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# A reassessment of the epidemiology of *Rice yellow mottle virus* following recent advances in field and molecular studies

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ABSTRACT

The available knowledge on the epidemiology of *Rice yellow mottle virus* (RYMV) is reassessed in the light of major advances in field and molecular studies of the disease it causes in rice. Previously undescribed means of transmission by mammals and through leaf contact have been discovered recently. Several agricultural practices, including the use of seedbed nurseries, have also contributed to a massive build-up of RYMV inoculum. Phytosanitation is now known to be critical to reduce disease incidence in rice. A new model of the ecology of RYMV in which man plays a central role has emerged. Furthermore, estimates of the evolutionary rate of change of RYMV provided a time-frame for its epidemiology, the first attempt for a plant virus. Earlier interpretations of the patterns of virus diversity which assumed a long-term evolution, and assigned a major role to adaptive events had to be discarded. In contrast, a wave-like model of dispersal of RYMV, which postulates its initial diversification in East Africa, followed by westward spread across the continent, was developed, refined and dated. The most salient – and largely unexpected – finding is that RYMV emerged recently and subsequently spread rapidly throughout Africa in the last two centuries. Diversification and spread of RYMV has been concomitant with an extension of rice cultivation in Africa since the 19th century. This major agro-ecological change increased the encounters between primary hosts of RYMV and cultivated rice. It also modified the landscape ecology in ways that facilitated virus spread.

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## 1. Introduction

Field epidemiology, based on glasshouse experiments and field studies, provides information on host range, means of virus transmission, and disease incidence at different locations and times. It also reveals information on the spatial and temporal dynamics of virus spread, and on environmental and agro-ecological conditions driving epidemics. Molecular epidemiology, which assumes that the genome sequences of virus isolates retain 'signatures' that reflect their history, gives additional information on the spatial and temporal patterns of the epidemics and provides an understanding of the underlying ecological and evolutionary processes involved. Although a distinction between field epidemiology and molecular epidemiology is somewhat arbitrary, it nevertheless reflects the fact that the two lines of epidemiological research are often disconnected, even though they are actually complementary. The patterns revealed by molecular epidemiology can be interpreted only if critical factors are known such as host range and means of transmission, as revealed by field epidemiology. Conversely, by an indirect approach, molecular epidemiology brings critical information on epidemiology, such as distant or rare past events, often well beyond the reach of field studies (Tibayrenc, 1998; De Meeus et al., 2007).

These principles are illustrated here by considering the results of studies on the spatial and temporal dynamics of *Rice yellow mottle virus* (RYMV) epidemics. The RYMV – rice pathosystem is one of the few plant-virus pathosystems to have been subjected to detailed studies involving both field and molecular epidemiol-

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ogy. The occurrence of RYMV, a member of the genus *Sobemovirus*, in East and West Africa was first established in the1960s and 1970s, respectively, shortly after trained and equipped virologists began operating in these regions and sensitive serological tools were developed (Bakker, 1974; Fauquet and Thouvenel, 1977). Previously, the detection of RYMV was hampered by the vague and non-specific symptoms expressed by infected plants. There is little direct evidence concerning the temporal component of the RYMV epidemiology. However, the diversification of RYMV follows a quasi-clockwise model and the rate of evolution has been estimated (Fargette et al., 2008a). Therefore, the time of the most recent common ancestor of current RYMV isolates in different regions can be assessed, and the history of its spread inferred. Field surveys suggested an overall increase of its incidence in most rice growing areas of Africa. This issue can now be addressed by reconstructing the demography of RYMV by coalescence analyses. However, the multiplicity of beetle and other animal vectors and the heterogeneous landscape ecology in Africa make the rate and the route of dispersal of RYMV difficult to determine. Fortunately, the relationship between geographic and genetic distances provides indirect but sound answers to such questions. Thus, information from field and molecular epidemiology can be combined to provide an in-depth view of RYMV epidemiology. They also shed light beyond the current epidemic phase, in particular on the earlier endemic stage when the virus was present in wild or cultivated plants before it caused severe epidemics. It also gives clues on the shift from wild to cultivated hosts. The different phases of emergence of RYMV could then be inferred: host range extension, dissemination of the pathogen, and increase in disease prevalence. Altogether, a reassessment of the epidemiology of rice yellow mottle disease is necessary in the light of the recent advances in the ecology and evolution of the virus.

## 2. Field epidemiology

### 2.1. Historical view

Since the symptoms of rice yellow mottle disease were first observed in rice (*Oryza sativa*) in 1966 (Bakker, 1974), studies on RYMV epidemiology have undergone two main phases. The first, which lasted until the late 1980s, dealt with primary features related to the etiology of the disease in different regions of Africa, the host range of RYMV and its means of transmission. RYMV was then thought to be spread exclusively by a few species of chrysomelid beetles, while experimentally the virus was transmitted mechanically (Bakker, 1974). The outcome of this phase laid the foundations for later, more thorough investigations. However, no significant and immediate follow-up occurred, largely due to the withdrawal of overseas agricultural research teams and the absence of trained local scientists at that time. The second phase started in the early 1990s, when active epidemiology was undertaken after the outbreak of severe RYMV epidemics in different rice growing areas, especially in West Africa (Coulibaly et al., 2001). The "knowledge gap" at that time was wide and farmers as well as scientists lacked effective control measures. In the second phase of research, previously unknown and unexpected means of transmission of RYMV were demonstrated (Sarra and Peters, 2003; Sarra et al., 2004). Detailed epidemiology, which reproduced rice agricultural practices, established the key role of man in RYMV epidemiology (Sarra, 2005; Traoré et al., 2006b, 2008). Therefore, the simplistic view, which placed beetle vectors as the sole means of RYMV transmission in the field, had to be reconsidered. This led to modification and extension of our understanding of its epidemiology.

### 2.2. Novel means of RYMV transmission

Because field observations could not relate RYMV incidence to the occurrence and population densities of beetle vectors, other means of transmission were suspected (Heinrichs et al., 1997; Sarra and Peters, 2003). The first to be considered was the involvement of livestock, which are often allowed to feed on rice regrowths before a new crop is planted directly after a previous rice crop. Such domestic animals are known to transmit another sobemovirus, *Subterranean clover mottle virus*, and trampling being more important than grazing in spreading this virus (Mckirdy et al., 1998). RYMV was later shown to be contact-transmitted by cows and donkeys, and also by rats populating rice fields and nearby habitats (Sarra and Peters, 2003). Wind-mediated transmission of the virus was also demonstrated (Sarra et al., 2004). This resulted from contact between leaves of diseased and healthy plants, whereby sand grains and guttation fluid act predominantly as abrasive and inoculum source, respectively. An up to 44% infection incidence was reported following close contact between infected and diseased leaves (Traoré et al., 2008). However, although guttation fluid is a highly infectious virus source (Bakker, 1974; Sarra, 2005; Traoré et al., 2008), its involvement in wind-mediated transmission is apparently its only major role in RYMV epidemiology (Traoré et al., 2008). RYMV transmission has been demonstrated through contaminated soil, especially after transplanting healthy rice seedlings into fields that previously contained RYMV-infected crops (Sarra, 2005; Traoré et al., 2008). These means of transmission ensure disease spread over short distances within and between fields in the same area. Unlike other sobemoviruses such as SCMoV in its clover hosts (Wroth and Jones, 1992), RYMV is not known to be seed-transmitted in cultivated rice or wild hosts (Konaté et al., 2001; Allarangaye et al., 2006). Consequently, the beetle vectors – despite their generally poor flying ability – provide the most likely means of occasional long-distance transmission (Fargette et al., 2006). In addition, the grasshoppers *Conocephalus merumontus* and *C. longipennis*, which are stronger flyers than beetles, have been suspected to transmit RYMV (Abo et al., 2000). However, their involvement in field transmission remains unproven. Moreover, the clear-cut spatial pattern of diversity shown in molecular studies (Traoré et al., 2005) indicates that long-distance spread is likely to be rare.

### 2.3. Role of man in RYMV epidemiology

Field observations of RYMV infecting rice crops in West and Central Africa indicated that its epidemics are influenced by rice growing environments. In Africa, rice is cultivated in three main agro-ecosystems according to the source of water used to grow the crop. Both upland and lowland rice are rain-fed, whilst irrigated rice uses water from rivers and dams. RYMV occurs in all three environments, but epidemics are less frequent in rain-fed rice (Awoderu, 1991; Rossel et al., 1982; O. Traoré and G. Konaté, unpublished results). Most epidemics occur in areas where irrigated rice is grown and also, to a lesser extent, when water is available for several months during the rainy season where lowland rice occurs. The permanent availability of water provides favourable conditions for establishment and persistence of insect vectors and alternative host plants.

Recently, several cultural practices were shown to favour RYMV epidemics. They place man at the centre of the epidemiological process. The effect of such practices in lowland and irrigated rice systems is threefold. It allows the build-up of virus inoculum for subsequent crops and also contributes to both primary and secondary spread of the disease. The build-up of RYMV inoculum results from two main cultural practices. Firstly, when rice is harvested by cutting the plants with a sickle, without distinguishing

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between healthy and diseased plants. This practice increases disease incidence in subsequent rice regrowths from the remaining stubbles and builds-up an important RYMV inoculum reservoir (Sarra, 2005). Secondly, farmers in rice growing areas often own livestock. Once the rice is harvested, cattle are allowed to feed on regenerating stubbles, so that the dung deposited will fertilise subsequent crops. Therefore, the virus reservoir build-up through cutting is further enhanced through animal transmission by cattle, donkeys, rats and beetle vectors which all contribute to the contamination of the rice seedbeds being used for subsequent crops.

The use of rice seedbeds also plays a critical role in RYMV epidemics. To increase productivity, their use has become a standard practice in almost all irrigated rice systems and direct seeding has been largely abandoned (Coulibaly et al., 2001). Use of transplants gives a competitive advantage to rice over competing weeds and favours the formation of additional rice tillers. In many rice-growing areas of Africa, rice production is done by small farmers on a subsistence agriculture basis with poor management of virus diseases. This is illustrated by the fact that the seedbeds are frequently established alongside wild grasses, some of which are hosts of RYMV. Moreover, regrowths from the previous rice crop or infected shoots of the rhizomatous rice *O. longistaminata* (a major wild host for RYMV (Bakker, 1974)), sometimes occur within seedbeds. These practices often lead to RYMV infections in seedbeds either from external sources by vectors or involving secondary spread within seedbeds by leaf contact. Uprooting and bundling rice seedlings from seedbeds for use in fields also provides an efficient means of transmission through contact between healthy and diseased seedlings (Sarra, 2005; Traoré et al., 2006b). Successive contact events between diseased and healthy plantlets when handled during bundling and transplanting are also involved. During each of these processes, hand contamination of the seedlings can result in infection incidences reaching 5% (Traoré et al., 2006b). Altogether, handling of infected seedlings by farmers and the resulting virus spread led to considerable amplification of the initial inoculum present in seedbeds. Consequently, even though the incidence of RYMV in seedbeds may be as low as 0.2%, spread from such sources could result in almost total infection of the crops (Sarra, 2005). The effect of using seedlings from seedbeds contaminated at widely differing levels probably explained why highly infected rice fields could be found next to slightly infected ones (Sarra and Peters, 2003). The current knowledge of RYMV field epidemiology is summarised in Fig. 1.

### 2.4. Intensification of rice culture and RYMV emergence

The disease caused by RYMV is commonly considered to be one favoured by agricultural intensification, as epidemics have only been reported in the last 40 years, a period when rice production increased greatly. Intensification of rice cropping in Africa incorporated several genetic and agronomic changes. First, general adoption of seedbeds and cultivation of rice all-year-round in irrigated conditions led to the persistence of RYMV, augmented the inoculum sources, and subsequently promoted its epidemics. Second, Asian high-yielding but susceptible cultivars (BG 90-2, IR 1529-680-3, and Jaya) were sown over more than 90% of the area cropped, contributing to virus dispersal and increased damage (Sy and Séré, 2001). Third, in the irrigation scheme of the 'Office du Niger' in Mali especially, the change from direct seeding to transplanting increased disease incidence, as uprooting the seedlings and transplanting them inevitably damaged roots which facilitated virus entry. Thus, RYMV epidemics were triggered by a combination of changes that resulted from intensification of rice culture in Africa.

The epidemiological features of RYMV have major implications for developing an understanding of the emergence of resistance-breaking virus isolates. Very few rice cultivars are resistant to RYMV. The highest level of resistance is controlled by the recessive gene *rymv*1 which maps on chromosome 4 and encodes the translation initiation factor eIF(iso)4G (Albar et al., 2006). In experimental conditions, a significant percentage of isolates can overcome this high resistance after mechanical inoculation (Traoré et al., 2006a). As infection is highly dependent on the viral dose inoculated (Sorho et al., 2005), it could be argued that the ability of RYMV to overcome the resistance was overestimated in experimental trials involving mechanical inoculation of large amounts of virus to rapidly growing young plants. This argument would be valid if RYMV was exclusively or mostly beetle-transmitted. It is now known, however, that most infection in irrigated rice occurs in the seedbeds through agricultural practices (see above). Thus, the growth stage of the seedlings, the virus content of the plants, the intimacy of contact between plants within bundles creates conditions as favourable to transmission as those occurring experimentally. Consequently, the results of our studies on emergence of resistance-breaking ability closely reproduced the field situation. The risk of emergence and spread of resistance-breaking isolates is by no means an "experimental artefact". Considering the quick rate of dissemination of RYMV, spread of the virulent isolates to other fields, especially if they are not counter-selected in susceptible cultivars, would be rapid.

### 2.5. Phytosanitation reduces RYMV epidemics

In practice, phytosanitation involving isolation of nurseries and removal of infected weeds and regrowths is now known to be critical to reduce disease incidence. A lower inoculum pressure also decreased the risk of emergence of virulent isolates. Accordingly, deployment of resistant cultivars should be combined with prophylactic measures within an integrated disease management approach. The critical role of phytosanitation in managing RYMV epidemics effectively has been suspected for a long time. It has now been substantiated by three large-scale field observations which showed how a shift from effective to ineffective disease management practices and vice versa influenced, for better or worse, the development of RYMV epidemics.

In the Bongor region of Chad, which is an important irrigated-rice growing area, disease incidence was estimated at 10% or less before 2000–2002, only exceptionally reaching 20% (Traoré et al., 2001). The generally low incidence was associated with appropriate cultural practices implemented by government-led agricultural extension teams dealing with all aspects of rice production in the area. Thus, major field works were synchronised and, especially, all rice producers in a locality were required to plant their seedbeds at a set time. Moreover, irrigation channels and fields were regularly cleared of neighbouring vegetation in which virus reservoirs would have been present. In 2006–2007, most of the organisation of management tasks was transferred to an association of farmers who did not require continued application of the former practices. Subsequently, highly infected wild rice *O. longistaminata* was observed along the irrigation canals and the roadsides. In some instances, seedbeds were planted next to harvested rice fields where the stubble was beginning to regenerate. As a consequence, in 2007 disease incidence increased to 50–80% in most fields (O. Traoré and M. Allarangaye, unpublished results). In Molodo, N'Débougou and Niono, in the centre of the vast 'Office du Niger' rice growing area in the north of Mali, phytosanitary practices have been implemented since the severe epidemics which developed in the mid-1990s. Irrigation channels and areas around rice fields have been regularly cleared of weeds and of other virus reservoirs. Consequently, very few seedbeds (less than 2%) are contaminated by RYMV. By contrast, at Selingué, an irrigated rice growing locality in the south

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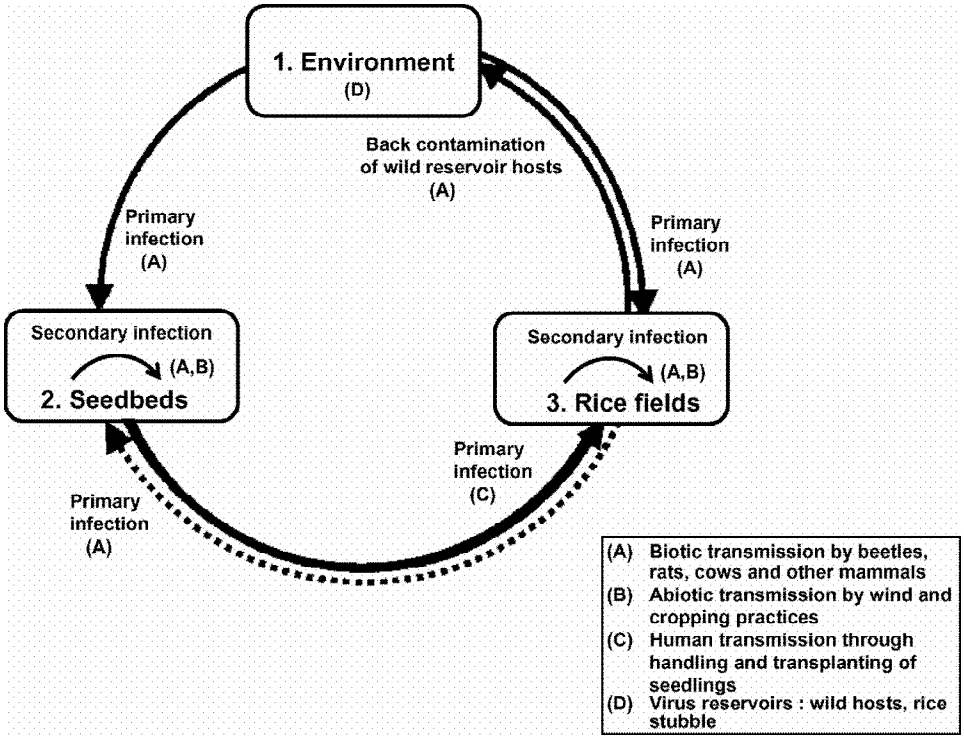



**Fig. 1.** Epidemiological model of RYMV. (1) In the environment, the virus present in wild reservoir hosts and in rice stubble is disseminated to rice seedlings in seedbeds by biotic agents (beetles, rats, cows and other mammals). (2). Once seedbeds are contaminated, secondary spread occurs through biotic and abiotic transmissions that are facilitated by close spacing. Handling rice seedlings from the seedbed to the field (uprooting, bundling, transplanting) leads to contamination of healthy seedlings and, ultimately, in amplification of the inoculum for primary infection to occur in the field. (3). As in seedbeds, secondary spread of the disease occurs through both biotic and abiotic means. The disease often spreads back to new seedbeds planted while growing crops are still present. Part of the primary infection in the field is due to virus transmission by biotic agents from sources in the environment. Reverse transmission from the field to alternative hosts in the environment occurs, especially when crops are near to harvest.

of Mali, more than 20% of the seedbeds are infected (Traoré et al., 2006b). This high disease incidence is associated with the presence of virus reservoirs (infected rice regrowths, wild *O. longistaminata* and *O. barthii*) alongside and sometimes even within seedbeds. In this region, rice is a secondary crop compared to sorghum and other crops. So, even when the farmers are aware of the disease, inadequate attention is devoted to control it. In the south of Guinea, a large agricultural settlement growing rice, rubber and oil palm trees to settle populations fleeing from political unrest in neighbouring countries was established in 1989. Rice was cultivated over hundred of hectares. The first symptoms of RYMV were observed in 1992, and disease incidence gradually increased to reach 20% in 2001. By 2003 the most susceptible cultivars were totally infected, and in 2005 rice cultivation had to be abandoned (E. Gout, personal communication). Sequencing revealed that the isolates belonged to the S2 strain (A. Pinel-Galzi, unpublished results), which is the most common in this region. Increasing incidence was associated with the use of inappropriate cultural practices, after experienced rice growers returned to their home countries. In 2007, strict phytosanitary measures were enforced, and in 2008 RYMV was seldom seen (A. Ghesquière, personal communication).

## 3. Molecular epidemiology

### 3.1. Advances in the RYMV phylogeography

Molecular epidemiology of RYMV was based originally on the analysis of sequences of isolates from only a few of the many rice-growing countries in Africa (Pinel et al., 2000; Abubakar et al., 2003; Fargette et al., 2004; Traoré et al., 2005). Since then, surveys have been completed in other countries and repeated sampling done in areas of special interest. These additional studies made it possible to test the epidemiological scenario proposed earlier on the centre of diversification of the virus, the route of dissemination and the overall spatial pattern of spread. Most importantly, the rate of evolution of RYMV was estimated (Fargette et al., 2008a), and the age of RYMV calculated (Fargette et al., 2008b). This information provides a timeframe for the epidemiology of the disease, to our knowledge the first attempt for a plant virus. Other important developments arose while the mechanisms involved in host changes were investigated (Pinel-Galzi et al., 2007), and when a host with differential susceptibility towards RYMV strains was first identified (Allarangaye et al., 2007). These results were used to assess the respective roles of ecological and genetic changes in the emergence of RYMV.

A first synthesis of the field and molecular epidemiology dealt with the relationship between RYMV spread and rice landscape ecology. It led to the proposal of a wave-like model of spread from East to West Africa (Fargette et al., 2006). The present review reconsiders the epidemiology of RYMV in the light of the more recent advances listed above. A phylogeographical approach was followed in which the biogeography of RYMV was revealed by comparing the phylogenetic trees of populations with the geographical distribution of each population (Holmes, 2008). This review benefits from the concepts and techniques developed recently that provide a better understanding of the phylogeography of animal and human viruses. The quite striking nature of these advances is apparent when comparisons are done between the two syntheses made by the same author on this subject only 4 years apart (Holmes, 2004, 2008). They reflect the rapid increase in the number of virus sequences available, especially full-length sequences, and the theoretical and algorithmic improvements made to extract phylogeographic information.

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### 3.2. A wave-like model of dispersal

Holmes (2008) listed a range of phylogeographic patterns of different human viruses. They basically reflect the relative rates of viral flow, the time of association with the host, and the mode of virus transmission. The same virus may have different spatial structures within specific localities. It may also possess multiple phylogeographic patterns, depending on the scale under consideration and on the conditions influencing epidemic development (Holmes, 2008). Therefore, interpretation and use of the phylogeographic patterns is a complex but informative exercise. RYMV is an appropriate plant virus to use for such an approach. This is because a comprehensive survey of RYMV diversity in Africa has been done over the last decade. The diversity is sufficient to be informative, but limited enough not to erase the phylogenetic signal. Moreover, the patterns of diversity are not blurred by genetic exchanges due to long-range transmission, plant movement or recombination events.

RYMV was shown to have a strong spatial subdivision. This first became apparent in studies with monoclonal antibodies with which several serotypes were identified in different countries (Konaté et al., 1997; N'Guessan et al., 2000). Sequencing of the coat protein (CP) gene confirmed the geographical distribution of the diversity, and further showed that the diversity decreased progressively from East to West Africa (Pinel et al., 2000). A strong link between genetic and geographic distance was revealed (Abubakar et al., 2003). This relationship means that dispersal and genetic differentiation of RYMV are both time-dependent processes with similar time-scales. Full sequencing of representative isolates allowed the reconstruction of RYMV phylogeny. The phylogeny was characterised by an asymmetric topology with a series of internal to external nodes orientated along an east to west geographical axis across Africa (Fargette et al., 2004). Collectively, these results suggested a wave-like pattern of dispersion. Such patterns arise when viruses radiate from a central starting point, thereby producing spatial subdivision and a relatively simple relationship between geographical and genetic distances (Holmes, 2008).

RYMV showed a wave-like pattern which suggested a westward expansion of the virus with a succession of founder effects and subsequent diversification phases. Genetic diversity was affected by recurrent bottlenecks occurring along the routes of colonisation. Host distribution discontinuity, limited vector flight ability and the non-persistent mode of virus transmission are likely to have contributed to the bottlenecks. The patchy rice cultivation in most regions in Africa inevitably led to a massive loss of inoculum as infective vectors failed to reach susceptible hosts. The range expansion of RYMV by its chrysomelid beetle vectors may proceed, as shown for the chrysomelid Colorado potato beetle (*Leptinotarsa decemlineata*), by a series of long-distance events and the establishment of outlying populations with local bottlenecks, instead of a simple advancing wave front with extensive gene flow (Grapputo et al., 2005). The non-persistent transmission of RYMV by beetles may result in the inoculation of a very limited number of particles, as found recently with non-persistent aphid transmission of viruses (Moury et al., 2007; Betancourt et al., 2008). Inevitably, only a small subset of the virus diversity in one region will be transferred by vectors into contiguous regions.

The Eastern Arc Mountain chain in eastern Tanzania, which had the highest intra- and inter-strain diversification and the most basal strains, was proposed as the centre of origin of RYMV. Consistently, the indel polymorphism, which is very rare indeed (only three indels in the CP gene) and ancestral (the indel polymorphism is associated with the basal splits between lineages) was restricted to this region. In a recent survey, the three distinct lineages were even found together only a few hundred metres apart (Kanyeka et al., 2007). This 'hot spot' of diversity, adjacent to the Eastern Arc Mountains, harboured most of the diversity of RYMV. As RYMV evolved under a quasi-clockwise model (Fargette et al., 2008b), the highest diversity in eastern Tanzania likely suggests a longer diversification of the virus in this area than elsewhere. This supports the assumption that the Eastern Arc Mountains, which are known to be one of the few world centres of plant and animal biodiversity (Myers et al., 2000), are the centre of origin of RYMV.

It became increasingly apparent that there may be at least one other major (Inner Niger Delta in Mali) and possibly more minor centres of diversification and dissemination of RYMV (Traoré et al., 2005). The Inner Niger Delta, an area where rice is cultivated intensively, harboured high virus diversity and the basal strains of West Africa. The Inner Niger Delta is apparently a secondary centre of diversification and dissemination. Reports in Togo, Benin and Niger of infection by strains not present in the two main centres of diversification suggest the existence of additional sources of infection in West/Central Africa. Despite the multiplicity of virus sources, the source-sink model, a variant of the wave-like model proposed for some human (Holmes, 2008) and plant viruses (Garcia-Andres et al., 2007), does not necessarily apply to RYMV. In the source-sink model, a limited number of geographical areas act as the source population, possibly by generating transmission waves, exporting viral lineages to sink populations where viruses might only survive in the short-term (Holmes, 2008). With RYMV, repeated field surveys revealed that once an area was contaminated, it remained infected in subsequent years, even if the disease incidence fluctuated from year to year. The virus persisted even in areas with patchy and seasonal rice cultivation where conditions are less favourable to virus survival. This persistence was attributed to widespread occurrence of perennial hosts such as *O. longistaminata* which, once infected, remain as a virus reservoir for years. The various different means of transmission ensured spread from these reservoirs to seedbeds. The inoculum is readily amplified, and the seedbeds become major sources of field infection.

### 3.3. Phylogeography of RYMV: a matter of scale

The wave-like pattern became apparent only when the diversity of RYMV was studied on a continental scale. In countrywide or even regional studies, spatial patterns were not apparent or were difficult to interpret. For instance, in the west of Africa (Sierra-Leone, Guinea and southern Côte d'Ivoire) or in Madagascar to the east, there was little diversity and consequently no structure was apparent. In other regions, such as Mali and to some extent Tanzania, a high diversity prevailed, but the patterns of spread were complex and prevented interpretation beyond the conclusion that frequent viral traffic must have occurred between localities. Phylogeographic structure was highly dependent on landscape ecology, as discussed earlier (Fargette et al., 2006). In particular, a single strain prevailed in the "rice belt" in West Africa, where rice is widely grown, with little differentiation and no isolation by distance. By contrast, in the "yam belt" of West-Central Africa, where rice is seldom grown, there is evidence of diversity and isolation by distance.

A regular network of sampling is necessary to assess viral diversity and to analyse the underlying ecological and evolutionary processes. RYMV is one of the plant viruses whose phylogeography is best known, as isolates from 17 countries in East, West and West-Central Africa have been characterised. However, a few countries in Central Africa, especially the Republic of Congo, have not been surveyed. The lack of information in some parts of Central-Africa was overcome by comparing the likelihood of competing hypotheses. The phylogeny of RYMV indicated that the scenario of spread from East to West Africa was more likely than the one assuming an origin in Central Africa followed by two independent routes of propagation to East and West Africa, respectively. A similar methodology was recently applied to elucidate the route of intro-

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duction of yellow fever virus into the Americas (Bryant et al., 2007). Even if isolates from all countries are considered, the wave-like pattern becomes fully apparent only when the complete sequences are analysed. Early scenarios based on coat protein genes of RYMV were partly biased (Pinel et al., 2000) because of incongruences in some lineages between this gene and the rest of the genome (Fargette et al., 2004).

When the wave-like transmission model was proposed for RYMV spread, it was emphasised that it could be interpreted "only when the time-scale of evolution of RYMV is known" (Fargette et al., 2006). Recently, the rate of evolution of RYMV was estimated from sequences of dated isolates (Fargette et al., 2008a). The time of the most common recent ancestor of the isolates was then inferred. It indicated that RYMV diversified c. 200 years ago (Fargette et al., 2008b). This short time-scale was quite unexpected, as low rates of evolution had been postulated earlier (Fargette et al., 2004). It drastically changed our view of the epidemiology of RYMV. It implied that RYMV diversification predated the first reports of disease symptoms by decades, and that the virus caused epidemics long before it was recognised and characterised. The short time-scale of RYMV evolution also excludes the possibility that divergence between isolates from Madagascar and from East Africa reflects vicariance events (Abubakar et al., 2003). Rather, introduction of RYMV in Madagascar was attributed to an influx of viruliferous chrysomelid beetle vectors from mainland Africa (Monaghan et al., 2005). The short time-scale also precluded a long-term host-related diversification. The African rice *O. glaberrima* was domesticated in West Africa approximately 3000 years ago, whereas the Asiatic rice *O. sativa* was introduced in the 10th and 16th centuries to East and West Africa, respectively (Sweeney and McCouch, 2007). Consequently, RYMV diversified centuries after rice was domesticated or introduced into Africa. This excluded the possibility that different strains reflected an origin in various species of Gramineae prior to rice culture, as postulated earlier (N'Guessan et al., 2000; Pinel et al., 2000).

Knowledge of the history of RYMV dissemination also provided an overall time frame for the phylogeography of the virus in Africa. Dispersal of RYMV across the continent over 5000 km within two centuries means that the disease spreads at an average rate of several 10s of kilometres per year. This speed of dispersal is unexpectedly high, considering the overall poor flight ability of the chrysomelid beetle vectors, and the patchy distribution of hosts in most regions in Africa. However, this is consistent with the rate of c. 30 km per year reported in surveys of the advance of the epidemic front of the severe pandemic strain of cassava mosaic virus in East Africa (Otim-Nape et al., 1997), although *Bemisia tabaci*, the whitefly vector of this virus, has a limited flight ability (Byrne and Bellows, 1991). This is also consistent with the rapid spread of the chrysomelid Colorado potato beetles throughout Europe within a few decades, although they are considered to be poor fliers (Grapputo et al., 2005).

The close relationship at the continental scale between geographic and genetic distances indicated that, on average, RYMV dispersal was gradual (Fargette et al., 2004). However, occasional long-range transmission events – attributed to beetles or other vectors (see above) – were suggested by molecular characterisation of strains invading islands such as Madagascar (Fargette et al., 2006). To achieve such a rapid dissemination in Africa, RYMV must follow efficient corridors where wild and cultivated hosts provide a continuous landscape ecology, and where there are no physical barriers to insect vector movement. Riverbanks and flood plains, which harbour wild and cultivated RYMV hosts, combine these features. It was earlier proposed that the rivers Benue and Niger formed a major east to west axis of dissemination of RYMV across Africa (Fargette et al., 2006). The Chari and Logone rivers in Central Africa, bordered by rice fields now known to be infected by RYMV, may have played a similar role in the dispersal of the virus along a south-north axis towards Lake Chad. In the most western part of Africa, the rivers Senegal and Gambia may be involved in the westward spread of RYMV (see below). Fig. 2 encapsulates the present state of knowledge of the phylogeography of RYMV. It incorporates a time-scale, and illustrates the main centres of diversification, bottleneck effects and routes of virus spread throughout Africa.

### 3.4. Phylogeography of RYMV: a dynamic process

The recent and rapid propagation of RYMV throughout Africa implies that the situation is dynamic and continually changing. The current distribution of strains differs from that prevailing only a few decades ago. Moreover, it is soon likely to be different again. Epidemiological observations support this assumption. The homogeneity of the isolates in countries like Madagascar, Benin, Togo and Niger suggests a recent colonisation, at most a few decades ago. Therefore, dispersal is still in progress and spread to as yet unaffected regions is likely to occur.

Repeated surveys in Tanzania, the country with the highest diversity, also suggest a dynamic situation. Surveys conducted in the late 1990s revealed that strains S5 and S6 were confined to the eastern part of the country, bordered to the west by the Eastern Arc Mountains and to the east by the Indian Ocean. By contrast, S4 strains were found elsewhere in the country, but never in eastern Tanzania. Surveys conducted 10 years later revealed rare occurrences of S4 isolates in eastern Tanzania, and conversely of S6 isolates in western Tanzania (Kanyeka et al., 2007; A. Mpnunami and A. Pinel, unpublished results). Unequivocal evidence of the displacement of other plant viruses or strains within a few years has been provided recently (Sanchez-Campos et al., 1999; Davino et al., 2006; Desbiez et al., this volume). It is unclear whether strain displacement contributes to current changes in the phylogeography of RYMV. Competition between strains occurred experimentally in glasshouse experiments (N'Guessan et al., 2000, 2001) and double infection with different strains was never found in naturally infected plants. However, co-existence of strains, even in the same fields, was common in the centres of diversification.

The major distinctions established in earlier studies (split between strains of East, West-Central, and West Africa) have not been altered, despite repeated sampling in several areas and surveys of previously un-prospected regions. Even at the country level, predictions of isolate identity were generally correct. This consistency suggests that the main features of the phylogeography are now understood. However, the identity of a circulating strain, its origin and its route of dissemination are sometimes difficult to anticipate, even if with hindsight it is possible to explain them retrospectively. For instance, S4 isolates spread recently from Lake Malawi into eastern Tanzania, despite the mountain chain which separates the two regions (Kanyeka et al., 2007). This was somewhat unexpected, as mountain chains are physical obstacles to vector spread. This underlines that given sufficient time, even unlikely events can occur. It is also difficult to predict, among a range of possibilities, the route of spread. Senegal and The Gambia form the westernmost part in the "rice belt" in Africa. In other parts of the "rice belt", isolates were exclusively of the S2 strain. Consequently, S2 isolates may have spread to The Gambia from the infected fields of neighbouring countries of the "rice belt". Actually, recent characterisation of Gambian isolates revealed that they belonged to an unknown strain, markedly different from S2 and related to the basal West African lineage found in the Inner Niger Delta in Mali (Séré et al., 2008). This suggested that RYMV infection in The Gambia originated in the Inner Niger Delta and dispersed in the savannah region across rice fields, possibly along the Niger, Gambia and Senegal rivers.

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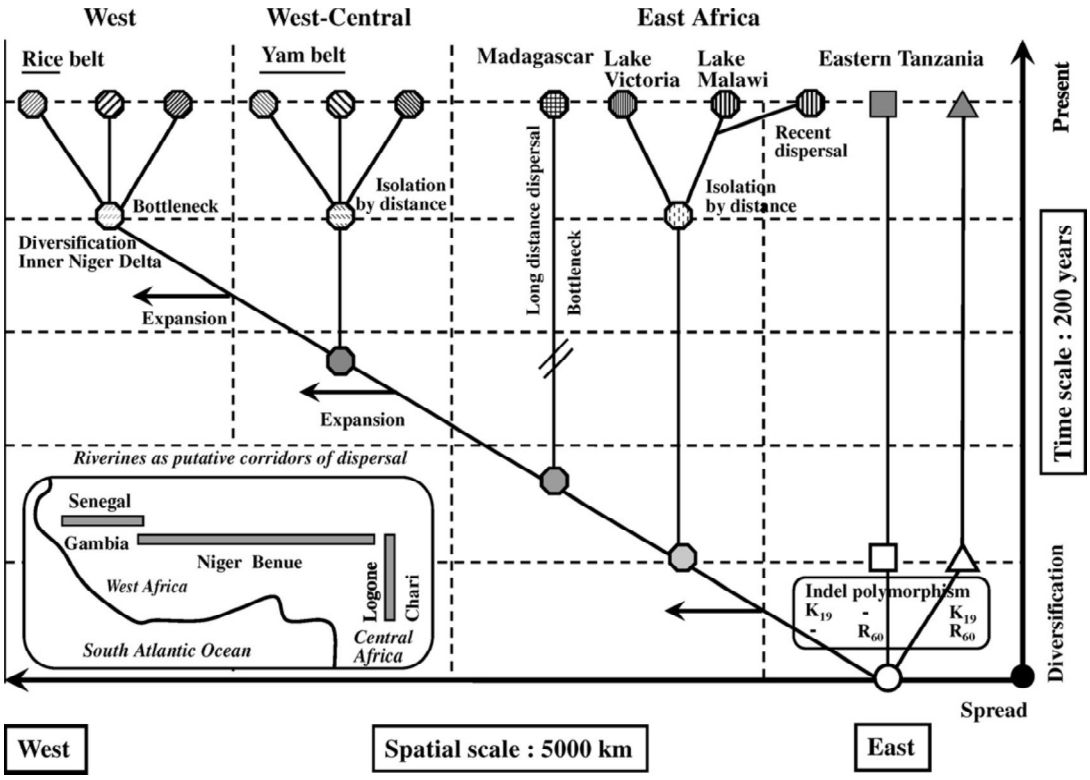



**Fig. 2.** Phylogeography of RYMV. The cladogram was reconstructed from full sequences of isolates representative of the genetic and geographic diversity of the virus from 17 countries: Bénin, Burkina Faso, Cameroon, Chad, Côte d'Ivoire, Ghana, Guinea, Kenya, Madagascar, Mali, Niger, Nigeria, Tanzania, Sierra-Leone, The Gambia, Togo, Uganda. The horizontal and vertical axes are indicative of, but not proportional to, the spatial and temporal scales, respectively. (1) RYMV diversified first in East Africa. The most basal strains occur in eastern Tanzania, within the Eastern Arc Mountains, and show the highest intra- and inter-strain genetic distances. The indel polymorphism supported the split between the basal lineages revealed by phylogeny. (2a) RYMV spread from eastern Tanzania into contiguous regions. This dispersal is characterised by a strong bottleneck effect as isolates elsewhere in Africa are derived from only one of the three basal strains. (2b) Isolation by distance was apparent in several regions in East Africa, especially around the Lake Victoria and Lake Malawi rice growing areas. There is evidence of recent spread of RYMV from Lake Malawi back to eastern Tanzania, despite the mountains which separate the two regions (dotted line). (2c) RYMV was introduced into Madagascar by long-distance spread (>400 km) and there is a strong bottleneck effect. (3) RYMV dispersed through Central Africa to West-Central Africa. Rice culture is patchy in most of this region (the "yam belt"), and strain differentiation among sites due to isolation by distance was apparent. (4) RYMV reached West Africa, possibly along the flood plains of the Benue-Niger rivers where wild rice occurs and cultivated rice is commonly grown. RYMV diversified in the Inner Niger Delta, an area where rice is widely grown. It then dispersed to other rice regions, especially in the "rice belt", and there was little evidence of strain differentiation through isolation by distance. The riverine areas which may have provided corridors of dispersal are indicated in the inset (bottom left).

## 4. Beyond epidemiology

### 4.1. Ecological changes and emergence of RYMV

The epidemiology of RYMV provides clues on the ecological and/or genetic changes involved in cross-host species transmission. The primary hosts of RYMV are wild species which likely belong to a genus within its natural or experimental host range. RYMV was estimated to have begun to diversify c. 200 years ago (Fargette et al., 2008b). The 19th century was a period of extension of rice cultivation in Africa (Porteres, 1950). Increased contacts between the virus reservoirs and rice favoured emergence of RYMV in cultivated rice. Several distinct emergence events may have occurred. Strains S4 and S6 are distantly related genetically and differ in geographical distribution. They are separated by the Eastern Arc Mountains with S4 to the west and S6 to the east (Kanyeka et al., 2007). These differences in genetic and geographic characteristics may reflect independent events of cross-species transmission from the same or from different primary reservoirs, possibly through intermediate hosts, to cultivated rice. Extension of rice cultivation, a major agro-ecological change, must have been critical in the transfer of RYMV from primary hosts to cultivated rice. A similar transfer from wild to cultivated plants in Africa was suggested for *Imperata yellow mottle virus*, a sobemovirus recently isolated in Africa. It is closely related to RYMV, and infects both indigenous *Imperata* sp. and an introduced crop, maize (Sérémé et al., 2008).

### 4.2. Genetic changes and emergence of RYMV

Emergence of RYMV in rice may be simply 'neutral' genetically, as suspected in the emergence of several animal and human viruses (Parrish et al., 2008; Holmes and Drummond, 2007). Comparisons of the CP genes of isolates from *O. sativa*, *O. barthii*, *O. longistaminata*, and a few wild grasses did not reveal any host-related strains (Traoré et al., 2005). This does not preclude, however, local adaptation to hosts by mutations that are restricted to a few sites. Three lines of evidence suggest that genetic characteristics are involved in host passages of RYMV. Adaptation of isolates from susceptible to resistant rice cultivars involved adaptive mutations (Pinel-Galzi et al., 2007). A polymorphism in the RYMV sequences at a site under diversifying selection was associated with contrasted preference to *O. sativa* or *O. glaberrima* (N. Poulicard, unpublished results). The most compelling evidence derived from the recent finding of a differential host, *Dactyloctenium aegyptium*. Isolates of two strains infecting rice were able to infect this species (Allarangaye et al., 2007). Comparison of the full viral sequences after passages in *D. aegyptium* and *O. sativa* did not reveal any difference (D. Sérémé, unpublished results). This suggests pre-existing specific genetic properties, still unknown, of the two strains able to infect both *D. aegyptium* and *Oryza* spp. Altogether, these observations indicate local adaptations during host changes. However, their significance in the emergence of RYMV from primary hosts to cultivated rice is still unknown.

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If RYMV evolves during host changes, conversely does rice adapt through acquisition of resistance under the selective pressure induced by the virus? *Oryza sativa* and *O. glaberrima*, which diverged over c. 500 000 years ago, are examples of parallel evolution in crop plants (Khush, 1997; Sweeney and McCouch, 2007). The wild progenitor of *O. sativa* is the Asian common wild rice, *O. rufipogon*. In a parallel path, *O. glaberrima* was domesticated in West Africa from the annual *O. barthii*, which in turn evolved from perennial *O. longistaminata*. The *rymv1-2* and *rymv1-4* alleles of resistance found in *O. sativa* and *O. glaberrima*, respectively, were caused by the same E to K substitution. Moreover, the 3D structure of the middle domain of the eIF4G gene revealed that the two mutated sites were located in the same small region of a long alpha-helical hairpin, on the surface, on the same side, and in the same direction (Albar et al., 2006). This suggests convergent evolution of resistance to RYMV in rice.

As mentioned above, it was estimated that RYMV diversified in rice c. 200 years ago (Fargette et al., 2008b). Yet severe epidemics developed only during the past few decades and their intensity has been highly variable in time and space. Under these conditions, can genetic convergence in resistant rice reflect the selection pressure imposed by RYMV? Genetic footprints for resistance to pathogens are detected consistently in their hosts, whereas evidence of strong and long-lasting selective pressure is lacking from their pathogens. This paradox was addressed recently by postulating highly localised responses to inoculum pressure and human intervention in the selection process of resistance (Salvaudon et al., 2008). Human intervention is best exemplified by selection of rice cv. Bekarosaka by Malagasy farmers, one of the two *O. sativa* cultivars with the *rymv1-2* allele. Cultivar Bekarosaka was selected by farmers in the north-west of Madagascar, a region only recently affected by RYMV (Rakotomalala et al., 2008). Overall, the hypothesis of convergent evolution in rice for RYMV resistance seems plausible, even over a short time-scale. However, alternative hypotheses cannot be excluded. For instance, the shared mutations in resistant cultivars *O. sativa* and *O. glaberrima* may be a consequence of an adaptation to other traits. Interspecific crosses between *O. sativa* and *O. glaberrima*, which are being found increasingly, may also explain the presence of similar resistance mutations in cultivars of the two species.

## 5. Conclusion

Recent advances in field and molecular epidemiology have clarified the epidemiology of RYMV. Simplistic views and preconceived ideas had to be abandoned as a more realistic understanding of RYMV epidemiology emerged. Nevertheless, much remains to be done to reach a stage where this knowledge can be applied to help promote efficient and durable control measures. A thorough understanding of RYMV epidemiology is critical to enhance the chances of success of current virus resistance breeding programmes. The risk of emergence of resistance-breaking isolates was established clearly, and shown to be highly dependent on the inoculum pressure imposed. Integrated disease management strategies, where prophylactic measures are applied when RYMV-resistant cultivars are grown, will therefore help to optimise the chances of successful control. Molecular epidemiology should exploit the recent rapid advances in evolutionary epidemiology. The underlying factors determining the epidemiology should be unravelled further. Although historically field and molecular epidemiology followed different paths and objectives, they are substantially complementary and mutually beneficial, as emphasised in this review. The major advances in field and molecular epidemiology of RYMV are summarised below, and priorities for future epidemiological studies are proposed.

### 5.1. Field epidemiology

Initially, only a few species of chrysomelid beetles were reported to transmit RYMV, whereas coccinelids and grasshoppers were suspected (Abo et al., 2000). Due to lack of additional studies, beetles were then considered to be the only means of RYMV transmission, although their epidemiological role was unclear. However, since the early studies of Bakker (1974), evidence accumulated on the lack of a close relationship between beetle occurrence and epidemics. This suggested that other means of transmission were involved. Recent studies have unravelled several previously undescribed and unsuspected biotic and abiotic means of RYMV transmission. Among them, the pivotal role played by man in development and control of RYMV epidemics through different cropping practices was established clearly. Among these practices, the use of seedbeds, where primary inoculum was amplified tremendously, was identified as being crucial.

Although the various means of transmission of RYMV are now better known, the conditions that lead to disease outbreaks are still poorly understood. Consequently, factors that influence epidemics locally should be investigated at the field scale. Studies of population dynamics of its insect vectors in relation to disease incidence will provide useful information on their roles in RYMV epidemiology. There is a need to investigate vector diversity in terms of virus transmission efficiency in order to determine their contribution to virus spread in farmers' fields. The climatic factors (temperature, light, relative humidity) favourable to disease development or completion of vector life cycles need to be elucidated. This should explain why RYMV is absent in rice grown at high altitude (>1500 m) in the high plateaux of central Madagascar (M. Rakotomalala, unpublished results) and in the mountains at the West of Cameroon (O. Traoré, unpublished results), but present at approximately the same altitude in Moshi in the Kilimanjaro region of northern Tanzania (Kanyeka et al., 2007). It also needs to be explained why disease incidence is generally higher in the rainy season than in the dry season (Traoré et al., 2006b). The effect of plant nutrition on disease development should also be investigated to understand why, for example, application of manure increases RYMV incidence (Sarra, 2005). By bridging these gaps, an understanding of RYMV epidemiology at the field level will emerge and facilitate disease control.

### 5.2. Molecular epidemiology

The genetic diversity of RYMV exhibits a clear and informative pattern. This is due to a possibly unique combination of biological and agronomic characteristics. The genetic diversity of RYMV is sufficient to be informative, but not so large as to be affected by homoplasy. Moreover, the phylogenetic signal is not blurred by genetic exchanges due to the movement of infected vegetative plant material, long-distance transmission, or recombination events. The patterns of diversity were assessed in surveys conducted all over Africa. The continental scale of the study allowed the underlying processes of the patterns found to be unravelled; this was not possible in previous smaller scale studies. The patchy distribution of host plants in most parts of Africa restricted the rate of spread. Consequently, the spatial spread and the genetic diversification operated on a similar scale, so that genetic distances between isolates provided information on the geographic split. The different phases of virus emergence could then be untangled: host range extension, dissemination of the pathogen, increased disease prevalence.

Recent surveys of the variability of RYMV – either in previously unsurveyed countries or in regions of special interest – supported and refined the scenario proposed of an initial diversification of the virus in East Africa, followed by spread westward across the continent (Fargette et al., 2004, 2006; Traoré et al., 2005). They pro-

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vide additional information on spatial patterns of diversity, centres of diversification, bottleneck events and routes of dissemination of RYMV. The recent estimate of the rate of evolution of RYMV gave renewed impetus to phylogeographic studies. Geographical isolation and divergence time can now be related through genetic distances. The most salient and largely unexpected finding is the recent emergence of RYMV (c. 200 years ago) and, subsequently, its rapid spread throughout Africa (c. 10s of kilometres per year). Earlier interpretations of the patterns of diversity, which assumed long-term evolution and assigned a major role to adaptive events, had to be abandoned. The previously suggested link between RYMV diversification and extension of rice cultivation since the 19th century is unlikely to be fortuitous. Rice extension greatly increased the opportunities for contacts between primary hosts and cultivated rice. Modified landscape ecology ensured rapid dissemination of the virus. Due to the current circulation of virus strains, the present situation is dynamic and the future is somewhat unpredictable. However, as rice cultivation increases in Africa, it seems inevitable that RYMV will soon be present throughout the continent. Whether RYMV – which is not seed-borne, but occasionally spreads over long distances – will disseminate outside Africa is still an open question.

The link between appearance and spread of RYMV and extension of rice cultivation illustrates that agro-ecological changes are critical for emergence of plant viruses. Recent observations indicate that host passages are not simply "genetically neutral". The significance of genetic changes in the emergence of RYMV is currently being investigated. Another step required will be simultaneous analysis of the temporal, spatial and demographic aspects of RYMV epidemiology, as advocated for animal and human viruses (Grenfell et al., 2004). This will utilise the current development of algorithms which co-estimate spatial, temporal and demographic dynamics. However, with time and the further increase of rice cultivation in Africa, it seems inevitable that the current phylogeographic pattern of RYMV will eventually disappear. This is already apparent through evidence of strain circulation. Altogether, we are following a rapidly evolving situation and a phylogeographic structure which is doomed to fade. For this very reason, it is revealing the processes which determine the epidemiology of the virus.

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