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Pathogen profile

Maize streak virus: an old and complex 'emerging' pathogen

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SUMMARY

Maize streak virus (MSV; Genus *Mastrevirus*, Family *Geminiviridae*) occurs throughout Africa, where it causes what is probably the most serious viral crop disease on the continent. It is obligately transmitted by as many as six leafhopper species in the Genus *Cicadulina*, but mainly by *C. mbila* Naudé and *C. storeyi*. In addition to maize, it can infect over 80 other species in the Family *Poaceae*. Whereas 11 strains of MSV are currently known, only the MSV-A strain is known to cause economically significant streak disease in maize. Severe maize streak disease (MSD) manifests as pronounced, continuous parallel chlorotic streaks on leaves, with severe stunting of the affected plant and, usuallly, a failure to produce complete cobs or seed. Natural resistance to MSV in maize, and/or maize infections caused by non-maize-adapted MSV strains, can result in narrow, interrupted streaks and no obvious yield losses. MSV epidemiology is primarily governed by environmental influences on its vector species, resulting in erratic epidemics every 3–10 years. Even in epidemic years, disease incidences can vary from a few infected plants per field, with little associated yield loss, to 100% infection rates and complete yield loss.

Taxonomy: The only virus species known to cause MSD is MSV, the type member of the Genus *Mastrevirus* in the Family *Geminiviridae*. In addition to the MSV-A strain, which causes the most severe form of streak disease in maize, 10 other MSV strains (MSV-B to MSV-K) are known to infect barley, wheat, oats, rye, sugarcane, millet and many wild, mostly annual, grass species. Seven other mastrevirus species, many with host and geographical ranges partially overlapping those of MSV, appear to infect primarily perennial grasses.

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Physical properties: MSV and all related grass mastreviruses have single-component, circular, single-stranded DNA genomes of approximately 2700 bases, encapsidated in 22×38-nm geminate particles comprising two incomplete $T = 1$ icosahedra, with 22 pentameric capsomers composed of a single 32-kDa capsid protein. Particles are generally stable in buffers of pH 4–8.

Disease symptoms: In infected maize plants, streak disease initially manifests as minute, pale, circular spots on the lowest exposed portion of the youngest leaves. The only leaves that develop symptoms are those formed after infection, with older leaves remaining healthy. As the disease progresses, newer leaves emerge containing streaks up to several millimetres in length along the leaf veins, with primary veins being less affected than secondary or tertiary veins. The streaks are often fused laterally, appearing as narrow, broken, chlorotic stripes, which may extend over the entire length of severely affected leaves. Lesion colour generally varies from white to yellow, with some virus strains causing red pigmentation on maize leaves and abnormal shoot and flower bunching in grasses. Reduced photosynthesis and increased respiration usually lead to a reduction in leaf length and plant height; thus, maize plants infected at an early stage become severely stunted, producing undersized, misshapen cobs or giving no yield at all. Yield loss in susceptible maize is directly related to the time of infection: infected seedlings produce no yield or are killed, whereas plants infected at later times are proportionately less affected.

Disease control: Disease avoidance can be practised by only planting maize during the early season when viral inoculum loads are lowest. Leafhopper vectors can also be controlled with insecticides such as carbofuran. However, the development and use of streak-resistant cultivars is probably the most effective and economically viable means of preventing streak epidemics. Naturally occurring tolerance to MSV (meaning that, although

plants become systemically infected, they do not suffer serious yield losses) has been found, which has primarily been attributed to a single gene, *msv-1*. However, other MSV resistance genes also exist and improved resistance has been achieved by concentrating these within individual maize genotypes. Whereas true MSV immunity (meaning that plants cannot be symptomatically infected by the virus) has been achieved in lines that include multiple small-effect resistance genes together with *msv-1*, it has proven difficult to transfer this immunity into commercial maize genotypes. An alternative resistance strategy using genetic engineering is currently being investigated in South Africa.

Useful websites: ⟨http://www.mcb.uct.ac.za/MSV/mastrevirus.htm⟩; ⟨http://www.danforthcenter.org/iltab/geminiviridae/geminiaccess/mastrevirus/Mastrevirus.htm⟩.

Fig. 1 Maize streak disease symptoms: chlorotic streaks on a maize leaf. Photo credit: Frederik Kloppers.

MAIZE STREAK IN AFRICA: A CENTURY OF PATHOLOGY

Maize streak disease (MSD) was first recorded in South Africa by Claude Fuller (1901), the Government Entomologist of Natal. Fuller also quoted personal sources who noticed the disease of 'mealie variegation', as it was then described, as early as the 1870s. The disease was therefore not new at the time, and had probably been around as long as maize had been grown in the region. Fuller's investigation of MSD was motivated by an increase in incidence of the disease, marked by a serious outbreak in 1896. Although Fuller was ignorant as to its cause—he thought it was caused either by a soil nutrient deficiency or a 'chemical enzyme' acquired from the soil—he accurately described many features of the disease as it manifests today. Thus, he noted: (i) that the streaks were composed of 'a series of elongated or almost circular spots' (Fig. 1); (ii) that severely affected plants had fewer green leaves at the base than mildly diseased plants; (iii) that some plants with pronounced chlorosis grew normally and yielded cobs, whereas others were severely stunted and yielded nothing; and (iv) that severely diseased plants could be found next to perfectly healthy ones. His illustrations were also excellent, and he showed that the diseased sections of leaves or the 'streaks' contained few or no chloroplasts. Sadly, he was probably one of the first victims of motor vehicle accidents in Africa: he was killed by a car in Lourenço Marques (now Maputo) in Mozambique in 1905.

Over 100 years later, MSD remains the most significant viral disease of Africa's most important food crop (Bosque-Pérez, 2000), costing between US$120M and US$480M per year according to one conservative estimate based on average annual yield losses of only 6%–10% (Martin and Shepherd, 2009). Despite considerable advances in control measures (see below), which could halve these monetary losses (Martin and Shepherd, 2009), poorer farmers continue to suffer serious crop losses to MSD.

DISEASE AETIOLOGY

The legendary H.H. Storey was the first to show that MSD was caused by a virus, and that this virus was obligately transmitted by a leafhopper (Storey, 1924, 1925). Storey and his colleagues subsequently elucidated the transmission cycle, latent periods in the vector, the fact that the vector's transmission ability was genetically determined, the existence of distinct strains of the virus and differential reactions of various host plants to the same viruses (Storey, 1928, 1931, 1932, 1938, 1939; Storey and McClean, 1930). McClean went on to describe streak virus infections in maize, sugarcane and wild grasses in South Africa (McClean, 1947)—work which reinforced the earlier finding that there were genetically distinct 'streak viruses' infecting maize, sugarcane and grasses.

Direct proof of the existence of maize streak virus (MSV) in infected tissue came with the visualization of virus particles in 1974. Bock *et al.* (1974) discovered that MSV virions have a novel, twinned, quasi-icosahedral (geminate) shape, from which the name 'geminivirus' was born (Fig. 2). This was followed by the unexpected discovery in 1977 that geminivirus particles contain circular single-stranded DNA (ssDNA), a genome type never before observed in plant viruses (Goodman, 1977a, b; Harrison *et al.*, 1977).

Despite being initially transmitted into phloem sieve tubes by the leafhopper, virus particles occur at high concentrations within the mesophyll cells of infected maize leaves (Lucy *et al.*, 1996). Infection of chlorenchyma cells causes malformation of chloroplasts and reduced chlorophyll production (Pinner *et al.*,

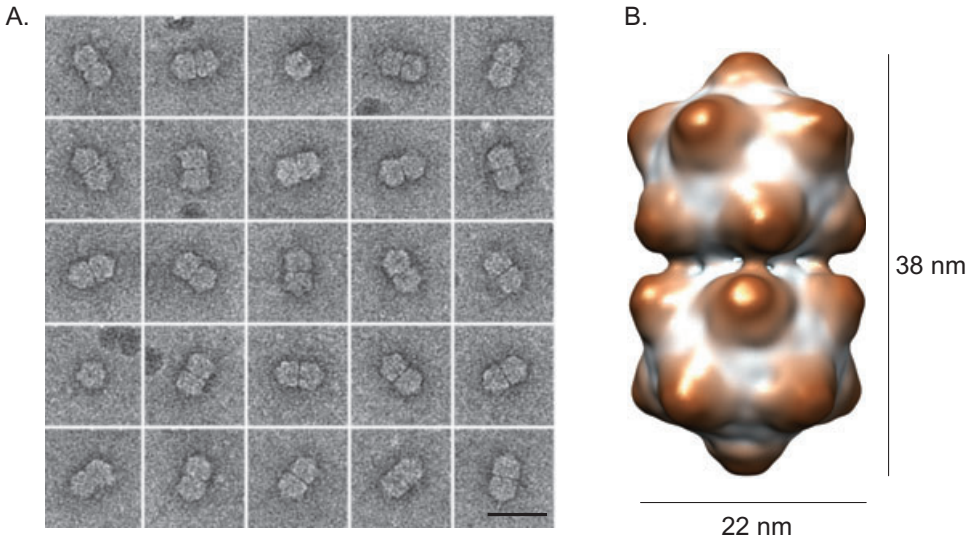

Fig. 2 (A) Negatively stained geminate particles in different orientations. Size bar, 30 nm. (B) Three-dimensional reconstruction of a geminate particle from cryoelectron microscopy data.

1993). The pattern of chlorotic lesions on infected maize leaves is directly correlated with the pattern of virus accumulation within the leaves (Lucy *et al*., 1996) and the virus can only be acquired by leafhoppers from these lesions (Peterschmitt *et al*., 1992; Storey, 1928).

MOLECULAR VIROLOGY

Each MSV virion contains a single, covalently closed, circular, ssDNA molecule (Francki *et al*., 1980) of approximately 2700 bases. It is generally accepted that geminiviruses replicate via double-stranded DNA (dsDNA) intermediates using a rolling circle replication mechanism (Heyraud *et al*., 1993a, b; Laufs *et al*., 1995a, b; Saunders *et al*., 1991, 1992). However, there is now good evidence that 'recombination-dependent replication' mechanisms also play an important role in geminivirus replication (Jeske *et al*., 2001; Jovel *et al*., 2007; Preiss and Jeske, 2003; Saunders *et al*., 1991).

In replicative dsDNA molecules, genes are expressed from both strands, and diverge from an intergenic region which contains the virion-sense origin of replication (Fig. 3). Transcription is thus bidirectional, with transcripts originating in the intergenic region and converging on the diametrically opposite side of the circular genome (Morris-Krsinich *et al*., 1985). Rolling circle replication is initiated by binding of the virus replication-associated protein (Rep) to the virion-strand origin of replication, where the protein initiates and terminates virion-strand DNA synthesis (Heyraud *et al*., 1993b; Heyraud-Nitschke *et al*., 1995; Laufs *et al*., 1995a, b; Stanley, 1995; Stenger *et al*., 1991; Willment *et al*., 2007).

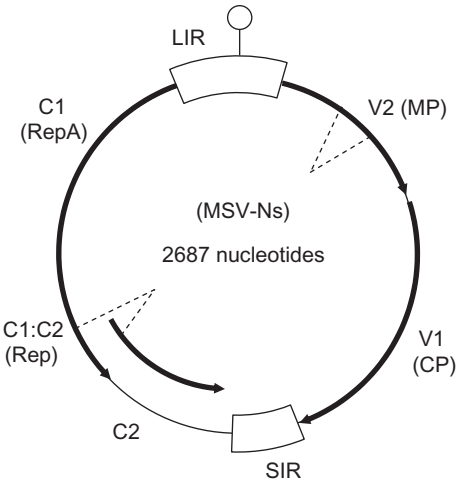

Fig. 3 Genome organization of a representative maize streak virus isolate from Nigeria (MSV-Ns). The virion-sense origin of replication is represented by a stem-loop symbol. Open reading frames (ORFs) are depicted by arrows in the direction of transcription, with broken lines indicating differential splice events. Non-coding genomic regions are shown with open boxes. ORFs are named according to whether they are encoded in the virion or complementary sense (they are assigned either a V or C, respectively). Functional names of proteins (in parentheses below the ORF name) and the names of non-coding genomic regions are abbreviated as follows: CP, coat protein; LIR, long intergenic region; MP, movement protein; Rep, replication-associated protein; SIR, short intergenic region.

MSV Rep is the translation product of two complementary sense open reading frames (ORFs), C1 and C2. The C1:C2 transcript, which contains an intron, is translated to yield either Rep (spliced transcript) or RepA (unspliced transcript). Although Rep

alone appears to be both necessary and sufficient for replication of mastreviruses in appropriate host cells (Liu *et al*., 1998; Schalk *et al*., 1989), RepA is thought to perform a variety of important additional functions during the mastrevirus life cycle. These include the modulation of host cell cycle regulation, and possibly other developmental pathways (Boulton, 2002; Gutierrez, 1999; Shepherd *et al*., 2005).

Apart from Rep and RepA, the MSV genome encodes only two additional proteins: the movement protein (MP) and the coat protein (CP). MP facilitates the movement of the virus out of the nucleus (the site of replication) and to adjacent cells (Boulton, 2002; Liu *et al*., 2001a). CP, as with most viruses, is responsible for the encapsidation of the viral nucleic acid, in this case ssDNA. However, mastrevirus CP performs a multitude of other functions: the CP of at least some leafhopper-transmitted geminiviruses determines vector specificity, implying specific interactions with unknown vector factors (Boulton *et al*., 1989; Briddon *et al*., 1990); MSV CP is capable of binding non-specifically to both ssDNA and dsDNA (Liu *et al*., 1997), and thereby facilitates the nuclear import of virus DNA (Liu *et al*., 1999); MSV CP is required for cell-to-cell and systemic spread of the virus in plants (Boulton *et al*., 1989, 1993; Lazarowitz *et al*., 1989; Liu *et al*., 2001b); moreover, MSV CP interacts specifically with MP to shuttle virus DNA out of the nucleus (Kotlizky *et al*., 2000; Liu *et al*., 2001a).

EPIDEMIOLOGY

Maize was first introduced to Africa via Ghana by Portuguese traders in the 16th century. The maize-adapted MSV-A strain can infect over 80 species in the Family *Poaceae* (Damsteegt, 1983), but it is probable that ancestral MSV-A viruses that first came into contact with maize were specifically adapted to infecting species in the Genus *Digitaria* (Varsani *et al*., 2008a). MSV also infects other introduced grass species, such as wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), rye (*Secale cereal*), oats (*Avena sativa*) and sugarcane (*Saccharum officinarum*), as well as cultivated indigenous species, such as finger millet (*Eleusine coracana*), pearl millet (*Pennisetum americanum*) and sorghum (*Sorghum bicolor*). Streak diseases in these crops are all probably of minor economic importance (Soto *et al*., 1982). One exception might be a sugarcane streak disease caused by the MSV-A strain (van Antwerpen *et al*., 2008), which is increasing in prevalence in southern Africa and could have a serious impact on sugar production across this region.

Yield losses in MSV-infected susceptible maize plants are directly related to times of infection: infected seedlings either die or produce no seed and, in one particular report, plants infected at the second, sixth and tenth leaf stages experienced approximately 55%, 40% and 25% losses in grain weight, respectively (Bock, 1982).

Rapid increases in virus populations and epidemic spread between crops are usually attributable to the convergence of factors, such as: (i) staggered growing seasons in which MSV-A populations can bulk up in early planted maize and devastate seedlings that germinate in successive plantings (Dabrowski *et al*., 1991; Fajemisin and Shoyinka, 1976); (ii) the population density of wild grasses that are reservoirs of both MSV-A and leafhopper vectors (Autrey and Ricaud, 1983); (iii) the presence within leafhopper populations of a high percentage of MSV transmitters; and (iv) environmental factors that drive the long-distance movement of leafhoppers (Rose, 1978).

An important consideration for commercial maize farmers is the severe impact of MSD on ultra-short season hybrids, which are becoming popular in southern Africa. The shorter growing time of these hybrids allows farmers to plant a second crop, such as wheat, during the winter, which also serves as a host for leafhoppers. Temporal overlap of these two crops provides a 'green bridge' (Kloppers, 2005), allowing survival of the leafhopper throughout the year. Although increased environmental virus titres towards the end of the growing season generally result in greater crop losses to MSD, ultra-short season hybrids are especially vulnerable because of their sensitivity to MSV and the density with which they are planted. This provides a favourable microenvironment for the proliferation of leafhoppers that subsequently spread the virus. In addition, shortened growing seasons provide little chance for corrective action and recovery. This is because insecticidal control of leafhopper populations cannot usually be implemented in time to effectively control the spread of the disease (Kloppers, 2005).

Although maize is a favoured host for leafhopper feeding, leafhoppers preferentially breed on annual wild grass species (P. Markham, John Innes Centre, Norwich, East Anglia, UK). Approximately 70% of the more than 138 grass species on which leafhoppers feed are also MSV hosts (Konate and Traore, 1992), and the density and composition of grass populations in any region almost certainly has a major influence on MSD epidemiology in that region. For example, the maize-adapted MSV-A strain and the closely related grass-adapted MSV-B strain appear to be particularly well adapted to the infection of grasses in the Genus *Digitaria* (Varsani *et al*., 2008a).

Although outbreaks of MSD are governed by leafhopper acquisition and movement of severe MSV isolates from infected to non-infected hosts, MSD epidemiology is complicated by the fact that different *Cicadulina* species have different proportions of individuals capable of transmitting the virus (ranging from 15% to 45%; Asanzi *et al*., 1995). In addition, not all of the 18 species of *Cicadulina* identified in Africa can transmit MSV (Bosque-Pérez, 2000; Lett *et al*., 2002; Mesfin *et al*., 1995). Early studies (Markham *et al*., 1984; Storey, 1938), indicating that the insect gut wall acts as a barrier to MSV transmission in non-vector species, were later confirmed using polymerase chain

reaction (PCR) detection in individual insect organs (Lett *et al*., 2002): MSV was detected in the gut, haemolymph and head of a vector species (*C. mbila*), but restricted to the gut of a non-vector species (*C. chinai*). In *C. mbila*, MSV crosses the gut in less than 3 h, indicating an active mechanism for transmembrane flow via a specific receptor (Lett *et al*., 2002). Although the main site for MSV accumulation is the alimentary canal (Ammar *et al*., 2009; Lett *et al*., 2002), amounts of viral DNA decrease considerably over time in both the gut and haemolymph. Interestingly, however, virus DNA copies remain stable over time in the head (presumably in the salivary glands, from which MSV is released into the phloem when the leafhopper feeds on a host plant), and it is probable that virus copies released by the salivary glands on feeding are continuously replaced from elsewhere, probably the haemolymph. As MSV is thought not to replicate in leafhoppers (Boulton and Markham, 1986; Reynaud and Peterschmitt, 1992), this flow towards the salivary glands, in addition to viral degradation, would explain the decrease in viral accumulation over time. Stable viral DNA levels in the salivary glands are also consistent with the observation that *C. mbila* can transmit the virus for 5 weeks after an acquisition access feeding period of only 3 h (Reynaud and Peterschmitt, 1992).

Although transmission to some hosts by *C. mbila* is remarkably efficient, studies of the feeding activities of this species by Mesfin *et al.* (1995) revealed vector preferences for certain hosts. On hosts from which the leafhoppers prefer not to feed, MSV transmission rates are decreased by reduced probing times (Bosque-Pérez, 2000), indicating that feeding behaviour on a maize genotype influences its resistance to MSV infection. Although studies have shown that *C. mbila* is the species most often implicated in MSD outbreaks (Dabrowski, 1987; Magenya *et al*., 2008), Oluwafemi *et al.* (2007) found that *C. storeyi* is the better transmitter, indicating that transmission ability alone does not make an efficient vector. Other considerations are distribution (*C. mbila* is the most widely distributed species throughout Africa) and the fact that a larger proportion of *C. mbila* populations have the ability to transmit MSV compared with other *Cicadulina* species (Markham *et al*., 1984; Storey, 1928, 1933). This is partly a result of the proportion of *C. mbila* females (which are better transmitters), being two to three times higher than in other species (Wambugu and Wafula, 2000).

An additional factor contributing to transmission efficiency is the mobility of leafhoppers. In the warm wet season, *C. mbila* develops a longer body morph. This morph flies less than 10 m and, consequently, only isolated pockets of disease develop. However, with the onset of crop maturity or under drought conditions—both causing the food plants of leafhoppers to dry out—the stronger flying, short-bodied *C. mbila* morph predominates. Extensive migration into irrigated crops occurs, spreading disease over great distances and resulting in widespread epidemics (Rose, 1978).

Environmental factors that have an influence on leafhopper population sizes also play an important role in MSD epidemiology. For example, MSD outbreaks are often associated with drought conditions, followed by irregular rains at the beginning of growing seasons (Efron *et al*., 1989), as in the savanna regions of West Africa in 1983 and 1984 (Rossel and Thottappilly, 1985), or in Kenya in 1988–89 (Njuguna *et al*., 1990). The relative abundance of various *Cicadulina* species with differing abilities to transmit the virus in different parts of Africa is influenced by altitude, temperature and rainfall (Dabrowski *et al*., 1987). In addition, late rainfall favours the development of leafhopper nymphs during the winter (Stanley *et al*., 1999). The interplay of all of these factors makes MSD epidemiology rather erratic, with the disease being devastating in some years and insignificant in others (Efron *et al*., 1989).

MSV DIVERSITY AND EVOLUTION

In addition to the demographics of grass and vector populations, the exact make-up of MSV-A populations in different parts of Africa probably also has a major influence on MSD epidemiology. Although there are fairly obvious differences in the genetic composition of MSV-A populations in eastern, western and southern Africa (Briddon *et al*., 1994; Martin *et al*., 2001; Willment *et al*., 2001), it is not currently known whether these translate into differences in disease epidemiology. Of the four main lineages of MSV-A currently circulating in Africa (MSV-A_1, MSV-A_2, MSV-A_3 and MSV-A_4; Fig. 4), MSV-A_1 and MSV-A_4 are apparently responsible for more than 95% of all MSD cases that have been analysed over the past 20 years. It is therefore safe to assume that these are the main lineages driving MSD epidemics throughout southern and East Africa where the bulk of virus sampling has been carried out (Martin *et al*., 2001; Owor *et al*., 2007; Varsani *et al*., 2008a; Willment *et al*., 2001).

Although MSV-A_4 is seemingly confined to southern Africa, MSV-A_1 has a geographical range that spans the whole of sub-Saharan Africa (Martin *et al*., 2001; Owor *et al*., 2007; Varsani *et al*., 2008a; Willment *et al*., 2001). A characteristic of MSV-A_4, which may have some bearing on its more restricted geographical range, is that it is apparently less severe in maize than MSV-A_1 (Martin *et al*., 1999). The widespread distribution of MSV-A_1 is, however, somewhat unusual in that no other group of similar MSV variants (i.e. a MSV lineage displaying the same depth of genetic diversity as MSV-A_1) has ever been found to be spread between major regions of the continent, such as between East and West Africa or East and southern Africa.

Although MSV-A is the only strain known to cause severe MSD (Martin *et al*., 2001; McClean, 1947; Storey and McClean, 1930), 10 non-maize-adapted MSV strains (MSV-B to MSV-K; Fig. 4) have also been identified (Martin *et al*., 2001; Schnippenkoetter *et al*., 2001a; Varsani *et al*., 2008a; Willment *et al*., 2002).

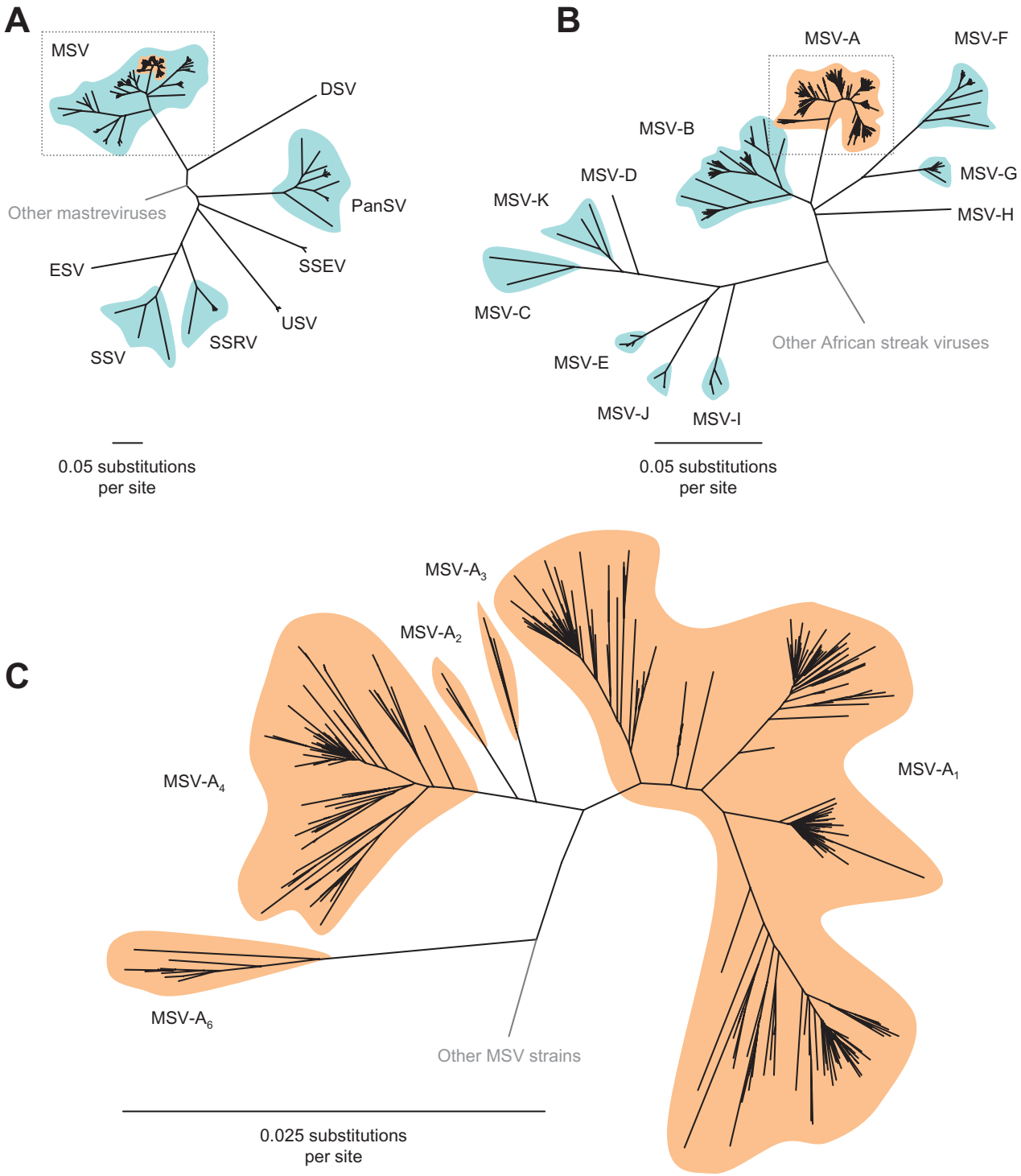

Fig. 4 Phylogenetic relationships between viruses related to maize streak virus (MSV). (A) In addition to MSV, there are six other known African streak virus species, including *Eragrostis* streak virus (ESV), sugarcane streak virus (SSV), sugarcane streak Réunion virus (SSRV), *Urochloa* streak virus (USV), sugarcane streak Egypt virus (SSEV) and *Panicum* streak virus (PanSV). *Digitaria* streak virus (DSV), from the island of Vanuatu in the Pacific, is closely related to the African streak viruses and is included here for reference purposes. This tree has been rooted on the virus *Chloris* striate mosaic virus (not shown). The boxed area is expanded in (B). (B) There are 11 known MSV strains but only one of these, MSV-A (in orange), is known to cause severe streak disease in maize. The boxed area is expanded in (C). (C) There are five major MSV-A variants: MSV-A_6 has only been found on islands in the Indian Ocean; MSV-A_2 has only been found in West Africa; MSV-A_3 has only been found in East Africa; and MSV-A_4 has only been found in southern Africa. MSV-A_1 is found throughout mainland Africa.

Although they are normally found infecting wild grasses, some of these (MSV-B, MSV-C, MSV-D and MSV-E) are also known to produce mild infections in MSV-susceptible maize genotypes (Martin *et al*., 1999, 2001). Although they have no known direct impact on African agriculture, these MSV strains have probably had a large indirect influence on the evolution of the economically significant MSV-A strain. For example, the main genetic feature differentiating MSV-A_4 from other MSV-A lineages is that it is the product of a recombination event between MSV-A and MSV-B viruses found in southern Africa (Martin *et al*., 2001).

As with other geminiviruses, recombination has featured prominently in the evolution of MSV. In general, intra-strain MSV recombination appears to have been far more prevalent during recent MSV-A evolution than inter-strain or inter-species recombination (Owor *et al*., 2007; Varsani *et al*., 2008a). In all three recorded examples of recent natural inter-strain recombination events involving MSV-A viruses, fewer than 200 nucleotides have been exchanged, possibly indicating that inter-strain recombination has little to offer in the way of substantial MSV-A fitness improvements. This notion is backed up by the fact that laboratory constructed MSV-A—MSV-B and MSV-A—MSV-C chimaeras have invariably been severely defective (Martin and Rybicki, 2002; Martin *et al*., 2005; Schnippenkoetter *et al*., 2001b; van der Walt *et al*., 2008b). When maize plants are co-infected with reciprocal MSV-A—MSV-B chimaeras (for example, laboratory constructed recombinants with *mp* and *cp* genes reciprocally swapped between MSV-A and MSV-B isolates), these viruses recombine very rapidly to produce MSV-A-like recombinants (van der Walt *et al*., 2009). Collectively, these observations indicate that the fitness of contemporary MSV-A genotypes cannot be easily improved through inter-strain recombination.

It is therefore somewhat surprising that the MSV-A strain is thought to have arisen via a large recombination event that merged the *mp* and *cp* genes of an ancestral MSV-B variant with the long intergenic region (LIR), short intergenic region (SIR) and *rep* genes of an ancestral virus resembling the progenitor of the MSV-G and MSV-F strains (Varsani *et al*., 2008a). It has been proposed that this recombination event may have triggered the emergence of MSV-A as an agricultural pathogen. Given that, together with other geminiviruses (Duffy and Holmes, 2008; Ge *et al*., 2007), MSV probably has an evolution rate somewhere between 10^{-4} and 10^{-3} substitutions per site per year (Isnard *et al*., 1998; van der Walt *et al*., 2008a), this recombination event can be dated to any time between 100 years ago, when MSD was first described, and 500 years ago, when maize was first introduced to Africa—times that fit well with the hypothesis that this recombination event was pivotal in the adaptation of MSV-A to maize.

Large inter-strain recombination events and smaller inter-species recombination events have also probably contributed substantially to the evolution of various MSV strains other than MSV-A. Four strains (MSV-F, MSV-H, MSV-J and MSV-K) apparently arose via the exchange of large genomic regions (more than 1000 nucleotides) amongst two or more distinct MSV strains (Varsani *et al*., 2008a).

Other recombination events that are detectable in the genomes of grass-adapted MSVs involve the introduction of short sequences (usually entirely within the SIR) from other streak virus species (Oluwafemi *et al*., 2008; Shepherd *et al*., 2008; Varsani *et al*., 2008b). These mostly perennial grass-infecting viruses include *Panicum* streak virus, sugarcane streak virus, sugarcane streak Réunion virus, sugarcane streak Egypt virus, *Eragrostis* streak virus and *Urochloa* streak virus (Fig. 4). All of these viruses share largely overlapping geographical ranges, host species and leafhopper vectors with MSV, and it is perhaps surprising that, given the rampant inter-species recombination observed in other geminiviruses (Lefeuvre *et al*., 2007a, b; Padidam *et al*., 1999), more inter-species recombination is not found amongst these so-called African streak viruses. As none of the African streak viruses other than MSV is considered to be a serious agricultural threat, and there is no evidence of genetic exchange between MSV-A and these other viruses, it is currently doubtful whether they have any influence on MSV-A epidemiology and evolution.

CONTROL

Suggested disease avoidance practices include barriers of bare ground between early- and late-planted maize fields to reduce leafhopper movement and subsequent spread of MSV (Bosque-Pérez, 2000), avoidance of maize plantings downwind from older cereal crops and the use of crop rotations that minimize invasion by viruliferous leafhoppers (Rose, 1978). The vector can be controlled by the application of systemic insecticides to the planting furrow during maize planting or, even more effectively, as seed treatments. However, expensive chemical seed treatments are generally not an option for poorer farmers—they provide only limited protection under severe pressure, and handling such treated seeds can be dangerous. The development and use of streak-resistant cultivars is probably the most effective and economically viable means of preventing streak epidemics.

Thirty years after the first report of MSD, resistance in maize was discovered in the variety 'Peruvian Yellow' (Fielding, 1933), and several other maize genotypes have since been found to have varying degrees of resistance. Resistance usually manifests as reduced symptom severity combined with low virus titres, leading to low virus incidence in the field. Resistant varieties are therefore much poorer sources of inoculum during secondary disease spread (Rodier *et al*., 1995). Some resistant varieties produce good yields despite being infected (Bosque-Pérez, 2000).

MSV-resistant maize genotypes include Tzi4, a partially resistant inbred line from Nigeria originating from the TZ-Y (Tropical Zea Yellow) population and developed at the International Institute of Tropical Agriculture (Kim *et al*., 1987), CML202, a subtropical white inbred line from CIMMYT-Zimbabwe (Welz *et al*., 1998), and D211 and CIRAD390, both from the Indian Ocean island of Réunion (Marchand *et al*., 1994; Pernet *et al*., 1999b; Rodier *et al*., 1995). The first resistant genotype to be mapped by molecular markers was Tzi4: a single, partially dominant gene was identified on the short arm of chromosome 1 and designated *msv-1* (Kyetere *et al*., 1995). As no other genomic region was associated with MSV resistance, the resistance was described as being monogenic. However, this resistant genotype would be better described as being MSV tolerant, with a rating in field tests of '3' on a '1–5' scale ('1' being completely immune; Kyetere *et al*., 1999).

Mapping of the other three resistant lines (CML202, D211 and CIRAD390) indicated that they all probably carry either *msv-1* or some allelic variation thereof. However, these three genotypes also seem to carry various additional small-effect MSV resistance genes that are apparently not found in Tzi4. These genes may account for observable differences in the degrees of resistance between genotypes: CML202 was given a score in field tests of '2' (Welz *et al*., 1998), whereas the two Réunion sources were rated as being completely immune to field infection by MSV (Pernet *et al*., 1999a, b).

There are currently active MSV resistance breeding programmes in South Africa, Zimbabwe, Nigeria, Kenya, La Réunion, and elsewhere. However, despite the past success of these efforts, there are several difficulties in producing conventionally bred maize genotypes having high degrees of MSV resistance. The first is that all MSV resistance so far reported appears to rely quite heavily on the *msv-1* gene. If the enormous evolutionary potential of MSV (Isnard *et al*., 1998; van der Walt *et al*., 2008a) eventually yields virus genotypes capable of breaking this resistance, all current commercially available MSV-resistant germplasm would be rendered largely ineffective. It is therefore imperative that alternative sources of MSV resistance be found.

A second problem is that natural resistance is not found in varieties with the best agronomic qualities. Current resistant sources are mostly tropical varieties with maturation and flowering characteristics that make them difficult to work with in the field. In addition, these resistant genotypes are not as high yielding as commercially favoured, albeit MSV-sensitive, genotypes.

The third and probably largest obstacle to transferring very high degrees of MSV resistance to agronomically favourable genotypes is the coordinated transfer of multiple resistance genes scattered amongst different chromosomes. Transferring numerous resistance alleles, some of which may be recessive and/or have only small effects, during multiple breeding cycles is extremely difficult, particularly when these genes need to be separated from undesirable genetic backgrounds.

An alternative strategy to using conventional breeding would be to directly engineer MSV-resistant maize genotypes using either natural maize MSV-resistance genes or resistance genes from other sources. Great successes have been achieved in the genetic engineering of resistance to geminiviruses (see Shepherd *et al*., 2009 and Vanderschuren *et al*., 2007 for reviews) using the pathogen-derived resistance concept (Sanford and Johnson, 1985), whereby pathogens themselves provide the genes for engineered resistance. This approach has already been successfully applied to the production of MSV-resistant maize in South Africa (Shepherd *et al*., 2007).

Although direct genetic engineering provides better prospects than conventional breeding for the development of novel, varied and durable resistance strategies, this technology also has some significant drawbacks. These include: (i) maize genotypes with commercially appealing agronomic properties are not easy to transform or easily regenerated in tissue culture; (ii) it is technically difficult to genetically engineer maize because the commonly used transformation techniques (such as particle bombardment) generally introduce an unacceptably large number of gene copies at random locations in the maize genome, which can potentially disrupt important regulatory or coding sequences; (iii) a lengthy and costly risk assessment needs to be carried out to ensure that genetically engineered maize is both safe to eat and poses no harm to the environment and non-target organisms; and (iv) public perception of genetically engineered foods can be unfavourable and they are banned in many African countries. This last obstacle may, however, become less formidable in the future. Since 2008, Burkino Faso, Egypt and Kenya have joined South Africa (which has permitted genetically modified crop farming since 1997) in allowing the production and use of genetically engineered crops. Meanwhile, several other African countries, such as Mali, Togo, Malawi, Zimbabwe and Cameroon, have the appropriate legal frameworks in place for the commercialization of genetically engineered crops, although they have yet to do so. For now, however, the use of conventionally bred resistant varieties, coupled with sound crop management practices, is still probably the best means of limiting the impact of MSD on maize yields.

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