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The role of Kenya in the trans-African spread of maize streak virus strain A

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ABSTRACT

Maize streak virus (MSV), the causal agent of maize streak disease (MSD), is the most important viral pathogen of Africa's staple food crop, maize. Previous phylogeographic analyses have revealed that the most widely-distributed and common MSV variant, MSV-A_1, has been repeatedly traversing Africa over the past fifty years with long-range movements departing from either the Lake Victoria region of East Africa, or the region around the convergence of Zimbabwe, South Africa and Mozambique in southern Africa. Despite Kenya being the second most important maize producing country in East Africa, little is known about the Kenyan MSV population and its contribution to the ongoing diversification and trans-continental dissemination of MSV-A_1. We therefore undertook a sampling survey in this country between 2008 and 2011, collecting MSD prevalence data in 119 farmers' fields, symptom severity data for 170 maize plants and complete MSV genome sequence data for 159 MSV isolates. We then used phylogenetic and phylogeographic analyses to show that whereas the Kenyan MSV population is likely primarily derived from the MSV population in neighbouring Uganda, it displays considerably more geographical structure than the Ugandan population. Further, this geographical structure likely confounds apparent associations between virus genotypes and both symptom severity and MSD prevalence in Kenya. Finally, we find that Kenya is probably a sink rather than a source of MSV diversification and movement, and therefore, unlike Uganda, Kenya probably does not play a major role in the trans-continental dissemination of MSV-A_1.

Abbreviations: MSV, maize streak virus; PanSV, panicum streak virus; IITA, international institute of tropical agriculture; MSD, maize streak disease; ML, maximum likelihood; GTR + I + G_4, general time reversible model with invariant sites and gamma distributed rate variation; BEAST, Bayesian estimation and analysis of sampling trees; ESS, effective sample sizes.

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1. Introduction

Infection with maize streak virus (MSV; Genus *Mastrevirus*, Family *Geminiviridae*), the causal agent of maize streak disease (MSD), is among the top ten constraints on maize yields in sub-Saharan Africa (FAO, 2007; Martin and Shepherd, 2009; Pingali and Pandey, 2000). The virus and its insect vectors – leafhopper species in the genus *Cicadulina* – are ubiquitous in Africa and are also found on the Indian Ocean islands of Madagascar, Mauritius, Réunion, and the Atlantic Ocean island of Sao Tomé (for a review see Shepherd et al. (2010)).

Eleven distinct strains of MSV are known (Muhire et al., 2013) but only one of these, MSV-A, causes serious MSD (Briddon et al., 1994; Martin et al., 2001). MSV-A is believed to have originated in southern Africa in the mid-1800s (Harkins et al., 2009; Monjane et al., 2011) following a recombination event between two viruses belonging to the MSV-B and MSV-F strains (van der Walt et al., 2009). MSV-A variants then likely spread to the Indian Ocean island of Réunion between 1880 and 1920, to West Africa between 1930 and 1968 and to East Africa between 1939 and 1980 (Monjane et al., 2011). While disseminating, it is apparent that the MSV-A lineages diverged into at least four genetically distinct subtypes: MSV-A_2 in West Africa, MSV-A_3 in East Africa, MSV-A_6 on Réunion Island and MSV-A_4 in southern Africa (Monjane et al., 2011). During this initial wave of dispersal a fifth subtype, MSV-A_1, arose probably somewhere in southern Africa between 1949 and 1971, and began a second wave of dissemination (Monjane et al., 2011). Whereas MSV-A_1 isolates are today found throughout sub-Saharan Africa, MSV-A_2 isolates have only ever been found in West Africa (Mullineaux et al., 1984), MSV-A_3 isolates in East Africa (Howell, 1985), MSV-A_4 isolates in southern Africa (Lazarowitz, 1988) and MSV-A_6 isolates on the Indian Ocean islands of Réunion and Mauritius (Peterschmitt et al., 1996).

Comparison of the genetic diversity and geographical distributions of the known MSV strains with those of the known strains of the closely related African mastrevirus, panicum streak virus (PanSV), has revealed that MSV in general appears to have a higher rate of dispersal than PanSV. It also showed that the MSV-A strain disperses more rapidly than other MSV strains, with MSV-A_1 likely having the highest rate of dispersal among the known MSV-A subtypes (Varsani et al., 2009, 2008a). The underlying cause of MSV-A dispersing more rapidly across Africa than other MSV strains is unknown but may be due to MSV-A having either a broader host range, or a greater propensity for spread by humans (within infected leaf material or viruliferous insects) than its wild-grass-adapted relatives (Martin and Shepherd, 2009; Varsani et al., 2008b). Further, MSV-A_1 appears to be the most pathogenic of the MSV-A subtypes (Martin et al., 2001) and this might explain why it is the only MSV-A subtype that has been found widely distributed throughout sub-Saharan Africa.

Crucially, within Africa there appear to be two discrete hotspots of MSV-A_1 diversification from which almost all of the detectable trans-continental MSV-A_1 dissemination events have occurred: one in southern Africa in a region encompassing Zimbabwe, Zambia, South Africa and Mozambique (the same region where the most recent common ancestors of both MSV-A and MSV-A_1 are believed to have originated), and another in East Africa in the region around Lake Victoria (Monjane et al., 2011).

While this second diversification hotspot potentially includes Kenya, considerably less is known about the diversity of MSV-A within this country than is known about MSV-A diversity in neighbouring Uganda. In the last major survey of MSV in Kenya, between 1996 and 1998, 29 MSV-A isolates were collected (Martin et al., 2001). Although only five full genome sequences were determined during this survey, partial genome sequencing and restriction fragment-length polymorphism analyses on additional isolates indicated that 27 isolates were MSV-A_1 variants and two were MSV-A_3 variants. A much larger Ugandan MSV diversity survey in 2005 (Owor et al., 2007a) genetically characterised 155 samples (all of them MSV-A_1 isolates), from which 62 genomes were fully sequenced. These Ugandan MSV sequences proved crucial for identifying the diversification and dissemination hotspot around Lake Victoria (Monjane et al., 2011).

Given that so little is presently known about MSV diversity in Kenya, and that the Lake Victoria region appears to play such an important role in the evolution and epidemiology of MSV in Africa, we conducted a survey of Kenyan MSV diversity between 2008 and 2011. We sequenced 159 complete Kenyan MSV-A genomes and, together with 463 previously determined MSV-A full genome sequences with known sampling dates and coordinates, analyzed the evolutionary and spatial movement dynamics of MSV in Kenya.

2. Materials and methods

2.1. Virus sampling

A total of 170 maize samples and 122 uncultivated grass samples were collected between 2008 and 2011, during May and June (the first maize cropping season) and November and December (the second cropping season). The four major maize growing areas of Kenya from which samples were obtained included the southern coastal region (hereafter referred to as the coastal region), the central region (including the Nairobi and Eastern provinces), the Rift Valley, and the western region (including the Nyanza and Western provinces; Fig. 1). For each sample, geographical coordinates and sampling dates were recorded (Supplementary Table S1 in the online version at DOI: 10.1016/j.virusres.2017.02.005).

Each of 119 farmers' fields was divided into four segments by two diagonal lines and, wherever possible, two samples were taken from the first diagonal line and a third from the second line randomly. In fields where fewer than three symptomatic plants were on the two diagonals, up to three symptomatic plants were sampled from elsewhere within the fields. All of the fields that were surveyed were separated by at least ten kilometers and contained maize plants ranging in age from 1 to 3.5 months. Whenever uncultivated grasses displaying streaking symptoms were found around the maize fields these were also collected for further analysis. Samples were press-dried and stored within individually sealed paper envelopes. The total time between sample collection and eventual processing in the laboratory ranged from two to four months.

2.2. Assessment of MSD prevalence and severity in the field

Disease severity in each field was scored using the Institute of Tropical Agriculture's subjective six-point MSV resistance rating system (where a score of 0 indicates symptomless, and 5 indicates extensive leaf chlorosis and extreme stunting; Soto et al., 1982) as an average score for the sampled leaves. In addition, MSD prevalence in each field was taken as the proportion of plants along the two diagonals that displayed MSD symptoms.

2.3. Cloning and sequencing of complete MSV genomes

Total plant DNA was isolated, and viral genomes were amplified and cloned using a previously described approach (Owor et al., 2007a; Shepherd et al., 2008 Shepherd et al., 2008). Briefly, circular viral DNA was preferentially amplified using rolling circle amplification with Phi29 DNA polymerase, and the concatenated amplicons were digested with *Bam*HI, *Bgl*II or *Kpn*I to yield ~2.7-Kb linearized unit length MSV genomes. Linearized products were then resolved on a 0.7% agarose gel, purified and ligated to the *Kpn*I