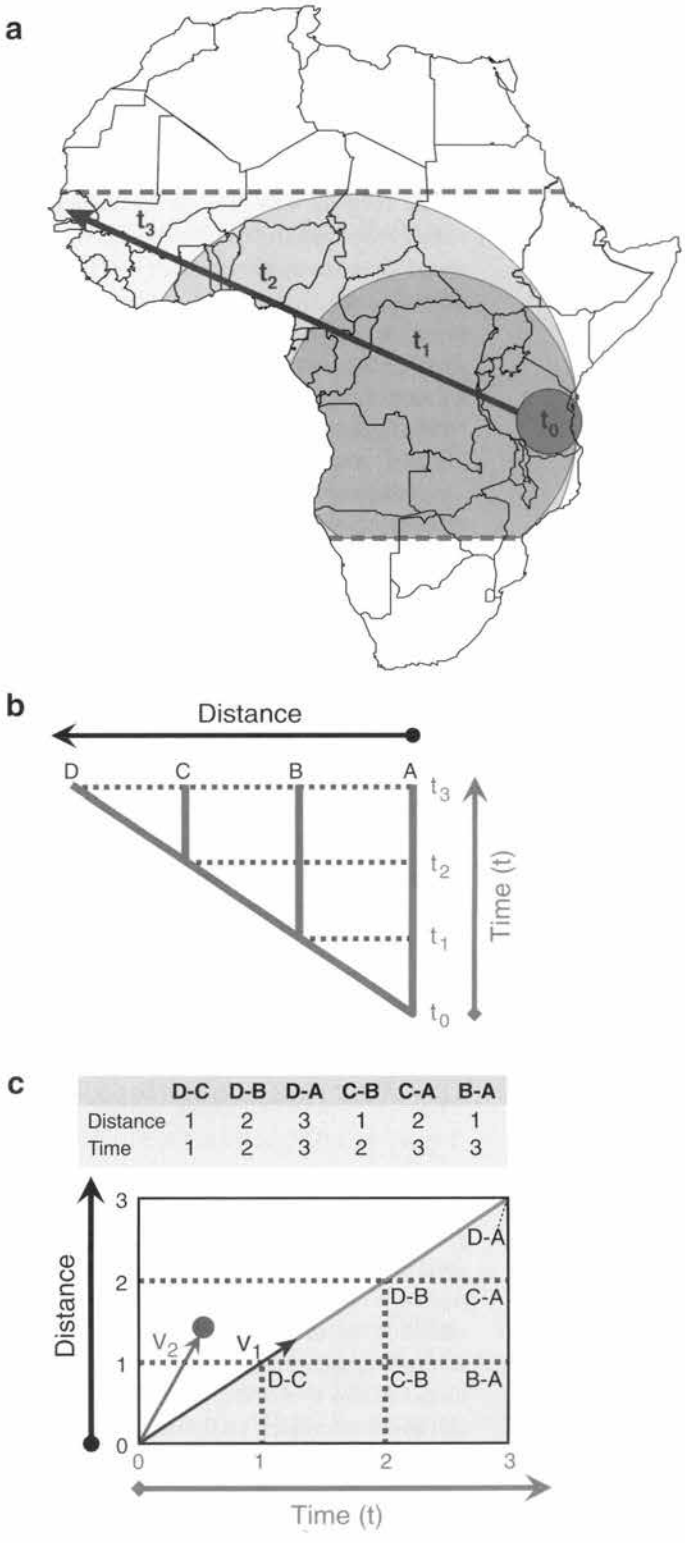
Field spread of several vector-borne plant viruses results from jump spread by windborne vectors that initiate new outbreaks, followed by radial spread from this source and additional satellite outbreaks (120). The relative importance of radial and jump spread depends on the pathosystem (64, 66, 67). Despite the major differences in time and space, there is a link between such epidemic progress within a field, consisting of both expansion of existing foci and initiation of new foci, and dispersive traveling waves in an assemblage of distant and scattered fields over several successive cropping seasons (132). At regional scales, fronts of propagation have been observed for a few plant viruses (26), including CMGs that were estimated to advance at circa 30 km per year (95). At continental scale, waves of propagation of several animal viruses have been inferred recently from phylogeographic studies (8, 22, 108, 134, 135), and sometimes confirmed by direct epidemiological observations (15). Such a traveling wave-like process on a continental scale is proposed here for plant viruses. This is apparent from phylogeographic studies of RYMV where spatial dispersal and genetic differentiation occur on similar timescales. With RYMV, the slow spatial dispersal (limited flight ability of the beetle vector, generally adverse west-to-east orientated wind currents, nonpersistent virus/vector relationship, patchy habitat) is consistent with the high diversification rate (up to 16% in nt diversity of the coat protein gene). **Figure 2** illustrates how a simple simultaneous dispersal and diversification process from eastern Africa across the continent restitutes the main characteristics of the phylogeography of RYMV. Development of evolutionary scenarios from phylogeographic studies is likely to be more complex and/or less informative for plant viruses with a low genetic diversity (40), a fast dispersion ability by vector or by human (121), or a high recombination rate (13).

Emergence

RYMV is often presented as an example of a virus causing an emergent disease triggered by agricultural intensification (3). Our studies indicate that a recent and massive long-range virus dissemination by vectors from Kenya since 1966 (when and where it was first reported) to the rest of Africa as often assumed or implied (3) is most unlikely. More likely, the virus was present unnoticed in the wild grasses and in subsistence rice after its dissemination across Africa. The limited areas of rice grown previously had been mainly in small, seasonal, rain-fed plantings that provide little opportunity for major outbreaks to develop. Epidemics developed only after rice intensification in the 1960s. *Indica* cultivars from

Figure 2

Phylogeographic implications of a dispersal from East Africa. (*a*) Dispersal of RYMV from a putative center of origin in East Africa. Four stages of the dispersal across intertropical Africa (t_o, t_1, t_2, t_3) are considered. The approximate north and south limits of rice cultivation are indicated by dotted horizontal lines. During each stage the virus diversified genetically, a fraction of the population dispersed westward, whereas the rest remained localized. The disease extension at the four stages was chosen to coincide approximately with the present limits of the distribution areas of the four lineages (eastern Tanzania, East Africa, West-Central Africa, and West Africa). We considered four representative isolates collected recently, one in each of the four areas (*A*, *B*, *C*, *D*). The cladogram was reconstructed (*b*) and the relationships between geographical and genetic distances calculated (*c*). (*b*) Cladogram. The horizontal axis is a spatial axis which represents the distances of the four isolates (*A*, *B*, *C*, *D*) from the putative center of origin. Genetic divergence was taken as a marker of temporal divergence. The vertical axis is a temporal axis which represents the genetic diversification of a putative ancestor isolate throughout the four periods t_o, t_1, t_2, t_3 leading to the four present isolates. The resulting cladogram showed the phylogenetic relationships between the four lineages. It restituted the characteristic asymmetrical ladder-like structure of the observed RYMV cladograms with a nested series of internal nodes from East to West Africa (17). Consequently, the diversity decreased from the east to the west. (*c*) Relationship between geographic and genetic distances. Geographical and genetic distances were calculated for each pair of isolates assigning one unit of temporal difference between two successive periods and one unit of spatial difference between two adjacent areas. They were plotted, respectively, on the X and Y axes of the diagram. [To a given distance of separation between two isolates is associated a minimal time of separation, the greater the distance, the greater this time.] The diagonal represents the average rate of expansion (V_1). Under the hypothesis of molecular clock for nucleotide substitutions and assuming a uniform speed of dispersal, all the experimental points should be below the diagonal. This was verified with a representative sample of isolates from each strain (1). When the full corpus of isolates was considered, however, deviations from this relationships were observed with clusters of points above the diagonal (D. Fargette, A. Pinel & G. Konaté, unpublished results). This reflects higher expansion rates at local or regional scales (V_2). Reasons for the changes in expansion rates are discussed in the text.



Asia that are very susceptible to RYMV were introduced in Africa. Irrigation allowed continuous cropping and an extensive growth of grasses, weeds, and self-sown rice plants that persisted through the dry season. These conditions facilitated the build-up of virus and beetle vectors. Rice area expansion also resulted in decreased interfield distances that favored spread to more remote or less favorable environments. Infection soon became prevalent in many areas, thereby changing RYMV from an endemic low-level disease that failed to attract attention to one causing recurrent severe epidemics.

Rice yellow mottle emergence thus resulted from a three-phase process: an extension of the host range, an expansion in geographical range, and an increase in disease incidence. It can be speculated that each phase was associated with different stages of rice intensification. Introduction of rice cultivation in the putative center of origin was followed by infection from the primary indigenous wild hosts. Development of rice cultivation allowed the propagation of the virus across Africa. Intensification of rice cultivation resulted in the present pandemy. This scenario can be confirmed only when the time-scale of the evolution of RYMV is known (see below).

CASSAVA MOSAIC DISEASE IN AFRICA

The first report of cassava mosaic disease (**Figure 1**) in Africa was in 1894 from what is now Tanzania. By the end of the 1940s, CMD had been reported in virtually all cassava-growing regions of the African continent and several islands, and the transmission of the disease by the whitefly *Bemisia tabaci* was established. In the late 1970s, Bock and collaborators, using classical virological methods including electron microscopy and virus purification techniques, established the viral etiology of CMD, geminate particles were described, and soon thereafter the first sequence of the African cassava mosaic virus (ACMV) was determined (72).

In 1993 on the basis of serology and genome sequencing, a second virus was described originating from East Africa (59, 118). The etiological situation of CMD in Africa was therefore simple with the occurrence only of two cassava mosaic geminivirus (CMG) species now referred to as *African cassava mosaic virus* and *East African cassava mosaic virus* (EACMV), respectively. The first full-length DNA-A sequences published were both from ACMV, collected from Kenya (116) and Nigeria (83). The situation became more complicated in the 1990s with more sampling and sequencing, but above all by the appearance of a cassava geminivirus outbreak in Uganda, leading to the discovery of geminivirus recombination and a wealth of epidemiological and molecular studies.

Importance of Geminivirus Recombination

An important development in the understanding of the molecular characterization of CMGs came in 1997 following reports of the rapid spread of an unusually severe form of CMD in central Uganda (45). Sequences determined for virus isolates obtained from severely infected plants suggested for the first time the occurrence of a CMG for which the DNA-A had arisen by interspecies recombination between two different begomovirus species (ACMV and EACMV) (24, 137). The recombinant virus had an EACMV-like genome, but showed in the capsid protein sequence a typical ACMV fragment of 459 nts. The recombinant virus was finally considered to be a strain of EACMV (24), and was definitively designated as EACMV-UG in a comprehensive review of the taxonomy of the family *Geminiviridae* (36, 115).

After this first evidence of geminivirus recombination, sequence comparisons of many geminivirus representatives of species and strains have shown that recombination is

a very common occurrence worldwide, and clearly plays a very important role in their evolution and emergence (96). For the CMGs, however, it appears that an important distinction can be drawn between ACMV, which shows a high degree of identity regardless of the origin of the isolates in Africa (above 94% identity, **Figure 3*a***), and EACMV-like viruses, for which variation is considerable and recombination very frequent (92, 103) (**Figure 3*a***).

The African CMD Pandemic

The epidemic of severe CMD that affected most of Uganda in the 1990s devastated the country's cassava production, causing losses estimated at more than U.S. $60 million annually between 1992 and 1997 (94) (**Figure 3*b*, *c***). Key characteristics of the CMD pandemic were the high incidences of severe CMD (45) (**Figure 3*b*, *c***), rapid vector-borne spread (94), and superabundant *B. tabaci* populations (74) (**Figure 3*d***). Since the 1990s, the disease has progressed from Uganda to Gabon in West Africa (red arrow in **Figure 3*a***), and a complete description of the pandemic has been published (72). Studies of the relationship between CMGs and the pandemic revealed a consistent association of the recombinant EACMV-UG (137), frequently in mixed infection with ACMV (56, 103, 104). However, despite the constant association of EACMV-UG with very severely affected plants, there is currently no clear explanation for the presence of the ACMV recombinant fragment in the capsid protein of EACMV-UG and the rapid CMD spread in Africa. Similarly, the presence of EACMV-UG in mixture with other CMGs does not always cause a pandemic of CMD. EACMV-UG does not seem to be unique in being able to elicit very severe symptoms in cassava, as similar symptoms have been described both for ACMV (103) and other EACMVs (72, 104). These facts suggest that the synergism between EACMV-like viruses and ACMV is indispensable but not sufficient for EACMV-like viruses to be spread by vectors. This is also related to the fact that single infections of EACMV-like viruses most often accumulate very low levels of viral DNAs, as exemplified by EACMCV in Cameroon (38).

Synergism Between Cassava Mosaic Geminiviruses

Mixed ACMV and EACMV-UG infections were shown to be a frequent feature of the severe CMD pandemic (56, 71,103, 104). Plants infected in the field with EACMV-UG expressed more severe symptoms than those infected with ACMV, but plants infected with both viruses showed more severe symptoms than any of the singly infected plants (56, 104). This observation was later confirmed in laboratory experiments where virus concentration evaluations in all three infection conditions demonstrated that ACMV and EACMV-like viruses interact synergistically (38, 104, 131) (**Figure 3*e–h***). For EACMCV and ACMV, the accumulation of DNA-A and -B for each virus can be boosted by 20- to 50-fold or more in a synergistic interaction compared with single infection (38, 130). This synergism is the only example known for geminiviruses and also for plant viruses of the same genus in the same family. This biological phenomenon seems to be of primary importance for the emergence of this new geminivirus disease and is certainly a key factor in the spread of the CMD pandemic in East and Central Africa (56, 71) (red arrow in **Figure 3*a***) but alone cannot explain the pandemic.

Synergism is Caused by the Combined Effect of Two Viral Suppressors of Gene Silencing

The molecular biological mechanism behind the synergism between ACMV and EACMV-like viruses has been elucidated recently and shown to result from a differential and combined action of two geminiviral suppressors of gene silencing (14, 130, 131). It was demonstrated previously that CMGs possess two

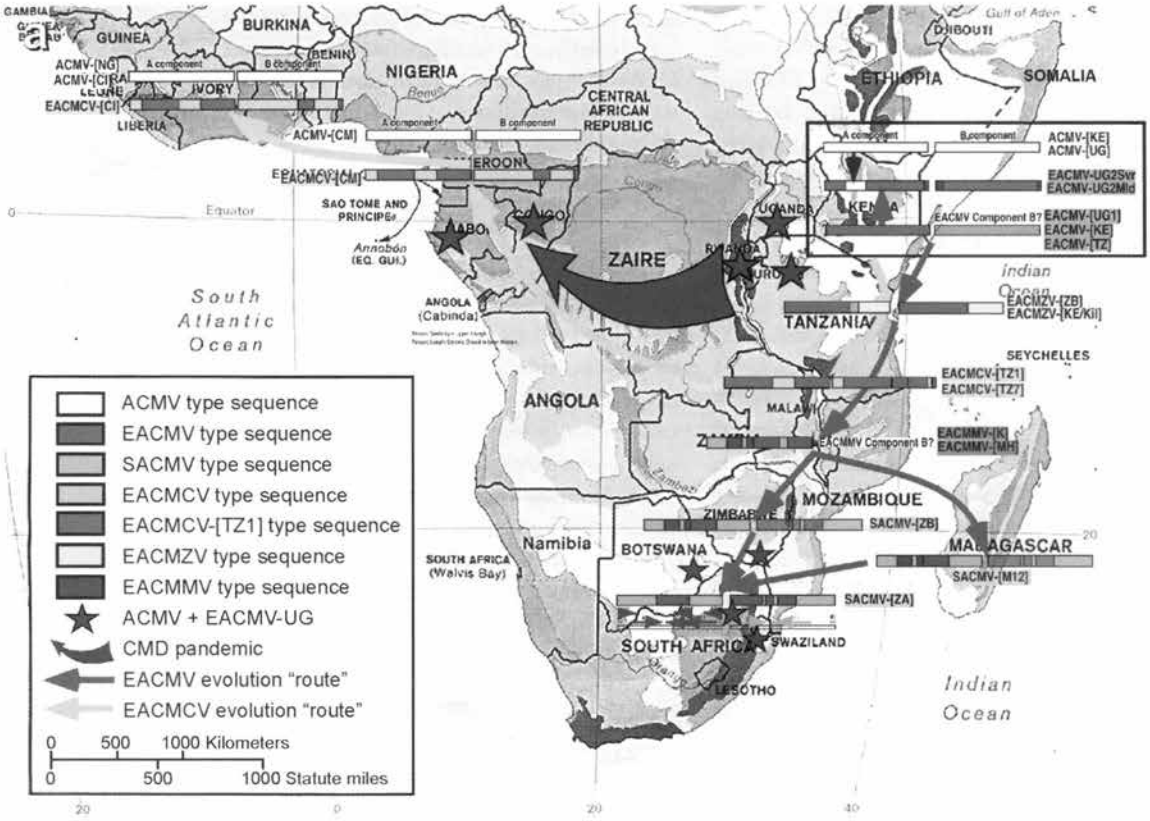


Figure 3

(*a*) Map of Africa depicting the putative inter-species recombinations of components A and B of CMGs identified in different parts of Africa from an analysis of GenBank accessions. The significance of the color codes is given in the figure. Where the component B of a particular virus has not been cloned it is indicated in letters for a different species representative, or as a faint shading for a different isolate. For simplification of the drawing, not all the ACMV isolates have been drawn, as they are very similar (all white in the diagram). Similarly, the EACMV-UGs associated with the CMD pandemic, now present in several central African countries, have been depicted as red stars, and the large red arrow represents the progress of CMD pandemic in East and Central Africa. The solid blue arrows represent the possible "route" of dissemination of the EACMCV viruses, and the pink arrows represent the possible "route" of dissemination of the EACMV-like viruses. (*b*, *c*) CMD synergistic-type symptoms on naturally infected cassava plants (*b* and *c*) in the field in Uganda (photo courtesy of James Legg). (*d*) Very high whitefly population density of adults on cassava in Uganda (photo courtesy of James Legg). (*e-b*) Symptom severity and levels of viral DNA accumulation associated with CMD induced by ACMV-[CM] and EACMCV or both. From left to right, cassava plants mock-inoculated (control, *e*); inoculated with ACMV-[CM] alone (*f*), EACMCV alone (*g*), and dual inoculation with ACMV-[CM] and EACMCV together (*b*). Southern blot strips next to each plant indicate relative amount of viral DNA accumulation, AC: ACMV-[CM], EC: EACMCV, and AC & EC accumulation in doubly infected plant.

posttranscriptional gene silencing (PTGS) suppressors named AC2 and AC4, respectively. ACMV uses the AC4 protein (known in some cases to enhance pathogenicity) as an efficient PTGS suppressor, whereas EACMV-UG and EACMV use the AC2 protein (a transcriptional activator protein). When a cassava plant is infected by both viruses, the AC4 of ACMV provides an immediate PTGS suppression. It benefits both viruses to enhance

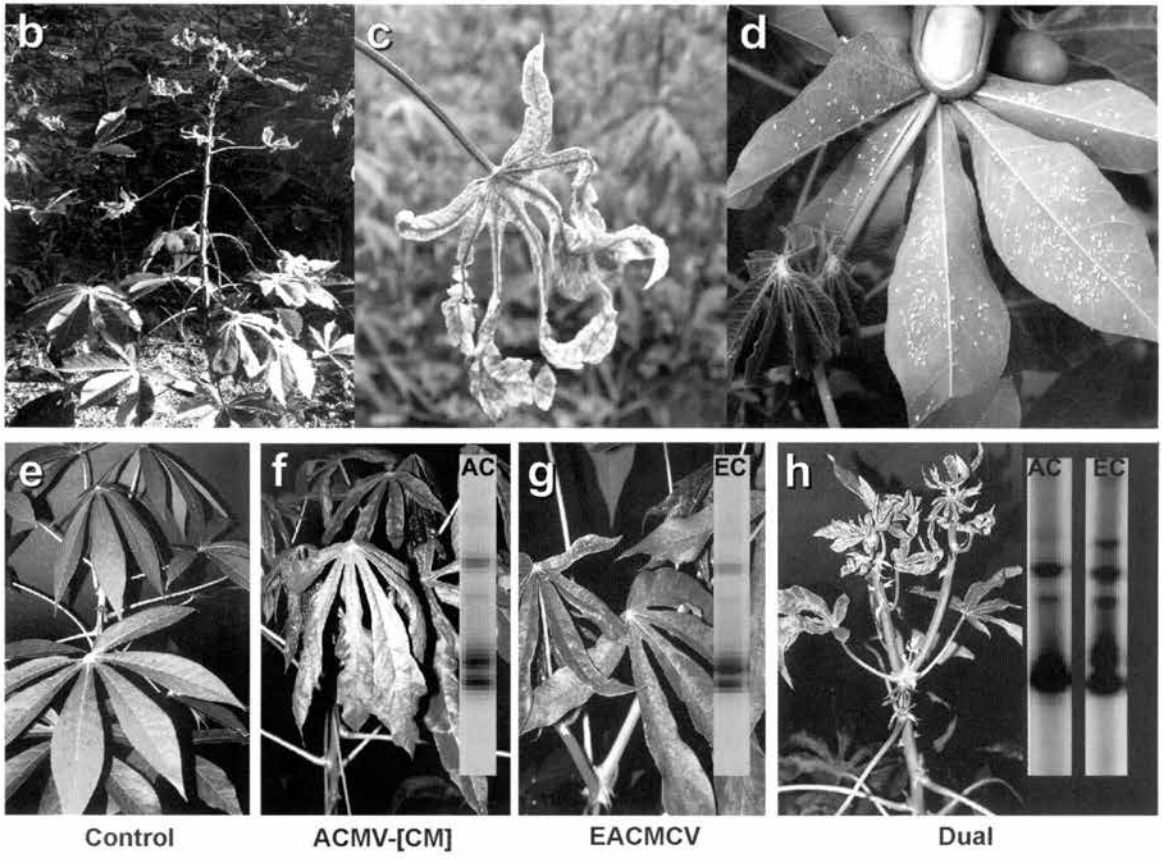


Figure 3

(*Continued*)

their replication and increase their concentration, which in turn produces more AC2 protein of the EACMV-like virus that will suppress PTGS more effectively for the rest of the plant's life. This combined effect will boost both ACMV and EACMV-like virus DNAs, accumulating 20 to 50 times more than each control (**Figure 3*f–h***). Consequently, the dually infected cassava plant will literally collapse from the virus load (14, 130, 131). The resulting symptoms are called candle-type symptoms (**Figure 3*h***) and are typical of synergistic interactions recorded in the fields with thick stems and leaves reduced to a fraction of their size with very thick veins (**Figure 3*h***). These findings provided the first indication of the importance of the control of gene silencing of the host plant for geminiviruses, despite the fact that geminiviruses are ssDNA viruses with no dsRNA phase in their cycle. This discovery enhances the importance of molecular studies in understanding ecological phenomena.

Molecular Characterization of *Bemisia Tabaci*

Bemisia tabaci has been recorded from more than 1000 crops and weed host species in the tropics and pan-tropics, and occupies a great diversity of niches with variable ecological conditions. Genetic markers have been used to separate populations (10, 11). Evidence for the occurrence of an invasive

pandemic-associated *B. tabaci* genotype based on MtCO1 sequence analyses of *B. tabaci* adults collected along a transect running perpendicular to the pandemic "front" has been reported (73). Studies in Uganda suggested that severely CMD-affected cassava plants interact synergistically with *B. tabaci* populations (93). This interaction is characterized by an increased colonization of CMD-infected plants by whiteflies, higher concentration of eggs on symptom-free leaves of diseased plants, and increased rates of fecundity on CMD-diseased plants. This important phenomenon could explain the greater spread of the viruses associated with the pandemic (18).

Conclusion

Since the 1990s, the use of molecular tools has greatly expanded our knowledge of viruses infecting cassava in the world, particularly in Africa. Although still limited, our appreciation of virus diversity in nature is much larger and much more detailed than previously. It is now clear that interspecies recombination of geminiviruses plays an important role in the evolution, spread, and survival of these viruses. This conclusion also applies to the whitefly geminivirus vectors, where information about the genetic and behavioral differences between geographically distributed vector populations is emerging. The molecular explanation for the synergistic interaction of geminiviruses and the role of gene silencing of the host plant in regulating or promoting these viruses helped in elucidating the epidemiological and economic consequences of such interactions. Although the causes of the CMD pandemic in Africa are incompletely understood, it seems that the synergistic interaction of ACMV with EACMV-like viruses, combined with the spread of the EACMV-like virus via newly adapted populations of whiteflies, has been crucial in the rapid development of this disease on a continental scale. Molecular ecology is therefore essential to understanding epidemiological and evolutionary phenomena.

STREAK DISEASES CAUSED BY MASTREVIRUSES IN AFRICA

"Streak" was the name given in 1925 to 30 virus diseases in Africa (**Figure 1**), affecting maize, sugarcane, and wild grasses, because of the typical symptoms consisting of chlorotic streaks developing on the leaves parallel to the veins (117). Until the late 1980s, all viruses causing streak disease in Africa were included as strains of an all-encompassing maize streak virus, mainly because they had twinned quasi-isometric virion morphology, similar transmission with leafhopper species of the genus *Cicadulina*, and the existence of serological relationships (7, 23, 99). However, using molecular tools, it appeared that the sequence nucleotide identities between members of the so-called strains of maize streak virus were sufficiently low, according to the current species demarcation criteria, to distinguish several virus species belonging to the genus *Mastrevirus*, family *Geminiviridae*. Besides *Maize streak virus*, three geminivirus species were identified from sugarcane (6, 111) and one from panicum (*Panicum streak virus*) (9). The species from sugarcane are the *Sugarcane streak virus* from South Africa, which was the first to be identified, and subsequently *Sugarcane streak Reunion virus* and *Sugarcane streak Egypt virus*. Two strains of *Panicum streak virus* were described, one from Kenya (PanSV-[Ken]) and one from South Africa (PanSV-[Kar]). Mastreviruses of *Panicum* sp. and sugarcane were less studied than those isolated from maize, mostly because they cause relatively mild symptoms on their original host and on maize.

Phylogenetic analysis revealed that these viruses form an intrageneric cluster of African streak viruses (110). Surprisingly, *Digitaria streak virus*, a mastrevirus species which was isolated from Vanuatu (east of Australia) and transmitted by a leafhopper

of a distinct genus (*Nesoclutha declivata*), groups with this African cluster and not with *Chloris striate mosaic virus*, an Australian mastrevirus species transmitted by a distinct leafhopper of the genus *Nesoclutha*, *N. pallida*.

Maize streak virus is the most studied mastrevirus species because it causes the most important virus disease of maize in Africa. Incidence of nearly 100% was recorded in Burundi (136). Yield loss of 33% was recorded in Kenya (48). Five *Maize streak virus* strains were identified: strain A containing mainly maize isolates, strain B found in wheat, rye, and grasses, and three rare strains detected in *Setaria* sp. (strain C), in *Urochloa* sp. (strain D), and in *Digitaria* sp. (strain E) (81). Six subtypes were distinguished within the A strain. Isolates from Réunion Island form subtype A6, which is the most divergent, probably because of a founder effect (98). Unlike the isolates of the maize strain that cause severe symptoms in maize, isolates of the other strains cause mild symptoms in maize. With the exception of the wheat strains that generally cause severe symptoms in wheat, the strains isolated from wild grasses cause mild symptoms in their original hosts.

Maize was introduced into Africa in the sixteenth century. Because maize is not a perennial host and *Maize streak virus* is not seed transmitted, the isolates detected on maize in different countries are predictably of local origin. Thus, isolates of the maize strain (strain A) collected over several thousand kilometer distances all around the African continent, from Nigeria, Kenya, Zimbabwe, and South Africa, and share 96% to 99% nucleotide identity on their whole genome (81, 98), it is suggested that highly similar isolates occurred in these countries in wild species and/or that different viruses have adapted in a similar fashion to maize, resulting in near-identical sequences. It was proposed that adaptation to annual grasses resulted in quick and pronounced symptoms. This would help to ensure leafhopper transmission before death of the host or, in the case of maize, before harvest time (6, 81). Different selection pressures are expected in vegetatively propagated weed grass hosts and in sugarcane, also an introduced host in Africa, because viruses can be perpetuated through the vegetative propagation of the host, even if the fitness of the virus is relatively low (6). This may explain why sugarcane was found to be infected by representatives of several distinct species of African streak geminiviruses, whereas maize was infected by only one (6). In addition, the geographic distribution of geminiviruses infecting sugarcane may have been influenced by the movement of infected cuttings. For example, it cannot be discounted that *Sugarcane streak Reunion virus* isolate that was detected in millet in Nigeria has not the same geographic origin as the *Sugarcane streak Reunion virus* sugarcane isolates from Mauritius and Réunion Islands.

BANANA STREAK VIRUS

Banana streak virus belongs to the genus *Badnavirus*, family *Caulimoviridae*. The importance and diversity of badnaviruses has been recognized only relatively recently and particularly in tropical plants, due to progress made in molecular diagnostic techniques. In addition to *Banana streak virus* (BSV), other badnaviruses commonly reported are *Cacao swollen shoot virus* (CSSV) in cocoa, *Dioscorea bacilliform virus* (DBV) in yam, *Taro bacilliform virus* (TaBV) in taro, *Sugarcane bacilliform IM virus* (SCBIMV) in sugar cane, and *Citrus mosaic bacilliform virus* (CMBV) in citrus species. Banana streak disease was first observed in Côte d'Ivoire in 1958. It was then reported from southern Morocco in 1986, and the bacilliform particles responsible for the disease were described (77). It was subsequently found in different *Musa* cultivars in many countries in Africa (**Figure 1**) and elsewhere (78). The realization that banana streak disease was widespread and that the causal agent could be carried in banana without showing symptoms has caused problems

for quarantine authorities and organizations involved in the international exchange of banana germplasm.

BSV is naturally transmitted to banana (*Musa* species) in a semipersistent manner by at least three mealybug species, of which *Planococcus citri* is the most prevalent. Field observations in many countries suggest that natural dissemination of BSV by mealybug vectors is limited in occurrence and does not play a major role in epidemiology. BSV mainly spreads by the vegetative propagation of asymptomatic infected source plants. Temperature fluctuation, rather than absolute temperature, may be a critical factor in expression of banana streak disease symptoms. Moreover, foliar symptoms appear sporadically during the year, and leaves showing symptoms may be succeeded by leaves expressing few or none.

Constraints to the Development of Molecular Studies

Molecular studies of BSV have been hampered by several factors. The tedious extraction process needed to obtain viral DNA from banana plants, the lack of alternate indicator hosts, and the inability of this virus to be transmitted by mechanical inoculation have been the main constraints on its molecular characterization.

Molecular characterization of BSV was boosted in 1990, due to the improvement of the techniques of purification (77). The first complete sequence of a BSV isolate was then determined in 1998 (52). As shown by several studies, badnaviruses are highly variable at both the genomic and serological levels, a feature that complicates the development of both molecular and antibody-based diagnostic tests (42, 79). Recently, many BSV sequences of the polymerase coding region of ORF 3 became available. Because of the important variability found between these sequences, the Caulimoviridae study group of the International Committee on Taxonomy of Viruses decided that BSV sequences having more than 20% of nucleotide differences in the polymerase (RT + RNAse H) region will now be considered as different badnavirus species (61). Three different BSV species are now clearly identified in the *Badnavirus* genus: *Banana streak Mysore virus* (BSMyV), *Banana streak GF virus* (BSGFV), and *Banana streak OL virus* (BSOLV). The term BSV used here is based on the symptoms observed on the banana from which sequences were obtained and not from a taxonomic standpoint.

Highly Original Ecology of BSV Revealed by Molecular Tools

Banana streak disease has only recently been considered to be a serious problem. The spontaneous appearance of the disease in certain tissue culture and breeding lines then caused increasing concern. The modern varieties of *Musa* are mainly interspecific hybrids of ploidy combinations of two parental species, *Musa acuminata* (A genome) and *Musa balbisiana* (B genome). A significant number of interspecific *Musa* genotypes, including newly created hybrids, showed a tendency to produce BSV-infected propagules when either virus-free source plants were propagated by tissue culture or virus-free mother plants are used for sexual hybridization (20, 75).

By combining different molecular tools (PCR, molecular hybridization, and in situ hybridization), it was possible to prove the existence in the *Musa* genome of integrated BSV sequences that can cause episomal infection under certain circumstances (54, 91). BSV integrated sequences appear to be relics of ancient infection events and probably arose from "illegitimate" recombination between host and viral DNA. For BSOLV (*Banana streak OL virus*), the integrated sequence found in *Musa* sp. cv. Obina l'Ewai (Musa AAB group) contains a rearranged but complete virus genome, and a model for activation to the episomal form has been proposed (91). The original finding of BSOLV sequences integrated in the *Musa* genome has since been extended, and this unique source of infection may

occur for other BSV-like viruses species (44). In all cases so far, these potentially activatable sequences have been found in only some B genome-containing *Musa*, although there are other badnavirus sequences, found in both *Musa* A and B genomes, that have not been associated with disease (43, 91). Genetic markers and BSOLV sequence integration linked to the expression of banana streak disease in interspecific *Musa* hybrids progeny have been identified together only in the *Musa* B parental genome (75).

This phenomenon has prevented the deployment of tissue cultures of improved interspecific hybrids of banana and plantain and has seriously hampered all *Musa* breeding based on utilization of the *Musa* B genome. A bacterial artificial chromosome (BAC) library of the banana *M. balbisiana* genome is now available as a valuable resource to study the integration and activation mechanisms of BSV sequences (112). A more detailed characterization of integrated badnavirus sequences is now required to define the roles of the different integrants in infections. *Musa* breeding programs need to find a parental genotype without an episomally expressible viral integrant. The use of IC-PCR (119), provided that appropriate negative and internal *Musa* PCR controls are used, avoids false positives due to integrated viral sequences and remains a potentially useful tool in diagnosing BSV infection.

Ecological Significance of Genetic Variation

As showed in **Figure 4**, the range of BSV-like sequence variability, estimated with a fragment of the RT gene, is very high compared to the variability of other badnaviruses studied so far. The high divergence between BSV isolates has led recently to the creation of three different BSV species despite their close biological properties (61). The list of BSV species will probably extend when new full sequences become available. The origin of BSV-like variability is still not clearly elucidated, but has most probably occurred from different sources (51). Indeed, BSV-like viruses from Uganda can be separated into three different clades, each more closely related to other badnaviruses than to BSV viruses in the other clades (**Figure 4**). The first clade included viruses derived originally from activatable integrated sequences within the B genome, episomal infections having been transmitted horizontally thereafter to other banana plants (51). However, it has not yet been proved that some of the sequences included in this first clade were integrated. The second clade contained sequences integrated in various *Musa* genotypes on the basis of comparisons with integrated sequences (43). The third clade containing 60% of the viral sequences identified in Uganda is hypothesized to have originated from other alternative hosts, but evidence is still lacking. Moreover, an SCBV isolate from Mauritius has been found closely related to BSOLV (clade I) (63). Many other SCBV sequences closely related to this clade have been detected from sugarcane in Guadeloupe (E. Muller, unpublished results). Complex relationships found between SCBV and BSV sequences reflect close ecological relationships between these two viruses. Additional studies are now needed to gain a better understanding of the origin of the BSV sequence diversity and to clarify the complex taxonomy of badnaviruses.

Currently, no obvious correlation has been established between a particular BSV clade, or individual sequence, to specific *Musa* genotypes, cultivars, or to disease severity and symptoms. There is only one geographical relationship demonstrated between a BSV cluster and a region of Uganda. A long history of banana cultivation, movement of vegetative material, and trade may explain this lack of association (51). Moreover, the recent infections arising from endogenous BSV-like sequences are responsible for the wide spread of still very homogenous sequences such as BSOLV sequence along with dissemination of hybrids. Molecular epidemiology of the BSV-like viruses is thus highly complicated

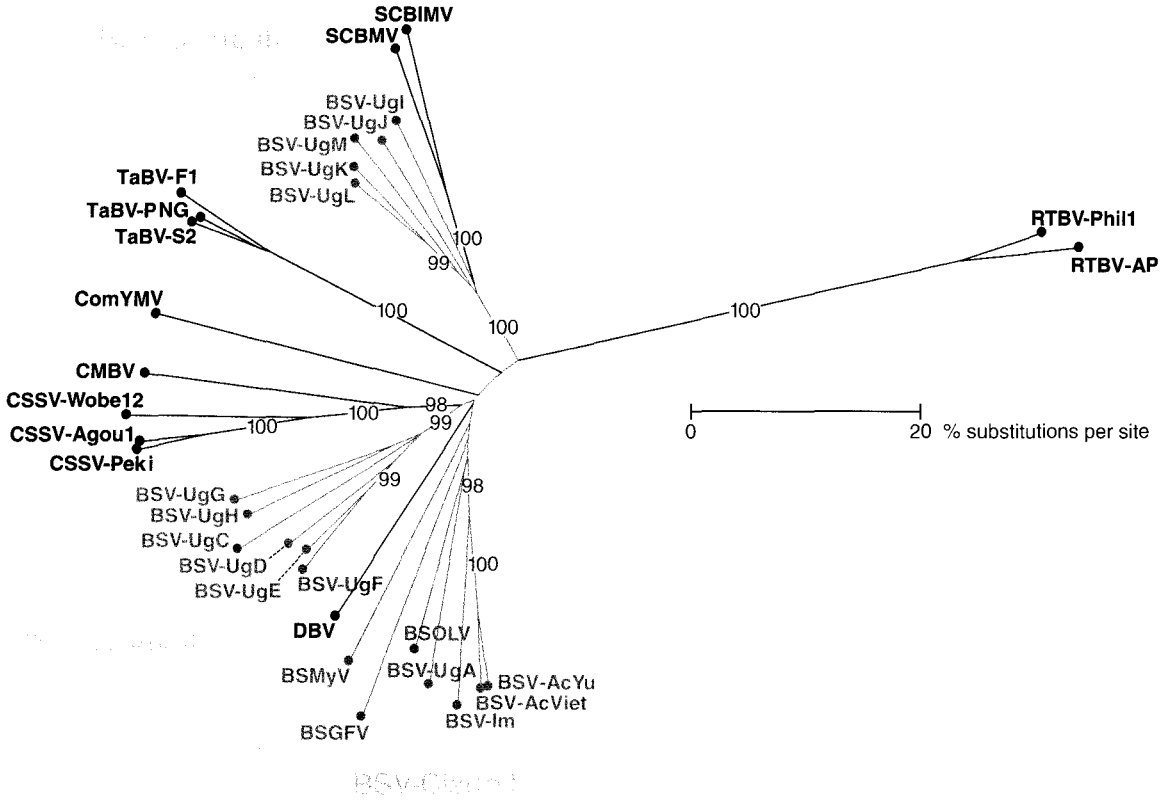


Figure 4

Phylogenetic neighbor-joining trees generated by the Darwin 4 program (97) based on nucleotide sequences of 543pb of the ORF3 RNase H region from BSV-like sequences, and other badnaviruses. Numbers at the nodes of the branches represent percentage bootstrap values (1000 replicates) exceeding 90%. Sequences used have the following accession numbers: AJ002234 (BSOLV), AF215814 (BSGFV), AF214005 (BSMyV), AY750155 (BSV-AcViet), DQ092436 (BSV-AcYu), AJ968458 (BSV-UgA), AJ968464 (BSV-UgC), AJ968465 (BSV-UgD), AJ968467 (BSV-UgE), AJ968469 (BSV-UgF), AJ968470 (BSV-UgG), AJ968472 (BSV-UgH), AJ968486 (BSV-UgI), AJ968501 (BSV-UgJ), AJ968504 (BSV-UgK), AJ968510 (BSV-UgL), AJ968542 (BSV-UgM), AJ968450 (BSV-Im), X52938 (ComYMV), AF347695 (CBMV), L14546 (CSSV-Agou1), AJ781003 (CSSV-Wobe12), AJ609019 (CSSV-Peki), X94576 (DBV), M89923 (SCBV), AJ277091 (SCBIMV), AF357836 (TaBV-PNG), AY186612 (TaBV-F1), AY186615 (TaBV-S2), AJ292232 (RTBV-AP), X57924 (RTBV-Phil1).

by the difficulty in distinguishing between episomal sequences derived from a classical viral evolutionary process and those derived from a long history of coevolution inside the banana genome. A better knowledge of the banana genome's content concerning BSV-like integrated sequences, based on the study of bacterial artificial chromosome (BAC) library of these banana genomes, would facilitate interpretation of the origin of BSV-like virus variability.

CONCLUDING REMARKS AND PERSPECTIVES

Analyses of the emergence of the plant viruses reported here, although globally referred to as molecular ecology, have applied tools and concepts of population genetics, landscape ecology, ecological genetics, ecological functional genomics, phylogeny, phylogeography, and molecular epidemiology. This underlines the need to combine different approaches

within interdisciplinary studies (37, 62) to address the issue of virus emergence. Moreover, the limits are increasingly blurred between evolutionary and ecological studies for RNA viruses whose rapid rates of nucleotide substitution means that the epidemiological processes that shape their diversity act on a similar timescale as mutations are fixed in viral populations (47, 88). A wide range of molecular techniques was applied, but they all involved analysis and comparison of partial or full-length sequences. Further accumulation of sequences, new statistical frameworks to analyze them, and development of user-friendly software to implement the analyses are opening new avenues for studies of ecological relevance. They are currently applied to assess the risk of emergence of virulent strains (see below).

This review illustrates, through a restricted number of examples, the variety of mechanisms, most unsuspected until recently, that have led to the emergence of plant viruses: recombination and synergism between virus species, new vector biotypes, genome integration of the virus, host adaptation and host range expansion, long-distance dispersal, and agro-ecological changes. The same term "emergence" covers contrasting situations, with, respectively, emphasis on ecological changes (RYMV), species interactions (CMGs), host adaptation (MSV), and host genetics (BSV). It partly reflects differences in the focus of the studies, and subsequent generalizations are difficult to make. For instance, symbiosis is critical in plant virus evolution (109) and may be decisive in virus emergence. This is apparent with new CMGs in Uganda whose emergence is driven by recombination and synergy, but not with RYMV, BSV, or MSV. Integrated sequences of BSV led to episomal viral infections, but geminiviral sequences found in plant genomes did not (53). Although, as stressed here, agro-ecological changes were critical in RYMV dissemination, host adaptation, as postulated for MSV, may also be involved in shifts from wild grasses to *Oryza* species, from wild to cultivated rice species, from *O. glaberrima* to *O. sativa*, from susceptible to resistant rice cultivars. This should be considered, as markedly distinct and nonoverlapping host ranges of most sobemoviruses signify a high degree of biological specificity and host adaptation (60). Accordingly, the differences in substitution rates across the genome among strains (31) is more likely associated with virus/host interactions than with the nonspecific transmission. Moreover, pathotypes of RYMV infecting rice cultivars and other gramineaceous species previously thought to be highly resistant or immune have been found recently (69; G. Konaté, O. Traoré & M. Allarangaye, unpublished results), and a single point mutation was sufficient to induce some of these host changes (32, 57).

Actually, emergence is multifactorial and remains poorly understood. It sometimes results from a combination of changes in the different components of the pathosystem (virus, vector, host, environment). For instance, emergence of new CMGs implied a complex interplay, not yet fully elucidated, of modifications of the virus, its vector and of their interaction. Several agricultural changes triggered the emergence of the epidemics. Massive dissemination of infected cassava cuttings enhanced mixed infection and favored recombination and synergism between different CMGs. The introduction of maize and sugarcane in Africa was followed by infection by indigenous streak viruses. The production of inter-specific *Musa* hybrids and tissue culture stress led to the expression and dissemination of BSV. Rice intensification favored RYMV dispersal throughout Africa. Additional work will further elucidate, and possibly enlarge, the range of underlying causes of emergence of plant viruses and will identify the role of the interactions operating at different scales. Overall, the range of processes of emergence and their increased likelihood due to further intensification of agriculture provide a likely explanation of why viruses are the pathogens responsible for the highest number of emergent diseases (3).

A major challenge is to complement the analysis of the causes by an assessment of the risks of emergence. Appreciation of these risks is most needed in tropical areas that rely heavily on agriculture for subsistence and rural livelihoods. Risk is defined as a combination of hazard and vulnerability (21, 50): hazard of the emergence of a pathogen, and host vulnerability to this pathogen. Such risk is high in tropical countries where the current phase of agricultural intensification increases the opportunities for disease emergence, whereas crops are still highly vulnerable owing to the lack of alternative resources to design and implement appropriate control measures. The properties of complex biological systems such as virus emergence are difficult to predict because of the intricate chain of distal and proximal underlying causes (133). This is quite apparent with new CMGs in Uganda whose emergence was impossible to anticipate. By contrast, significant efforts to assess and to reduce crop vulnerability have been made. Surveys of the progress of the epidemics in Uganda and knowledge of the general characteristics of the CMGs epidemics (30, 33) were used to define the neighboring regions and countries likely to be at risk within the next decade (71). Large-scale multiplication of highly resistant cultivars (27) helped to control CMGs in Uganda and provided an efficient way to decrease vulnerability of cassava in regions at risk (124).

Studies on the dissemination of RYMV showed that all rice culture in Africa is at risk to RYMV unless high, nonspecific and durable resistances are deployed. High resistance to RYMV of a few *O. sativa* and *O. glaberrima* cultivars, i.e., effective against all strains of RYMV (2, 90), offered promising prospects to control the disease (29). However, experimental inoculation of field isolates to highly resistant cultivars sometimes resulted in successful infections (32, 69, 125). Then, the durability of these resistances, once deployed in the fields, is questioned (114). Altogether, when resistances are available, crop vulnerability and risk are highly dependent on the durability of the resistances. This is important as high resistances to plant viruses are rare, and critical in tropical environments where additional control methods such as use of virus-free material, other phytosanitary measures, appropriate cultural practices, and insecticide treatment of the vectors are difficult to implement for socioeconomic reasons (123).

Accordingly, various criteria to predict the durability of resistance to plant viruses have been tested. They encompass a wide range of features including the ecology of the disease, the nature of the resistance, the spread and fitness of virulent strains, and the characteristics of the virulence gene (41, 55, 70, 123). As a corollary, it can be considered that the pathogen's life history provides clues to assess the durability of resistances. A virus with a fast substitution rate and clear molecular signatures of selection events would easily adapt to new environmental conditions, such as those caused by widespread cultivation of resistant plants (70). With animal RNA viruses, substitution rates were estimated by analysis of heterochronous sequences, and the rapid timescale of the changes was inferred (25, 65, 107). By contrast, no estimates of substitution rates are available for plant RNA viruses. This may be due to the shorter range of heterochronous sequences available, or reflect an intrinsic lower rate of substitution. Although the lack of timescale is a major deterrent to an appreciation of the evolutionary potential of RNA plant viruses, assessment of the relative substitution rates of genes among strains is still most informative (16, 84).

Another difficulty is the lack of neutral markers as both coding and noncoding regions are under selection pressure. However, advances in the analyses of the distribution of synonymous and nonsynonymous substitutions at the gene and codon levels revealed the selection forces that shaped RNA virus evolution. Applied first to animal viruses, they are increasingly being used for plant viruses (12, 49, 76, 84, 87, 128), and recently to relate

sites under positive selection with amino acid found experimentally to be determinants of pathogeny (84, 86). Inferences on the durability of resistances can be made, as resistance is expected to be durable if the matching virulence gene is highly conserved, but at risk if the virulence gene is variable, especially if sites under diversifying selection are detected (85). In *Beet necrotic yellow vein virus* (BNYVV), one codon was under high positive selection. This amino acid position was suspected to determine the ability to overcome partial resistance in recently introduced beet cultivars (113). With *Potato virus Y* and *Potato virus A*, correspondence was detected between sites under positive selection in the genome-linked viral protein (VPg) and amino acids involved in virulence to virus-resistant plants (86). With RYMV, a single amino acid under positive selection was detected in the VPg of isolates from West and Central Africa (D. Fargette, A. Pinel & E. Hébrard, unpublished results). It is adjacent to amino acids shown by mutagenesis experiments to be responsible of the breakdown of the high resistance (57). Past events of diversifying selection in a viral domain critical for virulence suggests a lack of durability of the resistance. Accordingly, a high percentage of virulent isolates have been found in the Sudan savannah zone of West and Central Africa where the highly resistant cultivars and their *O. glaberrima* ancestors have been grown (125). Following these lines of research, it can be anticipated that further linkage between populations genetics, evolutionary epidemiology, and experimental evolution will soon allow major advances in subjects of ecological relevance, including, but not limited to, the emergence of plant viruses.

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