Variable regions on the genome of Malawi isolates of African swine fever virus

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Restriction enzyme site mapping showed that most BamHI and all ClaI sites were conserved on the genomes of 17 African swine fever virus isolates from separate disease outbreaks that occurred between 1982 and 1989 in Malawi. However, frequent variation between virus genomes did occur due to addition or deletion of DNA sequences at various positions along the genome and 11 virus genotypes could thus be distinguished among the 17 isolates analysed. Length variations occurred at 10 different loci on the virus genome. These variable regions were located between the left DNA terminus and a position up to 48 kb from that terminus, in the centre of the genome 90 to 93 kb from the left DNA terminus and between the right DNA terminus and a position 22 kb from that terminus. Length variations in most of these regions were small (<1 kb) but variations of about 4 kb occurred in a region up to 20 kb from the left DNA terminus.

Introduction

African swine fever (ASF) is caused by a large, cytoplasmically located icosahedral virus particle containing a linear dsDNA genome which varies in length between 168 and 189 kb in different virus isolates (Blasco et al., 1989a). ASF virus (ASFV) has some similarities with poxviruses, including the genome structure which contains terminal cross-links and inverted terminal repeats (for review, see Viñuela, 1985, 1987; Dixon et al., 1990a; Costa, 1990).

In southern African countries a reservoir of ASFV is maintained in warthogs (Phacochoerus aethiopicus) and in the arthropod vector of ASFV, the soft tick Ornithodoros moubata, which inhabits their burrows. The tick vector plays an essential role in the transmission cycle involving warthogs (Plowright, 1981; Thomson et al., 1983), and both warthog and bushpig (Potamochoerus porcus) can be asymptomatic carriers of ASFV (Plowright et al., 1969). In contrast to the situation in warthogs, horizontal transmission of ASFV occurs readily between domestic pigs without the necessity for a tick vector (for reviews, see Wilkinson, 1981, 1984). Primary outbreaks of ASF in domestic pigs in Africa have been frequently associated with wild pigs, although the mechanism of transmission from wild to domestic pigs is uncertain (Plowright, 1981). In these outbreaks, the mortality rate usually approaches 100% (Hess, 1971) but a reduction in virulence has been observed in areas of Angola (Leite Velho, 1956) and Malawi (Haresnape et al., 1985, 1987), where the disease has become enzootic in domestic pigs.

An ASF enzootic area was first recognized in Malawi in 1931, on the border with Zambia in the vicinity of Mchinji (Turnbull, 1933). The present enzootic area covers about 8000 km² of the Central Region of Malawi and extends westwards in to the Eastern Province of Zambia and southwards into the Tete province of Mozambique (Haresnape et al., 1987). Devastating incursions of disease into the western part of the Central Region and into the Southern Region have occurred at intervals, although ASF has not become enzootic in domestic pigs in these regions. However, recent surveys (Haresnape et al., 1985, 1987) suggest enzootic foci may have become established in the southwestern Central Region, in Dedza and Ntcheu districts.

O. moubata are found in warthog lairs, domestic pig pens and human houses in many regions of Malawi but ASFV has been recovered only from O. moubata collected within the enzootic area (Haresnape et al., 1988) where they may act as virus reservoirs.

Restriction enzyme site mapping of genomes of ASFV isolates from O. moubata inhabiting warthog burrows in different areas of Zambia showed that these isolates were genetically very diverse (Dixon & Wilkinson, 1988), as were African isolates collected from various hosts in widely dispersed geographical locations (Wesley & Tuthill, 1984; Thomson, 1985; Blasco et al., 1989a, b). In

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Fig. 1. Map showing villages in the Central Region of Malawi from which ASFV isolates were obtained. In the right panel a complete map of Malawi is shown with the enzootic area (Haresnape et al., 1987) stippled. The boxed area is enlarged in the left panel. Isolates were collected from outbreaks in villages shown as filled circles and codes designated to these isolates are shown.

\textbf{Methods}

\textbf{Virus isolates.} Virus isolates, from outbreaks occurring between 1982 and 1989, were from spleen specimens collected from dead pigs at the time of outbreaks (BON 83, DED 86, MCH 87/1, MCH 87/2, ZOM 84, NAD 84, DOW 86, MCH 89/1, LIL 89/1, LIL 89/2, LIL 89/3, DED 89/1, DED 89/2, NTC 89/1), or from ticks collected no more than 1 month following outbreaks in November 1983 (TIK 82, KON 83, LIL 20/1; Haresnape et al., 1988) (Fig. 1). One isolate was examined from each of 17 outbreaks of ASF. Outbreaks were separated from each other by at least 25 km or by at least 1 year. The location of villages in Malawi from which isolates were collected are shown in Fig. 1.

Thirteen ASFV isolates were collected 3 weeks after the last known occurrence of ASF in pigs from pools of ticks (nine from pig pens, four from human huts) in the Chalaswa area in November 1983 (Fig. 1), and a further six isolates were obtained from individual ticks (three from pig pens, three from huts) collected from the same pig pens and huts in the Chalaswa area at intervals following the outbreaks (March 1984, two isolates; April 1984, one isolate; July 1984, three isolates). The Tengani 1962 virus was isolated by Cox & Hess (1962) from the spleen of a moribund pig from an outbreak in the lower Shire Valley, Southern Region, Malawi, where almost 100% of the pig population (about 7000) died within a 5 to 6 week period (W. R. Hess, personal communication).

\textbf{Cloned DNA.} DNA from Malawi ASFV isolate LIL 20/1 has been cloned in bacteriophage lambda and plasmid vectors, and \textit{Bam}HI, \textit{Cla}I and \textit{Sal}I restriction enzyme site maps of the genome were obtained (Dixon, 1988). A collection of plasmid clones containing DNA from a Spanish isolate (BA71V) of ASFV, adapted to grow in Vero cells (Ley et al., 1984), was a gift of Dr E. Viñuela (Universidad Autónoma, Madrid, Spain). Bacteriophage lambda and plasmid DNAs were prepared by standard methods (Maniatis et al., 1982).

\textbf{Growth of virus and purification of viral DNA.} Viruses in field material were identified by producing haemadsorption in primary cultures of pig bone marrow cells but the stock viruses, used for intravenous inoculation of pigs, were dilutions of suspensions prepared from original pig spleen or pools of ticks obtained from Malawi. The Tengani 1962 isolate was from the second pig passage. Virus was purified from the red blood cell fraction of viraemic pig blood by the method described by Wesley & Tuthill (1984), with the modifications described by Dixon & Wilkinson (1988). DNA was extracted from isolated virus by phenol extraction following lysis of virus with SDS and pronase.

\textbf{Enzyme reactions.} Restriction endonucleases were used according to the manufacturers' recommendations. End-labelling of restriction endonuclease digests with \([\text{P}\]dATP and 3 mm of the three other
unlabelled dNTPs, using the Klenow fragment of DNA polymerase, was performed using standard procedures (Maniatis et al., 1982).

Agarose gel electrophoresis and Southern blotting. Electrophoresis of DNA fragments was carried out in 0.6%, 0.8%, 1% or 1.5% agarose (Bethesda Research Laboratories electrophoresis grade) gels in 40 mM-Tris-acetate buffer (40 mM-Tris-acetate, 1 mM-EDTA pH 8.0) or Tris-borate buffer (89 mM-Tris-borate, 2 mM-EDTA pH 8.0). Bands were visualized by autoradiography of dried gels containing end-labelled restriction endonuclease fragments. DNA was transferred from gels (Southern, 1975) onto Hybond-N filters (Amersham) and attached to the filters by baking at 80 °C for 2 h.

Preparation of radioactively labelled DNA probes and hybridization conditions. DNA probes were labelled with [32P]dATP using the random oligonucleotide primer method (Feinberg & Vogelstein, 1983, 1984). Hybridization conditions were as described by Dixon & Wilkinson (1988). Filters were washed at 50 °C in 0.4 x SSC, 0.1% SDS for 30 min (low stringency) or 65 °C in 0.1 x SSC, 0.1% SDS for 30 min (high stringency), in each case following washes in 2 x SSC/0.1% SDS and 2 x SSC/0.1% SDS at room temperature. Radioactive probes were eluted from filters by washing for 45 min at 42 °C in 0.4 M-NaOH and for 30 min at 42 °C in 0.1 M-Tris-HCl pH 7.5, 0.2 x SSC, 0.2% SDS.

Results

Restriction enzyme analysis of the genomes of 17 ASFV isolates collected from separate disease outbreaks

Fragments produced by restriction enzyme digestion of the genomes of 17 ASFV isolates, collected from separate disease outbreaks in Malawi between 1982 and 1989 (Fig. 1) were end-labelled with [32P]dATP, separated by agarose gel electrophoresis and detected by autoradiography of dried gels (Fig. 2, 3, 4). Genomes of 10 isolates collected between 1982 and 1987 (Fig. 2) and seven isolates collected in 1989 were analysed with BamHI. Digests of six of these are shown in Fig. 3; a
Among the 17 isolates examined by analysis of the variation in the mobility of fragments and gain or loss of restriction enzyme sites, 11 distinct genotypes were distinguished. Of these, seven isolates had unique BamHI fragment patterns (BON 83, KON 83, TIK 82, LIL 20/1, DED 86, MCH 89/1 and LIL 89/1), whereas four types of BamHI fragment patterns were distinguished amongst the remaining 10 isolates. Indistinguishable BamHI fragment patterns were seen from two pairs of isolates, NAD 84 and ZOM 84, MCH 87/1 and MCH 87/2. A further pair of isolates, DOW 86 and LIL 89/3 had very similar BamHI fragment patterns, although these two isolates were not compared directly.

A fourth group, containing four isolates collected in 1989 (LIL 89/2, DED 89/1, DED 89/2, NTC 89/1) (Fig. 3), also had indistinguishable BamHI fragment patterns. The LIL 89/1 isolate had a very similar BamHI fragment pattern to the LIL 20/1 isolate but the two isolates could be distinguished because two fragments (BamHI t and u), did not migrate with the same mobility when the two genomes were compared (data not shown). Analysis with ClaI distinguished between the same isolates as BamHI.

### Genome mapping of variable length BamHI and ClaI restriction enzyme fragments

All restriction enzyme fragments which varied in length when different isolates were compared were mapped on the genome. Southern blots of BamHI or ClaI digests of virus DNA were hybridized sequentially to clones containing overlapping DNA inserts (Fig. 5). This enabled the map positions of variable length fragments to be deduced (Fig. 6, 7, 8). Fragments which were the same length as those of the LIL 20/1 isolate were assumed to have the same map position as in the LIL 20/1 isolate.

### Variation in the left terminal region up to 18 kb from the left DNA terminus

Terminal genome fragments were mapped with clones which hybridized either to both terminal fragments, because they contain part of the terminal inverted repeat sequences (RK' and RD'; Ley et al., 1984), or to only one terminal fragment. In some virus isolates, a BamHI site was present about 8.0 kb from the left DNA terminus and, in these isolates, the left terminal fragment BamHI a varied in length between 8.0 kb (NAD 84, ZOM 84) and 8.3 kb (DED 86) (Fig. 5, 6). In isolates which had lost this BamHI site, the terminal fragment a'b varied in length between 33 kb (BON 83, MCH 87/1, MCH 87/2 and five other 1989 isolates indistinguishable in this region) and 35 kb (KON 83). Left terminal ClaI a fragments varied in length between 14 kb (BON 83,
MCH 87/1 and 2, and five 1989 isolates) and 18.3 kb (TIK 82) in length. The 4.3 kb size variation in ClaI a fragments occurred in at least two separate loci. One of these was within the terminal BamHI a fragment, which varied in size by 0.3 kb possibly due to variation in the number of tandem repeats within the terminal inverted repeats as observed in poxviruses (Moss et al., 1981). The second variable locus was between the left end of BamHI b and the right end of ClaI a fragments (between approximately 8 and 18 kb from the left DNA terminus). This region varied in size by 1.35 kb when different isolates were compared. It was not possible to distinguish size variation at these two loci in virus isolates which had lost the BamHI site between fragments a and b. However, it is possible that the BamHI site was lost from isolates which had the shortest ClaI a fragment (14 kb) by deletion of a large DNA sequence which included this site (Fig. 5, 6).

Conservation of fragments between 18-3 and 32 kb from the left DNA terminus

Hybridization of clones containing sequences from ClaI b and c fragments to Southern blots of ClaI virus DNA digests showed that ClaI fragments b (7.2 kb) and c (6.5 kb) did not vary in length when different virus isolates were compared. This region of the genome, which in the TIK 82 isolate is between 18-3 and 32 kb from the left DNA terminus, is therefore conserved in length in different virus isolates (Fig. 6).

Variation in fragments between 32 and 48 kb from the left DNA terminus

The ClaI d fragment was 9.1 kb in length in DOW 86, LIL 89/3 and DED 86 isolates and 9.3 kb in length in other virus isolates. The ClaI e fragment varied in length by 0.7 kb between 12.5 kb (LIL 20/1, LIL 89/1, DOW 86, LIL 89/3, NAD 84, DOW 86, LIL 89/3 and DED 86) and 13.2 kb (TIK 82). BamHI c fragments had the same size variation between isolates as ClaI e fragments and thus this variation must be localized between the left end of ClaI e fragments and right end of BamHI c fragments, which are between 41-3 and 48.0 kb from the left DNA terminus in the TIK 82 genome (Fig. 5, 6).

Variable regions of ASFV genome

Fig. 5. Mapping of variable BamHI fragments. Virus DNAs were digested with BamHI, separated by agarose gel electrophoresis and blotted onto Hybond-N membranes. Panels (a) to (e) show hybridization to variable BamHI fragments. Lane 1, BON 83; lane 2, KON 83; lane 3, TIK 82; lanes 4 and 5, LIL 20/1; lane 6, DOW 86; lane 7, DED 86; lane 8, NAD 84. Probes used were clones containing ASFV DNA: (a) LMw1, (b) LMw4, (c) RK 1, (d) and (e) LMw22. Letters at the right of these panels indicate the fragment map position. (f) BamHI digest of LIL 20/1 DNA end-labelled with [32P]dATP. (g) Hybridization to variable ClaI m fragments probed with LMw10. Lanes 1 to 14 are BON 83, MCH 87/1, MCH 89/1, LIL 89/2, DED 89/1, NTC 89/1, KON 83, TIK 82, LIL 20/1, LIL 89/1, NAD 84, DOW 86, LIL 89/3 and DED 86, respectively. (h) Location of the probes used, on the genome of LIL 20/1. BamHI fragments are indicated above the genome, ClaI fragments below. Probe RK 1 contained DNA from the BA71V genome (Ley et al., 1984) whereas the other probes contained LIL 20/1 DNA (Dixon, 1988).
NAD located between 93 and 97 kb from the left DNA terminus between 3-7 kb (DED 86) and 4.0 kb (LIL 20/1).

Variation in the central region of the genome

In this region, length variation of 0·3 kb was detected by hybridization to Clal m fragments. This fragment is located between 93 and 97 kb from the left DNA terminus of the TIK 82 isolate (Fig. 7) and varied in length between 3·7 kb (DED 86) and 4·0 kb (LIL 20/1).

Variation in fragments located up to 28·7 kb from the right DNA terminus

When different isolates were compared, four BamHI fragments (t, u, v, w) located internally and adjacent to the right DNA terminus varied in size (Fig. 5, 8). The BamHI s fragment was conserved in length in all isolates except DOW 86 and LIL 89/3 in which the BamHI site between fragments s and t was lost, either due to a point mutation or small deletion. The BamHI t fragment, which is located between 17·8 and 22·4 kb from the right DNA terminus of the TIK 82 isolate, varied in length by 0·45 kb, from 4·45 kb (BON 83, MCH 87/1,2 and LIL 89/2) to 4·9 kb (DED 86) (Fig. 5, 8). Among the 11 virus genotypes analysed, six different lengths of BamHI t fragments were observed. The BamHI u fragment, which is located between 12·8 and 17·8 kb from the right DNA terminus of the LIL 20/1 genome, varied in length by 0·15 kb, between 4·8 kb (TIK 82) and 4·95 kb (LIL 20/1, DOW 86, LIL 89/3). Four different lengths of BamHI u fragments were distinguished and seven of the 11 genotypes had a u fragment of 4·85 kb. The BamHI v fragment, which is located between 10 kb and 12·8 kb from the right DNA terminus of the LIL 20/1 genome.

Fig. 7. BamHI and Clal site maps of Malawi ASFV genomes showing the genome locations of variable regions, including a central fragment. BamHI sites and fragments are indicated above the line and Clal sites below the line. Variable fragments are indicated in larger text. An enlargement of the central variable region which is located in the Clal m fragment is shown. B, BamHI site.

Fig. 8. BamHI and Clal site maps of the right terminal 30 kb showing variable fragments in Malawi ASFV genomes. BamHI sites are indicated above the line and Clal sites below. The size of fragments (kb) in the TIK 82 genome is indicated at the top of the figure and on other genomes when the fragment size differed from that of the TIK 82 genome. The complete genome length of the various isolates is indicated on the right. Asterisks indicate largest fragments and the difference between these and the smallest fragments is indicated at the bottom of the figure. △ indicates that Clal fragment sizes were not determined.

(BON 83, MCH 87/1,2, MCH 89/1, LIL 89/2, DED 89/1,2 and NTC 89/1)
and 2.9 kb (BON 83, MCH 87/1 and 2, and MCH 89/1). Of the 11 virus genotypes distinguished, eight had a 2.8 kb 
BamHI v fragment and two fragment sizes were 
distinguished in the remaining three genotypes. The 
right terminal BamHI w fragment varied by 0.7 kb, 
between 9.9 kb (NAD 86, ZOM 86) and 10.6 kb 
(DED 86). Most of this length variation was probably 
localized within the right terminal ClaI v fragment, 
which varied in length by 0.5 kb between 6.1 kb 
(NAD 84, ZOM 84, KON 83) and 6.6 kb (DED 86).

Both ClaI v and BamHI w fragments had six different 
lengths in the 11 genotypes.

In some virus isolates, fragments from both termini of 
the genome varied in size by the same amount. For 
example, both BamHI a and w fragments were 0.1 kb 
shorter in the NAD 84 and ZOM 84 isolates when 
compared to the a and w fragments in the LIL 20/1 
isolate. Similar size variation in both terminal fragments 
has been observed in other ASFV isolates (Blasco et al., 
1989a, b) and may indicate an interaction between the 
genome termini during replication.

Restriction enzyme analysis of the genomes of 19 ASFV 
isolates collected from O. moubata in Chalaswa villages 
shortly after an outbreak and at intervals thereafter

The results described above show that the genomes of 
ASFV isolates from separate disease outbreaks in 
Malawi between 1982 and 1989 were very similar 
because most BamHI and ClaI restriction enzyme sites 
were conserved. However, variation in the size of a 
number of restriction enzyme fragments enabled 11 
different virus genotypes to be distinguished from the 17 
isolates analysed. To investigate whether passage of 
virus in O. moubata ticks might result in genome 
variation, genomes of 19 ASFV isolates collected from 
O. moubata ticks in Chalaswa villages (Fig. 1) shortly 
after an outbreak and 4 to 8 months afterwards were 
analysed.

The BamHI fragment patterns of 12 ASFV isolates 
from pools of ticks collected shortly after the ASF 
outbreak in November 1983 were compared with each 
other and with the previously characterized Malawi 
isolates. Of these 12 isolates, 11 were indistinguishable 
by analysis with BamHI. The isolate which could be 
distinguished varied in the mobility of a single restriction 
enzyme fragment. This isolate, LIL 20/1, had a left 
terminal BamHI a fragment which was 0.1 kb shorter 
than that of other isolates (data not shown).

The 11 indistinguishable isolates were analysed with 
HindIII, which cuts the genome into a larger number of 
fragments (> 60) than BamHI or ClaI. Southern blots of 
HindIII digests were hybridized to clones containing 
inserts from the previously characterized variable 
regions of the genome. This analysis enabled the 
LIL 26/1 isolate to be distinguished from the other 10 
isolates because terminal fragments were reduced in size 
by 50 bp in the LIL 26/1 isolate compared to those in 
other isolates (data not shown). However, the presence of 
submolar fragments, the same size as terminal fragments 
in other genomes, indicated that this LIL 26/1 virus 
isoalte consisted of a mixture of virus genomes which 
differed in the size of their terminal HindIII fragments. 
The LIL 26/1 isolate was from a pool of three adult 
female ticks. Of adult females collected at the same time, 
21% were estimated to be infected with ASFV (Haressnape & Wilkinson, 1989). Thus, the virus populations 
present may either have been present in a single tick or 
more than one tick.

Genomes of six ASFV isolates, obtained from 
individual O. moubata nymphs between 4 and 8 months 
(between July and November 1984) after the outbreak, 
were compared to each other and to an isolate (LIL 28/2) 
obtained at the time of the outbreak, as described above. 
BamHI fragment patterns of these isolates were indistinguisiable and no variation was detected in the size of 
the terminal HindIII fragments (data not shown).

Comparison of the genome of an ASFV isolate from 
Malawi in 1962 with genomes of Malawi ASFV isolates 
collected between 1982 and 1989

Comparison of the genome of the Tengani 1962 isolate 
(see Fig. 1) with those of other African isolates showed 
that it was not closely related to the other isolates 
examined (Wesley & Tuthill, 1984; Blasco et al., 1989a, 
b). The BamHI pattern of the Tengani 1962 isolate was 
very different from that of the DED 86 isolate, which 
had a genotype typical of the 1982 to 1989 Malawi 
isolates (Fig. 9). At least 17 of 23 BamHI fragments of the 
Tengani 1962 isolate did not comigrate with those of 
DED 86. The results suggest that the Tengani 1962 
outbreak was caused by an isolate with a genotype not 
closely related to genotypes recently circulating in the 
encezootic area of the Central Region of Malawi.

Comparison of the total genome lengths of Malawi ASFV 
isolates

The total length of each genome was estimated by 
addition of BamHI or ClaI fragment sizes (Fig. 8). The 
TIK 82 isolate had the longest genome (182 kb), derived 
by summation of both BamHI and ClaI fragments, 
whereas BON 83 had the shortest genome (178 kb), 
estimated by analysis with both enzymes. The size of the 
LIL 20/1 isolate genome was calculated to be 181 kb with 
BamHI and 182 kb with ClaI. The Tengani 1962 genome
Fig. 9. Comparison of the BamHI fragment patterns of DED 86 (lane 2) and Tengani 1962 (lane 3) isolates. Virus DNA was digested with BamHI and end-labelled with [32P]dATP. Fragments generated were separated on 0.6% agarose gels and detected by autoradiography of dried gels. Lane 1, marker DNA (kb).

was estimated to be 181 kb which is close to the size (183 kb) estimated by Blasco et al. (1989a).

Discussion

Comparison of the genomes of ASFV isolates from 17 separate outbreaks between 1982 and 1989 in Malawi showed that these isolates were closely related to each other because most BamHI sites (21 out of 22 or 23) and all Clal sites were conserved on all these virus genomes. However, frequent changes in mobility of restriction enzyme fragments were observed due to addition or deletion of DNA sequences. These length variations were observed in seven BamHI fragments and six Clal fragments. Mapping with BamHI and Clal enabled 10 separate regions where length variation occurred to be mapped on the virus genome.

These variable regions were located between the left DNA terminus and a position 48 kb from that terminus in the longest virus genome (TIK 82), in the centre of the genome between 93 and 97 kb from the left DNA terminus and between the right DNA terminus and a position 22 kb from that terminus. These results differ from previous results (Blasco et al., 1989a) because we have identified additional regions of the genome where length variations occur.

Between about 8 and 18 kb from the left DNA terminus, length variations of greater than 1 kb were observed and eight isolates had a 4-3 kb deletion in this region of the genome when compared with the longest virus genome (TIK 82; Fig. 6). In previous studies, large additions or deletions (up to 8 kb) of DNA sequences were observed at this genome position (Blasco et al., 1989a, b) due to DNA rearrangements resulting in gain or loss of members of a multigene family encoding proteins of about 110 amino acids (Almendral et al., 1990). Virus clones were characterized which differed in length by up to 16 kb at this genome position and included two variants that did not contain any members of multigene family 110. However, all the clones caused severe disease and high mortality rates in pigs (Aguero et al., 1990). DNA rearrangements in sequences containing another multigene family, encoding proteins of 360 amino acids, resulted in genome length variation close to both termini (Gonzalez et al., 1990).

In poxvirus genomes, frequent changes in length of terminal fragments result from variation in the number of tandem repeats within terminal inverted repeats (TIRs) (Moss et al., 1981). In the Spanish Ba71V ASFV isolate the TIRs are 2-4 kb long (Sogo et al., 1984; Vifiuela, 1987) and also contain blocks of tandem repeats (Vifiuela, 1987). Some of the length variations we observed in terminal genome fragments probably resulted from variation in the number of tandem repeats in TIRs.

The additional variable regions of the genome which we have mapped are as follows: (i) at the left end of the genome, between 32 and 48 kb from the left DNA terminus, in the Clal d fragment, in a region between the left end of the BamHI c fragment and the left end of the Clal e fragment, (ii) in the centre of the genome in the Clal m fragment and (iii) at the right end of the genome between 12 and 22 kb from the right DNA terminus in the BamHI t and u fragments. Within the BamHI t fragment, tandemly repeated sequence arrays, similar to chromosomal minisatellite sequences, have been identified and length variation in this region results from variation in the number of repeat units within these repeat arrays (Dixon et al., 1990b). The molecular basis for the length variation at the other newly characterized variable loci is not known.

Of 19 isolates collected from O. moubata over an 8 month period in villages following an outbreak, 17 had indistinguishable genomes and the remaining two isolates had small variations in the size of terminal fragments. These results indicate that genome variation similar to that observed between genotypes collected
from separate disease outbreaks does not occur during the course of a single disease outbreak or during virus multiplication in ticks over an 8 month period. One virus isolate collected in 1989 (LIL 89/3) was indistinguishable from an isolate collected in 1986 (DOW 86), which also suggests that virus genotypes may be relatively stable during passage in pigs. The 11 virus genotypes distinguished in isolates from separate outbreaks therefore probably diverged during virus circulation in domestic pigs or *O. moubata* over long periods of time.

The identification of some virus groups with indistinguishable genomes may, in some cases, indicate a direct epizootiological link between disease outbreaks. One such case is the outbreak which occurred outside the enzootic area in Zomba in 1984. The genome of an isolate (ZOM 84) collected from this outbreak was indistinguishable from that of the NAD 84 isolate, and it is probable that the Zomba outbreak was caused by transfer of infected material from in or around Nadulu.

A previous study showed that virus genomes from *O. moubata* ticks in warthog burrows, both in geographically separate locations and from within a small locality, had very different restriction enzyme site maps (Dixon & Wilkinson, 1988). All the isolates from outbreaks of disease which we examined had conserved restriction enzyme sites which indicates that these isolates are closely related and that ASFV is persisting in Malawi by circulating in domestic pig populations from a primary focus within the enzootic area by either continual reintroduction into domestic pig populations from *O. moubata* or warthogs in National Parks.

Genomes of isolates from outbreaks which occurred at similar times outside the enzootic area were often indistinguishable. For example, three isolates from outside the enzootic area in 1989 (DED 89/1, DED 89/2, NTC 89/1) had genotypes indistinguishable from that of an isolate from an outbreak within the enzootic area (LIL 89/2). The temporal occurrence and geographical location of these outbreaks suggests that disease spread from a primary focus within the enzootic area by either separate introductions from that focus or from secondary foci outside the enzootic area.

Genomes of Malawi isolates were very different from those of the closely related isolates from Europe and West Africa (Dixon, 1988; Ekue, 1989; P. J. Wilkinson, L. K. Dixon, K. J. Sumption & F. Ekue, unpublished results), as well as from the Tengani 1962 isolate. The Malawi viruses therefore constitute a separate, related virus group.

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**References**


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