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The relative infectivities and genomic characterisation of three distinct mastreviruses from South Africa

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Summary. The genomic nucleotide sequences of the cloned agroinfectious genomes of three South African mastreviruses obtained from *Zea mays*, a *Setaria* sp., and *Panicum maximum* (designated MSV-Kom, MSV-Set, and PanSV-Kar respectively), were determined. Additionally, their relative infectivities and virulence were analysed in a range of differentially susceptible wheat, maize, and barley genotypes. MSV-Kom produced moderate to severe streak symptoms in all maize genotypes tested, but only moderate to very mild symptoms in the wheat and barley genotypes. MSV-Set infected only the susceptible to tolerant maize genotypes, but was generally more severe in the barley and wheat genotypes than MSV-Kom. PanSV-Kar was incapable of infecting any of the wheat and barley genotypes and only produced very mild symptoms on the three most sensitive maize genotypes. Genomic characteristics in common with related mastreviruses were identified. Phylogenetic analysis indicated that while MSV-Kom was closely related to previously sequenced MSV isolates, MSV-Set and PanSV-Kar represented distinctly novel strains of MSV and PanSV respectively. In the case of MSV-Set, this is the most distantly related MSV strain yet characterised.

Introduction

Maize streak virus (MSV), *Panicum streak virus* (PanSV) and *Sugarcane streak virus* (SSV) are members of an "African streak virus group" within the *Mastrevirus* genus of the family *Geminiviridae* [6, 13], and occur only in Africa and Indian Ocean territories. While most mastreviruses infect only monocotyledonous hosts and are transmitted by leafhoppers in the genus *Cicadulina*, all have monopartite single stranded circular genomes encapsidated within twinned (geminate) quasi-icosahedral virions [17]. The genomic organisation and molecular biology of the mastreviruses has been recently reviewed by Palmer and Rybicki [23].

Maize streak disease, caused by MSV, is arguably the most significant viral disease of maize in sub-Saharan Africa. As maize is an introduced crop in Africa, it could only have encountered African mastreviruses from the 1600s onwards; MSV genotypes were presumably pathogens of indigenous African grasses at the time maize was introduced. The most divergent MSV isolate sequenced to date was obtained from the Indian Ocean island of La Réunion, and its genomic sequence differs from other sequenced isolates by less than 5% [25]. The next most related viruses known are *Digitaria streak virus* (DSV) [7], PanSV [5] and SSV [11], all of which share less than 65% sequence identity with the MSV isolates. In order to clarify some of the recent evolutionary history of the MSV genotypes responsible for maize streak disease, it is essential that viral genotypes that share between 70 and 90% sequence identity with MSVs be discovered and sequenced. Our laboratory has an ongoing programme aimed at elucidating the genomic diversity of African maize- and grass-infecting mastreviruses [11–13, 27, 28]: here we report the complete nucleotide sequences of three distinct African streak viruses from South Africa. One of these, MSV-Set, was isolated from an indigenous *Setaria* sp.; one is an MSV variant obtained from maize (MSV-Kom), and the other is a new PanSV strain (PanSV-Kar) isolated from *Panicum maximum*. Their relative virulences in a range of maize, wheat and barley genotypes are also described.

Materials and methods

Virus isolates and maize, barley, and wheat genotypes

Cloning and restriction site analysis of MSV-Kom, MSV-Set and PanSV-Kar RF-DNAs from *Zea mays* (pKom500), a *Setaria* sp. (pSet100), and *Panicum maximum* (pPS100) plants showing typical streak symptoms have been described previously [12, 13]. Seed for the sweetcorn cultivars Jubilee and US More was obtained from Starke Ayres Nursery (Cape Town, South Africa); popcorn seed used was a commercial supermarket variety; seed for the Vaalharts Wit and Vaalharts Geel composite maize varieties was provided by J.B.J. van Rensburg (Summer Grains Centre, Potchefstroom, South Africa); seed for the maize hybrids PNR 6549, PNR 6552, PNR 7541 and PNR 7563 was provided by Pioneer Seed Co. (Greytown, South Africa); seed for the maize hybrids PAN 6191, PAN 6166, PAN 6133, PAN 6363, PAN 6480, PAN 6043, PAN 6564, PAN 6474, PAN 6481, PAN 6496, PAN 6099, PAN 6549, PAN 6578, PAN 6552, PAN 6195, PAN 6528, PAN 6364, PAN 6462, PAN 6479 and PAN 6141 was obtained from Pannar Seed Co. (Greytown, South Africa); and seed for the wheat genotypes SST 66, SST 44, Marquis, Dias, and Agent, and barley genotypes Palmiet, Adam, Tas, Chokka, Festiquay, and Clipper was obtained from the Elsenburg Agricultural Development Institute (Elsenburg, South Africa).

Subcloning and sequencing of cloned virus genomes

An ordered series of deletion clones and subclones derived from pSet100, pPS100 and pKom500 were prepared in pBluescript (Stratagene, CA, USA) and pUC18 using standard cloning techniques [30] and were used to sequence the entire genomes of MSV-Set, PanSV-Kar and MSV-Kom, respectively. Selected plasmid templates were subjected to bidirectional Sanger dideoxynucleotide chain termination plasmid sequencing using either the Sequenase II kit and protocols (United States Biochemical Corporation, CA, USA) or the TaqTrack *Taq*

polymerase method of sequencing (Promega, WI, USA). On average the sequence of both strands of all viruses were each read twice.

Phylogenetic and sequence analyses

Sequence data was assembled and analysed using GCG (version 7.1, Genetics Computer Group, Inc., WI) and DNAMAN (Version 2.2, Lynnon Biosoft, Quebec, Canada). Multiple nucleotide and putative amino acid sequence alignments were produced using DNAMAN. Phylogenetic trees were produced by the neighbour joining method of Saitou and Nei [29].

Agroinfectious clones and agroinoculation of maize seedlings

Agroinfectious clones of MSV-Kom, MSV-Set and PanSV-Kar were constructed essentially according to Grimsley et al. [9]. Using standard cloning techniques, partial tandem repeats of the genomes containing two full long intergenic regions (LIRs) were constructed in pUC18 and inserted into the binary cloning vector pBI121 (Clontech, Palo Alto, CA). Agroinfectious constructs were transformed into *Agrobacterium tumefaciens* C58C1 (pMP90) [14] using the freeze-thaw transformation method of An et al. [1]. Because the sweetcorn cultivar Jubilee could be both easily inoculated and was highly susceptible to all three viruses examined in this study, it was used for testing the relative infectivity and severity of the viruses. Three day old seedlings were agroinoculated and maintained as described previously [18]. The infection rates, degrees of stunting and chlorotic leaf streaking elicited by the three viruses were quantified according to Martin et al. [18]. DNA was isolated from plants displaying streak symptoms [24], digested with *Pvu*II, separated on an agarose gel and Southern blotted onto a nitrocellulose membrane using standard techniques [30]. Viral DNA was detected using a DIG labelled probe (Boehringer-Mannheim, Germany; [13]) prepared from one of pPS100, pSet100, and pKom500, and the identities of the viruses were confirmed on the basis of their banding patterns.

Leafhopper maintenance and transmissions

Cicadulina mbila adults and nymphs used in MSV transmission experiments were obtained from MSV-free colonies maintained here [13]. The three viruses were transmitted by *C. mbila* to a range of differentially MSV sensitive maize, wheat and barley genotypes. Non-viruliferous leafhoppers were allowed to feed for between 48 and 96 h on agroinoculated maize plants showing streak symptoms. Viruses were transmitted to two week-old uninfected maize, wheat and barley seedlings. Viruliferous leafhoppers were allowed to feed on the plants for 5–10 days. All transmissions of each virus into particular host genotypes were repeated four times. Streak symptoms were rated on a 5 point rating scale (0 = no streaks and no stunting, 4 = 80–100% of leaf area chlorotic with severe stunting).

Accession numbers

The nucleotide sequences of the infectious clones of MSV-Set, MSV-Kom and PanSV-Kar have been submitted to the GenBank nucleotide sequence database, and have been assigned the accession numbers AF007881, AF003952, and L39638 respectively.

Results

Complete nucleotide sequences and phylogenetic analysis

The full genomic sequences of MSV-Kom, MSV-Set and PanSV-Kar revealed a number of features similar to those identified in other mastreviruses. The

Table 1. Genomic features of MSV-Kom, MSV-Set, and MSV-Kom as determined by analogy with other mastreviruses

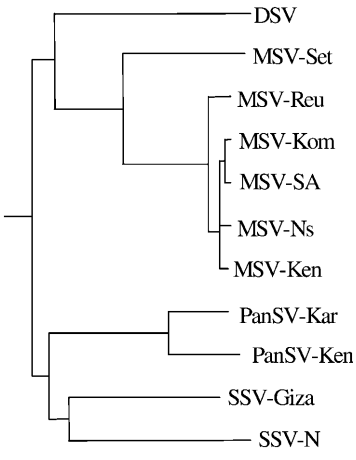
Genomic feature	Nucleotide positions			Reference
	Kom	Set	PanSV	
ORFs				
V1	150–453	152–455	145–466	[21]
V2	466–1198	468–1199	479–1223	[21]
C1	2528–1712	2526–1710	2564–1705	[21]
C2	1815–1371	1813–1369	1830–1413	[21]
Intergenic regions				
LIR	2528–150	2526–152	2564–145	[21]
SIR	1200–1371	1183–1369	1225–1413	[21]
Introns				
V1	241–316	242–316	254–341	[29]
C1/C2	1795–1886	1793–1884	1810–1901	[1]

MSV-Kom genome consists of 2690 nucleotides; this is exactly the same size as the MSV-SA genome [16], with which it shares 99% homology. MSV-Set and PanSV-Kar sequences were 11 and 15 nucleotides larger than MSV-Kom at 2701 and 2705 nt, respectively. MSV-Set was most closely related to, and shared 78% sequence identity with, the sequenced MSVs obtained from maize. PanSV-Kar shared 87% sequence identity with PanSV-Ken from Kenya [5] and 60% identity with MSV-Set and the other sequenced MSV isolates from maize [16, 20, 25].

A neighbour-joining tree constructed using full aligned genomic nucleotide sequences (Fig. 1A) showed that while MSV-Kom and MSV-Set grouped with other MSVs, the MSV-Set sequence was distinct, and it is the most divergent MSV genome yet sequenced. PanSV-Kar was most closely related to PanSV-Ken; however, at only 87% sequence identity, these viruses almost certainly represent different PanSV strains [22, 26].

Genomic features of the three viruses, including ORFs, introns and intergenic regions, were identified by analogy with other mastreviruses, and are summarised in Table 1. The predicted MP, CP, and Rep amino acid sequences of representative African mastreviruses, including MSV-Kom, MSV-Set and PanSV-Kar, indicated that while CP sequences are most conserved within this group, MP sequences are least conserved (Fig. 1B). MSV-Kom, MSV-Set and PanSV-Kar all had potential intron donor and acceptor sites in both their C1/C2 and MP ORFs. However, while a characteristic CTGAC "lariat" sequence was identifiable in the C1/C2 ORF of MSV-Kom and PanSV-Kar, the analogous sequence in MSV-Set was CTGAT, the same as that found in the non-infectious clone of SSV-N [11].

Sequence elements within the LIRs of MSV-Kom, MSV-Set and PanSV-Kar that are believed to be functionally significant were inferred by analogy with other mastreviruses, and are described in Fig. 2. The most prominent feature of the



A

B

	98.9									
MSV-Kom	98.4	98.0								
	97.5		96.9							
MSV-Reu	99.2	97.0	98.8	95.0						
	81.7		81.4		81.7					
MSV-Set	83.5	75.0	84.0	75.0	83.5	74.0				
	64.2		63.9		64.5		66.2			
PanSV-Kar	68.6	56.0	69.0	57.1	68.2	54.9	66.9	54.9		
	65.6		65.6		66.5		65.3		67.6	
SSV-Giza	68.7	51.6	68.3	52.6	67.9	51.6	67.9	52.6	77.1	60.4
	MSV-Ns		**MSV-Kom**		**MSV-Reu**		**MSV-Set**		**PanSV-Kar**	

Key
Rep
CP **MP**

Fig. 1. The phylogenetic relationship between MSV-Set, MSV-Kom and PanSV-Kar and other African streak viruses. **A** A neighbour-joining tree constructed using full genomic sequences. Horizontal distances are proportional to distances; vertical distances are arbitrary. All nodes supported by less than 60% of a 1000 repeat bootstrap iteration are collapsed. The tree was rooted on the *Wheat dwarf virus* sequence (not shown). **B** Pairwise sequence identity matrices for the deduced Rep, CP, and MP amino acid sequences of selected African streak viruses. Virus sequences used included DSV from Vanuatu [7], MSV-Reu from La Réunion [25], MSV-SA [16] and SSV-N [11] from South Africa, MSV-Ns [20] from Nigeria, MSV-Ken [10] and PanSV-Ken [5] from Kenya, and SSV-Giza from Egypt [2]

LIRs of these viruses is the characteristic geminivirus stem-loop structure, with the sequence in the loop containing the invariant TAATATTAC nonanucleotide. The LIRs of MSV-Kom, MSV-Set and PanSV-Kar contain sequences capable of forming such a stem loop. While the putative stem sequences of MSV-Kom and MSV-Set are 18 nt, those of PanSV-Kar are 15 nt. PanSV-Kar's loop sequence is 14 nt and those of MSV-Kom and MSV-Set are 12 nt. While there are extensive differences in the stem sequences of PanSV-Kar and MSV-Kom, there are only

Fig. 2. Sequence features within the LIR of MSV-Kom, MSV-Set, and PanSV-Kar. Shaded regions represent iterated sequences (called iterons) that potentially interact with Rep. The hatched region marks the characteristic TAATATTAC nonanucleotide found in every mastrevirus sequenced to date. RPE refers to putative rightward promoter element sequences

Table 2. Amino acid sequence motifs within the Rep proteins of MSV-Kom, MSV-Set and PanSV-Kar

Motifs	Isolate	Sequence	Position	Reference
RCR[a] Motif 1 (FLTYp)	Kom	FLTYP	18–22	
	Set	FLTYP	18–22	[16]
	PanSV	FLTYS	26–30	
RCR Motif 3 Y(U)xK	Kom	YILK	100–103	
	Set	YITK	100–103	[16]
	PanSV	YILK	108–111	
Rb Motif (LXCXE)	Kom	LLCNE	198–202	
	Set	LLCNE	198–202	[30]
	PanSV	LLCNE	205–209	
NTP Binding ($EGX_4GKTX_{32}DD$)	Kom	$VGX_4GKSTX_{29}DD$	227–268	
	Set	$VGX_4GKSTX_{29}DD$	227–268	[11]
	PanSV	$VGX_4GKTSX_{29}DD$	234–275	

[a] RCR = Rolling circle replication

4 nt differences in the stem sequences of MSV-Set and MSV-Kom. Portions of the stem and, in the case of PanSV-Kar, loop sequences iterated between the C1 start codon and TATA box that are believed to play a role in regulation of C1 expression [3], were identified (Fig. 2). These "iterons" were 8 nt, 6 nt and 5 nt long in MSV-Kom, MSV-Set and PanSV-Kar, respectively. At the C1 side of the stem base PanSV has the sequence GGGCCGG, that is analogous to the rightward promoter element (RPE) identified by Fenoll et al. [8]. In the same position in MSV-Kom and MSV-Set and potentially also comprising a RPE in these viruses, is a double repeat of this sequence (Fig. 2).

Four amino acid motifs found in the predicted Rep protein of the 3 viruses studied which are analogous to those identified in the Rep proteins of other mastreviruses are described in Table 2. Besides the rolling circle replication (RCR) motifs 1 and 3 described in the Table 2, Koonin and Ilyana [15] identified an additional RCR motif sequence (motif 2) in the Rep proteins of geminiviruses. Although such a motif was identifiable in the predicted Rep proteins of MSV-Kom and PanSV-Kar, only one of the expected histidine residues was found at the appropriate positions in MSV-Set.

Infectivity and relative severity of agroinfectious virus clones

Of the three viruses, MSV-Kom was most agroinfectious and induced both the highest degrees of stunting and streaking in infected maize plants. While almost as agroinfectious as MSV-Kom, MSV-Set induced far less stunting in infected maize plants and produced chlorotic lesions covering substantially smaller leaf areas. PanSV-Kar was the least agroinfectious, produced very mild chlorotic streaking and did not stunt infected plants (see Figs. 3 and 4). The streak symptoms of

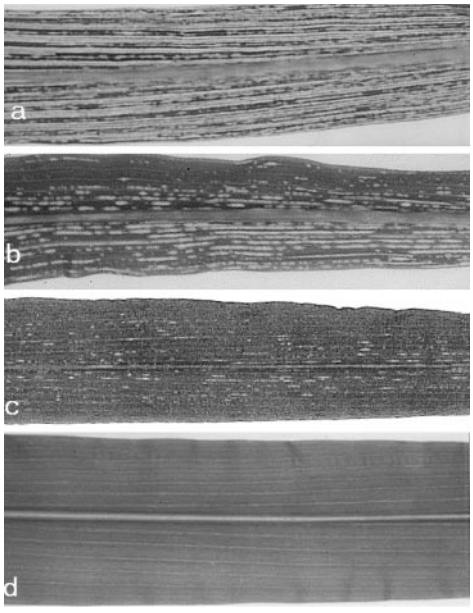


Fig. 3. Photograph of typical symptoms in sweetcorn cv. Jubilee agroinoculated with MSV-Kom, MSV-Set and PanSV-Kar. **a** MSV-Kom; **b** MSV-Set; **c** PanSV-Kar; **d** healthy leaf

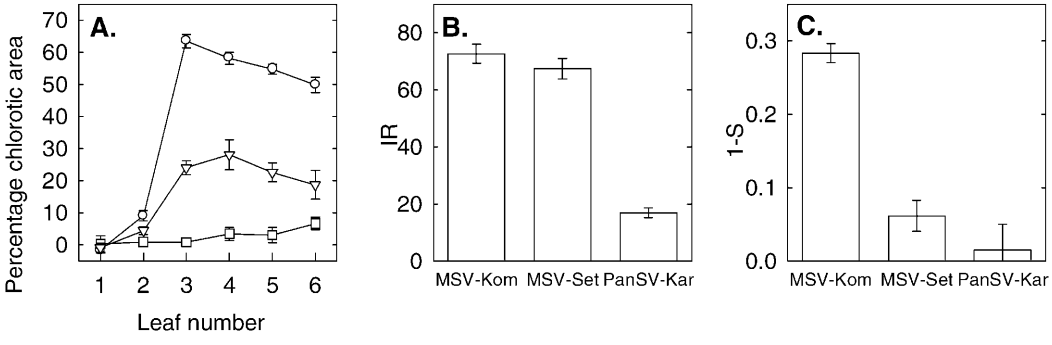


Fig. 4. Quantitation of symptoms in sweetcorn cv. Jubilee. **A** Percentage chlorotic areas occurring on the first six leaves of agroinoculated plants: PanSV-Kar (□), MSV-Set (▽), and MSV-Kom (◯). **B** Infection rate (*IR*) calculated as the mean percentage of infected plants observed 5, 8, 11 and 14 days post agroinoculation [20]. **C** Virus induced stunting expressed as 1-S [19]. S is calculated as the mean height of symptomatic plants (measured from coleoptilar node to tip of 4th leaf 15 days post agroinoculation) expressed as a proportion of the mean height of uninfected control plants [19]. In all cases error bars represent 95% confidence intervals

the three viruses were very distinct from one another. PanSV-Kar caused white, very narrow streaks never more than 3cm in length, MSV-Set produced broader white stippled streaks and MSV-Kom caused severe continuous yellow streaks that often coalesced into large areas of continuous chlorosis.

Southern blot analysis of DNA extracted from infected agroinoculated plants indicated that symptom severity appeared directly correlated with the amount of viral DNA present. *Pvu* II digested DNA extracts from infected plants confirmed

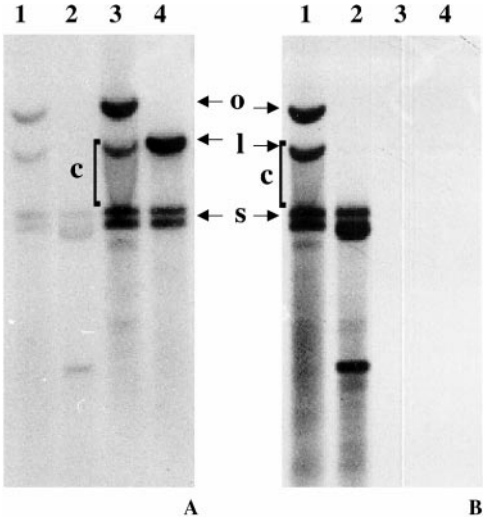


Fig. 5. Southern blots for the detection of viruses in agroinfected plants. **A** Blot hybridised with MSV-Set DNA probe; **B** same blot stripped and rehybridised with MSV-Kom DNA probe. *1* and *2*: undigested and *Pvu* II-digested DNA from a MSV-Kom agroinfected plant displaying severe streak symptoms; *3* and *4*, undigested and *Pvu* II-digested DNA from MSV-Set agroinfected plants displaying moderate streak. One μg of total plant DNA extract was loaded in each lane. X-ray film was exposed to the membrane for chemiluminescent detection for 30 min for MSV-Set detection (**A**), and 15 min for MSV-Kom detection (**B**). *o* ds open circular DNA; *c* closed circular (supercoiled) viral DNA forms; *l* linear dsDNA; *s* ssDNA forms. **A**, *4* MSV-Set RF-DNA contains one *Pvu* II site, resulting in one ds 2701 bp fragment. **B**, *2* MSV-Kom RF-DNA contains two *Pvu* II sites resulting in linear dsDNA fragments of 1767 bp and 923 bp respectively. Blot in **A** was stripped and hybridised with DIG-labelled pBI121 resulting in a clear blot with no positive signals

the identity of the viruses (Fig. 5; PanSV not shown). Use of pBI121 as a probe indicated that the binary vector was not associated with infections (data not shown).

MSV-Kom, MSV-Set and PanSV-Kar infectivity characteristics and symptoms

Following leafhopper transmission from agroinfected sweetcorn, MSV-Kom caused severe stunting and produced continuous leaf-long streaks in most of the maize genotypes tested. Although stunting was less marked and streaking was less severe, it was still moderately severe in the "MSV resistant" maize genotypes. While MSV-Set was moderately severe in susceptible maize genotypes, it caused either very mild or no streak symptoms in maize genotypes bred for MSV resistance (Table 3). PanSV-Kar could not be leafhopper transmitted from agroinoculated sweetcorn to any of the wheat or barley genotypes, and amongst the maize genotypes could only be transmitted to the susceptible Jubilee, Kalahari Early Pearl and popcorn.

In general, MSV-Set produced more severe streak symptoms in barley and wheat genotypes than MSV-Kom. Although MSV-Kom caused streak symptoms at least as severe as MSV-Set in SST 66 (a wheat genotype), Adam Tas and Clipper (barley genotypes), MSV-Kom was milder than MSV-Set in wheat genotypes such

Table 3. Relative infectivity and symptom severity as determined by leafhopper transmissions

Genotype	PSV-Kar	MSV-Set	MSV-Kom
Z. mays			
Jubilee	1	2.5	3.5
US More	0	3	4
Popcorn	0.5	2	3.5
Kalahari Early Pearl	0.5	2.5	3.5
Vaalhartz Wit	0	0	3
Vaalhartz Geel	0	0	3
PNR 6549	0	0.5	3
PNR 6552	0	0	3
PNR 7541	0	0	3
PNR 7563	0	0.5	3
PAN hybrids[a]	–[b]	0	3
H. vulgare			
Clipper	0	3	3
Chokka	–	3	1
Festiquay	–	2	0
Adam Tas	–	3	3
T. aestivum			
SST 44	0	1	0
SST 66	0	2.5	3
Marquis	–	0.5	0
Agent	–	0.5	0
Dias	–	3	1

[a]See list of hybrids in Materials and methods
[b]Leafhopper transmissions not performed

as SST 44, Marquis, Agent, and Dias and barley genotypes such as Chokka and Festiquay. Wheat and barley genotypes severely infected with MSV-Kom and MSV-Set often had deformed and stunted leaves.

Discussion

The sequences of MSV-Kom, MSV-Set and PanSV-Kar showed that these viruses have genomic characteristics typical of other mastreviruses. Phylogenetic analysis of these sequences together with those of the currently sequenced mastrevirus genomes indicated that MSV-Kom, MSV-Set and PanSV-Kar are all clearly members of the "African streak virus" group. Because PanSV-Kar and PanSV-Ken share only 87% sequence identity these viruses should probably be considered as distinct PanSV strains. However, with 78% sequence identity between MSV-Set and the other MSVs currently sequenced, it is uncertain whether MSV-Set should be considered as a new streak virus species, or simply as a distinct strain of MSV. Resolution of this question will depend on whether MSV-Set and MSV-Kom (or any of the other more familiar MSV genotypes) are capable of *trans*-replicating

one another – an issue which is the focus of studies presently being carried out in our laboratory, and which will be published elsewhere. Preliminary indications are, however, that African grass mastrevirus are capable of *trans*-replicating each other across quite wide sequence differences (WH Schnippenkoetter, JA Willment and EP Rybicki, unpublished results), for which reason we refer to MSV-Set as a strain of MSV.

With one exception, the inferred Rep protein sequences of the three viruses described in this study contained all the amino acid sequence motifs currently identified in mastrevirus Rep proteins. The exception was that the Rep protein of MSV-Set contained only a partial RCR motif 2 [15] in that it lacked one of two otherwise highly conserved histidine residues believed to be involved in metal ion binding. The fact that MSV-Set is both highly infectious and insect-transmissible indicates either that both conserved histidine residues are not essential for Rep activity, or that if a functional RCR motif 2 is required, its structure is more flexible than would be suggested by its conservation in replicons as remotely related as eubacterial plasmids [15].

Cloned full-length virus genomes examined in this study were all infectious and transmissible by *C. mbila*. While MSV-Set and MSV-Kom were infectious in a range of maize, wheat and barley genotypes, PanSV-Kar was only capable of infecting a small number of highly susceptible maize genotypes. Besides being less virulent in the genotypes investigated, there are other factors that may have influenced PanSV-Kar's infectivity in the leafhopper transmission tests. It is possible that some species of *Cicadulina* may be more efficient vectors of PanSV than *C. mbila* [5]. It is also likely that the efficiency of virus acquisition by leafhoppers from particular hosts is positively correlated with the severity of streak caused by the virus in that host. Since virus distribution has been found to correlate with symptom expression in susceptible hosts [5], mild symptoms in the PanSV-Kar infected Jubilee from which PanSV-Kar was acquired by leafhoppers during transmission experiments may have resulted in low virus titres in viruliferous leafhoppers. However, the fact that certain genotypes could be infected indicates that PanSV was present in the salivary glands of leafhoppers during feeding. The leafhopper transmission tests also indicated that while viruses closely related to PanSV-Kar and MSV-Set are unlikely to pose a significant threat to maize production, viruses closely related to MSV-Set and MSV-Kom could potentially threaten wheat and barley production in South Africa.

The phylogenies of MSV-Kom, MSV-Set and PanSV-Kar strongly correlate with the infectivity characteristics of the viruses in the maize, barley and wheat genotypes examined. Based on the range of genotypes infected by the viruses and the intensity of symptoms, it was clear that, in accordance with their closer phylogenetic relationship, MSV-Kom and MSV-Set were also biologically more similar to one another than to PanSV-Kar.

All of the MSV isolates identified to date have been isolated from annual grasses such as *Setaria* spp., *Eleusine* spp., wheat and maize [4, 13, 27]. Conversely, the SSV and PanSV isolates that have been studied have all, with one exception from millet [3], been isolated from perennial grasses such as

sugarcane and *Panicum* spp. There is a distinct possibility that the progenitor of the MSV isolates that cause serious disease in maize today was specifically adapted to infecting annual grasses at the time maize was introduced into Africa in the 1600s. It is unlikely that the progenitor resembled MSV-Set more closely than it resembled the MSV isolates from maize. In support of this view, we have recently reported the isolation and partial sequence analysis of a closely related group of MSV isolates from wheat and wild annual grasses that, while distinctly different from MSV isolates from maize, resemble the maize isolates more closely than MSV-Set [27]. Despite the fact that the majority of African streak viruses examined have been isolated from annual grasses, the greatest diversity of genotypes has been obtained from perennial grass species [21]. If this sampling of diversity were roughly representative of the diversity of streak virus genotypes in natural African grass populations, it would indicate that annual grass adapted African streak viruses probably evolved from ancestral perennial grass adapted viruses. This would suggest that there is a direct causal link between the prehistoric evolution of African streak virus infectivity in annual grasses and the disease caused by MSV isolates in cultivated annual crops in Africa during modern times.

We have recently described use of the agroinfectious MSV-Kom and MSV-Set clones characterised in this study in an extremely sensitive technique for evaluating the resistance of maize genotypes to MSV [19]. We are currently conducting reciprocal genomic component replacement experiments between MSV-Kom and MSV-Set in order to study determinants of replication specificity, host range, and virulence. These experiments will, among other things, clarify whether MSV-Set is similar enough to the maize genotypes to be considered an MSV strain.

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