`Title: APOBEC3 deaminase editing in mpox virus as evidence for sustained human transmission since at least 2016

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Abstract: Historically, mpox has been characterised as an endemic zoonotic disease that transmits through contact with the reservoir rodent host in West and Central Africa. However, in May 2022, human cases of mpox were detected spreading internationally beyond countries with known endemic reservoirs. When the first cases from 2022 were sequenced, they shared 42 nucleotide differences from the closest mpox virus (MPXV) previously sampled. Nearly all these mutations are characteristic of the action of APOBEC3 deaminases; host-enzymes with antiviral function. Assuming APOBEC3-editing is characteristic of human MPXV infection, we develop a dual process phylogenetic molecular clock that — inferring a rate of ~6 APOBEC3 mutations per year — estimates MPXV has been circulating in humans since 2016. These observations of sustained MPXV transmission present a fundamental shift to the perceived paradigm of MPXV epidemiology as a zoonosis and highlight the need for revising public health messaging around MPXV as well as outbreak management and control.

Main Text:

Since 2017, the Nigeria Centre for Disease Control has been reporting cases of MPXV (mpox virus) infection in humans (Fig. S1)(*1*). MPXV, a DNA virus in the genus *Orthopoxvirus*; Family *Poxviridae*, is often described as being endemic in West and Central Africa as a zoonotic disease that transmits through contact with the rodent reservoir host. Since the first human cases were observed in the 1970s, MPXV infections have been predominantly associated with infants and children (*2–4*). However, of the cases observed in Nigeria since 2017 very few have been in children, with the virus mainly affecting adults aged 20 to 50 (79%), 27% of which were in women (*5*). Genome sequencing of viruses revealed enough genetic diversity between cases that distinct zoonotic events were not ruled out. In May 2022, cases of MPXV infection were detected spreading widely across Europe and subsequently across the globe. The first MPXV genome sequences from these 2022 cases showed they had descended from the clade characterised by cases diagnosed in Nigeria and Israel, Singapore, and the UK (*6*) associated with travel from Nigeria (Fig. S2; Table S1, in bold). These early 2022 genomes are indicated as a triangle within Clade IIb in Fig. 1A and represent lineage B.1 as per the nomenclature proposed by Happi et al. (*7*). Isidro et al., (*6*) noticed that sequences within lineage B.1 shared 42 single nucleotide differences from the closest earlier MPXV genomes from 2018. From a 2017 outbreak of MPXV in chimpanzees, the evolutionary rate of MPXV was estimated to be 1.9×10^{-6} substitutions per site per year ($1.2 – 2.7 \times 10^{-6}$) translating to ~1 nucleotide change every 3 years (*8*). 42 substitutions in the space of 3-4 years is an unexpectedly large number.

Under the paradigm that MPXV is a zoonotic virus with limited human to human transmission, one interpretation of this long branch might be that it represents adaptation to humans facilitating the sustained transmission that is now observed. However, as we show here, and as was quickly seen when the first genomes from 2022 were sequenced, it is clear that these mutations are not the result of errors by the virus's replication machinery and occur at much higher rate than would be expected for an orthopoxvirus (*6*, *9*). Specifically, the majority of observed nucleotide changes appear to be of a particular type – a dinucleotide change from TC→TT or its reverse complement, GA→AA (*9*, *10*). This particular mutation is characteristic of the action of the APOBEC3 (Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 3) family of cytosine deaminases. These act on single stranded DNA (ssDNA) to deaminate cytosine to uracil causing a G→A mutation in the complementary strand when it is synthesised. Most human APOBEC3 molecules have a strong bias towards deaminating 5'TC dinucleotides and APOBEC3-driven deamination has been demonstrated with many DNA viruses and retroviruses; (*11–18*). Furthermore, a recent study has specifically demonstrated APOBEC3F editing in cell culture and during human MPXV infection (*19*).

We assess the extent to which APOBEC3 has acted on MPXV and explore whether this is the source of the elevated mutation rate observed since 2017. We also explore the evolutionary consequences of this mechanism driving evolution in MPXV and model the distinct processes underpinning the evolution of MPXV in the human population.

APOBEC3 editing as a signature of MPXV evolution in the human population

The known diversity of MPXV is decomposed into three major clades; Clades I, IIa and IIb (Fig. 1A; Happi et al., (*7*)). Clade I represents MPXV sampled in Central Africa and Clade IIa is composed of viruses from human and non-human animal samples taken in or connected to West Africa. Both of these clades include virus genomes spanning from the 1970s to present day, although the majority of samples were collected within the last 20 years (Fig. S3). Clade IIb has an early sample taken in 1971 (Genbank accession KJ642617) but most of the sequences in Clade IIb are more recent virus genomes from 2017-2022 that (*7*) have labelled as hMPXV-1 (Fig. 1A, labelled phylogeny Fig. S2). Within the recent diversity of Clade IIb (indicated as the darker box within IIb in Fig. 1A), we catalogued transmitted mutations that occurred between 2017 and 2022 with only a single representative from the 2022 global lineage B.1 (n=44, Fig. S2)(*20*).

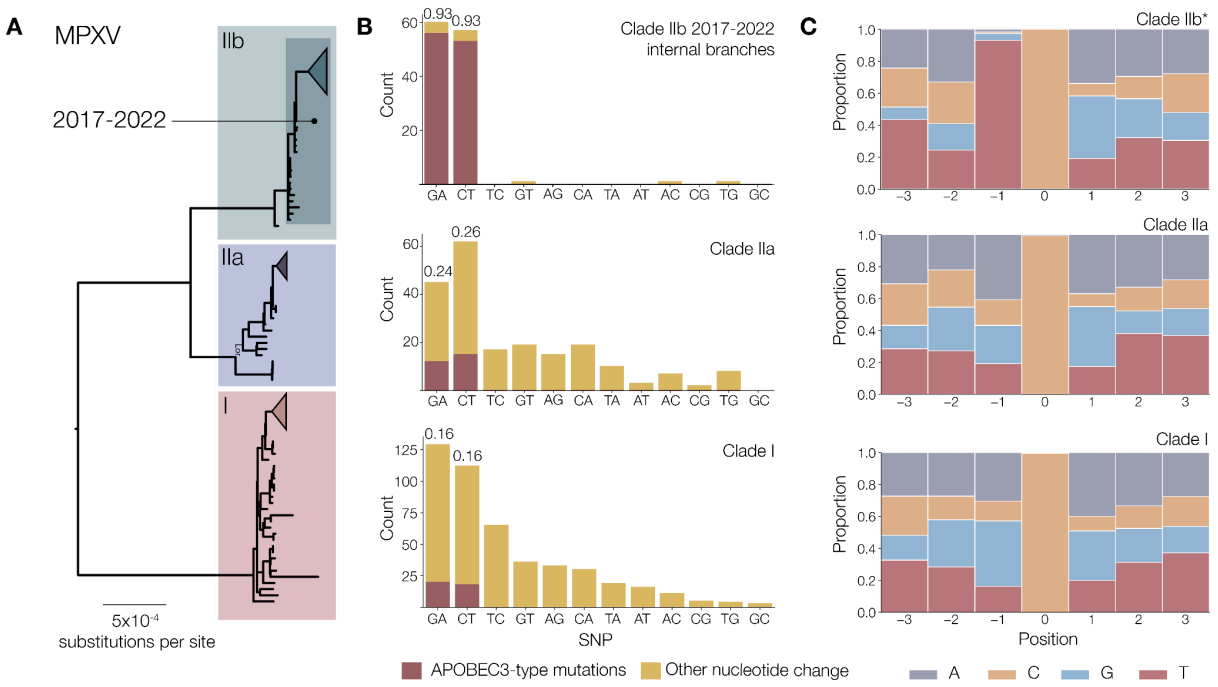

Fig. 1. Specific enrichment of APOBEC3-type mutations in MPXV samples collected since 2017. **(A)** MPXV genetic diversity is categorised into Clade I (predominantly sequences from the DRC), Clade IIa (predominantly West African sequences) and Clade IIb. Within Clade IIb is a sub-clade of genomes sampled from 2017-2022 that show distinct mutational patterns to the other two clades. **(B)** We catalogue single nucleotide mutations across the phylogenies of Clade IIb, Clade IIa and Clade I (top to bottom). For Clade IIb, we include samples from 2017-2022 and only a single representative of the global lineage B.1. Of 120 reconstructed mutations that occurred on internal branches of the Clade IIb phylogeny (so are observed transmitted mutations), 109 are consistent with APOBEC3 editing (90.8% of mutations). Individual proportions of G→A and C→T mutations shown above the respective bars. Ancestral state reconstruction performed across Clade IIa and Clade I does not produce the same enrichment of mutations consistent with APOBEC3 editing, with only 27 of 207 observed mutations (13%) and 38 of 463 Clade I mutations (8%) fitting the dinucleotide pattern. **(C)** Observed heptamers of C→T or G→A mutated sites of Clade IIb, IIa and I phylogenies (top to bottom). Heptamers associated with G→A mutations have been reverse-complemented to reflect deamination on the negative strand. For Clade IIb, most C→T mutations are present in a TC dimer context, consistent with APOBEC3 editing (107 of 115 mutations, or 93%). However the same is not seen for Clades IIa and I, in which 29 of 149 (19%) mutations and 42 of 256 (16%) have the dinucleotide context of APOBEC3 respectively, which is what we would predict under standard models of nucleotide evolution.

*Only mutations occurring on internal branches of the Clade IIb phylogeny included.

Within MPXV Clade IIb, we observe rates of molecular evolution far greater than that expected for double-stranded DNA viruses and indeed that observed in Clades I and IIa of MPXV (*8*) and see this excess accumulation of mutation in samples as early as 2017. The great majority of these mutations are of the type G→A or C→T (90.8%) (Fig. 1B), regardless of sample host species (Fig. S4). Comparing MPXV Clade IIb with Clade I and

IIa emphasises that this pattern is not seen outside of Clade IIb (Fig. 1B, labelled phylogenies Fig. S5), nor is it seen when looking at reconstructed mutations within a phylogeny of 46 Variola Virus (VARV) genomes, the human virus responsible for smallpox (Fig. S6). For the other MPXV clades, APOBEC-type mutations are observed at between 8 and 13% frequency, which fits with the expected proportion under standard models of nucleotide evolution (*21*, *22*) (Fig. 1B). Strikingly, the heptamers of C→T and G→A mutations that occurred across the Clade IIb phylogeny show this is a specific enrichment of APOBEC3-type dimer mutations of the type TC→TT and GA→AA (Fig. 1C). Similarly, this enrichment of TC→TT and GA→AA mutations is observed within the B.1 lineage (Fig. S7), where 84.8% of observed SNPs are consistent with APOBEC3 editing (Fig. S8). Observed heptamers around the observed C→T and G→A mutations show a striking enrichment in TC and GA target sites in the genomes sampled from 2017-2022 in contrast with the rest of MPXV diversity (Fig. 1C), and this enrichment is also reflected within lineage B.1 (Fig. S9).

Our analysis highlights that evolution within Clade IIb prior to the emergence of lineage B.1 mirrors that within lineage B.1, but is distinct from MPXV Clade I or IIa. Since 2022, the B.1 lineage has been sampled and sequenced internationally in the global epidemic of MPXV. Lineage B.1 is known to be circulating by sustained human-to-human transmission and as such, mutations that have accumulated in B.1 can be considered characteristic of this mode of transmission. We suggest that the APOBEC3-driven evolution of recent Clade IIb MPXV is a signature of a switch to sustained transmission within the human population. Within the B.1 lineage, believed to be entirely the result of human infection and transmission, we continue to see the same pattern of predominantly APOBEC3 mutations accumulating at a similar rate to that seen in A lineage genomes since 2017. It is unlikely that, by chance, MPXV evolved to become susceptible to APOBEC3 action within the putative rodent reservoir prior to the emergence of cases and to retain that susceptibility to human APOBEC3 molecules once transmitting in humans. Given that all human cases sequenced since 2017 share substantial numbers of APOBEC3 mutations, including nine on the stem branch leading to hMPXV-1 it is very unlikely these represent multiple zoonotic introductions. APOBEC3 genes emerged in placental mammals from a duplication of the ancestral AID gene and have a dynamic recent evolutionary past, with gene duplication and loss across phyla (*23*, *24*). APOBEC3 genes in primates have undergone recent expansion, with primate genomes now having 7 paralogs of 3 ancestral genes (*25*, *26*). Rodents, the reservoir of MPXV, have only a single functional APOBEC3 protein resulting from gene loss and fusion events (*25*). Rodent APOBEC3 has been shown to be

expressed preferentially in spleen and bone marrow with limited expression observed in other tissues (*27*, *28*).

APOBEC3 has a limited repertoire to generate variation in the MPXV genome

If we assume this observed evolution within hMPXV-1 is APOBEC3-driven, this may have implications for its sustained transmission in the human population. Considering all GA and TC dimer sites in the Clade II reference genome (Accession number NC_063383) – i.e. those that could be the target of APOBEC3 editing but had not been by that point – we assessed what amino acid changes a deamination mutation at these sites would bring about (Fig. 2). Of the 23,718 such dimers, 61.6% (14,618) would produce amino acid replacements, 21% (4990) would be synonymous, 2.9% (692) would induce stop codons, and 14.4% (3418) would occur outside of coding regions. For the Clade IIb genomes, of the 633 mutations at these dimers that did occur, 38.7% (245) were amino acid replacements and 35.7% (226) were synonymous, 4.7% (30) were nonsense, and a further 132 APOBEC3 mutations were in intergenic regions (20.8%). The probability of getting 226 or greater synonymous mutations out of 663 under a simple binomial distribution with 21.0% chance of a context being synonymous is $P=7.6\text{x}10^{-18}$. We do not see the same enrichment for synonymous mutations in the mutations that are not APOBEC3-like, although the quantity of these mutations is considerably lower (Fig. S10). There are also more mutations outside of protein coding regions than we would expect based on the location of target dimers (probability of $4.5\text{x}10^{-6}$ of getting at least 132 non-coding mutations given only 14.4% of targets are in these regions). This supports the hypothesis that what we are observing are the residual least harmful APOBEC3 mutations after natural selection has eliminated those with substantial fitness costs to the virus. By comparing the density of observed C→T and G→A mutations and the density of APOBEC3 target sites (TC or GA dinucleotides) across the reference genome, we see that the distribution of mutations is not simply a product of the availability of target sites (Kolmogorov-Smirnov test statistic=0.07, P-value=0.0002; Fig. S11A-B). When considering synonymous and non-synonymous APOBEC3-like mutations separately, there is a significant difference between the density of target sites across the MPXV genome and the distribution of observed APOBEC3-like synonymous and non-synonymous mutations respectively (Fig. S11C-D).

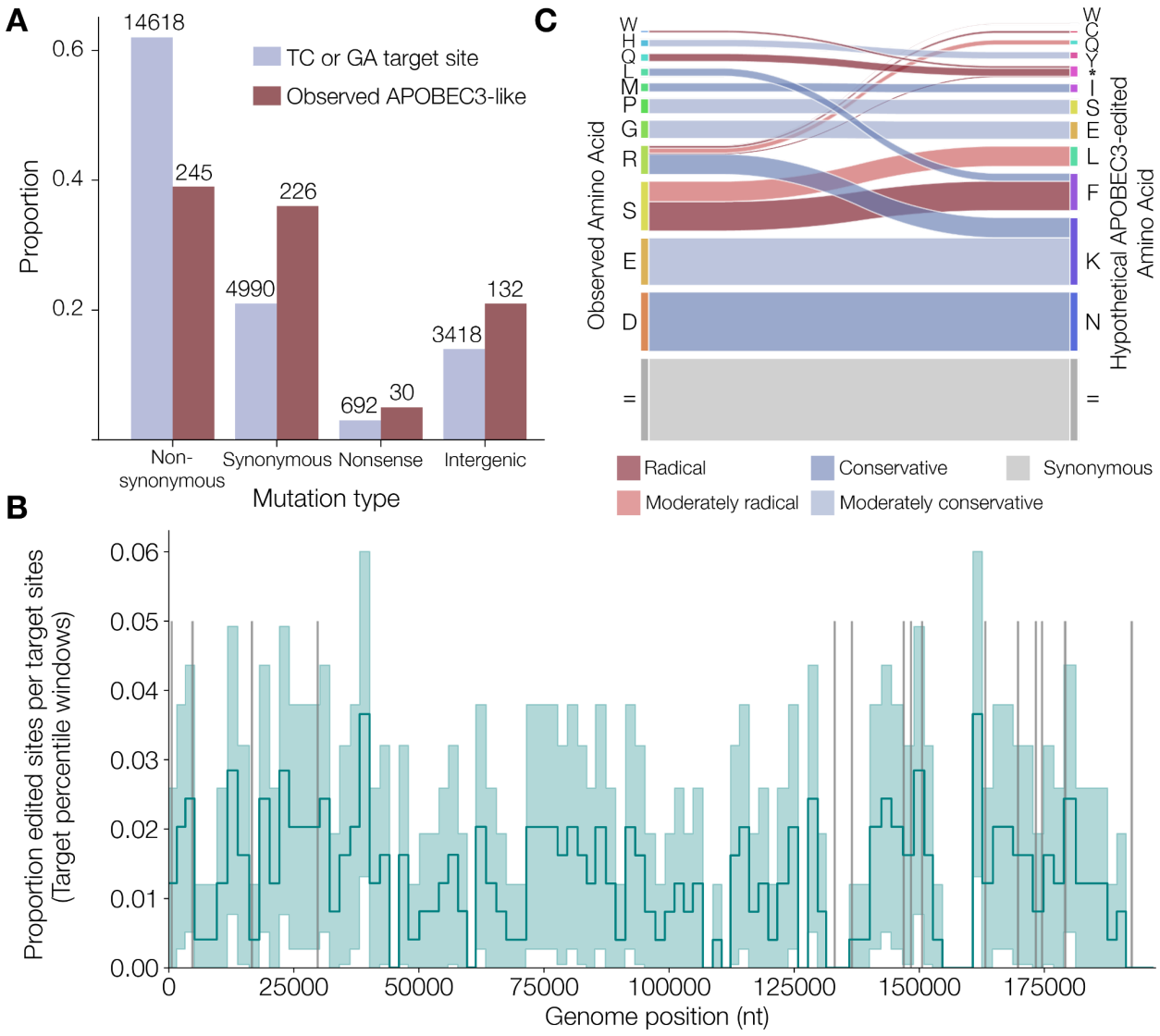

Fig. 2. Observed APOBEC3-type mutations are not merely a product of available target sites. (A) Consequence of hypothetical APOBEC3 mutations at target dimer site (either the C in the TC target site or G in the GA target site) in the coding regions of the NCBI reference MPXV genome for Clade II (Accession NC_063383) and those observed APOBEC3 mutations across the coding regions of the Clade IIb phylogeny (not including the outgroup branch leading to the 1971 genome sequence). These are categorised into non-synonymous (altered amino acid), synonymous (amino acid remaining unchanged), nonsense (editing producing a stop codon) and intergenic (not present in a coding sequence). **(B)** The proportion of target sites edited for each target site percentile window across the MPXV genome. The teal shaded regions represent the binomial confidence interval around observations. Masked regions indicated by vertical grey bars. Observed edits include data from the Clade IIb phylogeny, with a single representative of lineage B.1 and not including the branch leading to the outgroup 1971 genome sequence. **(C)** Hypothetical amino acid changes for codons overlapping with TC and GA target sites in a reference MPXV genome (Genbank accession number: NC_063383) if APOBEC3 edited those dimers to TT and AA. Amino acid changes are coloured by Grantham Score (0-50 conservative: dark blue; 51-100 moderately conservative: light blue; 101-150 moderately radical: light red; >150 radical: dark red; synonymous: grey).

The ‘repertoire’ of mutations that APOBEC3 is able to provide as genetic variation on which natural selection can act is severely restricted. Only a limited number of dinucleotide contexts are present, and the repertoire of amino acid changes that APOBEC3 editing can induce is also limited (Fig. 2C, Fig. S12). Only 13 different amino acid replacements are possible, and three that give rise to stop codons, and they are not reversible by the same mechanism. This means that given the restricted set of positions at which these mutations occur and the limited amino acid changes they can result in, the elevated rate won’t necessarily facilitate adaptation of the virus.

APOBEC3 hypermutation is a host-mediated antiviral mechanism. These molecules act as the viral genome is being replicated and single strands are exposed. During repeated rounds of replication either strand can be deaminated leading to both C→T and G→A changes on the positive strand as seen here. Thus it is likely that the genomes that are extensively mutated by APOBEC3 will simply not be viable and will not be transmitted further. MPXV replicates in the cytoplasm likely by means of rolling-circle amplification (*29*) and this facilitates extensive continuous genome replication (*30*). The high processivity of this mechanism efficiently produces high copy numbers of MPXV genome molecules in the cell, potentially saturating the APOBEC3 enzyme action if the concentration of MPXV DNA molecules is high enough. This likely means that many MPXV genomes are unaffected by APOBEC3 action. Occasionally however a genome, modestly mutated by APOBEC3, may remain viable and be transmitted. We see this in the enrichment of observed synonymous and intergenic mutations relative to available targets in the MPXV genome. Given the non-reversibility of the APOBEC3 action, sustained evolution within the human population may result in a depletion of lower-consequence target sites (i.e., synonymous or conservative amino acid changes) and thus expedite a decrease in fitness of MPXV over time. This could be both through a reduction in the number of viable offspring viruses produced by infected cells and as a result the accumulation of moderately deleterious mutations by genetic drift (i.e., mutation load). However the timescale on which this might happen is uncertain and other evolutionary forces such as recombination may act to restore fitness, and we do not address this further in this study. A further uncertainty arises when considering the variable repertoire of genes associated with virus infectivity or host immune modulation in poxvirus genomes. Mutations that alter or abrogate the function of these genes may have little direct effect on virus replication machinery, but may disrupt the virus/host interaction. There is precedent for the naturally occurring inactivation of genes in VARV contributing to host specificity, and consequently the loss of function of some MPXV genes through APOBEC3 activity may potentially have adaptive value for the virus as it replicates and transmits in a new host (*31*, *32*).

Even if the mutations that accumulate through this process are simply the neutral residue of a sub-optimal antiviral host defence, they have produced sufficient variability for the phylogenetic analysis of the epidemic over the short term. The initial lineages proposed by Happi et al (*7*) have now expanded with the 2022 epidemic B.1 lineage now encompassing 17 sublineages at time of writing (*33*). The rapid and temporally linear accumulation of mutations means that genomic epidemiological models and tools (*34*, *35*), usually employed for RNA viruses, may also have utility to hMPXV-1.

The linear accumulation of APOBEC3-type mutations since the emergence and spread of MPXV in humans

Since 2017 the genomes thus far sampled from Clade IIb have accumulated APOBEC3-type single nucleotide mutations approximately linearly over time (Fig. 3A-B, labelled phylogeny in Fig. S13). We applied Bayesian regression analysis on the root-to-tip plot of sequences in Fig. 3A, which includes one representative B.1 genome, and also separately on the B.1 lineage (B.1 phylogeny in Fig. S1)(*20*). To ensure the elevated temporal signal is unique to APOBEC3 data, we show combinations of APOBEC3 and non-APOBEC3 mutations on Clade IIb data in Fig. S14. The estimated rate of accumulation was 6.18 per year (95% credible intervals of 5.20, 7.16). For the B.1 lineage the rate was 5.93 per year (2.95, 8.92) – suggesting that despite widespread and rapid transmission within MSM networks, the rate of accumulation of APOBEC3 mutations remained the same as the rest of the Clade IIb. It is notable that the regression line for B.1 lies substantially above that for the rest of Clade IIb suggesting that this lineage accumulated more mutations than expected prior to the emergence of B.1. However most of these mutations are also present in the genome from Maryland, USA (Accession number: ON676708) from November 2021(*10*), indicating they arose and circulated for some months prior to the B.1 epidemic (Fig. S15-S16). Extrapolating back to when the APOBEC3-type mutations started to accumulate provides an estimate of when the first APOBEC3 mutations occurred in the stem of the branch leading to the 2017 epidemic. If we assume all these mutations are due to APOBEC3 in humans then we estimate this date of emergence to be 20-Jun-2015. However, we expect a few APOBEC3-like mutations to actually be due to replication errors during the earlier pre-emergence epoch. The number of mutations we ascribe to this period will affect our estimate linearly – i.e., if 3 mutations were not due to APOBEC3 then the estimate would shift to 14-Dec-2015.

To accommodate this uncertainty in our estimates we have developed a more explicit model of APOBEC3-mediated evolution in the BEAST software package (*20*, *34*). We estimate the action of APOBEC3 on the MPXV population is driving evolution at a rate ~28 times faster than that of the background evolutionary rate (Fig. S17). Gigante et al (*10*) also described an elevated overall rate of evolution in the A lineage but did not decompose the APOBEC3 and non-APOBEC3 contribution to this. The time of the most recent common ancestor of the post 2017 genomes is estimated to be 23-Feb-2016 (28-Jun-2015, 28-Sep-2016) with the transition to sustained human to human transmission to be 14-Sep-2015 (21-Aug-2014, 31-Jul-2016; Fig. 3C, Fig. S17). Unlike the assumption in Fig. 3B that all APOBEC3 mutations occurred post-emergence, the BEAST analysis estimates the transition point integrating over all possibilities. This allows for the fact that a few of the APOBEC3-like mutations may actually be due to replication errors in the earlier evolutionary epoch and this might explain the slightly more recent date. We also see evidence of exponential growth in the number of infections in the epidemic prior to the emergence of lineage B.1 in 2022 (Fig. 3D), despite the decline in cases reported in 2020 (Fig. S1C), albeit the growth rate is relatively slow reinforcing the indication from the demographics of the cases that this is not a generalised epidemic.

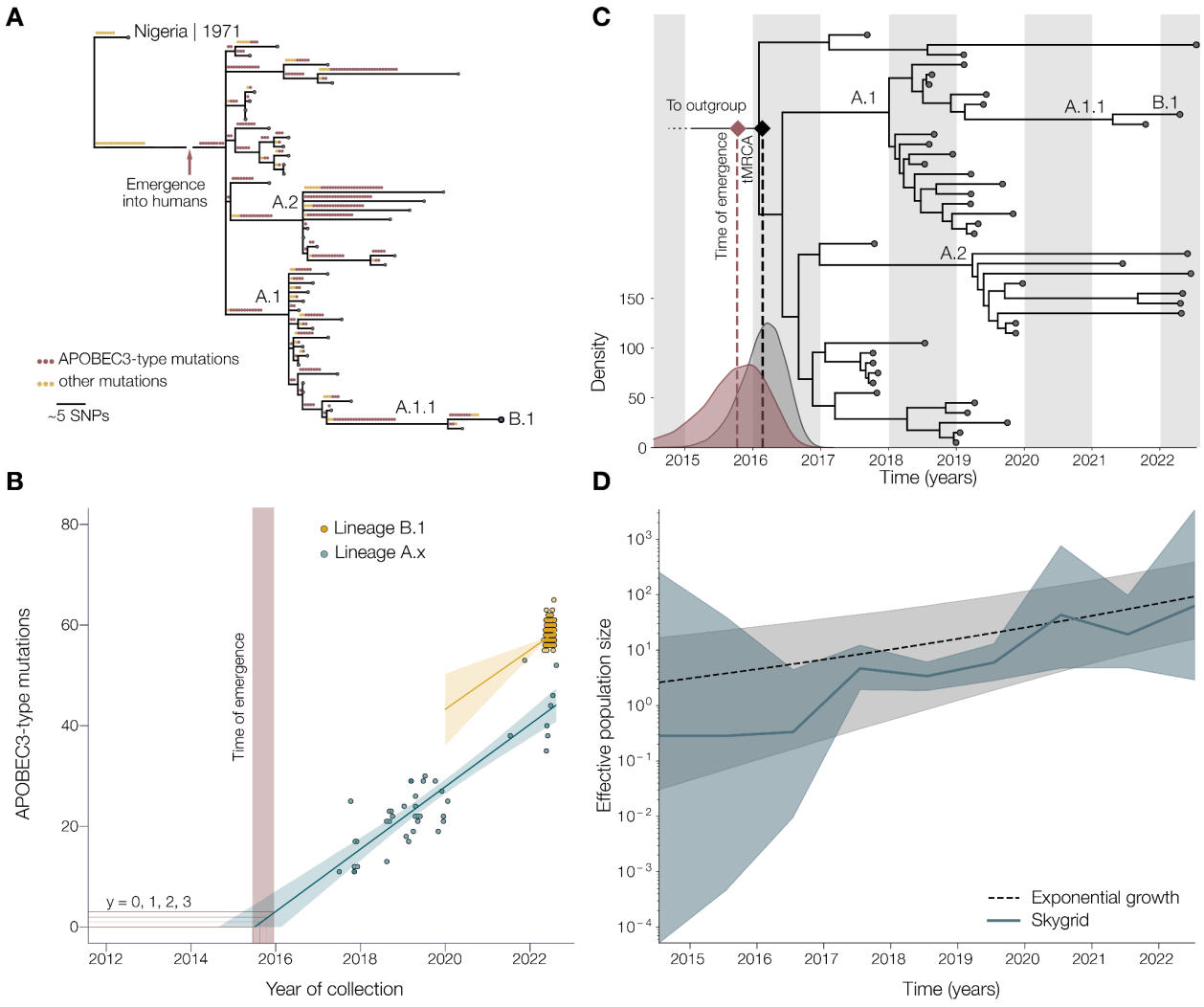

Fig. 3. Estimating the time of MPXV emergence into the human population from the accumulation of APOBEC3-type mutations. (A) MPXV genomes sampled from human infections from 2017-2022, with an outgroup sequence from an outbreak in Nigeria in 1971 (n=44 including outgroup). Lineages indicated as per nomenclature proposed by Happi et al., (*7*). Mutations along each branch are indicated with circles coloured by whether it is putatively APOBEC3 edited (TC→TT and GA→AA; red) or whether it is another mutation type (yellow). The break in the basal branch illustrates the assumption made in the regression model in panel B, that all APOBEC3 mutations occurred after emerging into the human population, however we do not know the precise distribution of red or yellow mutations. **(B)** APOBEC3 mutations from MPXV genomes sampled since 2017. The reconstructed most recent common ancestor (MRCA) of the panel A phylogeny is used as the root in the root-to-tip plot and the y-intercept is used as a proxy for time of emergence which is inferred by fitting a Bayesian regression to the sequence dates from panel A. Intersects with y=1, 2, & 3 are also shown as it is likely that a small number of the APOBEC3-type mutations are actually earlier replication errors and not induced by APOBEC3. **(C)** Maximum Clade Credibility (MCC) phylogeny of MPXV Clade IIb with absolute time shown on the X-axis. We separated the alignment into an APOBEC3 and a non-APOBEC3 partition and modelled the substitution process in each independently. We used an epoch model with two outgroup sequences (not shown in panel) representing the first epoch and hMPXV-1 ingroup sequences representing MPXV post-emergence into the human population with an exponential growth

model. The probability density distributions show the estimated time of the most recent common ancestor (tMRCA) of the ingroup as well as the estimated transition time that represents the time of emergence into the human population. **(D)** Estimated effective population size of the outbreak using a non-parametric coalescent Skygrid model with 11 change points over a period of 8.5 years. This reconstruction falls within the bounds of the exponential growth model estimated from the second epoch in panel C, suggesting that the MPXV population has been exponentially growing since at least 2016.

Implications for the global public health response to mpox cases

Since the identification of the B.1 lineage, a number of countries have reported other lineages that lie outside the diversity of B.1, including USA, UK, Portugal, India and Thailand. In almost all instances these cases are reported as having a history of international travel. The lineages these are placed in (designated as A.2.1, A.2.2, A.2.3 and A.3) can all be phylogenetically traced back to the epidemic in Nigeria (Fig. 3A). This suggests that at least one instance of sustained human to human transmission is still ongoing outside of the recognised MSM networks that were the focus of the 2022 global epidemic. Stopping transmission in these communities, whilst necessary, will not be sufficient to eliminate the virus as a human epidemic. There are large portions of the globe without the surveillance to detect MPXV cases and if sustained human to human transmission has been ongoing since 2015-2016 it is plausible there are other populations that are currently enduring epidemics.

Historically, mpox was considered a zoonotic disease and cases have been treated as independent spillover events with low levels of circulation in the human population. Thus far, this continues to be an accurate characterization of Clade I in Central Africa. For Clade IIb, whilst some non-B.1 lineage cases may be new zoonotic infections, the majority of cases since 2016 are likely the result of human to human transmission. Although the B.1 lineage across the world is now diminished – though not yet eradicated – the human epidemic from which it arose continues unabated. It is critical that global public health affords MPXV cases in countries that are historically considered to have endemic reservoir species equal attention and concern to those elsewhere. Surveillance needs to be global if MPXV is to be eliminated from the human population and then prevented from reemerging.

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Supplementary Materials Contents

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Authors declare they have no competing interests.

Data and materials availability:

Data, code, and materials used in the analysis are available from Zenodo: 10.5281/zenodo.8146689 and http://github.org/hmpxv/apobec3/. Tables S1 and S2 include details of accession numbers and author lists for all source genome sequence data used. All except a single genome sequence were sourced from Genbank on NCBI. Data partitions of the alignments have been included in the XML files on Zenodo and GitHub for ease of reproduction. A single sequence was sourced from GISAID (gisaid.org) and as such was removed from the XMLs. Supplementary tables include the GISAID identifier, which can be used to independently source the data and instructions for constructing the alignment and addition to the xml are included.

Supplementary Materials for

APOBEC3 deaminase editing in mpox virus as evidence for sustained human transmission since at least 2016

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The PDF file includes:

Materials and Methods

Nomenclature

A recently published paper (*7*) proposed an updated, systematic, nomenclature for the phylogenetic structure of MPXV to replace previous geographical clade names. In this nomenclature the 2022 global epidemic lies in Clade IIb with all genomically characterised cases since 2017 designated with lineage labels similar to SARS-CoV-2. The recent human epidemic, provisionally named hMPXV-1 by Happi et al. (*7*), comprises a hierarchy of lineages starting with 'A' currently represented by genomes from 2017 cases in Nigeria. The bulk of 2022 genomes are part of a lineage denoted 'B.1' but other lineages ('A.2' and 'A.3') have also been reported in the USA, UK and Portugal, in most cases in individuals with international travel history. We follow this nomenclature here.

Data Processing and Alignment

We compiled high-quality MPXV genomes from human and non-human animal outbreaks sampled from as early as 1965 and up to the current 2022 outbreak. The dataset consisted of 94 genomes with representatives from Clades I, IIa and IIb (Fig. S2-S3, Fig. S6). A second dataset comprises all Clade IIb genomes from 2017-2022 but with a single representative of lineage B.1 of hMPXV-1 and two earlier sequences from Nigeria from 1971 and 1978 (accession numbers KJ642617 and KJ642615, respectively). The genome from 1978 (accession KJ642615) was used as an outgroup, due to its greater divergence despite being sampled more recently, used to root the tree and then not analysed further. A separate analysis was run specifically on lineage B.1 where all high-quality B.1 genomes with an exact date of collection on Genbank were downloaded on 2022-08-22. The 2021 genome from Maryland, USA (lineage A.1.1; accession number: ON676708) was used as an outgroup to root the B.1 tree (n=769). Accession numbers and acknowledgments for genome sequences used in this study are provided in Table S1.

All the MPXV genome datasets were aligned against the Clade II reference genome (which is an early hMPXV-1 genome from Nigeria, accession: NC_063383) using minimap2 v2.17 (*36*) to generate a single coordinate system. Genome sequences were trimmed from position 190,788 to the end, corresponding to the 3' terminal repeat region of the MPXV genome. A series of repetitive or low-complexity regions were masked from the alignment (Table S3). This alignment, extraction and masking pipeline is available at https://github.com/aineniamh/squirrel.

The full MPXV phylogeny of Clade I, IIa and IIb was estimated using maximum likelihood in IQ-TREE v2.0 using a Jukes-Cantor substitution model (*37*) and subsequently midpoint

rooted. For the ancestral reconstruction analysis, we estimated phylogenies for each of Clade I, Clade IIa and Clade IIb. The MPXV Clade IIb phylogeny was estimated with the same parameters, but with the outgroup specified as the 1978 Nigerian MPXV genome sequence (Accession KJ642615) and zero-length branches collapsed. Similarly, we estimated phylogenies independently for the hMPXV-1 coding sequence alignment and for Clade I and Clade II of MPXV using the above parameters with genome sequence KJ642617 as an outgroup.

We performed the ancestral state reconstruction using IQ-TREE2 on all individual Clade phylogenies of MPXV. We parsed out all reconstructed sites that vary unambiguously across the phylogeny (i.e. we exclude missing data) and mapped the single nucleotide mutations that occurred to the relevant branch of the phylogeny. Using the reconstructed node states we also catalogued the dimer and heptamer context of all C->T or G->A mutations that occurred across the phylogenies. Scripts are available from Zenodo: 10.5281/zenodo.8146689.

We compared each recent Clade IIb sequence with the reconstructed most recent common ancestor of the Clade IIb phylogeny and collated the mutations down each branch from root-to-tip and their dinucleotide context. Using only APOBEC3-type mutations, we constructed an APOBEC3 root-to-tip linear regression for Clade IIb and similarly for lineage B.1. We first performed a linear regression of the number of APOBEC3 mutations from the root of the tree to each tip against the date of collection of the tip. The slope is an estimate of the APOBEC3-specific mutation rate and assuming that APOBEC3 mutations are characteristic of infection in the human host, we use the X-intercept of this regression to estimate the date of emergence into a human host. This approach does not adjust for phylogenetic correlation and, in particular, the upward shift in B.1 is the result of a single shared branch with a higher than average number of mutations, but one that is not necessarily beyond expectations.

To model the evolutionary processes in MPXV, we created two data partitions of the Clade IIb alignment. The first partition contains only sites with putative APOBEC3 modifications (i.e. observed C->T or G->A substitutions in the appropriate dinucleotide context) and such target sites that are entirely conserved (i.e. all C or all G). All other sites in the alignment are masked out as ambiguous nucleotides. As such, we have a partition that represents APOBEC3 mutations as a function of target APOBEC3 sites (“APOBEC3 partition”). The second data partition is simply the inverse of this – i.e., contains all the sites with only the APOBEC3 target sites masked. The APOBEC3 alignment has 24,704 unmasked sites and the non-APOBEC3 alignment has the inverse with 172,504 across the

44 taxa included in the analysis. We use a standard nucleotide GTR+G substitution model with 4 rate categories to represent the non-APOBEC3 partition. We model the substitution process of the APOBEC3-only partition as a two-state continuous time markov chain with an asymmetric rate, allowing only C->T mutations and not the reverse.

To estimate the time of the initial emergence of MPXV into the human population (the time of the primary case) we employed a 2-epoch molecular clock such that for the APOBEC3-only partition the rate transitions from background (non-APOBEC3) rate of evolution to the APOBEC3 rate of evolution at a time t_p. This transition time is parameterised as t_p = tMRCA(lineage A) + x, where x is a free parameter in BEAST that represents the unsampled transmission history prior to the MRCA of the sampled lineage A viruses. The non-APOBEC3 partition is given the background rate over the entire tree. We also employed a two-phase coalescent model with the tree from the MRCA(lineage A) onwards having a model of exponential growth, with an inferred rate of growth, and the earlier phase being a constant-population size coalescent model.

We further examined the pattern of epidemic growth during the lineage A phase using a non-parametric coalescent Skygrid model (*38*) with 11 change points spanning 8.5 years. For each model we ran two replicate chains with 10 million states to check for convergence, removed 1 million states as burn-in, and then combined the samples. BEAST XML files for these analyses and a script to generate the APOBEC3 and non-APOBEC3 data partitions are available from Zenodo: 10.5281/zenodo.8146689.

To investigate the target site context of mutations occurring in the hMPXV-1 phylogeny, we extracted the relevant nucleotide heptamers for all C->T or G->A mutations that occurred across the MPXV Clade IIb phylogeny (260 of 296 mutations) and for G->A mutations we took the reverse complement of the nucleotide sequences to normalise for strand. We also calculated the heptamer context for just the backbone of the phylogeny (i.e. the branches leading to the 2022 outbreak), which included 55 of 57 mutations on the respective branches. Using the reconstructed mutations and branch states in the phylogeny, we collected the ancestral dimer context for mutations that were either G->A or C->T to assess which mutation occurred in a context consistent with APOBEC3 editing.

We used gene coordinates from the MPXV Clade II genbank reference (Accession number: NC_063383) to categorise the observed APOBEC3-like SNPs into synonymous, nonsynonymous or nonsense mutations. Similarly, for APOBEC3 target sites we used the MPXV Clade II reference record to predict whether an edit would produce a change to the amino acid. For these observed APOBEC3-like mutations and the APOBEC3 target sites, we wanted to look for any spatial patterns of APOBEC3 edits along the genome. For windows that included 1% of APOBEC3 target sites (i.e. each window contains 197 target

sites out of 19700 total targets), we compiled a count of mutations consistent with APOBEC3 editing and represented it as a proportion of available target sites for that window, with a binomial confidence interval around that proportion. Scripts available from Zenodo: 10.5281/zenodo.8146689.

Root-to-tip regression

The model for the linear regression of number of APOBEC3-type mutations against time was as follows:

$m_i \sim \text{Normal}(\mu, \sigma)$

$\mu = \alpha + \beta\ (t_i - t_{mean})$

$\alpha \sim \text{Normal}(11, 100)$

$\beta \sim \text{Lognormal}(0, 1)$ and

$\sigma \sim \text{Normal}(0, 20)$,

where m_i is the number of mutations for genome i, α is the y-intercept and has a prior centred on the minimum number of mutations observed over all genomes, β is a strictly-positive evolutionary rate per year, t_i is the time of collection of the sample and σ is the model error standard deviation.

The model was fitted to the data in a Bayesian framework using a quadratic approximation to the posterior distribution implemented in the rethinking package in R (*39*). Posterior estimates and 97% highest posterior densities of the parameters for the lineage A data are α: 25.24 (23.80, 26.66), β: 6.18 (5.19, 7.16), σ: 4.76 (3.74, 5.78) and for the lineage B.1 data are α: 57.67 (57.49, 57.85), β: 5.93 (2.95, 8.91), σ: 1.77 (1.64, 1.90). An R script performing this analysis and generating the graphic seen in Fig. 3B is available at http://github.org/hmpxv/apobec3/

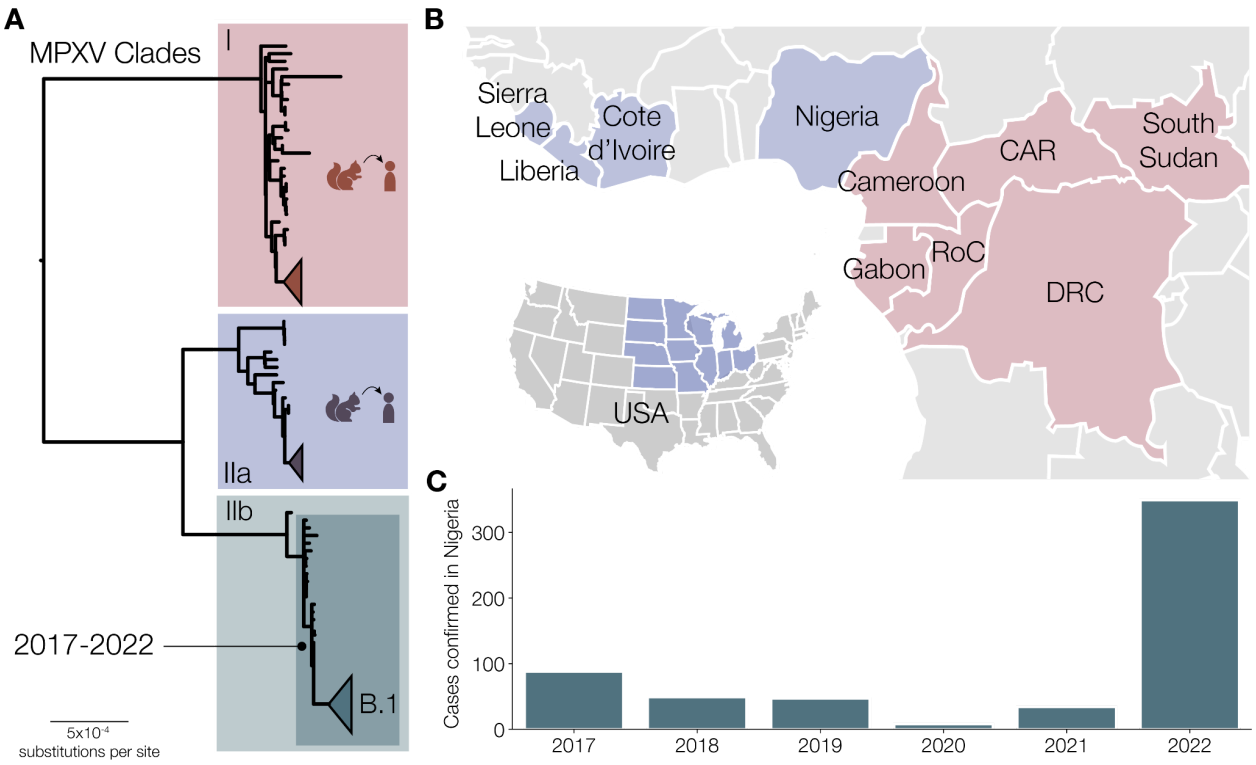

Fig. S1.

(A) The genetic diversity of MPXV is split into two major clades (I and II). Historically, outbreaks have been low-level zoonoses and could be traced back to contact with a rodent reservoir host species. Cases of MPXV have been reported in Nigeria since 2017 and significant genetic diversity within the outbreak suggested independent introductions from a rodent reservoir. May 2022 saw the emergence of a global lineage of Clade IIb, lineage B.1, that had widespread human-to-human transmission in MSM (men who have sex with men) contact networks. **(B)** Past isolates of MPXV from Central Africa fall into Clade I and isolates from West Africa predominantly fall into Clade II, however the true geographic ranges are not clear. **(C)** Low numbers of MPXV cases were reported in Nigeria since 2017, with a decline in case numbers in 2020. By May 2022, there was a large increase in the number of cases of MPXV reported in Nigeria. Case count data from Nigeria CDC situation reports (*1*).

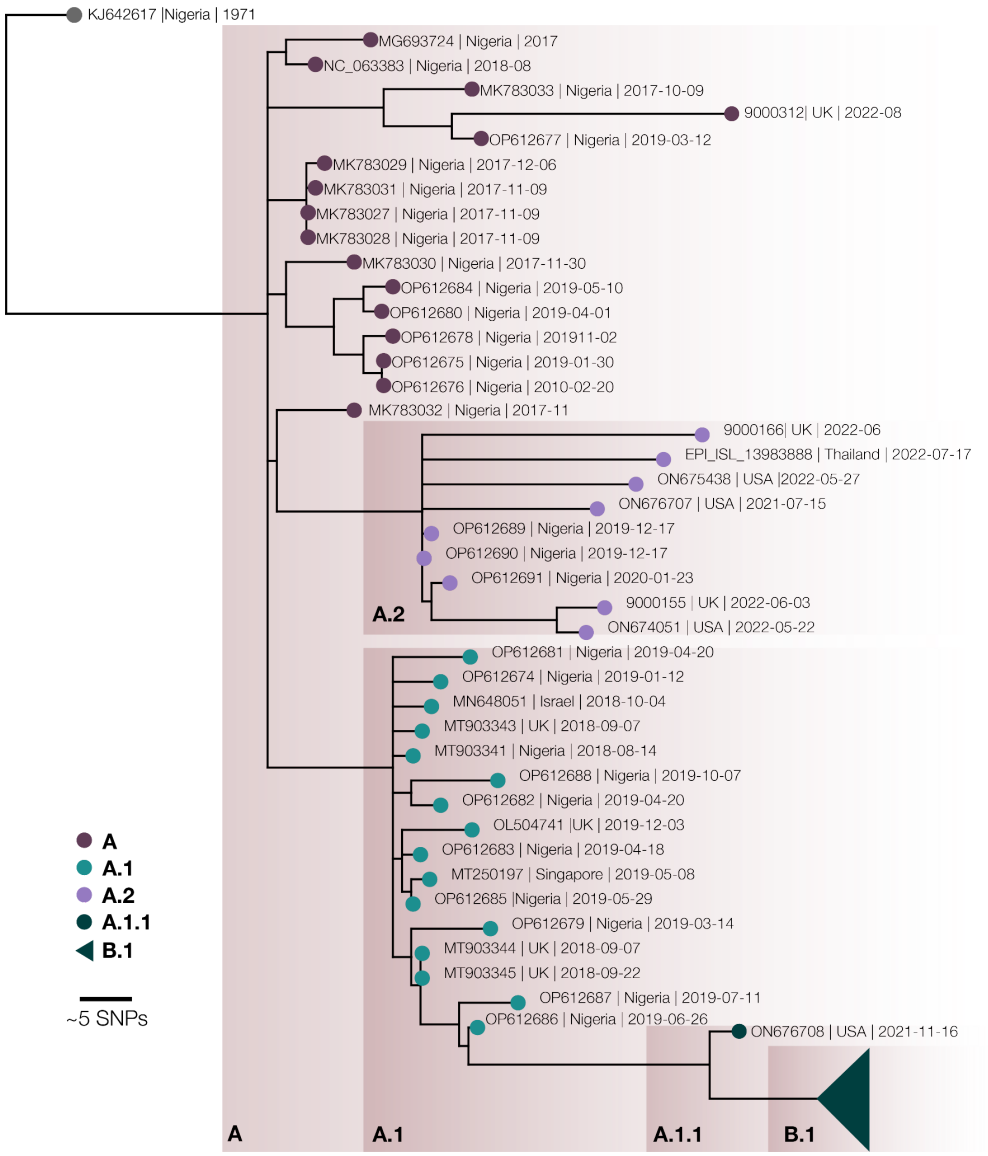

Fig. S2.

Clade IIb phylogeny illustrating sampled diversity since cases were first reported in Nigeria in 2017. Lineage A includes cases from 2017-2019 in Nigeria, A.1 includes diversity from Nigeria from 2018-2019 and A.2 includes a number of international cases with known travel history from Nigeria (e.g. UK, USA and Thailand). Lineage A.1.1 contains a single sequence, sampled in the USA in 2021. The large, global lineage B.1 that emerged in 2022 is represented by a triangle.

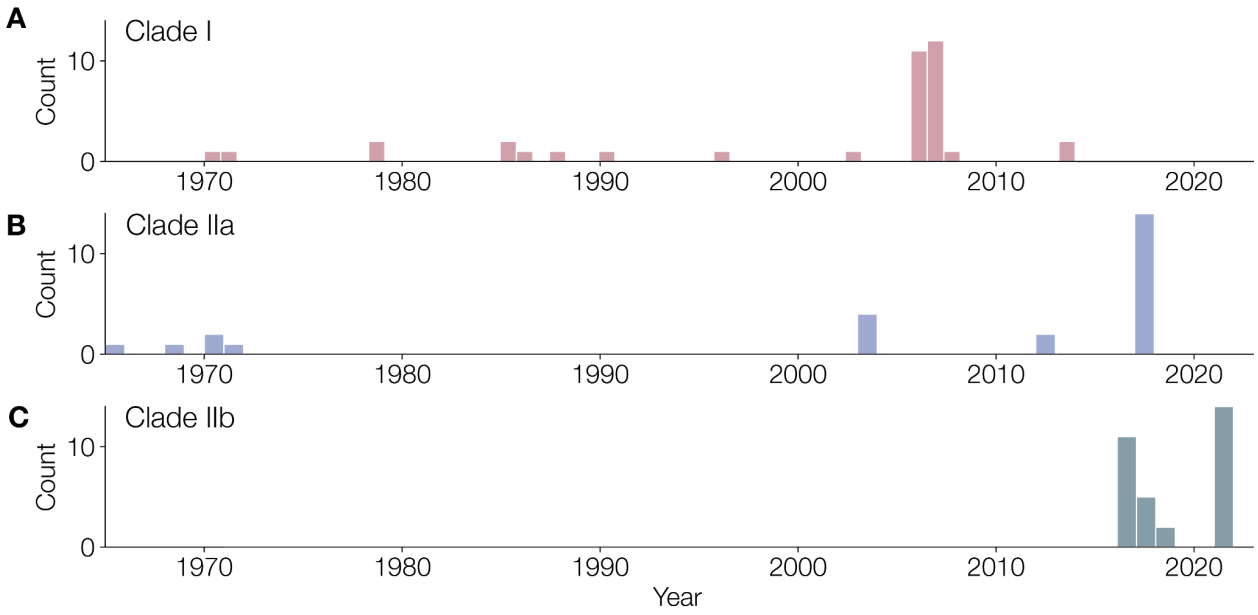

Fig. S3.

Sequences included in analysis from Clades I **(A)**, IIa **(B)** and IIb **(C)**, listed in Table S1. To account for intensive sampling in 2022, we include just a single representative of Clade IIb lineage B.1 (the global lineage that spread in MSM contact networks in 2022) in Clade IIb analyses and treat lineage B.1 separately unless otherwise stated.

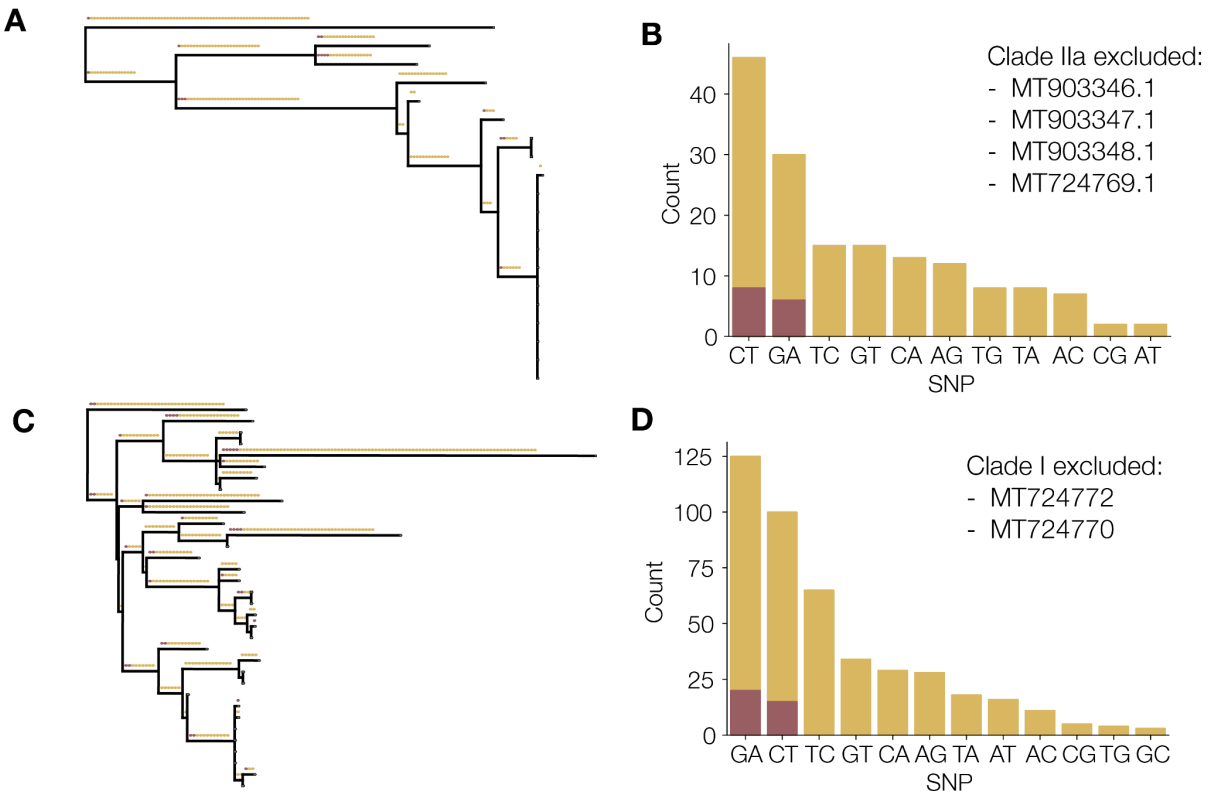

Fig. S4.

There are a small number of MPXV sequences in Clade I and Clade IIa that were sampled directly from rodent hosts. We believe that most, if not all evolution observed across Clades I and IIa has arisen while the virus resides in the rodent reservoir as there has not been any evidence of sustained human to human transmission until the recent outbreak of Clade IIb. Despite this, we investigated whether including MPXV sequences sampled from a rodent host introduces any artefacts into our observations and analysis. **(A)** For Clade IIa, we excluded sequences with accession numbers MT903346.1, MT903347.1, MT903348.1 and MT724769.1 from the alignment and estimated the maximum likelihood phylogeny and ancestral state reconstruction as described in the main text. **(B)** We then assessed the types of SNPs that occurred across the phylogeny and whether they were in an APOBEC3 context or not. **(C)** For Clade I, we excluded sequences MT724772 and MT724770 from the alignment, estimated the maximum likelihood phylogeny, and reconstructed mutations across each branch of the phylogeny. **(D)** We categorised the Clade I SNPs by whether they had occurred in an APOBEC3 context or not. APOBEC3-like SNPs are shown in red and other SNPs in yellow. As expected, we find that excluding the small number of rodent-derived MPXV samples makes no difference to the observed pattern, although counts of observed SNPs are slightly reduced.

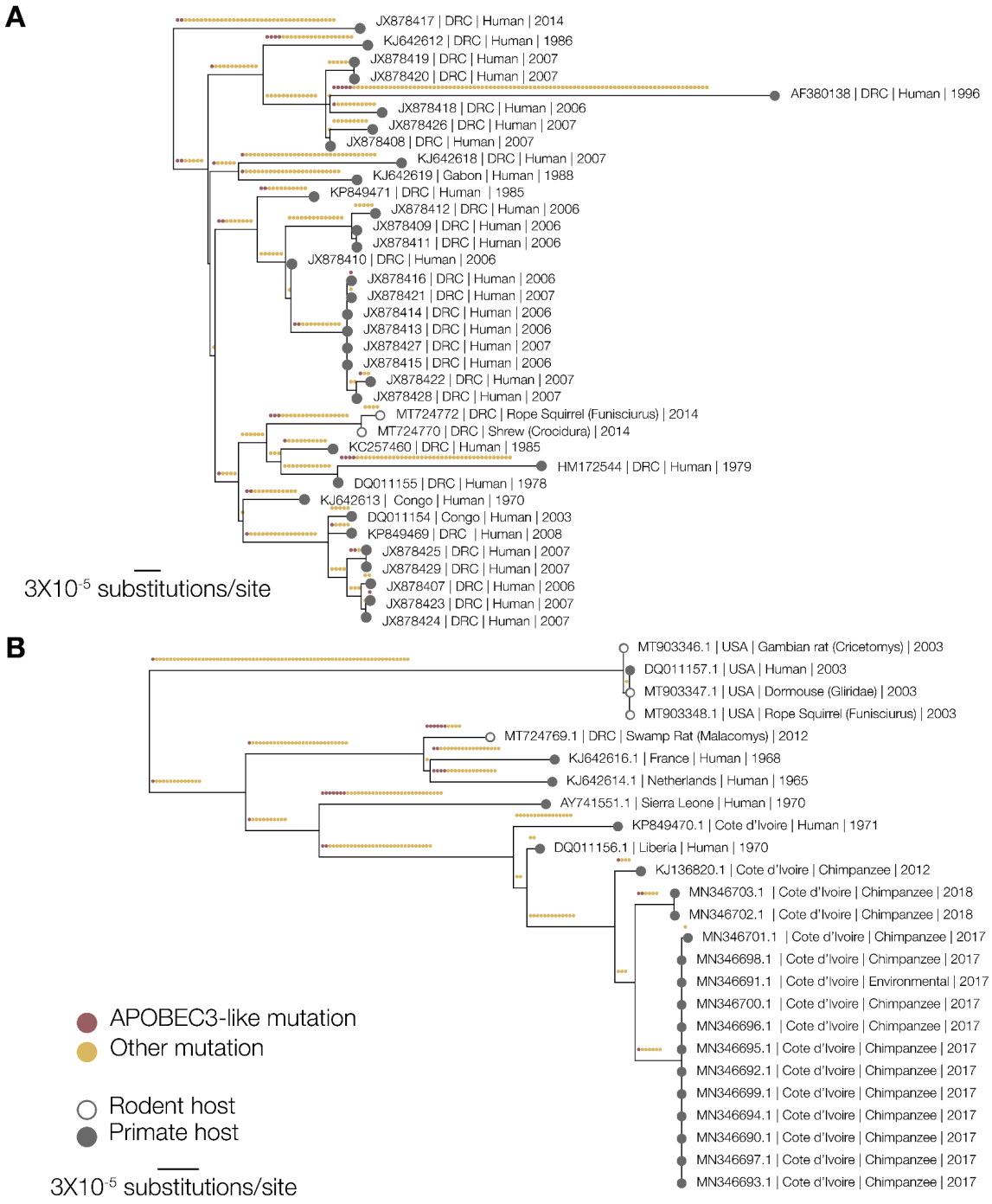

Fig. S5.

Tip-labelled phylogeny of Clades I and IIa of MPXV. Reconstructed SNPs are coloured by whether or not the mutation type is consistent with APOBEC3 editing. Tips are coloured by whether the host is rodent or primate (including human and chimpanzee samples).

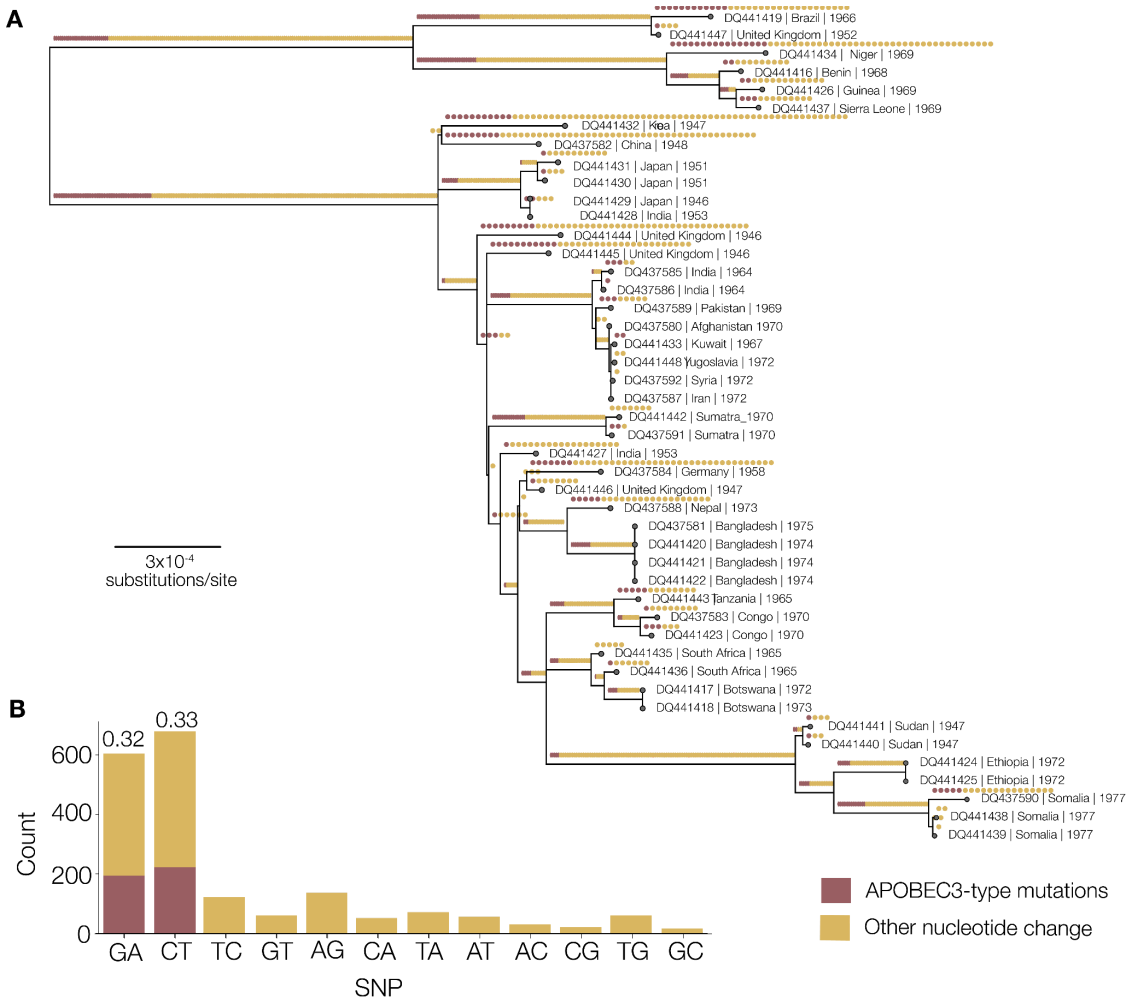

Fig. S6.

(A) To investigate whether an enrichment of APOBEC3-consistent mutations was seen in the human pathogen Variola Virus (VARV), we constructed a maximum likelihood phylogeny with 47 publically available VARV genomes using IQTREE2 with the VD21 sequence from the 17th Century (Genbank accession number: KY358055) set as the outgroup sequence. After pruning out the outgroup, we ran ancestral state reconstruction using IQTREE2 and mapped the reconstructed SNPs to respective branches. 1,835 SNPs were reconstructed across the phylogeny and categorised as APOBEC3-type mutations (red) and other (yellow). The SNPs are plotted along each respective branch and branches that have a large number of SNPs have the proportions of APOBEC3-like/ other represented as a bar. **(B)** We reconstructed the mutations across the VARV phylogeny and – of 1,835 SNPs – see only 414 that are consistent with APOBEC3-editing and so do not see any enrichment for APOBEC3-type mutations.

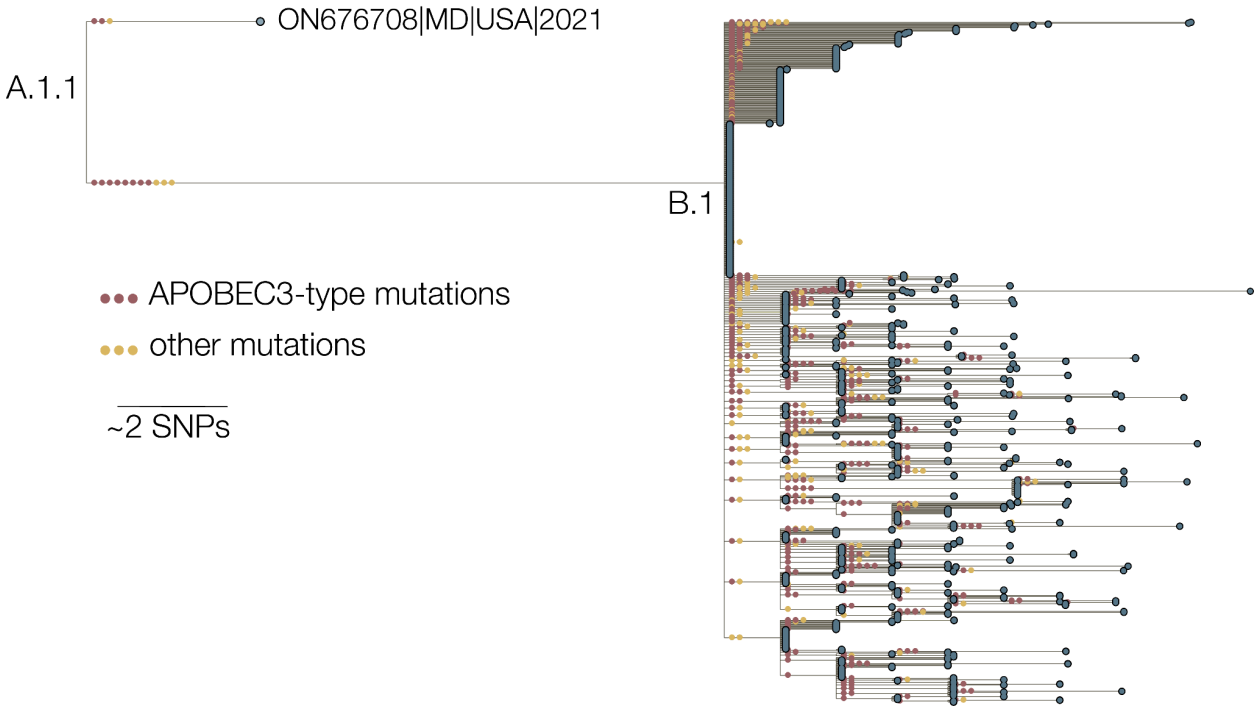

Fig. S7.

Phylogeny containing all high-quality hMPXV-1 B.1 genomes shared on Genbank as of 2021-08-22, with the 2021 Maryland sample (ON676708) used as an outgroup (n=769). Sequence names and authors are listed in Table S2.

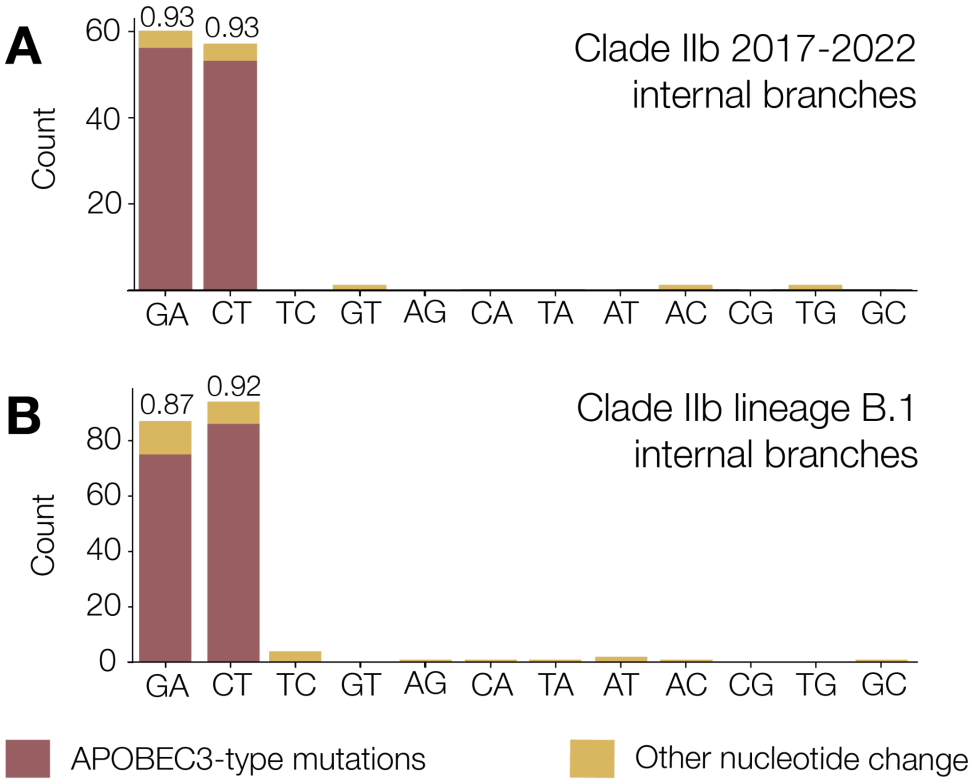

Fig. S8.

(A) We reconstructed single nucleotide mutations across the Clade IIb phylogeny, including only a single representative of the global lineage B.1. Of 120 SNPs that occur on internal branches across the phylogeny, 109 are consistent with APOBEC3 editing (90.8%). **(B)** Similarly, we reconstructed mutations that occurred across internal branches of the B.1 lineage. Of 191 mutations, 162 are consistent with APOBEC3 editing (84.8%).

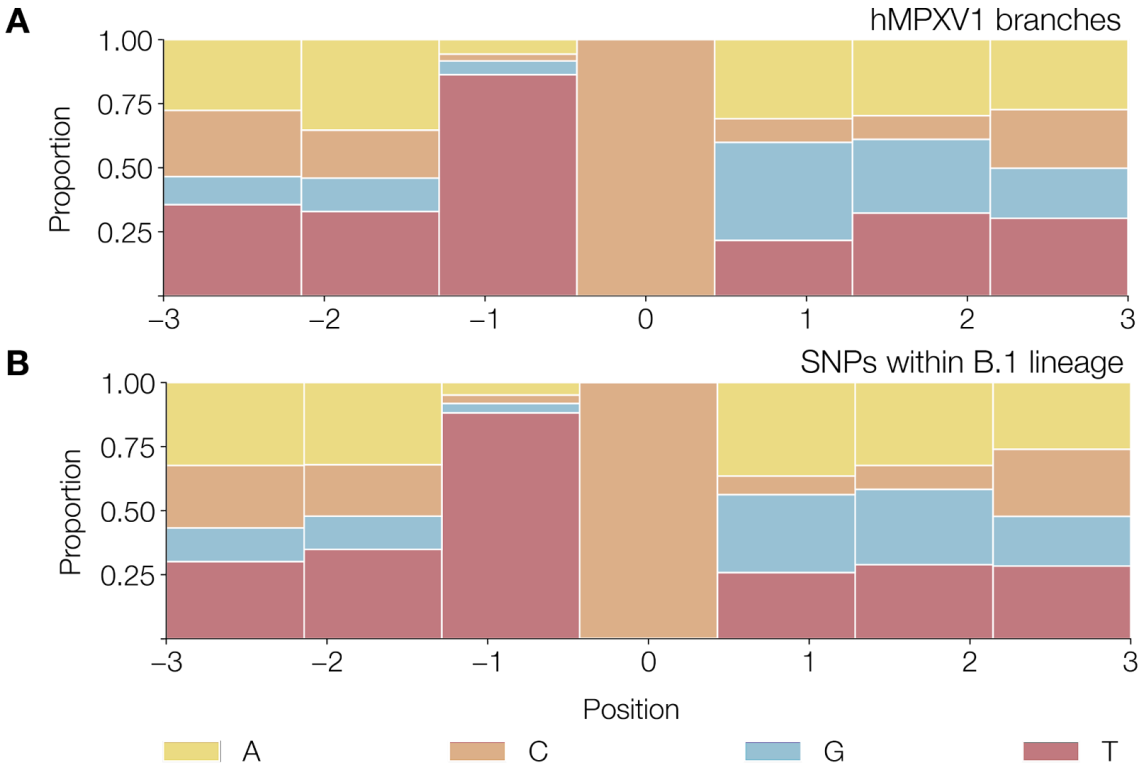

Fig. S9.

(A) Observed heptamers of C->T or G->A mutated sites in 337 of 383 unambiguous mutations that occur along branches in the MPXV phylogeny in Fig.4A, excluding the mutations that occur on the 1971 branch. Heptamers associated with G->A mutations have been reverse-complemented to reflect deamination on the negative strand. **(B)** Observed heptamers of C->T or G->A mutations in the B.1 lineage of Clade IIb (677 of 836 mutations), which we know is being transmitted human-to-human.

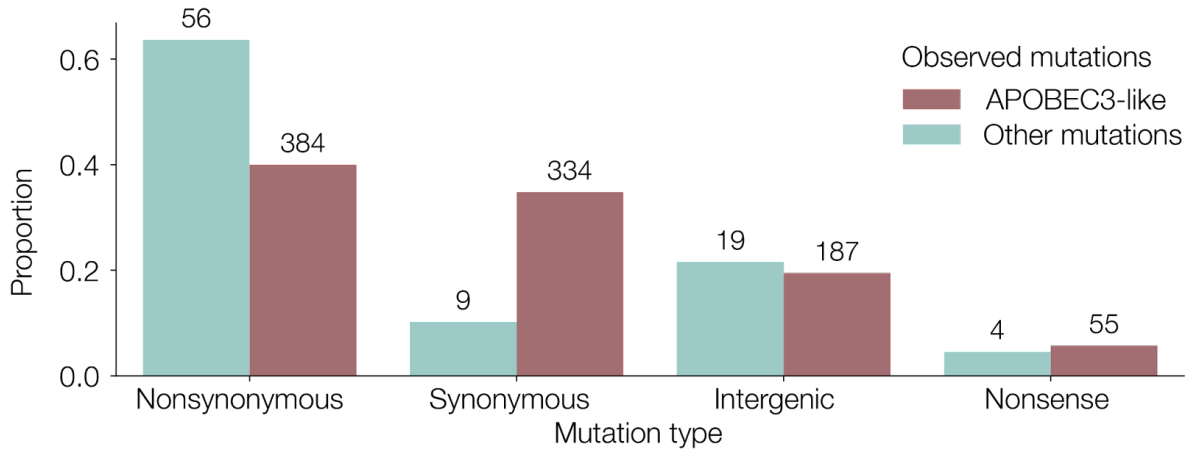

Fig. S10.

Observed mutations across the Clade IIb phylogeny (including all B.1 genomes, and not including the outgroup branch leading to the 1971 genome sequence). These are categorised into non-synonymous (altered amino acid), synonymous (amino acid remaining unchanged), nonsense (editing producing a stop codon) and intergenic (not present in a coding sequence), and coloured by whether they have occurred in an APOBEC3-like context or not.

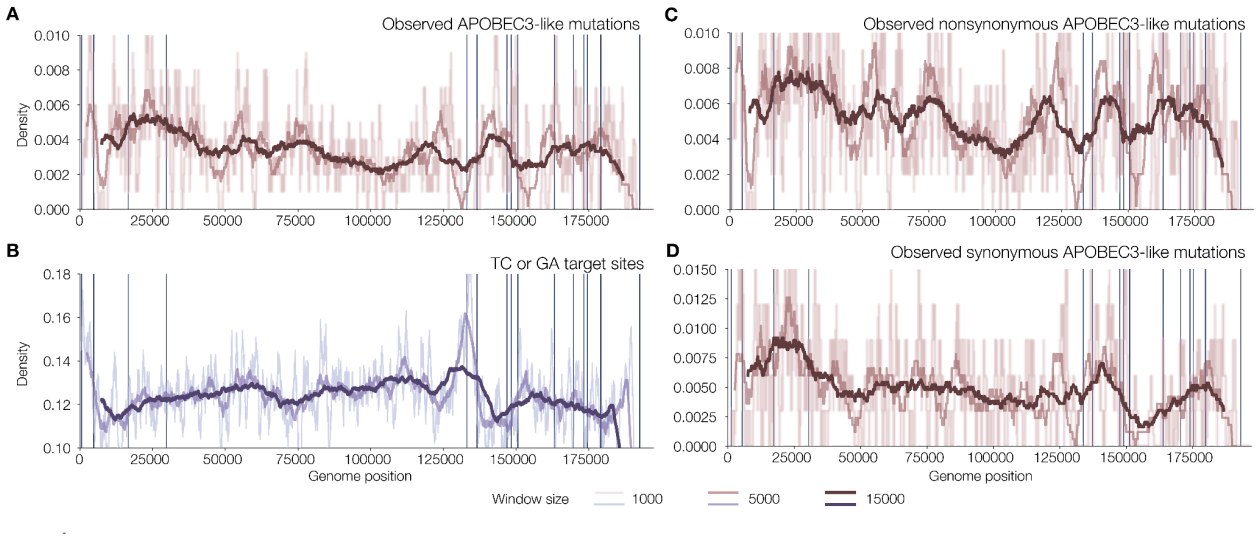

Fig. S11.

The density (moving average linear convolution with window sizes of 1000, 2500 and 15000) of **(A)** observed APOBEC3-like mutations across the MPXV genome, **(B)** available APOBEC3 target sites in the Clade IIb reference genome (Accession NC_063383), **(C)** observed APOBEC-like mutations that result in synonymous changes and **(D)** observed APOBEC-like mutations that result in nonsynonymous changes. We see the same difference between target density for both observed synonymous and non-synonymous distributions (Kolgorov-Smirnov test statistic synonymous vs target = 0.1133, P-value=0.0003; Kolgorov-Smirnov test statistic non-synonymous vs target = 0.067, P-value=0.059). Repetitive/ low-complexity masked regions indicated by vertical bars.

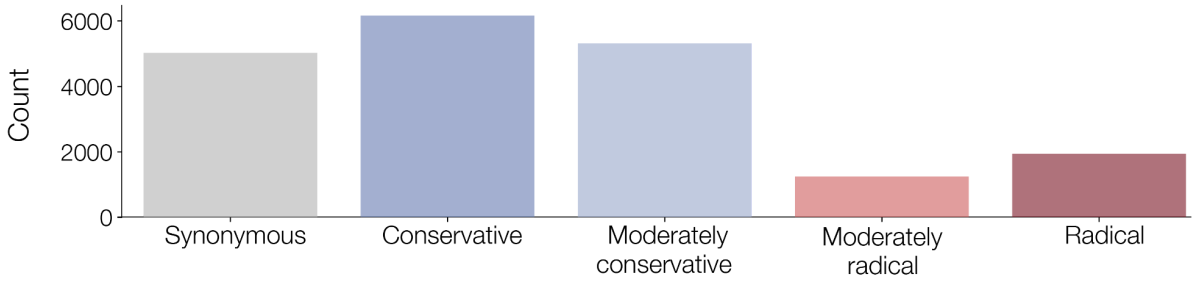

Fig. S12.

Amino acid mutations at TC and GA dimer sites in a reference MPXV genome (accession number NC_063383) that could occur through APOBEC3 editing. Barplot of amino acid changes categorised by Grantham Score (0-50 conservative, 51-100 moderately conservative, 101-150 moderately radical, >150 radical).

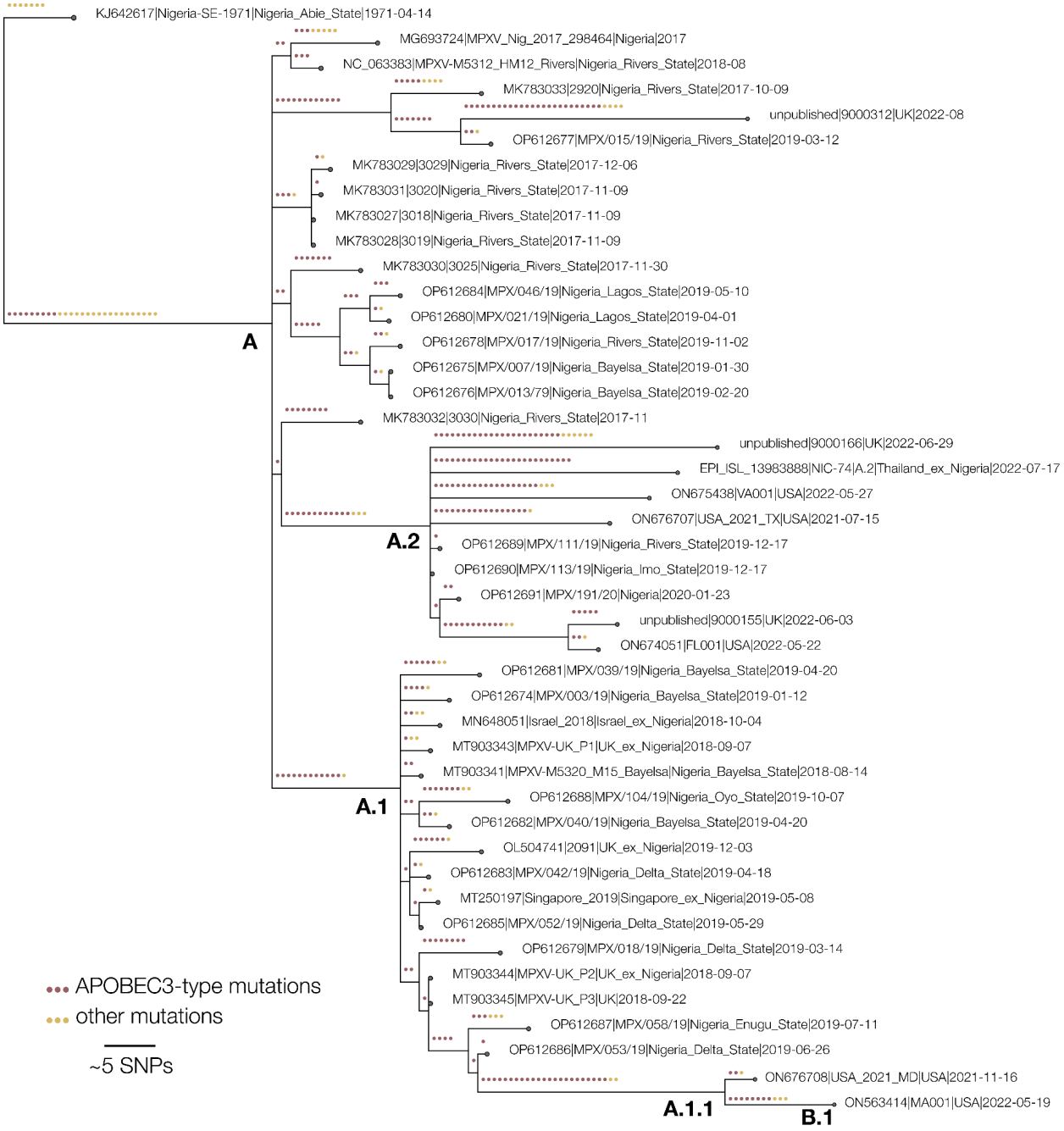

Fig. S13.

Tip-labelled phylogeny of Clade IIb of MPXV. Reconstructed SNPs are coloured by whether or not the mutation type is consistent with APOBEC3 editing. Lineages are labelled on the tree and only a single B.1 lineage representative is present.

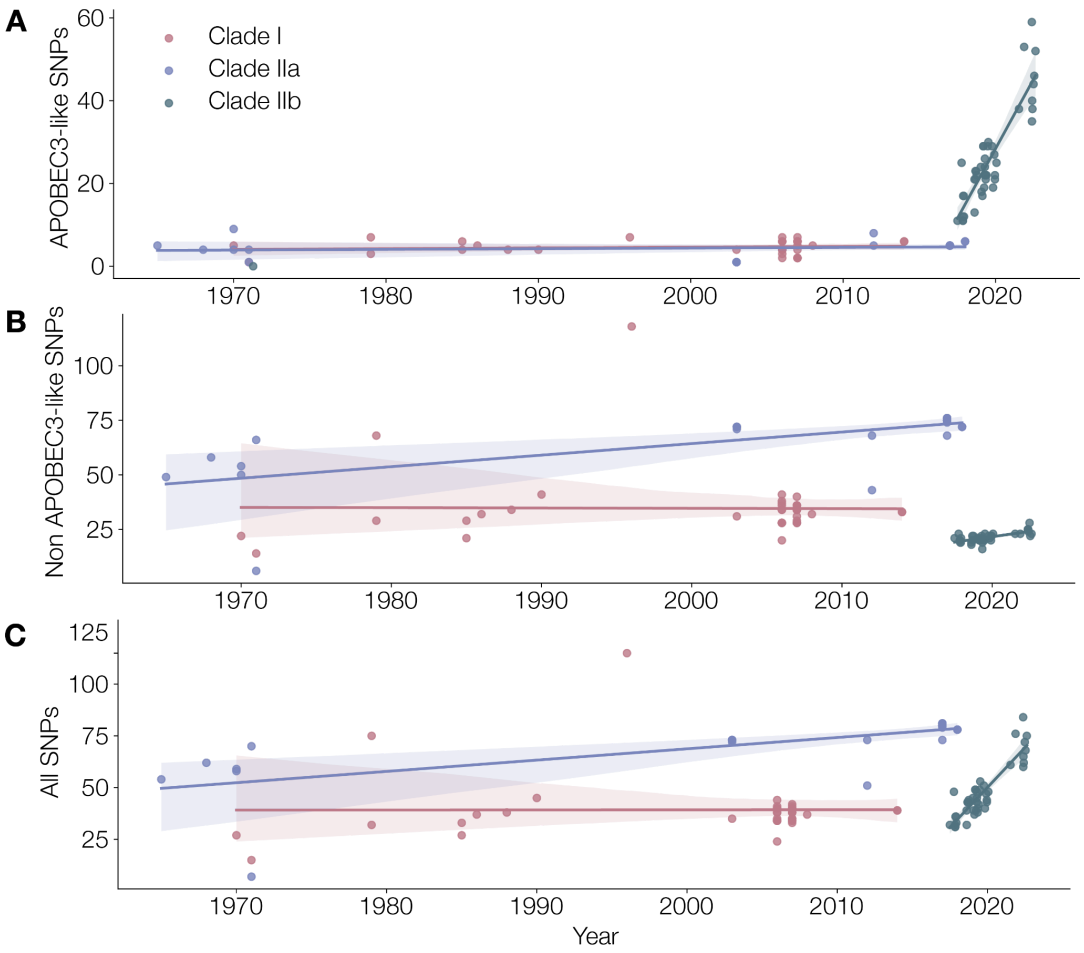

Fig. S14.

Root-to-tip of **(A)** APOBEC3-like, **(B)** non-APOBEC3-like and (**C)** all SNPs for each of the Clade I, Clade IIa and Clade IIb phylogenies. We fit a simple linear model through this data and the distinction between the accumulation of APOBEC3-like SNPs in Clade IIb in comparison to Clade I and IIa is striking, however there is uneven and sparse sampling for Clades I and IIa over greater time periods than for Clade IIb.

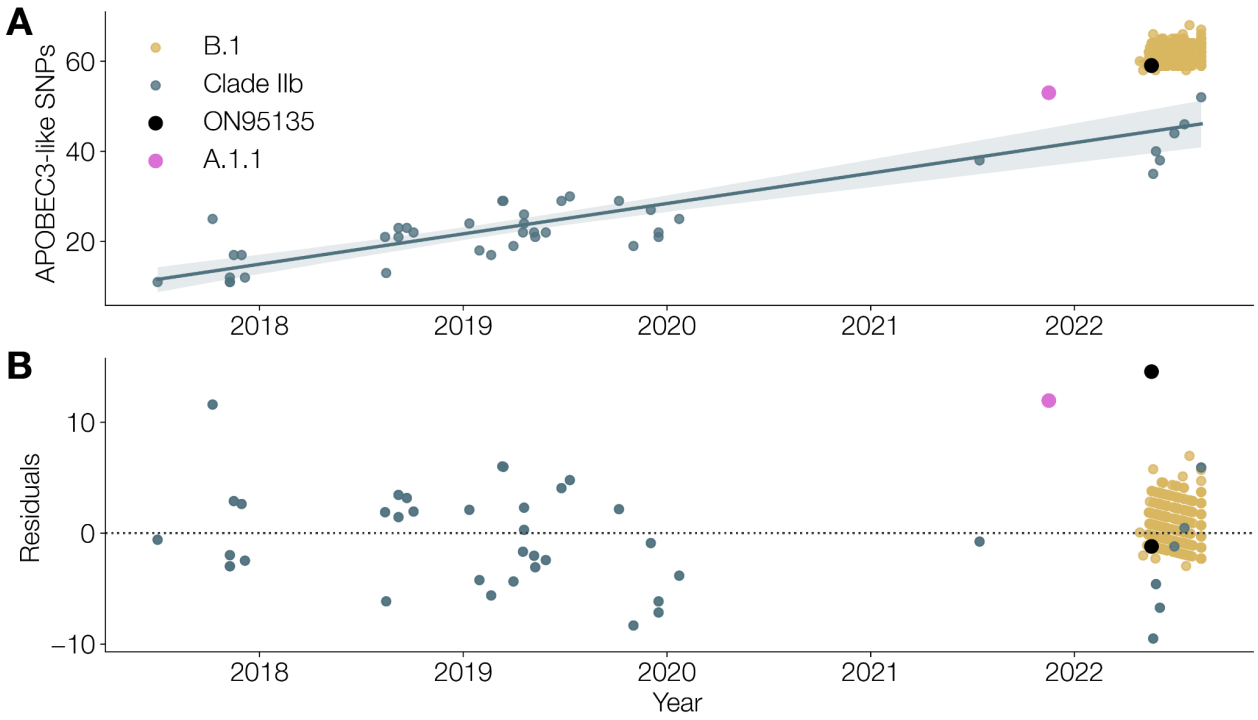

Fig. S15.

(A) Independent linear regressions of APOBEC3 accumulation from root to tip of Clade IIb 2017-2022 and lineage B.1 respectively. The overlapping data point (sequence MA001 – an early B.1 genome, accession number ON563414) is indicated. **(B)** Residuals of each linear regression in panel A demonstrate this upward shift in APOBEC3 accumulation in the B.1 lineage. However, this is mostly shared with lineage A.1.1, indicated, and this transient increase is not one that is not necessarily outside the bounds of expected noise about the regression.

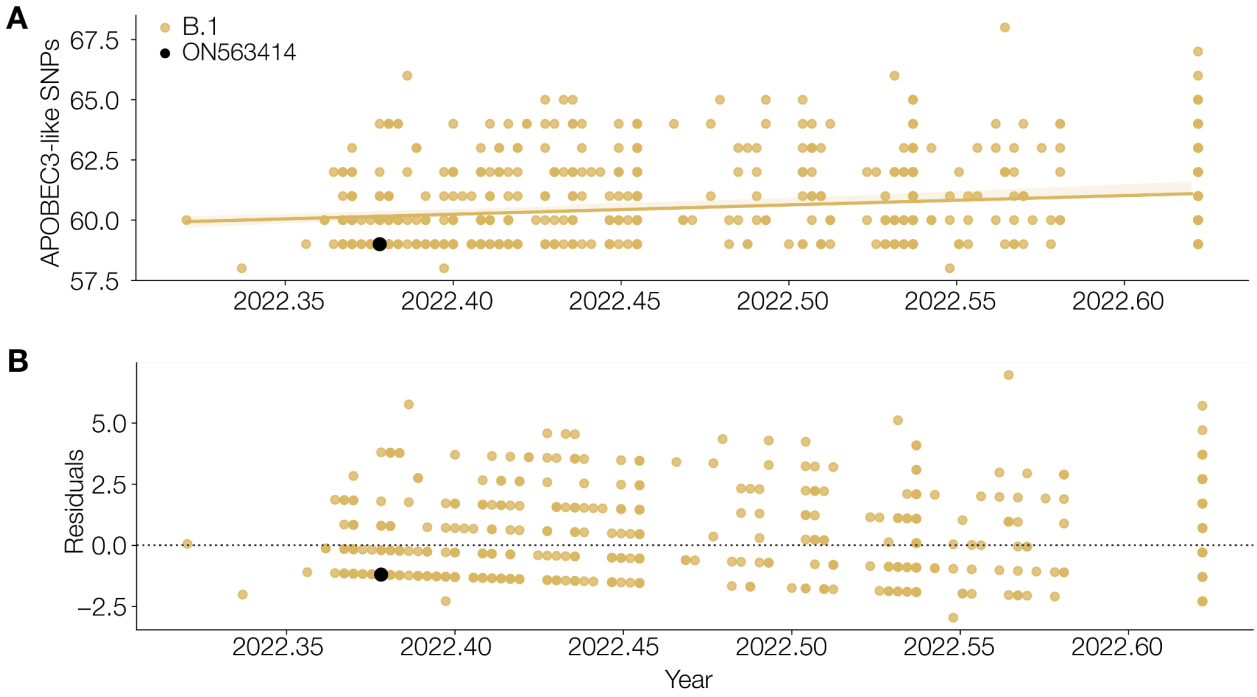

Fig. S16.

(A) Linear regression of APOBEC3 accumulation from root to tip of lineage B.1 with respect to the root of hMPXV. The overlapping data point used in both phylogenies (Sequence MA001, accession number ON563414) indicated. **(B)** Residuals of the linear regression in panel A about the B.1 lineage.

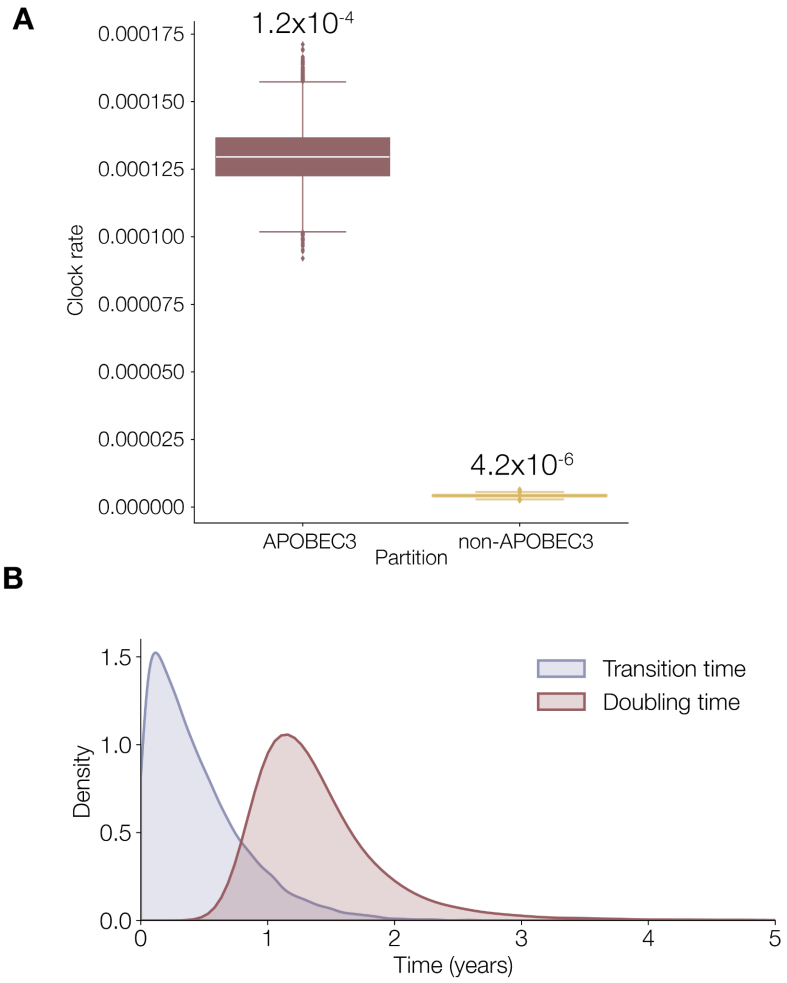

Fig. S17.

(A) Clock rate of APOBEC3 and non-APOBEC3 partitions, estimated using the epoch model. **(B)** The data suggests that the primary case (tPC) occurred less than a year before the most recent common ancestor (tMRCA) of the MPXV samples. The likely doubling time of the outbreak is ~1.5 years.

Table S1.

Authors, Institute and Accession numbers for sequences in analysis of hMPXV-1. Accession numbers in bold highlight the sequences from samples taken between 2018 and 2022 from individuals with travel history from Nigeria. The table includes 25 Clade IIa sequences, 44 Clade IIb sequences and 36 Clade I sequences.

Authors	Institute	Accessions	Clade
Nakazawa Y, Mauldin MR, Emerson GL, Reynolds MG, Lash RR, Gao J, Zhao H, Li Y, Muyembe JJ, Kingebeni PM, Wemakoy O, Malekani J, Karem KL, Damon IK, Carroll DS.	Poxvirus Program, Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, Atlanta, GA 30333, USA	KJ642615	IIb
Faye O, Pratt CB, Faye M, Fall G, Chitty JA, Diagne MM, Wiley MR, Yinka-Ogunleye AF, Aruna S, Etebu EN, Aworabhi N, Ogoina D, Numbere W, Mba N, Palacios G, Sall AA, Ihekweazu C.	Center for Genome Sciences, USAMRIID, 1425 Porter Street, Fort Detrick, Frederick, MD 21701, USA	MG693724	IIb
Yinka-Ogunleye A, Aruna O, Dalhat M, Ogoina D, McCollum A, Disu Y, Mamadu I, Akinpelu A, Ahmad A, Burga J, Ndoreraho A, Nkunzimana E, Manneh L, Mohammed A, Adeoye O, Tom-Aba D, Silenou B, Ipadeola O, Saleh M, Adeyemo A, Nwadiutor I, Aworabhi N, Uke P, John D, Wakama P, Reynolds M, Mauldin MR, Doty J, Wilkins K, Musa J, Khalakdina A, Adedeji A, Mba N, Ojo O, Krause G, Ihekweazu C	NCEZID/DHCPP/PRB, CDC, 1600 Clifton Rd, Atlanta, GA 30333, USA	MK783027 MK783028 MK783029 MK783031 MK783030 MK783032 MK783033	IIb
Cohen Gihon,I., Israeli,O., Shifman,O., Erez,N., Melamed,S., Paran,N., Beth-Din,A. and Zvi,A.	Institute for Biological Research, Reuven 24, Ness Ziona 74100, Israel	**MN648051**	IIb
Yong SEF, Ng OT, Ho ZJM, Mak TM, Marimuthu K, Vasoo S, Yeo TW, Ng YK, Cui L, Ferdous Z, Chia PY, Aw BJW, Manauis CM, Low CKK, Chan G, Peh X, Lim	National Public Health Laboratory, National Centre for Infectious Diseases, 16 Jln Tan Tock Seng, Singapore, Singapore 308442, Singapore	**MT250197**	IIb

PL, Chow LPA, Chan M, Lee VJM, Lin RTP, Heng MKD, Leo YS			
Mauldin MR, McCollum AM, Nakazawa YJ, Mandra A, Whitehouse ER, Davidson W, Zhao H, Gao J, Li Y, Doty J, Yinka-Ogunleye A, Akinpelu A, Aruna O, Naidoo D, Lewandowski K, Afrough B, Graham V, Aarons E, Hewson R, Vipond R, Dunning J, Chand M, Brown C, Cohen-Gihon I, Erez N, Shifman O, Israeli O, Sharon M, Schwartz E, Beth-Din A, Zvi A, Mak TM, Ng YK, Cui L, Lin RTP, Olson VA, Brooks T, Paran N, Ihekweazu C, Reynolds MG.	NCEZID/DHCPP/PRB, CDC, 1600 Clifton Rd, Atlanta, GA 30333, USA	MT903341 **MT903343** **MT903344** MT903345 NC_063383	IIb
Gigante,C.M., Myers,R., Seabolt,M.H., Wilkins,K., McCollum,A., Hutson,C., Davidson,W., Rao,A., Blythe,D. and Li,Y.	Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30329, USA	ON563414 ON674051 ON675438 ON676707 ON676708	IIb
Pilailuk Okada; Siripaporn Phuygun; Nuttida Thongpramul; Thanutsapa Thanadachakul; Kazuhisa Okada; Archawin Rojanawiwat; Chakkarat Pitayawonganon; Supakit Sirilak	Bangkok Hospital Phuket, 2/1 Hongyok Utis Road, Muang District, Phuket, 83000, Thailand | National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand, 88/7 Tiwanon Road, Amphoe Muang, Nonthaburi 11000, Thailand	**EPI_ISL_13983888**	IIb
Ndodo,N., Ashcroft,J., Lewandowski,K., Yinka-Ogunleye,A., Chukwu,C., Ahmad,A., King,D., Akinpelu,A., Maluquer de Motes,C., Ribeca,P., Summer,R.P., Rambaut,A., Chester,M., Maishman,T., Babatunde,O., Mba,N., Babatunde,O., Aruna,O., Pullan,S.T., Gannon,B., Brown,C., Ihekweazu,C.,	Nigeria Centre for Disease Control, National Reference Laboratory, Gaduwa, Federal Capital Territory, Abuja | Defence Science and Technology Laboratory, Porton Down, Salisbury SP4 0JQ, Wiltshire	OP612674 OP612675 OP612676 OP612677 OP612678 OP612679 OP612680 OP612681 OP612682 OP612683 OP612684 OP612685 OP612686	IIb

Adetifa,I. and Ulaeto,D.O.		OP612687 OP612688 OP612689 OP612690 OP612691	
Groves,N., Osman,K.L., Lewandowski,K.S., Carter,D.P., Pullan,S.T., Myers,R., Vipond,R. and Chand,M.	Research and Evaluation, UKHSA, Porton Down, Salisbury, Wiltshire SP4 0JG, UK	OP331335 OP331336 OP415257	IIb
Atkinson,B., Pottage,T., Ngabo,D., Crook,A., Pitman,J., Summers,S., Pullan,S., Lewandowski,K., Furneaux,J., Davies,K. and Brooks,T.	UKHSA, Manor Farm Road, Porton, Wiltshire SP4 0JG, UK	**OL504741**	IIb
Kugelman,J.R., Johnston,S.C., Mulembakani,P.M., Kisalu,N., Lee,M.S., Koroleva,G., McCarthy,S.E., Gestole,M.C., Wolfe,N.D., Fair,J.N., Schneider,B.S., Wright,L.L., Huggins,J., Whitehouse,C.A., Wemakoy,E.O., Muyembe-Tamfum,J.J., Hensley,L.E., Palacios,G.F. and Rimoin,A.W.	United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, USA	JX878407 JX878408 JX878409 JX878410 JX878411 JX878412 JX878413 JX878414 JX878415 JX878416 JX878417 JX878418 JX878419 JX878420 JX878421 JX878422 JX878423 JX878424 JX878425 JX878427 JX878426 JX878428 JX878429	I
Likos,A.M., Sammons,S.A., Olson,V.A., Frace,A.M., Li,Y., Olsen-Rasmussen,M., Davidson,W., Galloway,R., Khristova,M.L., Reynolds,M.G., Zhao,H., Carroll,D.S., Curns,A., Formenty,P., Esposito,J.J., Regnery,R.L. and Damon,I.K	National Center for Infectious Disease, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Mailstop G43, Atlanta, GA 30333, USA.	DQ011155 DQ011154	I

Farlow,J., Ichou,M.A., Huggins,J. and Ibrahim,S.	The United States Army Medical Research Institute for Infectious Diseases (USAMRIID), 1425 Porter Street, Frederick, MD 21702, USA	HM172544	I
Shchelkunov,S.N., Totmenin,A.V., Babkin,I.V., Safronov,P.F.,Ryazankina,O .I., Petrov,N.A., Gutorov,V.V., Uvarova,E.A., Mikheev,M.V., Sisler,J.R., Esposito,J.J., Jahrling,P.B., Moss,B. and Sandakhchiev,L.S.	Department of Molecular Biology of Genomes, SRC VB Vector, Koltsovo, Novosibirsk Region 630559, Russia	AF380138	I
Nakazawa,Y., Emerson,G.L., Carroll,D.S., Zhao,H., Li,Y., Reynolds,M.G., Karem,K.L., Olson,V.A., Lash,R.R., Davidson,W.B., Smith,S.K., Levine,R.S., Regnery,R.L., Sammons,S.A., Frace,M.A., Mutasim,E.M., Karsani,M.E., Muntasir,M.O., Babiker,A.A., Opoka,L., Chowdhary,V. and Damon,I.K.	Biochemistry and Microbiology, University of Victoria, 3800 Finnerty Road, Victoria, BC V8P 5C2, Canada	KC257460 KP849469 KJ642612 KJ642613 KJ642618 KJ642619 KP849471	I
Marien,J., Laudisoit,A., Patrono,L.V., Calvignac-Spencer,S., Leendertz,F., Leirs,H. and Verheyen,E.	University of Antwerp, Universiteitsplein 1, Antwerpen 2000, Belgie	MT724770 MT724772	I
Likos,A.M., Sammons,S.A., Olson,V.A., Frace,A.M., Li,Y., Olsen-Rasmussen,M., Davidson,W., Galloway,R., Khristova,M.L., Reynolds,M.G., Zhao,H., Carroll,D.S., Curns,A., Formenty,P., Esposito,J.J., Regnery,R.L. and Damon,I.K	National Center for Infectious Disease, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Mailstop G43, Atlanta, GA 30333, USA.	DQ011156.1 DQ011157.1	IIa
Nakazawa,Y., Emerson,G.L., Carroll,D.S., Zhao,H., Li,Y.,	Biochemistry and Microbiology, University of Victoria, 3800 Finnerty	KP849470.1 KJ642616.1 KJ642614.1	IIa

Reynolds,M.G., Karem,K.L., Olson,V.A., Lash,R.R., Davidson,W.B., Smith,S.K., Levine,R.S., Regnery,R.L., Sammons,S.A., Frace,M.A., Mutasim,E.M., Karsani,M.E., Muntasir,M.O., Babiker,A.A., Opoka,L., Chowdhary,V. and Damon,I.K.	Road, Victoria, BC V8P 5C2, Canada		
Marien,J., Laudisoit,A., Patrono,L.V., Calvignac-Spencer,S., Leendertz,F., Leirs,H. and Verheyen,E.	University of Antwerp, Universiteitsplein 1, Antwerpen 2000, Belgie	MT724769.1	IIa
Patrono,L.V., Pleh,K., Samuni,L., Ulrich,M., Roethemeier,C., Sachse,A., Muschter,S., Nitsche,A., Couacy-Hymann,E., Boesch,C., Wittig,R.M., Calvignac-Spencer,S. and Leendertz,F.H.	Project Group Epidemiology of Highly Pathogenic Microorganisms, Robert Koch Institute, Seestrasse 10, Berlin 13353, Germany	MN346698.1 MN346691.1 MN346700.1 MN346696.1 MN346695.1 MN346692.1 MN346699.1 MN346701.1 MN346694.1 MN346690.1 MN346697.1 MN346693.1 MN346703.1 MN346702.1 KJ136820.1	IIa
Chen,N., Li,G., Liszewski,M.K., Atkinson,J.P., Jahrling,P.B., Feng,Z., Schriewer,J., Buck,C., Wang,C., Lefkowitz,E.J., Esposito,J.J., Harms,T., Damon,I.K., Roper,R.L., Upton,C. and Buller,R.M.	Biochemistry and Microbiology, University of Victoria, Petch Building, University of Victoria, P.O. Box 3055 STN CSC, Victoria, B.C. V8W 3P6, Canada	AY741551.1	IIa
Mauldin,M.R., McCollum,A.M., Nakazawa,Y.J., Mandra,A., Whitehouse,E.R., Davidson,W., Zhao,H., Gao,J., Li,Y., Doty,J., Yinka-Ogunleye,A., Akinpelu,A., Aruna,O., Naidoo,D.,	CDC, 1600 Clifton Rd, Atlanta, GA 30333, USA	MT903346 MT903348.1 MT903347.1	IIa

Lewandowski,K., Afrough,B., Graham,V., Aarons,E., Hewson,R., Vipond,R., Dunning,J., Chand,M., Brown,C., Cohen-Gihon,I., Erez,N., Shifman,O., Israeli,O., Sharon,M., Schwartz,E., Beth-Din,A., Zvi,A., Mak,T.M., Ng,Y.K., Cui,L., Lin,R.T.P., Olson,V.A., Brooks,T., Paran,N., Ihekweazu,C. and Reynolds,M.G.

Table S2.

Authors, institute and accession numbers for sequences in Fig. S7.

Authors	Institute	Accession numbers
Zakotnik,S., Vlaj,D., Suljic,A., Zorec,T.M., Skubic,C., Rozman,D., Korva,M., Poljak,M. and Avsic Zupanc,T.	Laboratory for Diagnostics of Zoonoses and WHO Centre, Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloska 4, Ljubljana 1000, Slovenia	ON609725;ON631241;ON754987;ON754986;ON754985;ON754984;ON838178
Groves,N., Osman,K.L., Lewandowski,K.S., Pullan,S.T., Carter,D.P., Crook,J.M., Myers,R., Vipond,R. and Chand,M.	Research and Evaluation, UKHSA, Porton Down, Salisbury, Wiltshire SP4 0JG, UK	OP205140;OP205139;OP205138;OP205137;OP205136;OP205135;OP205134;OP205133;OP205132;OP205131;OP205130;OP205129;OP205128;OP205127;OP205126;OP205125;OP205124;OP205123;OP205122;OP205121;OP205120;OP205119;OP205118;OP205117;OP205116;OP205115;OP205114;OP205113;OP205112;OP205111;OP205110;OP205109;OP205108;OP205107;OP205106;OP205105;OP205104;OP205103;OP205102;OP205101;OP205100;OP205099;OP205098;OP205097;OP205096;OP205095;OP205094;OP205093;OP205092;OP205091;OP205090;OP205089;OP205088;OP205087;OP205086;OP205085;OP205084;OP205083;OP205082;OP205081;OP205080;OP205079;OP205078;OP205077;OP205076;OP205075;OP205074;OP205073;OP205072;OP205071;OP205070;OP205069;OP205068;OP205067;OP022170;OP022171;ON619838;ON619837;ON619836;ON619835
Chmel,M., Pajer,P., Nagy,A., Zlamal,M., Jirincova,H., Dresler,J. and Bartos,O.	Military Heatlh Institute in Prague, Military Heatlh Institute, U Vojenske nemocnice 1200/1, Prague 16200, Czech Republic	ON983168
Israeli,O., Guedj-Dana,Y., Lazar,S., Shifman,O., Erez,N., Weiss,S., Paran,N., Israely,T., Schuster,O., Zvi,A., Beth-Din,A. and Cohen Gihon,I.	Biochemistry and Molecular Genetics, Israel Institute for Biological Research, Reuven 24, Ness Ziona 7410001, Israel	ON649879
Licastro,D., DeGasperi,M., Negri,C., Piscianz,E., Koncan,R., Dal Monego,S., Segat,L. and D'Agaro,P.	Genomics and Epigenomics, AREA Science Park, km 163,5 Strada Statale 14, Basovizza, Friuli Venezia Giulia 34149, Italy	ON644344

Authors	Institute	Accession numbers
Hammerschlag,Y., MacLeod,G., Papadakis,G., Adnan Sanchez,A., Druce,J., Savic,I., Taiaroa,G., Roberts,J., Caly,L., Williamson,D.A., Cheng,A.C. and McMahon,J.H.	Victorian Infectious Diseases Reference Laboratory Doherty Institute 792 Elizabeth Street Melbourne Victoria 3000 Australia	ON631963
Rhie,G.-E.	Division of High-risk Pathogens Korea Disease Control and Prevention Agency 187 Osongsaengmyeong2-ro Osong-eup Heungdeok-gu Cheongju Chungcheongbuk-do 28159 Republic of Korea	OP204857
De Baetselier,I., Van Dijck,C., Kenyon,C., Coppens,J., Smet,H., de Block,T., Coppens,S., Vanroye,F., Bugert,J., Girl,P., Liesenborghs,L., Selhorst,P., Arien,K., Van den Bossche,D., Florence,E., Rezende,A.M., Vercauteren,K. and Van Esbroeck,M.	Department of Clinical Sciences Institute of Tropica Medicine Nationalestraat 155 Antwerp Antwerp 2000 Belgium	ON950045;OP144217;OP144216;OP144215;OP144214;OP144213;OP144212;OP144211
Sereewit,J., Xie,H., Roychoudhury,P. and Greninger,A.L.	Laboratory Medicine UW Virology 1616 Eastlake Ave East Suite 320 Seattle WA 98102 USA	OP055801;OP123055;OP123054;OP123053;OP123052;OP123051;OP123050;OP123049;OP123048;OP123047;OP123046;OP123045;OP123044;OP123043;OP123042;OP123041;OP123040;OP055809;OP055808;OP055807;OP055806;OP055805;OP055804;OP055803;OP055802;OP055800;OP257267;OP257266;OP257265;OP257264;OP257263;OP257262;OP257261;OP257260;OP257259;OP257258;OP257257;OP257256;OP257255;OP257254;OP257253;OP257252;OP257251;OP257250;OP257249;OP257248;OP257247;OP257246;OP257245;OP257244;OP257243;OP184765;OP184764;OP184763;OP184762;OP184761;OP184760;OP169346;OP169345;OP169344;OP169343;OP169342;OP169341;OP169340;OP169339;OP169338;OP169337;OP169336
Gigante,C.M., Hughes,S., Seabolt,M.H., Zhao,H., Wilkins,K., Respress,J., Howard,D., Batra,D., McCollum,A., Hutson,C., Davidson,W., Rao,A., Baumgartner,J. and Li,Y.	Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30329, USA	OP185716;OP185715;OP185714;OP185713;OP225967;OP225965;OP185712;OP225969;OP225966;OP225964;OP225962;OP185711;OP185710;OP225960;OP185709;ON959136;ON959135;ON959134;ON959133;ON959132;ON959131;ON954773;ON676708;ON676706;ON676705;ON676704;ON67670

Authors	Institute	Accession numbers
		3;ON563414;OP225968;OP225963;O P225961;OP185717;OP150927;OP15 0926;OP150925;OP150924;OP15092 3
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Table S3.

Regions masked in alignment.

Name	Type	Minimum	Maximum	Length
repetitive region	misc_feature	16609	16622	14
repetitive region	misc_feature	29749	29762	14
homopolymeric run	misc_feature	148334	148351	18
repetitive region	misc_feature	163190	163214	25
repetitive region	misc_feature	174518	174545	28
repetitive region	misc_feature	173274	173317	44
repetitive region	misc_feature	169721	169774	54
repetitive region	misc_feature	136513	136569	57
repetitive region	misc_feature	146848	146912	65
repetitive region	misc_feature	150542	150628	87
repetitive region	misc_feature	192404	192529	126
repetitive region	misc_feature	4680	4806	127
repetitive region	misc_feature	179057	179245	189
homopolymeric run	misc_feature	592	596	5
homopolymeric run	misc_feature	133071	133115	45

Table S4.

BEAST estimated statistics

Summary Statistic [95%HPD]	Epoch Model	Skygrid Model
Time of ingroup MRCA[1]	2016.147 [2015.489, 2016.744]	2016.548 [2015.951,2017.000]
Human emergence time[2]	2015.703 [2014.638, 2016.583]	N/A
APOBEC3 clock rate	1.298E-4 [1.099E-4, 1.502E-4]	1.407E-4 [1.197E-4, 1.609E-4]
non-APOBEC3 clock rate	4.224E-6 [3.215E-6, 5.177E-6]	6.692E-6 [4.824E-6, 8.626E-6]
Exponential Growth rate	0.550 [0.2374, 0.8686]	N/A

[1] The time of the most recent ancestor of the ingroup comprising all Clade IIb genomes other than those from the 1970's (the outgroup).

[2] The time, in years, prior to the MRCA of the ingroup, of the transition point to the APOBEC3 clock rate