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Monkeypox virus emergence in wild chimpanzees reveals distinct clinical outcomes and viral diversity

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Monkeypox is a viral zoonotic disease on the rise across endemic habitats. Despite the growing importance of monkeypox virus, our knowledge on its host spectrum and sylvatic maintenance is limited. Here, we describe the recent repeated emergence of monkeypox virus in a wild, human-habituated western chimpanzee (*Pan troglodytes verus*, hereafter chimpanzee) population from Taï National Park, Ivory Coast. Through daily monitoring, we show that further to causing its typical exanthematous syndrome, monkeypox can present itself as a severe respiratory disease without a diffuse rash. By analysing 949 non-invasively collected samples, we identify the circulation of at least two distinct monkeypox virus lineages and document the shedding of infectious particles in faeces and flies, suggesting that they could mediate indirect transmission. We also show that the carnivorous component of the Taï chimpanzees' diet, mainly consisting of the sympatric monkeys they regularly hunt, did not change nor shift towards rodent consumption (the presumed reservoir) before the outbreaks, suggesting that the sudden emergence of monkeypox virus in this population is probably due to changes in the ecology of the virus itself. Using long-term mortality surveillance data from Taï National Park, we provide evidence of little to no prior viral activity over at least two decades. We conclude that great ape sentinel systems devoted to the longitudinal collection of behavioural and health data can help clarify the epidemiology and clinical presentation of zoonotic pathogens.

The heightened activity of monkeypox virus (MPXV) in endemic areas[1] and recent identification of imported monkeypox cases in Eurasia[2–4] are challenging its definition of a rare zoonosis occurring in African rainforests. MPXV is a member of the genus *Orthopoxvirus*, family *Poxviridae*. The virus independently circulates in Western and Central Africa, as shown by the existence of two distinct genomic lineages in these regions, the West African (WA) and Congo Basin clades. In Central Africa, MPXV infections have a higher incidence and viruses of the Congo Basin clade seem to be more virulent[5,6]. Monkeypox is known as an exanthematous syndrome hardly distinguishable from smallpox, a disease eradicated through vaccination in the late 1970s caused by another orthopoxvirus, variola virus. Smallpox immunization has partially contributed to protecting the population born before 1980 from infections with other orthopoxviruses[7]. The global waning immunity, improved surveillance and increased contact with potential reservoirs due to habitat encroachment have been jointly considered as factors contributing to the recent rise of monkeypox case notifications[1,8,9]. More than 60 years after its discovery in captive crab-eating macaques (*Macaca fascicularis*)[10], MPXV ecology in wildlife however remains poorly understood[11]. To date, MPXV has been isolated from only two wild animals, a Thomas's rope squirrel (*Funisciurus anerythrus*) in the Democratic Republic of the Congo[12] and a sooty mangabey (*Cercocebus atys atys*) in Taï National Park (TNP)[13]. African rodents of several species are considered likely reservoirs, although serological and molecular evidence is still sparse[11,14].

Since 2000, we have performed veterinary monitoring of three neighbouring communities (north, south and east; Fig. 1) of wild chimpanzees in TNP[15]. This chimpanzee population has been habituated to continuous human presence and the object of a long-term behavioural study[16] since 1979, resulting in 93 recognized individuals (as of 2018). Chimpanzees living in rainforests represent a unique sentinel system, inasmuch as our closest genetic and behavioural relatives are exposed to pathogens of public health importance[17]. Through daily health observations by trained staff and non-invasive sampling of chimpanzee faeces, alongside necropsies performed on all chimpanzees and other wildlife found dead in the research area, we have been investigating the circulation of infectious agents in this habitat, a number of which were directly relevant to human health. After 16 years of anthrax mortalities[18] and outbreaks caused by human respiratory pathogens[19], in 2017 we started observing signs compatible with MPXV infection appear in all communities over a 16-month period. Making use of the unique collection of samples and observations gathered over decades of research at TNP, we investigated the epidemiology of MPXV outbreaks.

Results and discussion

MPXV infection in wild chimpanzees can cause severe respiratory distress and absent to diffuse cutaneous rash. In mid-January 2017, an infant and an adolescent chimpanzee in the south community ($n = 36$) showed severe respiratory signs that resolved by early February. These included coughing, breathing with the mouth open, and nasal and ocular discharge (Supplementary Videos 1 and 2

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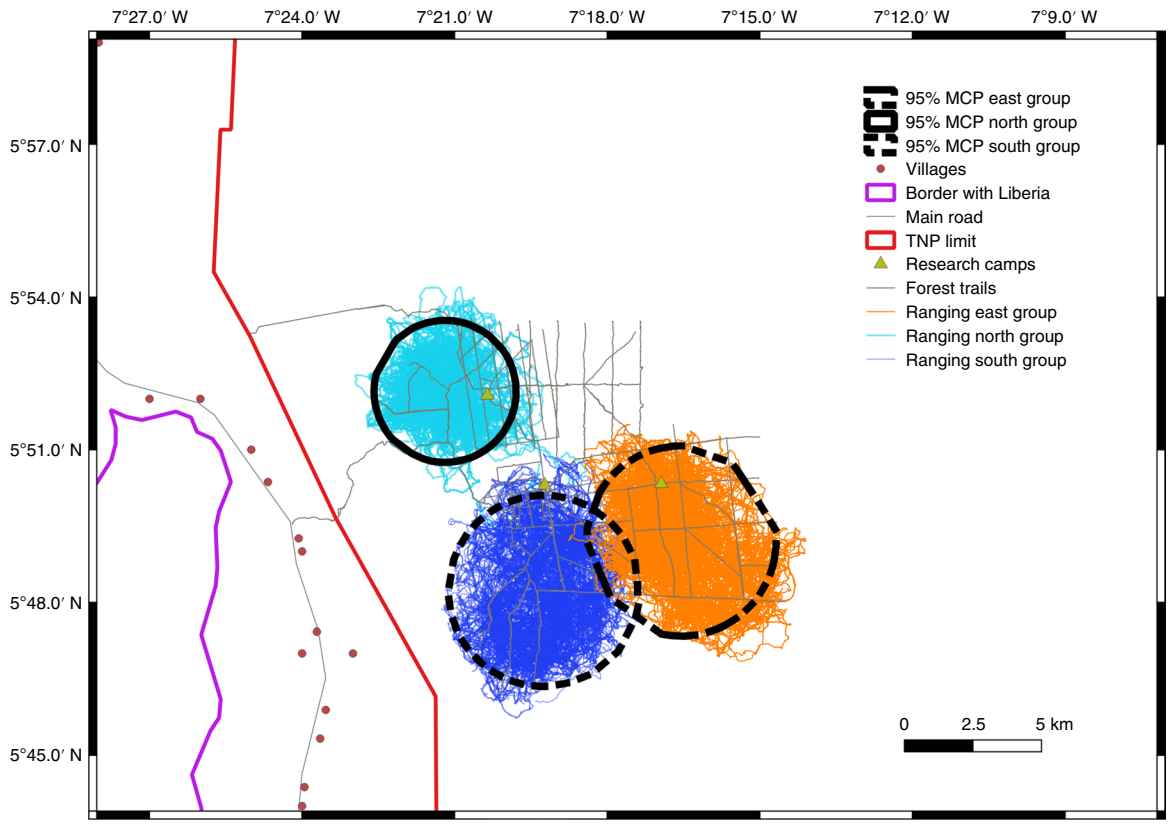

Fig. 1 | Map of the chimpanzee territories in TNP. Shown are the home ranges of the north (turquoise), south (blue) and east (orange) chimpanzee communities over a one-year period. The axes refer to the position of the research site with respect to the equator (N) and the Greenwich meridian (W). The black circles indicate the 95% minimum convex polygon (MCP), which represents the simplest approach to draw home-range boundaries.

and Supplementary Information). A single vesicular lesion was visible on their lips (Fig. 2a and Supplementary Video 1 and 2). In spite of our awareness of the presence of MPXV in this habitat[13], the repeated occurrence of acute disease caused by human respiratory viruses in this population[19], and the absence of diffuse exanthema, delayed the suspicion (and diagnosis) of MPXV to early March. At this time, a major outbreak hit the north community ($n=21$) with remarkably different clinical presentations over its 1-month duration (Supplementary Information). Three infant chimpanzees, including a fatal case, manifested the classical course characterized by a 1- to 3-day prodromal phase followed by a centrifugal cutaneous rash. A uniquely respiratory syndrome ranging from mild coughing to severe respiratory distress was observed in seven additional chimpanzees. The latest emergence consisted of a single case, observed in May 2018 in the east community ($n=36$). This infant chimpanzee developed systemic signs and a cutaneous rash (Fig. 2b, Extended Data Fig. 1, Supplementary Information and Supplementary Videos 3–5). Overall, in two of three instances, cases presenting predominantly respiratory syndromes were observed (Extended Data Fig. 2). Upon discarding a viral determinant (see below), individual susceptibility and transmission route may partially explain these observations. Infection with MPXV can occur via respiratory, mucosal or percutaneous exposure. These routes of exposure have been shown to influence disease manifestation and illness severity in both natural and experimental infections[20], with percutaneous exposure resulting in more severe illness and exanthema[21]. We can thus speculate that further to whether chimpanzees were exposed to a primary source of infection (for example, reservoir or accidental host) or acquired the infection from another chimpanzee, the route by which the virus entered the body may have determined the clinical manifestation. Current guidelines on monkeypox recognition in humans are centred on the presence of a centrifugal cutaneous rash[22]. The 2003 outbreak in the United States of America caused by a MPXV of the WA clade provided preliminary evidence of cases with a milder rash than in the classical definition[23]. Our observations show that in our closest relatives, natural infection with viruses of the WA clade can cause a respiratory syndrome with limited to absent exanthema. In endemic countries, MPXV infection in humans might be contributing to respiratory syndromes of unknown origin and therefore be markedly under-reported.

Genomic characterization reveals involvement of distinct MPXV lineages. Onset of clinical signs in the three communities suggested independent introductions. Accordingly, the eight (north), four (south) and two (east) complete viral genomes that we characterized via in-solution hybridization capture and Illumina sequencing from a necropsy and a variety of non-invasive samples collected during the outbreaks (see below) identified three distinct MPXVs (GenBank accession numbers: north MN346690-697; south MN346698-701; east MN346702-703). These genomes all belonged to the WA clade and differed from the MPXV isolated in 2012 from a sooty mangabey from TNP. No genomic rearrangement was detected. A 16-base-pair (bp) deletion in the *O1L* gene of the virus in the north community, leading to its disruption and the predicted loss of 90% of the encoded protein, was the sole difference from the virus in the south community; accordingly, the viruses in the north and south communities showed identical sequences in the alignment used for phylogenomic analyses (indels were removed from this alignment) and clustered together in a maximum likelihood (ML) tree (Fig. 3a,b). Despite emerging closely in time and being nearly identical, the two viruses did not show evidence of polymorphism for this deletion in any of the individual cases. Combined with the

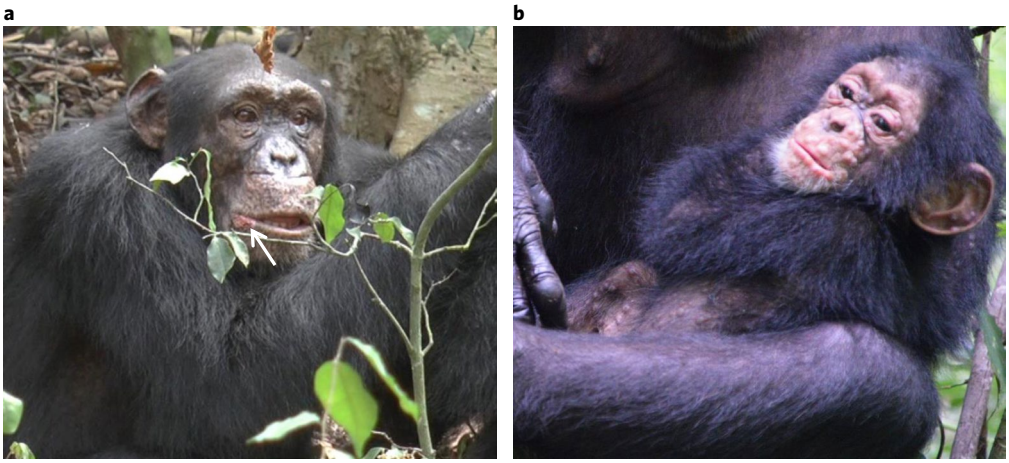

Fig. 2 | Monkeypox lesions in two wild chimpanzees. a, A single vesicular lesion present on the right corner of the mouth (white arrow) of a chimpanzee from the south community infected with MPXV in 2017. **b**, A diffuse rash present on the face and body of the chimpanzee from the east community infected with MPXV in 2018.

fact that no inter-group encounter was reported during this period, this suggests that the two outbreaks are not epidemiologically linked but rather represent separate introductions. Genomic evidence was less ambiguous concerning the virus circulating in the east community, which was separated from the viruses in the north and south communities by 11 single nucleotide polymorphisms and 3 indels, clearly demonstrating that this outbreak corresponded to a distinct emergence event (Fig. 3a,b and Supplementary Information). In the south and east communities, viral genomes identified in different individuals within each community were identical. In the north community, two genomes detected in the late stage of the outbreak presented distinct deletions in the left variable end (Supplementary Information). The two ends of orthopoxviruses' genomes are known to be highly variable with frequent indels[5]. These deletions might either have occurred during the outbreak or reflect multiple introductions from the reservoir(s). Irrespectively, the identification of at least two distinct MPXV lineages (north/south and east)—and most likely three (north, south and east)—is suggestive of measurable genomic variation of MPXV in its natural host(s), even at very small geographical scales (170 km^2).

Molecular clock analyses suggest that MPXV was present in TNP at least since the early 2000s. To investigate the tempo of MPXV diversification and determine the minimum age of the lineage currently circulating in TNP, we ran molecular clock analyses using a genomic sequence dataset restricted to viruses belonging to the WA clade. Preliminary exploration conducted using TempEst (ref. [24]) suggested a molecular-clock-like signal, as indicated by the correlation between tip dates and root-to-tip distances in a ML tree ($R^2 = 0.3894$). To thoroughly explore this, we first used BEAST (ref. [25]) to compare four different models, using our dataset with tip dates. These models essentially associated a molecular clock component (strict or relaxed lognormal) and a component describing the diversification process (tree prior; here, two coalescent models: constant population size or Bayesian skyride). Marginal likelihood estimates (MLEs) of the four models did not differ decisively (2lnBF (Bayes factor) < 10, Supplementary Table 1). Therefore, we selected the simplest model (a strict molecular clock and a coalescent model assuming a constant population size) to run a Bayesian evaluation of temporal signal[26]. This approach is a fully Bayesian model testing strategy aimed at demonstrating that sufficient molecular evolution happened over the considered sampling period to allow for a proper estimation of the substitution rate. It relies on the comparison of the fit of the original heterochronous model (with tip dates) and the corresponding isochronous model (without tip dates). Here, when comparing the MLE of the model with or without tip dates, we found the latter to be decisively lower (2lnBF = 40.6, Supplementary Table 2), thus confirming the presence of a temporal signal in our dataset. Node ages in the heterochronous model are therefore truly informed by the tip-date-derived substitution rate (Fig. 4). We estimated a genome-wide mean substitution rate of 1.93×10^{-6} ($1.21–2.66 \times 10^{-6}$ 95% highest posterior density (HPD)) substitutions per site per year. This estimate falls within the range of what is expected for double-stranded DNA viruses in general[27] and poxviruses in particular[28], but is slower than those recently estimated for myxoma (1×10^{-5})[29] and smallpox (8.5×10^{-6})[30,31] viruses. Focusing on TNP sequences, we found that the median divergence time between the MPXV isolated in 2012 from a sooty mangabey and the MPXVs recently found in the chimpanzees was 2001 (1991–2008 95% HPD) and that between the MPXVs circulating in the north/south and the virus found in the east community was 2006 (1999–2012 95% HPD). These results indicate that the presence of MPXVs in TNP probably preceded the laboratory-confirmed identification in the sooty mangabey[13] by at least a decade.

On a different note, from our Bayesian analyses we observed that all MPXV sequences from Nigeria fell basal in our chronogram. A similar pattern has been described for Lassa virus (LASV), where Nigerian sequences branch off early with respect to all other West African isolates[32]. When comparing the median divergence times of Nigerian MPXVs and LASVs from the rest of the respective WA sequences, these were estimated to be around 1717 (1589–1809 95% HPD) and 1623 (1513–1713 95% HPD)[32], respectively. Both viruses recently caused outbreaks of unprecedented proportions in humans in Nigeria and have an either suspected or well-known rodent reservoir. Additional genomic and epidemiological data might reveal whether these parallels are purely random or reflect ecological similarities between the two viruses.

Non-invasive sampling reveals MPXV DNA shedding in symptomatic and asymptomatic chimpanzees. To investigate the onset and spread of MPXV infection, we analysed 492 faecal, 55 urine and 26 fruit-wedge samples collected before, during and after each outbreak (Extended Data Fig. 3). Faeces and urine are collected immediately after direct observation of excretion by trained field personnel, who are capable of recognizing each chimpanzee individually. Fruit wedges are leftover pieces of fruit (not provided by researchers)

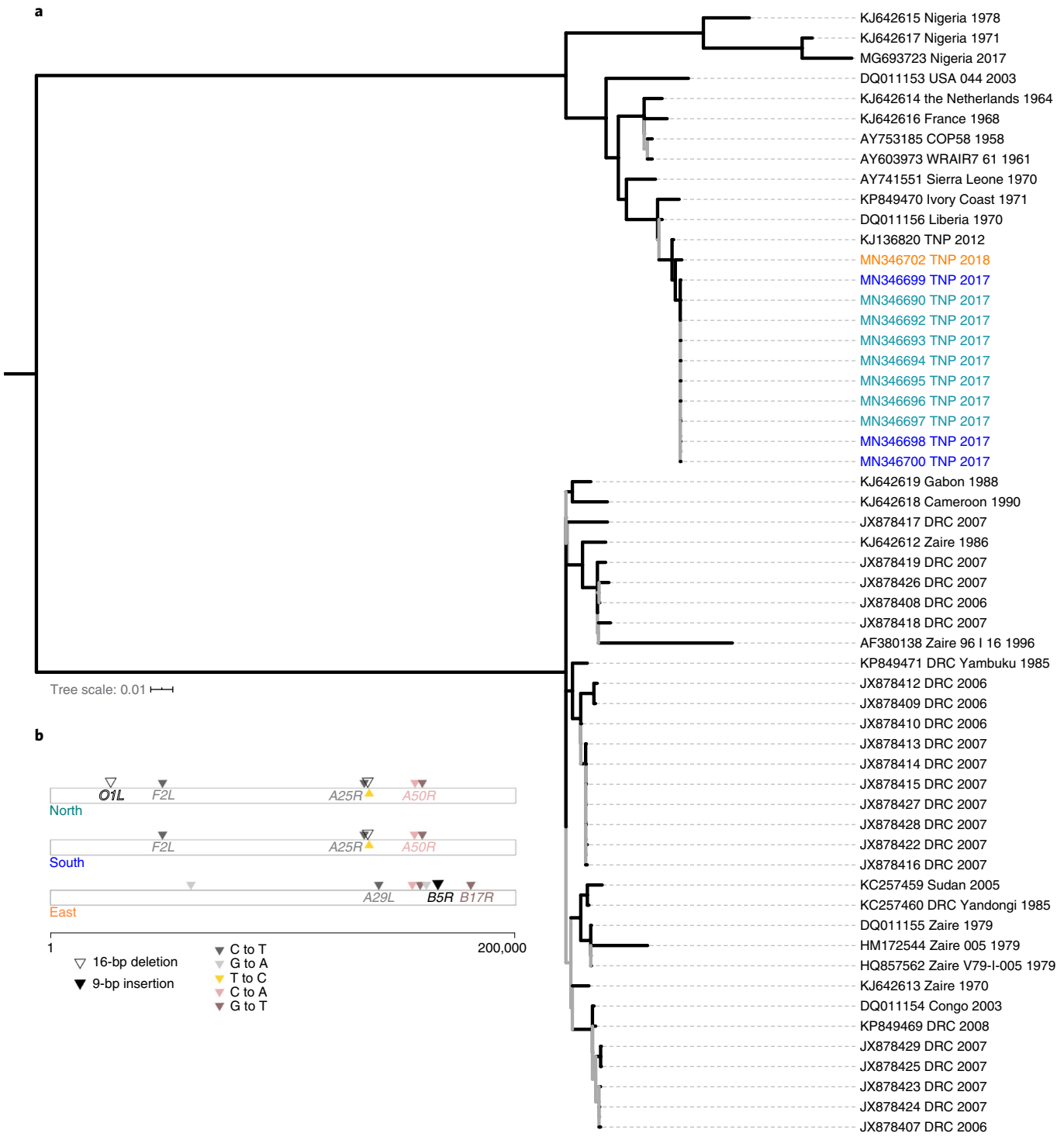

Fig. 3 | Genomic characterization of MPXVs found in the Taï chimpanzee communities. a, A ML tree of MPXV. Viral genomes identified in chimpanzees in TNP are in blue (south 2017), turquoise (north 2017) and orange (east 2018). The scale bar is in substitutions per variable site. The inner branch colours represent bootstrap support (grey is <0.90; black is ≥0.9). DRC, Democratic Republic of the Congo. **b**, A schematic representation of the three MPXV lineages detected in the chimpanzee communities of TNP. The triangles indicate deletions, insertions or single nucleotide polymorphisms. The colour coding of the gene names matches the respective mutation. The absence of gene names indicates non-coding regions. The locations provided correspond to positions in a MPXV reference genome (GenBank accession number KP849470).

previously fed on by chimpanzees, therefore potentially containing saliva. Viral DNA was detected in 12.6% of faecal, 20% of urine and 19.2% of wedge samples collected respectively from 19 (7 symptomatic, 12 asymptomatic), 3 (2 symptomatic, 1 asymptomatic) and 4 individuals (all symptomatic) (Fig. 5a, Extended Data Figs. 4–6, Supplementary Table 3 and Supplementary Information). Despite MPXV DNA detection in all age groups (from infant to adult), severe disease was developed only by infants ($n=5$), a juvenile and

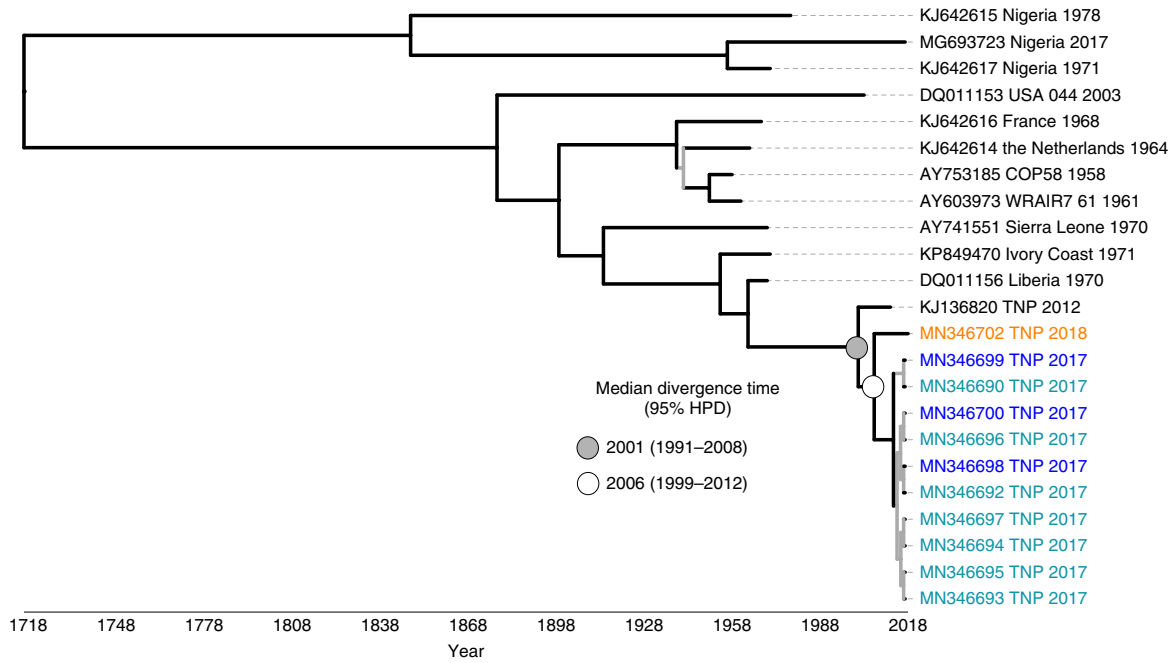

Fig. 4 | Bayesian chronogram of West African MPXVs. Viral genomes identified in chimpanzees in TNP are in blue (south 2017), turquoise (north 2017) and orange (east 2018). The inner branch colours represent posterior probabilities (grey is <0.95; black is ≥0.95). The coloured nodes are discussed in the text and refer to estimates for the median divergence times and 95% HPD of the TNP MPXVs.

an adolescent (13 years old). MPXV presence in this rainforest may have resulted in prior exposure/infection of older individuals, conferring them some immunity. In line with this notion, disease signs compatible with MPXV infection were observed by primatologists in the north community in the 1980s[33]. Detection of MPXV DNA in faeces spanned a time window of 12 days before and 2 months after the first observation of disease signs. To verify whether positivity outside the symptomatic window could reflect a higher frequency of (asymptomatic) exposure/infection than observed with syndromic surveillance, we randomly tested 276 faecal samples collected from the 3 communities between 2011 and 2014. None was positive (Supplementary Table 3), which indicates that chimpanzees are not frequently exposed to/infected with MPXV.

Signs compatible with MPXV infection were evident in a different number of chimpanzees in each community, namely 2 in the south, 11 in the north (of which 7 were only occasionally observed coughing) and 1 in the east community. Exposure to a carcass with high viral loads in the skin and major organs (Supplementary Table 3) may have contributed to the higher number of cases in the north community. The *O1L* gene has been associated with host range and its loss through frame-shifting indels has been identified in poxviruses with a restricted host range, such as taterapox and camelpox viruses[34]. Similarly, complete *O1L* deletion appeared in the modified vaccinia virus Ankara (MVA) during serial passaging on chicken embryo fibroblasts of its parental strain, the chorioallantois vaccinia virus Ankara (CVA)[35]. In light of the reductive evolutionary strategy of poxviruses[36,37], it seems plausible that this feature may have facilitated transmission, whether from reservoir to chimpanzee in a multiple-source scenario or from chimpanzee to chimpanzee in an epizootic paradigm.

Grooming activity is associated with asymptomatic shedding of MPXV DNA. To further investigate viral spread, we analysed social networks over a time frame spanning 90 days before and after the outbreak in the north community. Due to the lack of longitudinal behavioural data collection from infants and juveniles, we could not test whether their degree of connectivity could predict MPXV spread. We therefore characterized the grooming network of asymptomatic adult individuals to verify whether MPXV DNA detection in their faeces could be explained by their grooming behaviour. We acknowledge that under these circumstances we could not determine whether detection in healthy individuals reflects asymptomatic infections or a simple transit of viral particles through the gastrointestinal tract, but we also note that in both cases detection pinpoints exposure to MPXV. Our analyses focused on two parameters of the grooming network, namely the degree and strength. The degree describes the total number of connections of an individual. This is further categorized into connections towards an individual (in-degree), which corresponds to the number of individuals that groomed this specific individual, and connections from an individual (out-degree), which corresponds to the number of individuals that were groomed by the individual. The strength is the sum of all grooming rates that an individual has with the grooming partners and can also be divided into received (in-strength) and given (out-strength) grooming. During the outbreak, we recorded a significant reduction (Mann–Whitney test, $P=0.011$) in the overall strength of the grooming network of the group when compared to the previous trimester (Fig. 6a). Furthermore, we found that the strength, degree and out-degree were significantly lower (Mann–Whitney test, $P=0.016$, $P=0.033$ and $P=0.016$, respectively) in adults who tested negative in faeces (when compared to positive ones) and this, only during the outbreak (Fig. 6a,b, Extended Data Fig. 7d and Supplementary Information). Grooming, which included ingestion of skin lesions (Supplementary Videos 3–5), may have been a determinant of exposure to MPXV, and its reduction may have contributed to the limited spread of the disease.

Faeces and flies contain viable MPXV. Human-to-human transmission of MPXV is known to occur via respiratory droplets or contact with body fluids and lesion material, either directly or indirectly[11]. To investigate whether shedding in faeces may also be a source of infection, we attempted virus isolation from ten faecal

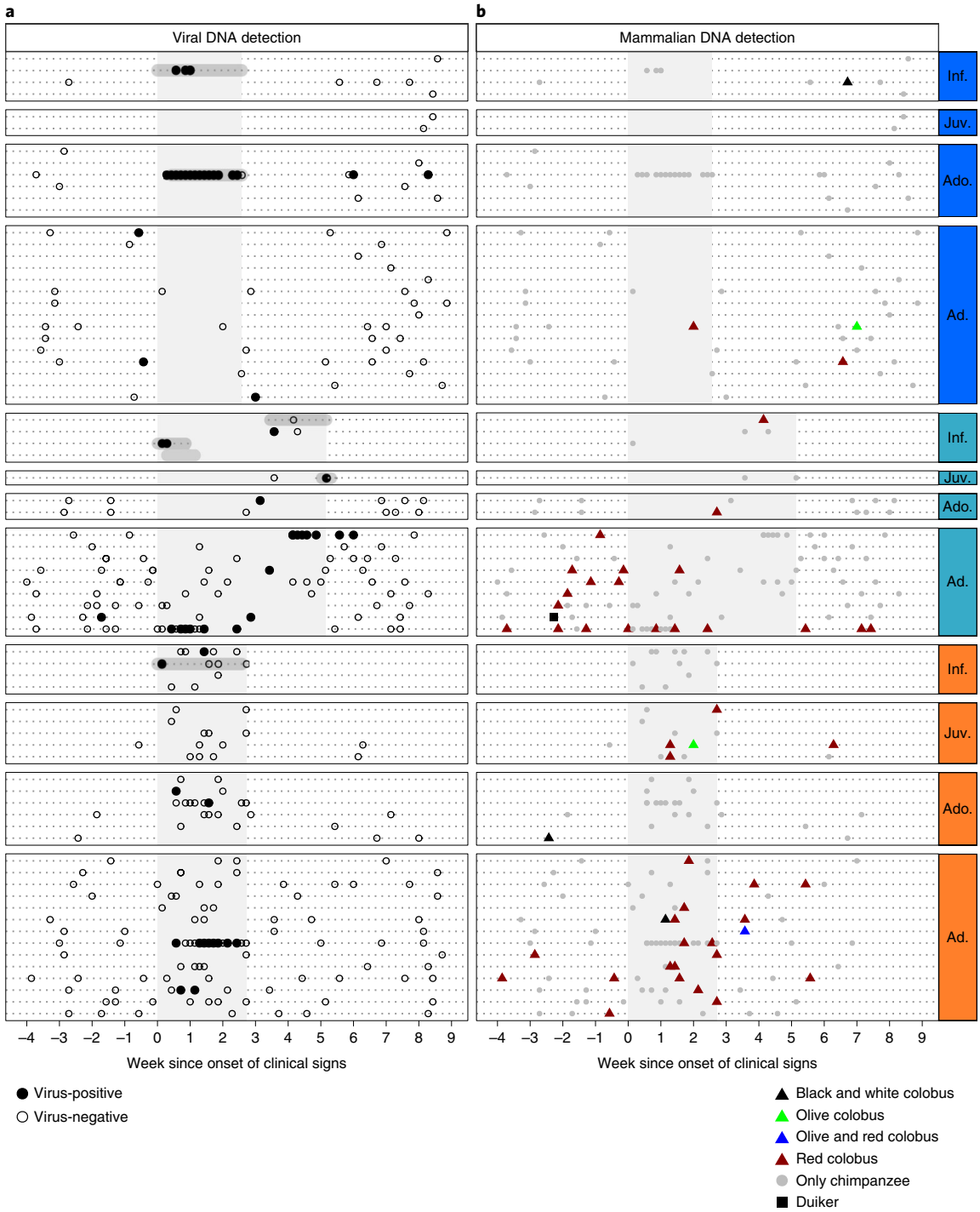

Fig. 5 | Snapshot of molecular analyses in faecal samples. a,b, Detection of MPXV DNA (**a**) and non-chimpanzee mammalian DNA (**b**) in chimpanzee faecal samples before, during and after each outbreak. Each line represents a chimpanzee. The boxes represent the chimpanzee community (south in blue, north in turquoise and east in orange) and age groups (infant (Inf.), juvenile (Juv.), adolescent (Ado.) and adult (Ad.)). The vertical light grey shadowing indicates the period in which clinical signs were observed in the group. The horizontal dark grey shadowing indicates the period in which clinical signs were observed in severely ill chimpanzees.

samples (Supplementary Table 3). Viable virus was found in one sample, suggesting that faeces might be a source of infectious MPXV. Due to their carrion- and faeces-feeding habit, flies can be used for assessing biodiversity and detecting vertebrate-infecting micro-organisms in difficult landscapes[38]. Recently, we have shown that blowflies (Calliphoridae spp.) and flesh flies (Sarcophagidae spp.) collected in the Taï Forest can harbour viable *Bacillus cereus* biovar *anthracis*, the causative agent of sylvatic anthrax[18]. To explore alternative pathways for indirect MPXV transmission, we also trapped flies during two outbreaks. In the outbreak in the north community, MPXV DNA was detected in maggots present on the corpse, in 1 out of 8 flies collected around it, and in all 12 swabs taken from leaves where flies had regurgitated/defaecated (visible as a dark spot on the leaf) in proximity of the carcass (Supplementary Table 3 and

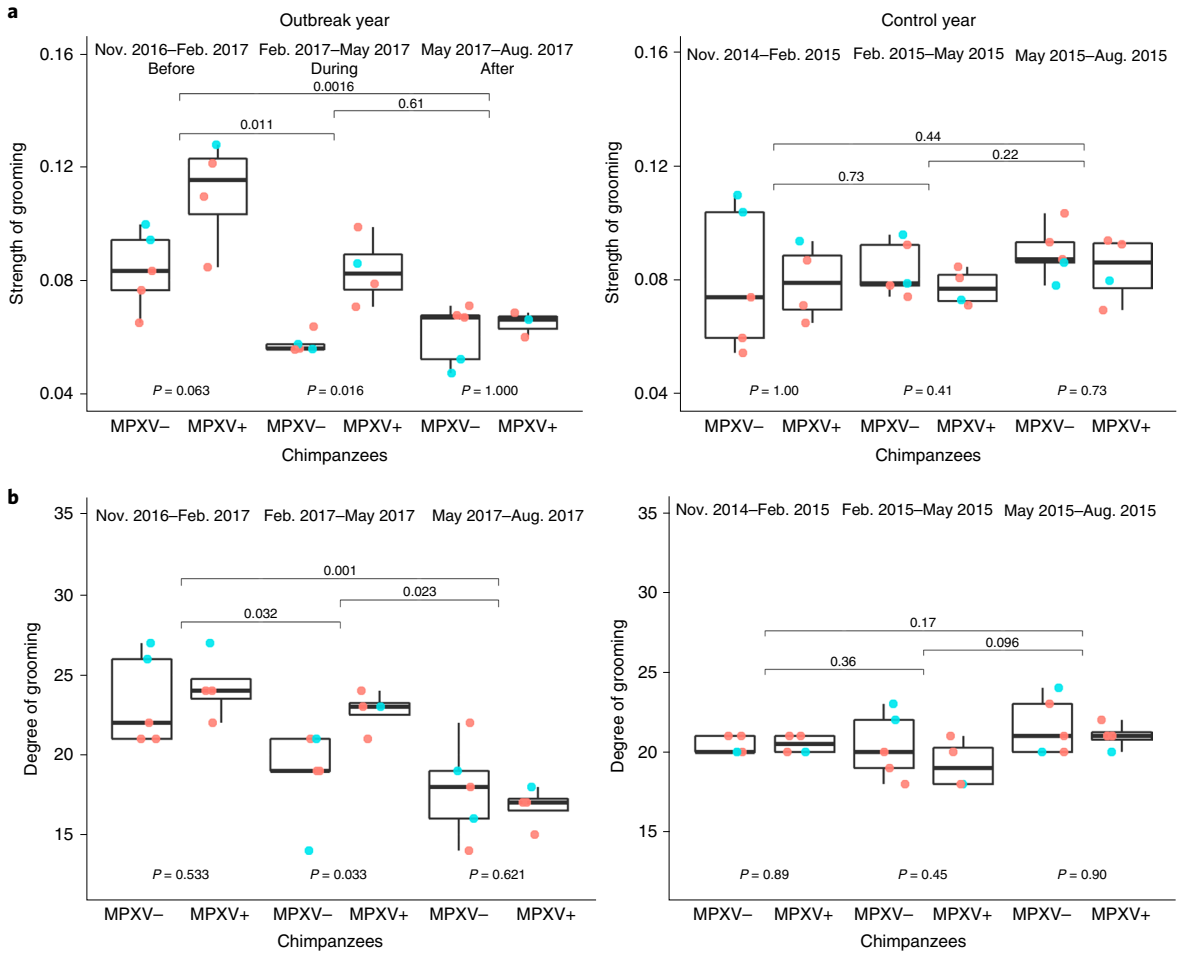

Fig. 6 | Social network metrics. a,**b**, Strength (**a**) and degree (**b**) of grooming for individuals in the north community in different periods of 2017 and 2015. Individuals ($n = 9$) are classified as MPXV negative (−) and positive (+) according to the PCR results from faecal samples. The time frames before, during and after the outbreak are based on the first and last positive PCR result. The horizontal line in the whisker plots represents the mean. The lower and upper bounds of the boxes indicate the first and third quartiles, respectively. The vertical lines are the upper and lower whiskers representing a maximum of the largest/smallest value, but not over 1.5 times the interquartile distance. The *P* values were calculated with a two-sided Mann–Whitney test. The network metrics are also shown for 2015 as the control year (red circle, female; blue circle, male).

Supplementary Fig. 1). A total of 3 out of 80 flies in the east community also contained MPXV genetic material (Supplementary Table 3); all three had been collected around excrements of the mother and sister of the MPXV-infected chimpanzee. Virus isolation was successful from 1 of the 12 swabs collected during the outbreak in the north community (GenBank MN346691). Species identification conducted on a subset of flies ($n = 12$, collected from faeces and around the carcass) and on the leaf swabs ($n = 2$) revealed a species composition in line with previous studies led in TNP: sequences from 7 fly samples and the 2 swabs could be assigned to the family Calliphoridae (4 to the species *Chrysomya putoria*, 4 to *Chrysomya albiceps* and 1 to *Chrysomya inclinata*); for the remaining 5 flies, assignment could not even be narrowed down to the family level (very little is known about tropical fly genetic diversity). Together, these data support the possibility of faeces- and fly-mediated MPXV contamination of the environment, which might occasionally lead to indirect infection of chimpanzees and other susceptible species.

No change in predation regime, nor rodent consumption detected before MPXV emergence. While wild rodents and in particular squirrels have been repeatedly suspected[14], it remains unclear which species act as MPXV reservoir(s). Exposure to potential reservoirs includes contact, which may lead to respiratory or percutaneous transmission, and meat consumption. Chimpanzees share their habitat with arboreal rodents such as squirrels, which allows for sporadic contact and hunting. To investigate a potential dietary source of infection, we performed DNA metabarcoding of 363 (out of the 492) faecal extracts. The approach we applied has previously been shown to detect a wide range of vertebrate DNA, including rodents[18,39,40]. We detected non-chimpanzee mammalian DNA in 16.8% of the samples (42.4% of tested individuals; (Fig. 5b, Extended Data Figs. 4–6 and Supplementary Information). The predation activity shown by these analyses reflected behavioural observations[41], with DNA of sympatric monkeys of four different species and a Maxwell's duiker (*Philantomba maxwellii*) detected. As expected[16], the western red colobus (*Piliocolobus badius*) was clearly over-represented. No rodent DNA was found. Therefore, the repeated emergence of MPXV in this population does not appear to be due to a recent dietary change. The MPXV reservoir(s) is unlikely to be frequently hunted; transmission to chimpanzees might be mediated by contamination of the environment or transmission of MPXV to more frequently hunted intermediate hosts. We also examined inter-individual variation in meat consumption frequency, since higher frequency may lead to increased exposure, especially considering that predators focus on weakened prey (for example, MPXV-infected monkeys). In our adult-biased sample,

we detected a clear inter-individual variation in meat consumption frequency but found no correlation with MPXV detection (Mann–Whitney test, $P=0.35$; Extended Data Fig. 8). Mothers of infants infected with MPXV, however, consumed meat at a higher frequency than mothers of infants who did not show signs of infection (Mann–Whitney test, $P=0.048$) (Extended Data Fig. 9). In spite of the tenuous nature of our observation, individual differences in access to meat[41] may determine MPXV primary exposure risk in chimpanzees.

Long-term mortality surveillance suggests recent emergence was preceded by years of limited viral activity. Previous observations of monkeypox-like infection in this chimpanzee population in 1983 and 1987[33] suggest endemicity. However, in the upcoming 29 years of monitoring no case was documented. By testing 36 chimpanzee carcasses and 9 bones bridging the temporal gap between the late 1980s and recent outbreaks, we confirmed to the best of our possibilities this apparent absence (Supplementary Table 6). As exemplified by the detection of cowpox virus in humans, cattle, carnivores, elephants and rodents[42], the host spectrum of poxviruses can be broad. To investigate potentially undiagnosed MPXV-related deaths in sympatric fauna, we extended the analysis to 111 carcasses and 17 bones recapitulating 25-year mortalities in 19 species (Supplementary Table 6). No additional case was found. Given the overall low lethality associated with infections with MPXVs of the WA clade, we cannot exclude that the virus has been circulating without causing deaths in non-monitored species. Yet, our long-term observations in habituated primates rather suggest that MPXV has remained largely silent in TNP until these recent flares. The years 2017–2018 have seen monkeypox reappear in humans in countries where it had last been seen in the 1970s, such as Nigeria[9] (1978) and Liberia[43] (1971). This resurfacing may be partially explained by previous under-reporting, probably ameliorated by the increasing notifications arising from Central African countries. However, the contemporary re-emergence in humans and non-human primates throughout Western Africa may reflect a simultaneous epizootic in the reservoir host(s) and/or a change in its demography, causing a sudden increase in spillovers. While natural seasonal and interannual variation (particularly in terms of resource availability) has been shown to play an important role in changing viral dynamics (for example, for the orthohantavirus Puumala virus[44]), human-induced changes might also have their part. African rainforests have been largely encroached on over the last century[45] and this ongoing process is still leading to considerable habitat destruction and environmental changes. Such changes can have strong effects on the ecology of host species (for example, via increased competition or predator release). These ecological changes can in turn affect host density or probability of contact among conspecifics and thereby pathogen prevalence and transmission. Host species diversity itself has been shown to influence pathogen circulation in their reservoir hosts. For rodent-borne hantaviruses, high host species diversity has, for example, been shown to result in a dilution or amplification effect depending on the system studied[46]. Together with the above-mentioned waning of population immunity, changing ecosystems might be contributing to the changing epidemiology of MPXV.

Outlook. In this study, we took advantage of the long-term behaviour and health monitoring of chimpanzees in TNP to detect and study three outbreaks of MPXV, during which the affected animals sometimes presented with only respiratory signs. MPXV genomic analyses revealed that these outbreaks probably all corresponded to independent transmission events from a still unknown reservoir host; diet metabarcoding did not reveal any notable change in chimpanzee predatory behaviour, nor any consumption of rodent. As expected, we found that exposure to MPXV was greater in those individuals that maintained a higher grooming activity during the outbreak, suggesting that MPXV was transmitted directly between chimpanzees. We also found that indirect pathways of transmission might exist, since viable virus could be obtained from faeces and flies associated with the chimpanzees. Finally, a retrospective analysis of samples accumulated over the last three decades did not suggest a high, unnoticed activity of MPXV in the area. Accordingly, we speculate that these three outbreaks might be linked to the same ecological changes that probably prompted the recent increase in monkeypox incidence in humans across Western Africa.

In conclusion, this study is an example of how wildlife health surveillance can inform public health systems on pathogen distribution and activity, increase awareness on atypical clinical presentations, and provide the opportunity to investigate the epidemiology and ecology of zoonotic pathogens. While systematic surveillance in humans is clearly of the uttermost importance, further investigations in wildlife will be instrumental in identifying the factors underlying MPXV ecology and ultimately, emergence.

Methods

Health monitoring and non-invasive sampling. The long-term health monitoring programme of the Taï Chimpanzee project includes continuous collection of faecal and urine samples from adult, individually recognized wild-living chimpanzees. Faeces are collected in 2-ml Cryotubes with the aid of a plastic spatula right after observing defaecation. Urine is collected in 2-ml Cryotubes from leaves that accidentally collect it when the chimpanzees are on a tree and urinate on the underlying vegetation. This is carried out using a fine Pasteur pipette. In outbreak situations, field assistants and researchers attempt collecting samples from all symptomatic individuals. Nevertheless, sampling from infant chimpanzees is difficult. Here, faeces and urine were collected from symptomatic and asymptomatic individuals, where possible. To intensify sampling in the presence of respiratory symptoms, fruit wedges were collected from symptomatic individuals to increase the chances of diagnosing an infection. This is done by collecting pieces of bitten and chewed fruit left behind by the chimpanzees and placing them in 2-ml Cryotubes. All samples were stored in liquid nitrogen tanks in the field until shipment on dry ice to Germany. To have a broad picture of virus circulation in the three communities, all available faecal samples collected a month before, during and up to two months after each outbreak were tested. For the south group, a total of 122 faecal samples collected between 1 December 2016 and 29 April 2017 were tested. Ten urine samples and ten wedges collected from symptomatic individuals between 17 and 31 January were also included. For the north group, a total of 108 faecal samples collected between 28 January 2017 and 19 May 2017 were tested. Additionally, 16 fruit wedges and 42 urine samples collected during the time frame of observation of symptoms (3 March 2017–8 April 2017) were also tested. For the east group, 262 faecal samples collected between 1 April and 25 August were tested. Only one urine sample and no fruit wedge could be collected from the symptomatic infant chimpanzee. Two urine samples from his mother were also included. These numbers refer to the individual samples collected. In some instances duplicate aliquots from the same sample were tested but counted as one (Supplementary Table 3). The health monitoring programme of the Taï Chimpanzee project includes wildlife mortality surveillance. To this end, necropsies are performed by a trained veterinarian on all wildlife found dead in the research area. Tissue samples of all inner organs are taken, as far as the state of carcass decomposition allows. Necropsies follow a standardized protocol, including the use of full personal protective equipment due to the occurrence of anthrax, Ebola virus disease and monkeypox in the area. The necropsy site is subsequently decontaminated according to World Health Organization guidelines, involving burial of the carcass and incineration or disinfection with 10% formalin of all contaminated materials. In the field, samples are stored in liquid nitrogen and formalin and subsequently shipped on dry ice to the Robert Koch Institute for analyses. Following the MPXV death, a full necropsy was performed under high-level safety measures. Additionally, maggots were collected from the carcass.

A total of 353 necropsy samples collected from 147 carcasses belonging to animals of 20 species sampled between 1998 and 2019 were included. Additionally, 26 bones collected between 1994 and 2013 from primates of 7 species were included but tested only via next-generation sequencing. Details are provided in Supplementary Table 6.

Fly trapping. Flies were caught on the ground in proximity to the carcass or around chimpanzee faeces using custom-made traps as previously described[18]. Trapping was performed for 60 min or until 20 flies were collected. Flies were euthanized with ether and stored at −20 °C in 2-ml Cryotubes (Carl Roth) containing up to 10 flies or at ambient temperature on silica in 50-ml Falcon tubes (Thermo Fisher Scientific) containing up to 20 flies. During the necropsy, 8 flies were trapped and 12 swabs were taken from leaves in proximity of the corpse

where potentially corpse-fed flies had been regurgitating/defaecating. During the outbreak in the east community, a total of 80 flies were collected from the faeces of 12 chimpanzees (23 faecal samples).

DNA extraction and orthopoxvirus DNA detection. Extractions were conducted in the BSL-3 facility of the Robert Koch Institute. Viral DNA was extracted from 40 mg of faeces using the GeneMATRIX Stool DNA Purification Kit (Roboklon). The same kit was used to extract DNA from fruit wedges and flies (entire). Each fly was cut into small pieces using sterilized scissors before being homogenized using a Fast Prep (MP Biomedicals), and subsequently the manufacturers' instructions were followed. For urine and leaf swabs, the Viral RNA Mini Kit (Qiagen) was used without performing the DNase step. Dry leaf swabs were immersed in 500 µl of phosphate-buffered saline (PBS) and incubated at 37 °C for 30 min on a shaking device. A 100-µl volume of urine and 140 µl of PBS were used as the input volume, respectively. For tissue samples, DNA was extracted from 20 mg of each tissue using the DNeasy Blood and Tissue kit (Qiagen). Extracts from bones were available from previous studies[18]. A TaqMan Real-time PCR targeting the *P4A* gene of all orthopoxviruses was performed[47]. To distinguish between orthopoxviruses, a conventional PCR targeting a 270-bp fragment of the haemagglutinin (*HA*) gene[48] was conducted and the resulting amplicons were Sanger sequenced using the BigDye Terminator chemistry. For weakly positive samples collected outside the window of the outbreak (that is, February and May, north community), extraction and PCR were repeated to confirm the result. Fly extracts were pooled, and in the case of a positive result single flies from a positive pool were individually tested. For weakly positive flies or fly pools, real-time *P4A* PCR results were further confirmed with the conventional *HA* PCR and Sanger sequencing.

Fly species identification. Species (or lowest possible taxon) identification was attempted on the basis of a barcoding approach. For this, we performed a PCR targeting a 710-bp region of the mitochondrial cytochrome *c* oxidase unit COI using the primers LCO1490 (ggTCAACAAATCATAAAgATATTgg) and HCO2198 (TAAACTTCAgggTgACCAAAAAATCA)[49] and the resulting amplicons were Sanger sequenced. After primer removal and quality control with Geneious v10.0.5 (ref. [50]), each sequence was compared to the non-redundant National Center for Biotechnology Information database using BLAST (ref. [51]).

Virus isolation. To investigate the viability of MPXV in non-invasive samples, isolation attempts were conducted as described previously[42]. A total of ten faecal samples, one fruit wedge and one leaf swab were tested. These samples were selected on the basis of the higher viral genome copy number. Briefly, samples were homogenized in PBS and added at different dilutions to confluent layers of Vero cells (ECACC, catalogue number 85020206) cultivated with medium with 2% fetal calf serum supplemented with penicillin/streptomycin in 24-well cell culture plates. Once cytopathic effects were evident under the microscope, passaging was initiated to increase the viral titre for shotgun sequencing.

Next-generation sequencing and hybridization capture. Illumina-compatible dual-index libraries were generated from up to 250 ng of DNA extracts (skin and spleen from the deceased chimpanzee, 1 fruit wedge, 14 faeces, 1 leaf swab, 26 bones and 1 virus isolate). With the exception of bones, each sample was fragmented using a Covaris S220 Focused-ultrasonicator in 130 µl of low-EDTA TE buffer using settings to generate a 400-bp fragment size (intensity = 4, duty cycle = 10%, cycles per burst = 200, treatment time = 55 s, temperature = 7 °C). Fragmented DNA was concentrated using the MinElute PCR purification kit and eluted into 50 µl low-EDTA TE buffer. Libraries were built from fragmented DNA using the NEBNext Ultra II kit according to the standard protocol. Where more than 50 ng was used as input, a 400-bp size selection was performed following adapter ligation using Ampure XP magnetic beads. Final libraries were quantified using the KAPA HiFi library quantification kit and stored at −20 °C until further use. Skin, spleen and virus isolate libraries were shotgun sequenced on a MiSeq platform (V3 chemistry, 2 × 300-bp reads). Libraries from faeces, the fruit wedge and leaf swab were subject to target enrichment via hybridization capture. For this purpose, libraries were pooled (250 ng of each) and concentrated using the MinElute PCR Purification kit (Qiagen) to a final volume of 10 µl. Two rounds of 24-h hybridization capture at 65 °C were performed using twofold tiling 80-nucleotide RNA baits (MYBaits) designed to target orthopoxviruses. The bait design was proposed by the service provider and validated by our team. The MYBaits Sequence Enrichment for Targeted Sequencing protocol (Version 2.3.1) was followed, with the exception that only a quarter of the recommended bait quantity was used per capture round. After each round of capture, the captured library pool was amplified using the KAPA Hot Start Library Amplification system to reach a minimum of 200 ng of library and quantified using the KAPA HiFi Library Quantification Kit. The enriched pool was diluted to 4 nM and sequenced on a MiSeq platform using the V3 chemistry (2 × 300 bp).

Next-generation sequencing data analyses. Raw reads were pre-processed using Trimmomatic v0.36 (ref. [52]), with the following settings: LEADING:30 TRAILING:30 SLIDINGWINDOW:4:40 MINLEN:40. Trimmed reads from a skin sample of the dead chimpanzee were first mapped to a MPXV WA reference genome (GenBank accession number KP849470) using BWA-MEM v0.7.15-r1140 (ref. [53]). Subsequently reads from all other samples were mapped to this first TNP chimpanzee-infecting MPXV genome. We sorted mapping files and removed duplicates using the SortSam and MarkDuplicates tools from Picard v1.113 (http://broadinstitute.github.io/picard). In parallel, reads were de novo assembled into contigs using metaSPAdes v3.12.0 (ref. [54]). Contigs were blasted in Geneious v10.0.5 against a MPXV database using blastn and selecting the option 'bin into hit and no hit'. Hits were then re-blasted against the same database using megablast and selecting the option 'create a hit table'. On the basis of the hit table, contigs were then mapped to the closest genome to obtain a linear consensus. The consensus sequence resulting from assembling the contigs was used as a reference sequence for a new mapping of the filtered reads using the pipeline described above. Consensus sequences were generated from the initial reference-based mapping and the mapping to the consensus resulting from the de novo assembly using Geneious. Base calling was set to 20 reads and 95% agreement. Where coverage was lower, base calling was set to two reads. Consensus sequences were aligned using MAFFT v7 (ref. [55]). When comparing consensi, ambiguous and unambiguous bases were manually checked. Differences observed within local tandem repeat regions were not taken into account because of the known error-prone difficulties in assembly[5,56]. For each sample, the two consensi were compared to check for accordance (no difference found). The de novo-generated consensus was finally used for downstream analyses due to better coverage. For some samples, the ends of the genomes were completed using the information from the reference-based consensus. Annotations were transferred from a reference genome (GenBank accession number KP849470) using a 50% similarity threshold. CDS annotations were exported to compare the number of genes present in the TNP and reference genomes and correct potential annotation duplications in the TNP genomes. Whole-genome alignment was performed with ProgressiveMauve v2.4.0 and genome co-linearity was checked.

Phylogenetic analyses. A dataset comprising all available MPXV complete genomes was downloaded from the National Center for Biotechnology Information database. Sequences ($n = 64$) were aligned using MAFFT v7 and Gblocks v0.91b was run under stringent settings in Seaview v4 (ref. [57]) to select conserved blocks. The online tool FaBox v1.4 (ref. [58]) was used to collapse the alignment to unique sequences ($n = 56$). Identical sites and sites containing ambiguities were stripped using Geneious; therefore, only variable sites were included in the analysis (1,071 bases). The best model of nucleotide evolution was identified using jModelTest v2.1.10 (ref. [59]), applying the Bayesian information criterion (SYM). Phylogenetic analyses were performed in ML and Bayesian frameworks. ML analyses were conducted using PhyML v3 (ref. [60]) as implemented in Seaview; branch robustness was assessed using bootstrapping (500 pseudo-replicates). The resulting tree was visualized and edited in iTOL v5.5 (ref. [61]; https://itol.embl.de/). Initial analyses of temporal signal were conducted using TempEst v1.5 (ref. [24]). Bayesian Markov chain Monte Carlo analyses were run in BEAST v1.10.4 (ref. [25]). For all following analyses, multiple Bayesian Markov chain Monte Carlo runs were performed and chain convergence as well as appropriate sampling of the posterior space (effective sample size >200) was checked. Using MLE (path sampling and stepping-stone sampling[62]), we first assessed the performance of four models corresponding to all possible combinations of two different tree priors and molecular clocks: a coalescent model assuming a constant population and a lognormal relaxed clock; a coalescent model assuming a constant population and a strict clock; a dynamic model as implemented in the Bayesian skyride method and a lognormal relaxed clock; and a dynamic model as implemented in the Bayesian skyride method and a strict clock. The Bayesian skyride method was chosen over the skygrid method because of no a priori knowledge on the timescale of the chronogram. Tip dates were provided for this analysis. BF comparisons were considered to convincingly support a model when $2\ln BF > 10$. Since none of the models clearly superseded the others, we selected the simplest model (strict clock and constant population size) for an additional analysis aimed at showing the presence of a genuine temporal signal in the dataset. This analysis consisted of estimating the fit of a last model in which all sequences were assumed to have been obtained contemporaneously. The BF comparison with the model with tip dates allows for a Bayesian evaluation of temporal signal[26]. We generated the final chronogram presented in Fig. 4 by combining post burn-in trees from multiple chains using LogCombiner v1.10.4, and then summarizing this posterior set of trees onto the maximum clade credibility tree identified with TreeAnnotator v1.10.4 (both software programs are distributed with BEAST). Branch robustness was assessed through posterior probabilities. We visualized the resulting chronogram in FigTree v1.4.0 (distributed with BEAST) and subsequently edited it in iTOL. The genome-wide mean substitution rate was calculated by multiplying the substitution rate observed by the ratio of our alignment length (1,071 variable sites) to the average length of the genomes used in our analyses (197,394).

Diet analysis. The taxonomy of mammalian DNA present in chimpanzee faeces was searched for in 363 faecal DNA extracts (south = 121, north = 105, east = 137) using a metabarcoding approach, as previously described[18,39]. Briefly, amplicon deep sequencing was conducted on PCR products generated by targeting a 130-bp

fragment of the 16S ribosomal RNA (16Smam1 5′-CGGTTGGGGTGACCTC GGA-3′, 16Smam2 5′-GCTGTTATCCCTAGGGTAACT-3′) in the presence of human and swine blocking primers (16Smam_blkhum 5′-CGGTTGGGGCG ACCTCGGAGCAGAACCC—spacerC3, 16Smam_blkpig 5′-CGGTTGGGGTGAC CTCGGAGTACAAAAAAC—spacerC3). Illumina adapters were subsequently incorporated using fusion primers (16S mam1 and 16S mam2 with Illumina overhangs) and a final indexing PCR was performed using Nextera XT indices to assign individual barcodes to each sample. A custom pipeline using OBITools v1.1.8 (ref. [63]) was used for downstream taxonomic assessment of each read to the family, genus and order level (all commands mentioned in the following without specific mention of a software package are OBITools commands). In a first step, paired-end reads were merged with the command illuminapairedend setting the minimum alignment score to 40. Primers were then removed using the programme Cutadapt v1.2.1 (ref. [64]), before quality trimming was conducted using Trimmomatic v0.36 (same settings as above). For dereplication of identical sequences and filtering for identical sequences that occurred at least ten times within the sample we used the obiuniq and obigrep commands. A reference database was built by performing an in silico PCR on all mammalian and vertebrate sequences available at Genbank using the programme ecoPCR v0.2 (ref. [65]). This database contained the reference sequences themselves and a unique taxid linking each sequence to a taxonomy database including broader taxonomic information. Read assignment was performed using the command ecotag. Ecotag implements the global alignment algorithm Needleman–Wunsch to find the most similar sequence to the query sequence in the reference database with a minimum identity level of 0.95 (primary reference sequence). The query sequence is assigned to the most common recent ancestor of the primary reference sequence and the secondary reference sequence that is the most similar reference sequence to the primary reference. To calculate the frequency of mammal DNA detection, we included only individuals for which four or more samples were analysed throughout the study period. This ratio was calculated by comparing the number of samples containing non-chimpanzee mammalian DNA with the number of samples containing only chimpanzee DNA. For the calculation of this ratio in female chimpanzees, only offspring who showed overt signs of MPXV infection and were confirmed by PCR were considered as positive.

Social network analyses. Behavioural data were collected by field assistants of the Taï Chimpanzee Project. The chimpanzees are observed throughout the year and the duration and direction of each behaviour are recorded in a full-day continuous focal animal sampling. The focus is on adolescent and adult individuals. We used the grooming data to calculate the grooming rates for each dyad in specified time frames before, during and after the MPXV outbreak (Supplementary Information and Supplementary Table 4). The grooming rate is the sum of time in which one individual groomed another one per total observation time of both in the specified time frames. Using the R package igraph (ref. [66]), we calculated weighted and directed networks based on dyadic grooming rates and the corresponding individual-level network metrics (degree and strength). The degree describes the total number of connections of an individual. The degree is further categorized into connections towards an individual (in-degree), which corresponds to the number of individuals that groomed this specific individual, and connections from an individual (out-degree), which corresponds to the number of individuals that were groomed by the individual. The strength is the sum of all grooming rates that an individual has with the grooming partners and can also be divided in received (in-strength) and given grooming (out-strength). The degree and strength of the network were used to compare chimpanzee behaviour before and during the outbreak. For analysing behavioural differences, we distinguished between MPXV-positive and MPXV-negative individuals on the basis of viral DNA detection from faeces. As a control of the network metrics during the outbreak year, we investigated the behavioural observations of the previous years and selected the year 2015 for comparison, as no other documented disease outbreak occurred in this year and the group composition was comparable to 2017. The same time frames were selected in the control year as in the outbreak year to calculate the network metrics strength and degree based on the grooming data.

Statistics. All statistical analyses performed in this study were conducted in RStudio v1.2.1335. The Mann–Whitney test (two-sided) was used to assess statistical significance (significance level ≤ 0.05).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Raw reads of 16S amplicons are available in the European Nucleotide Archive (ENA) under project accession number PRJEB34040 and sample accession numbers ERS3658904–ERS3659288. Raw reads resulting from MPXV target enrichment in non-invasive samples are available in the ENA under project accession number PRJEB34056, sample accession numbers ERS3659494–ERS3659510. Consensus sequences are available in GenBank, accession numbers MN346690–MN3466703. Annotated MPXV genomes, as well as sequences derived from the fly species identification, are available in Zenodo (https://zenodo.org record number 3373886 and 3606577, respectively). Source Data for Fig. 6 and Extended Data Figs. 7–9 are provided with the paper.

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References

1. Durski, K. N. et al. Emergence of monkeypox — West and Central Africa, 1970–2017. *Morb. Mortal. Wkly Rep.* **67**, 306–310 (2018).
2. Vaughan, A. et al. Two cases of monkeypox imported to the United Kingdom, September 2018. *Eurosurveillance* **23**, 1800509 (2018).
3. Erez, N. et al. Diagnosis of imported monkeypox, Israel, 2018. *Emerg. Infect. Dis.* **25**, 980–983 (2019).
4. Ng, O. et al. A case of imported monkeypox in Singapore. *Lancet Infect. Dis.* **19**, 1166 (2019).
5. Nakazawa, Y. et al. A phylogeographic investigation of African monkeypox. *Viruses* **7**, 2168–2184 (2015).
6. Chen, N. et al. Virulence differences between monkeypox virus isolates from West Africa and the Congo basin. *Virology* **340**, 46–63 (2005).
7. Parker, S., Nuara, A., Buller, R. & Schultz, D. Human monkeypox: an emerging zoonotic disease. *Future Microbiol.* **2**, 17–34 (2007).
8. Sklenovská, N. & Van Ranst, M. Emergence of monkeypox as the most important orthopoxvirus infection in humans. *Front. Public Health* **6**, 241 (2018).
9. Yinka-Ogunleye, A. et al. Reemergence of human monkeypox in Nigeria, 2017. *Emerg. Infect. Dis.* **24**, 1149–1151 (2018).
10. von Magnus, P., Andersen, E., Petersen, K. & Birch-Andersen, A. A pox-like disease in cynomolgus monkeys. *Acta Pathol. Microbiol. Scand.* **46**, 156–176 (1959).
11. Petersen, E. et al. Monkeypox — enhancing public health preparedness for an emerging lethal human zoonotic epidemic threat in the wake of the smallpox post-eradication era. *Int. J. Infect. Dis.* **78**, 78–84 (2019).
12. Khodakevich, L., Jezek, Z. & Kinzanzka, K. Isolation of monkeypox virus from wild squirrel infected in nature. *Lancet* **327**, 98–99 (1986).
13. Radonić, A. et al. Fatal monkeypox in wild-living sooty mangabey, Cote d'Ivoire, 2012. *Emerg. Infect. Dis.* **20**, 1009–1011 (2014).
14. Tiee, M. S., Harrigan, R. J., Thomassen, H. A. & Smith, T. B. Ghosts of infections past: using archival samples to understand a century of monkeypox virus prevalence among host communities across space and time. *R. Soc. Open Sci.* **5**, 171089 (2018).
15. Leendertz, F. H. et al. Pathogens as drivers of population declines: the importance of systematic monitoring in great apes and other threatened mammals. *Biol. Conserv.* **131**, 325–337 (2006).
16. Wittig, R. M. in *Encyclopedia of Animal Cognition and Behavior* (eds Vonk, J. & Shackelford, T.) 1–7 (Springer, 2018).
17. Gillespie, T. R., Nunn, C. L. & Leendertz, F. H. Integrative approaches to the study of primate infectious disease: implications for biodiversity conservation and global health. *Am. J. Phys. Anthropol.* **137**, 53–69 (2008).
18. Hoffmann, C. et al. Persistent anthrax as a major driver of wildlife mortality in a tropical rainforest. *Nature* **548**, 82–85 (2017).
19. Köndgen, S. et al. Pandemic human viruses cause decline of endangered great apes. *Curr. Biol.* **18**, 260–264 (2008).
20. Reynolds, M. G. et al. Clinical manifestations of human monkeypox influenced by route of infection. *J. Infect. Dis.* **194**, 773–780 (2006).
21. Saijo, M. et al. Virulence and pathophysiology of the Congo Basin and West African strains of monkeypox virus in non-human primates. *J. Gen. Virol.* **90**, 2266–2271 (2009).
22. *Monkeypox Fact Sheet* (World Health Organization, 2019); https://www.who.int/news-room/fact-sheets/detail/monkeypox
23. Huhn, G. D. et al. Clinical characteristics of human monkeypox, and risk factors for severe disease. *Clin. Infect. Dis.* **41**, 1742–1751 (2005).
24. Rambaut, A., Lam, T. T., Max Carvalho, L. & Pybus, O. G. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). *Virus Evol.* **2**, vew007 (2016).
25. Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**, 1969–1973 (2012).
26. Duchene, S. et al. Bayesian evaluation of temporal signal in measurably evolving populations. Preprint at *bioRxiv* https://doi.org/10.1101/810697 (2019).
27. Duffy, S., Shackelton, L. A. & Holmes, E. C. Rates of evolutionary change in viruses: patterns and determinants. *Nat. Rev. Genet.* **9**, 267–276 (2008).
28. Babkin, I. V. & Babkina, I. N. A retrospective study of the orthopoxvirus molecular evolution. *Infect. Genet. Evol.* **12**, 1597–1604 (2012).
29. Kerr, P. J. et al. Genomic and phenotypic characterization of myxoma virus from Great Britain reveals multiple evolutionary pathways distinct from those in Australia. *PLoS Pathog.* **13**, e1006252 (2017).

30. Duggan, A. T. et al. 17th century variola virus reveals the recent history of smallpox. *Curr. Biol.* **26**, 3407–3412 (2016).
31. Porter, A. F., Duggan, A. T., Poinar, H. N. & Holmes, E. C. Characterization of two historic smallpox specimens from a Czech museum. *Viruses* **9**, 2–5 (2017).
32. Andersen, K. G. et al. Clinical sequencing uncovers origins and evolution of Lassa virus. *Cell* **162**, 738–750 (2015).
33. Boesch, C. & Boesch-Achermann, H. The Chimpanzees of the Taï Forest (Oxford Univ. Press, 2000).
34. Smithson, C., Purdy, A., Verster, A. J. & Upton, C. Prediction of steps in the evolution of variola virus host range. *PLoS ONE* **9**, e91520 (2014).
35. Schweneker, M. et al. The vaccinia virus O1 protein is required for sustained activation of extracellular signal-regulated kinase 1/2 and promotes viral virulence. *J. Virol.* **86**, 2323–2336 (2012).
36. Hendrickson, R. C., Wang, C., Hatcher, E. L. & Lefkowitz, E. J. Orthopoxvirus genome evolution: the role of gene loss. *Viruses* **2**, 1933–1967 (2010).
37. Reynolds, M. G., Guagliardo, S. A. J., Nakazawa, Y. J., Doty, J. B. & Mauldin, M. R. Understanding orthopoxvirus host range and evolution: from the enigmatic to the usual suspects. *Curr. Opin. Virol.* **28**, 108–115 (2018).
38. Hoffmann, C., Stockhausen, M., Merkel, K., Calvignac-Spencer, S. & Leendertz, F. H. Assessing the feasibility of fly based surveillance of wildlife infectious diseases. *Sci. Rep.* **6**, 37952 (2016).
39. Calvignac-Spencer, S. et al. Carrion fly-derived DNA as a tool for comprehensive and cost-effective assessment of mammalian biodiversity. *Mol. Ecol.* **22**, 915–924 (2013).
40. Gogarten, J. F. et al. Fly-derived DNA and camera traps are complementary tools for assessing mammalian biodiversity. *Environ. DNA* **2**, 63–76 (2020).
41. Samuni, L., Preis, A., Deschner, T., Crockford, C. & Wittig, R. M. Reward of labor coordination and hunting success in wild chimpanzees. *Commun. Biol.* **1**, 138 (2018).
42. Stagegaard, J. et al. Seasonal recurrence of cowpox virus outbreaks in captive cheetahs (*Acinonyx jubatus*). *PLoS ONE* **12**, e0187089 (2017).
43. ProMED. Monkeypox - Africa (07): Liberia; archive no. 20180411.5740756. *ProMED* http://www.promedmail.org/post/5740756 (2018).
44. Reil, D. et al. Puumala hantavirus infections in bank vole populations: host and virus dynamics in Central Europe. *BMC Ecol.* **17**, 9 (2017).
45. Aleman, J., Jarzyna, M. & Staver, A. Forest extent and deforestation in tropical Africa since 1900. *Nat. Ecol. Evol.* **2**, 26–33 (2018).
46. Luis, A. D., Kuenzi, A. J. & Mills, J. N. Species diversity concurrently dilutes and amplifies transmission in a zoonotic host–pathogen system through competing mechanisms. *Proc. Natl Acad. Sci. USA* **115**, 7979–7984 (2018).
47. Schroeder, K. & Nitsche, A. Multicolour, multiplex real-time PCR assay for the detection of human-pathogenic poxviruses. *Mol. Cell. Probes* **24**, 110–113 (2010).
48. Kurth, A. et al. Rat-to-elephant-to-human transmission of cowpox virus. *Emerg. Infect. Dis.* **14**, 670–671 (2008).
49. Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **3**, 294–299 (1994).
50. Kearse, M. et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649 (2012).
51. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410 (1990).
52. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
53. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
54. Nurk, S., Meleshko, D., Korobeynikov, A. & Pevzner, P. A. metaSPAdes: a new versatile metagenomic assembler. *Genome Res.* **27**, 824–834 (2017).
55. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).
56. Zhao, K., Wohlhueter, R. M. & Li, Y. Finishing monkeypox genomes from short reads: assembly analysis and a neural network method. *BMC Genomics* **17**, 497 (2016).
57. Gouy, M., Guindon, S. & Gascuel, O. Sea view version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol. Biol. Evol.* **27**, 221–224 (2010).
58. Villesen, P. FaBox: an online toolbox for FASTA sequences. *Mol. Ecol. Notes* **7**, 965–968 (2007).
59. Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. jModelTest 2: more models, new heuristics and high-performance computing. *Nat. Methods* **9**, 772 (2015).
60. Guindon, S. et al. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* **29**, 307–321 (2010).
61. Letunic, I. & Bork, P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res.* **47**, W256–W259 (2019).
62. Baele, G. et al. Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Mol. Biol. Evol.* **29**, 2157–2167 (2012).
63. Boyer, F. et al. obitools: a unix-inspired software package for DNA metabarcoding. *Mol. Ecol. Resour.* **16**, 176–182 (2016).
64. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* **17**, 10–12 (2011).
65. Ficetola, G. F. et al. An in silico approach for the evaluation of DNA barcodes. *BMC Genomics* **11**, 434 (2010).
66. Csárdi, G. & Nepusz, T. The igraph software package for complex network research. *InterJournal Complex Systems* http://igraph.org (2006).

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Author contributions

Data and samples from the three outbreaks were collected by K.P. and L.S. The field investigations as well as diagnostic and research activities were coordinated by L.V.P., S.C.-S. and F.H.L. Molecular laboratory analyses were performed by L.V.P., C.R., A.S. and M.U. Social network analyses were performed by M.U. Virus isolation experiments were conducted and coordinated by S.M. and A.N. C.B., R.M.W. and E.C.-H. coordinated the field work and provided the behavioural data. The data were analysed by L.V.P. and S.C.-S. and the manuscript was drafted by L.V.P., S.C.-S. and F.H.L. The manuscript was revised and approved by all authors.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41564-020-0706-0.

Supplementary information is available for this paper at https://doi.org/10.1038/s41564-020-0706-0.

Correspondence and requests for materials should be addressed to F.H.L.

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Extended Data Fig. 1 | Clinical course of MPXV infection in an infant East chimpanzee. Pictures **a-h** show the evolution of the skin lesions over a 19-day period. Day 1 corresponds to the first day of symptoms' observation by researchers. **a** Papular lesions covering the entire body. **b** Lesions in vesiculopustular stage, also evident on eyelids. **c** Ulceration and umbilication of facial lesions, some crusts already visible. **d** Most facial lesions have ulcerated and crusted, lesions on limbs are still at a vesicular stage. **e** Most facial lesions have crusted and scabbed, lesions on the abdomen and extremities are still in previous stages. **f** Facial lesions mostly scabbed and crusts fallen off, on the abdomen and extremities crusting and scabbing are underway. **g**, **h** Lesions on the entire body have resolved.

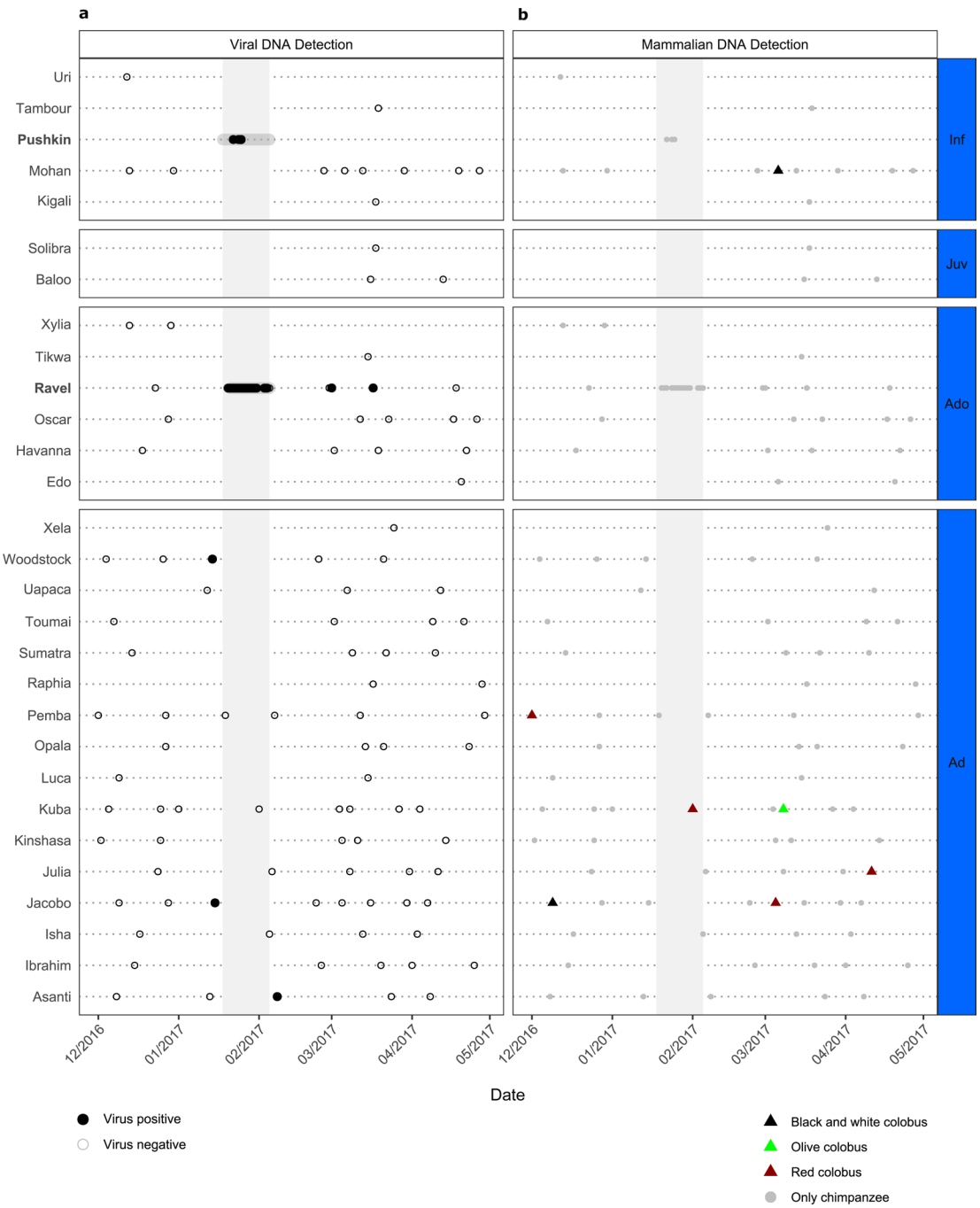
	Severe respiratory signs and diffuse rash	Severe respiratory signs and limited* to absent rash	Occasional coughing	Asymptomatic MPXV+ faeces
South Group (36) (18.01.2017-05.02.2017)				
Infant (10)		1		
Juvenile (4)				
Adolescent (4)		1		
Adult (18)				3
North Group (21) (03.03.2017-08.04.2017)				
Infant (9)	3		6	
Juvenile (1)		1		
Adolescent (2)				1
Adult (9)			1	3
East Group (36) (10.05.2018-29.05.2018)				
Infant (9)	1			
Juvenile (9)				1
Adolescent (3)				1
Adult (15)				2

Extended Data Fig. 2 | Clinical signs associated with MPXV infection during the 3 outbreaks in wild chimpanzees. The number following the group name is the total number of chimpanzees in the group. Dates below each group indicate the timeframe when clinical signs were observed. Numbers in brackets after each age group correspond to the number of chimpanzees belonging to that age group. Numbers under disease manifestation categories indicate the number and age class of chimpanzees who manifested those clinical signs. The number of asymptomatic chimpanzees in which MPXV DNA was detected in faeces is also reported in the last column.

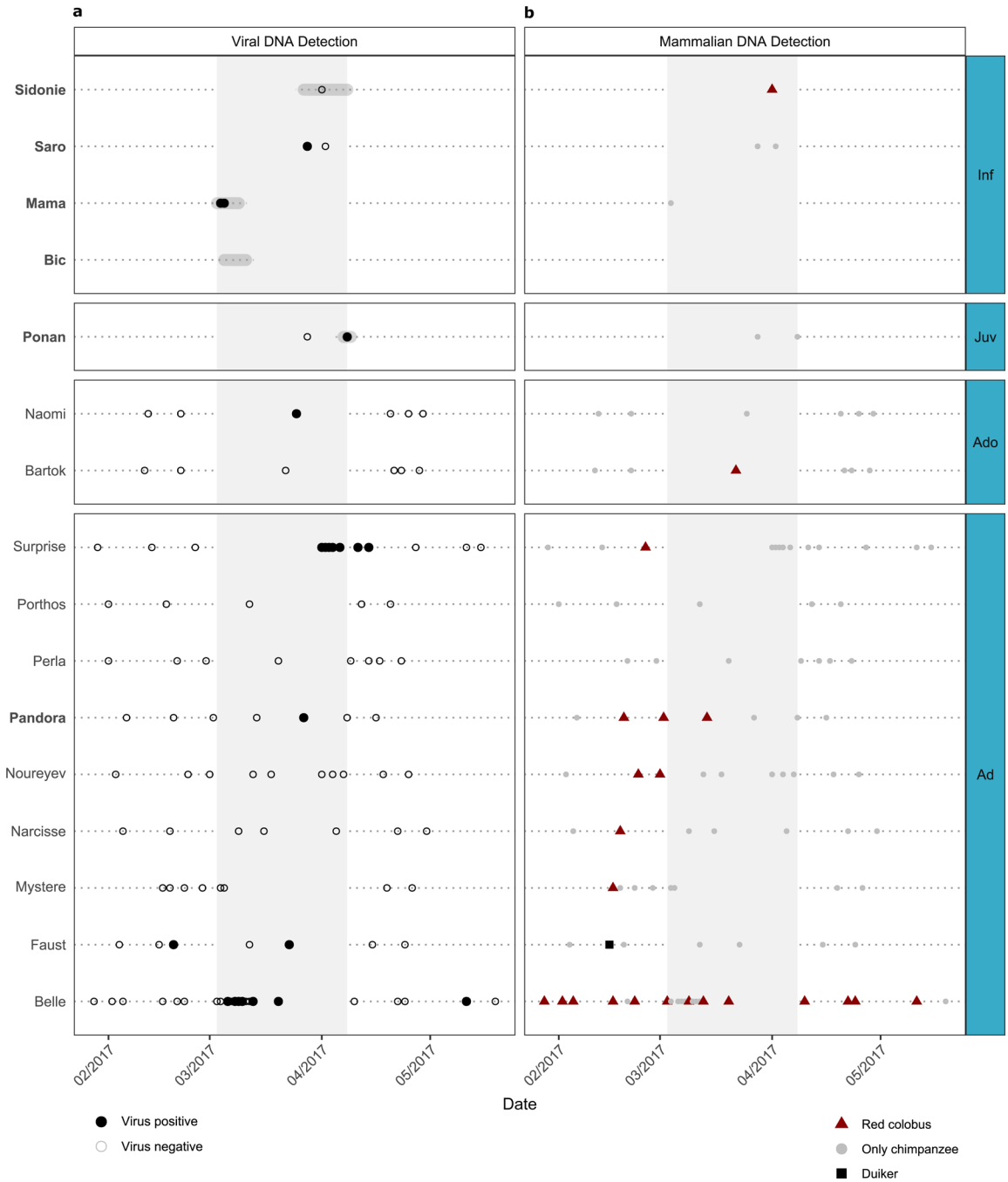
Type of sample	Faeces		Urine		Fruit wedge	
Group	Total	MPXV+ (%)	Total	MPXV+ (%)	Total	MPXV+ (%)
South Group	122	**23 (18.9%)**[1]	10	**5 (50%)**[2]	10	**3 (30.0%)**[3]
before (01.12.2016-17.01.2017)	31	2 (6.5%)				
during (18.01.2017-05.02.2017)	22	18 (81.8%)	10	5 (50%)	10	3 (30.0%)
after (06.02.2017-29.04.2017)	69	3 (4.3%)				
North Group	108	**24 (22.2%)**[4]	42	**6 (14.3%)**[5]	16	**2 (12.5%)**[6]
before(28.01.2017-02.03.2017)	34	1(2.9%)				
during (03.03.2017-08.04.2017)	43	20 (46.6%)	42	6 (14.3%)	16	2 (12.5%)
after (09.04.2017-19.05.2017)	31	3 (9.7%)				
East Group	263	**15 (5.7%)**[7]	3	0	nc	
before (01.04.2018-09.05.2018)	38	0	1	0		
during (10.05.2018-29.05.2018)	85	15 (17.6%)	2	0		
after (30.05.2018-25.08.2018)	139	0				
Total	492	**62 (12.6%)**	55	**11 (20.0%)**	26	**5 (19.2%)**

[1]5 chimpanzees (2 symptomatic, 3 asymptomatic), [2]1 chimpanzee (symptomatic), [3]2 chimpanzees (both symptomatic),
[4]8 chimpanzees (2 symptomatic, 2 asymptomatic mothers of symptomatic infants, 2 chimpanzees that coughed occasionally and are related to chimpanzees with severe signs, 2 asymptomatic unrelated),
[5]2 chimpanzees (1 symptomatic, 1 asymptomatic mother of symptomatic infant), [6]2 chimpanzees (both symptomatic),
[7]6 chimpanzees (1 symptomatic, 5 asymptomatic). nc= not collected

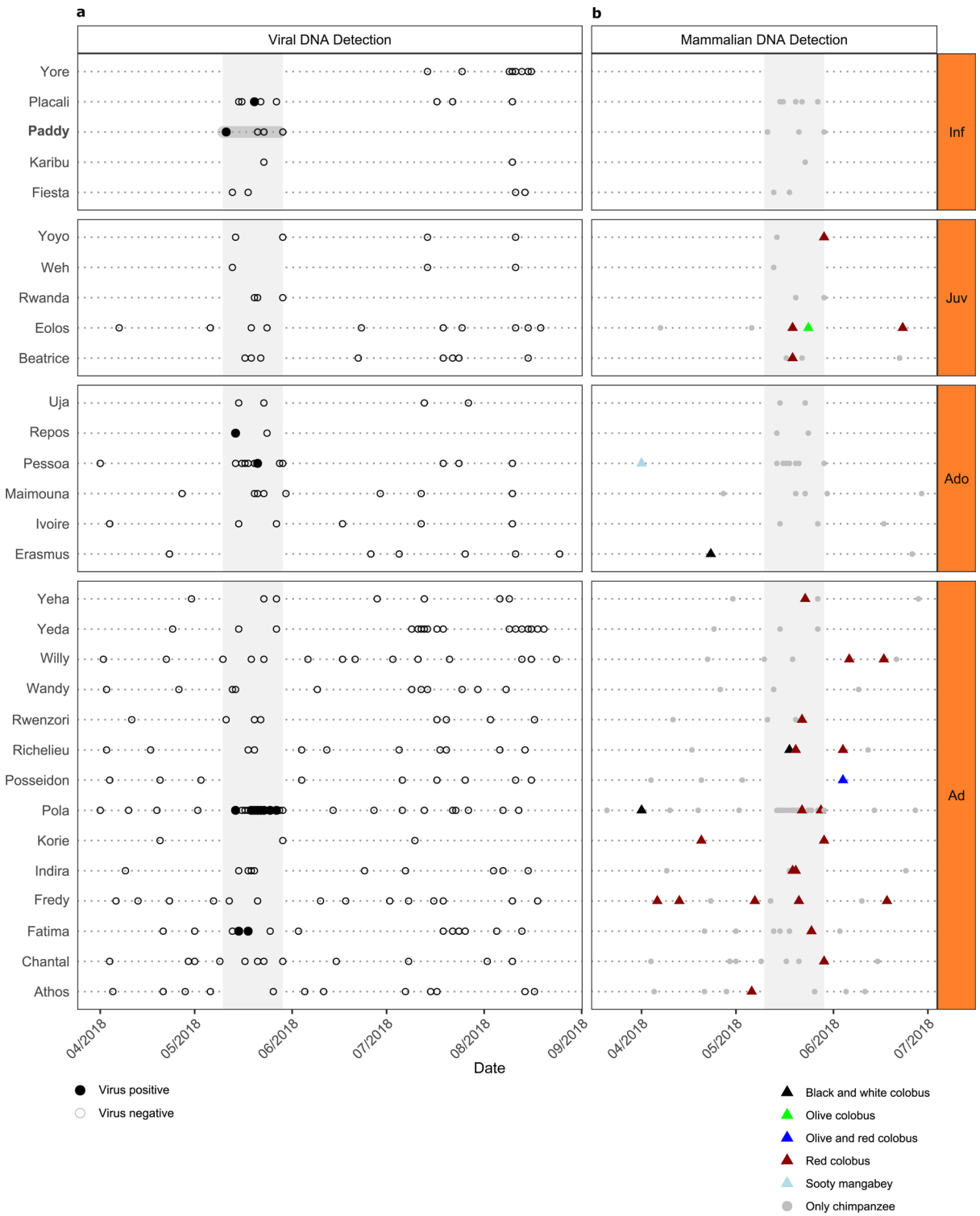
Extended Data Fig. 3 | Summary of samples types and MPXV DNA detection in all non-invasive samples collected before, during and after the appearance of clinical signs associated with MPXV infection in the three chimpanzee communities. Collection periods are reported for each group.



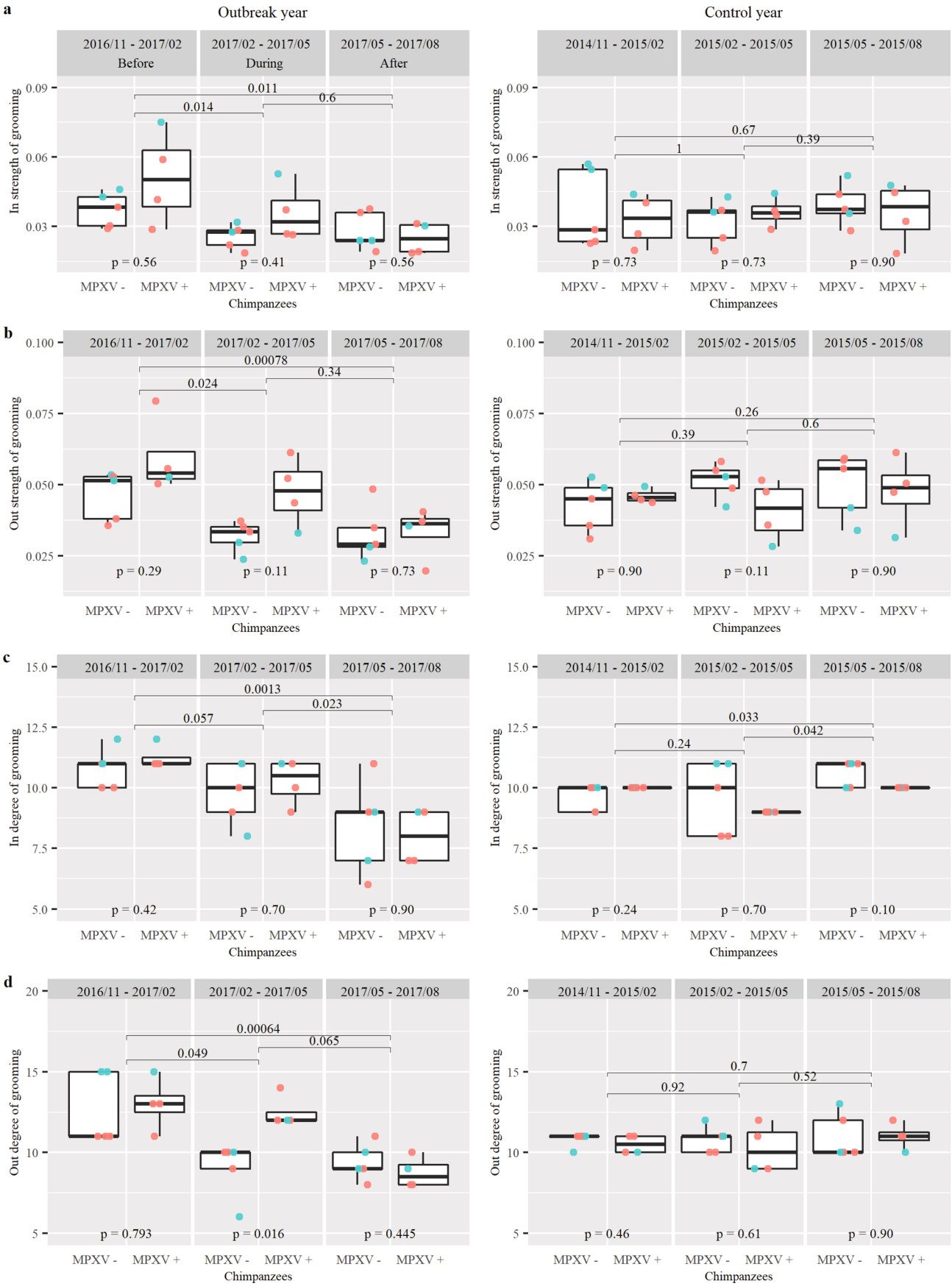
Extended Data Fig. 4 | Molecular analyses in faecal samples. a, Detection of MPXV DNA and non-chimpanzee mammalian DNA **b**, in chimpanzee faecal samples from the South community over time. Each line represents a chimpanzee. Bold names correspond to chimpanzees in which mild to severe signs compatible with MPXV infection were observed. Boxes represent age groups (infant, juvenile, adolescent and adult). Vertical light grey shadowing indicates the period in which clinical signs were observed in the group. Horizontal dark grey shadowing indicates the period in which clinical signs were observed in severely ill chimpanzees.



Extended Data Fig. 5 | Molecular analyses in faecal samples. a, Detection of MPXV DNA and non-chimpanzee mammalian DNA **b**, in chimpanzee faecal samples from the North community over time. Each line represents a chimpanzee. Bold names correspond to chimpanzees in which mild to severe signs compatible with MPXV infection were observed. Boxes represent age groups (infant, juvenile, adolescent and adult). Vertical light grey shadowing indicates the period in which clinical signs were observed in the group. Horizontal dark grey shadowing indicates the period in which clinical signs were observed in severely ill chimpanzees.

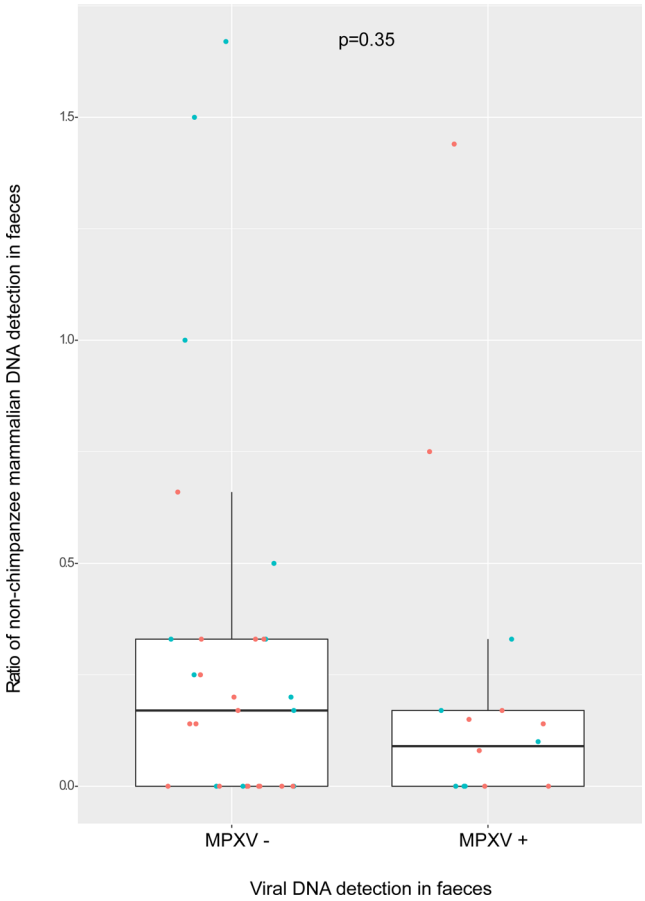


Extended Data Fig. 6 | Molecular analyses in faecal samples. a, Detection of MPXV DNA and non-chimpanzee mammalian DNA **b**, in chimpanzee faecal samples from the East community over time. Each line represents a chimpanzee. Bold names correspond to chimpanzees in which mild to severe signs compatible with MPXV infection were observed. Boxes represent age groups (infant, juvenile, adolescent and adult). Vertical light grey shadowing indicates the period in which clinical signs were observed in the group. Horizontal dark grey shadowing indicates the period in which clinical signs were observed in severely ill chimpanzees.

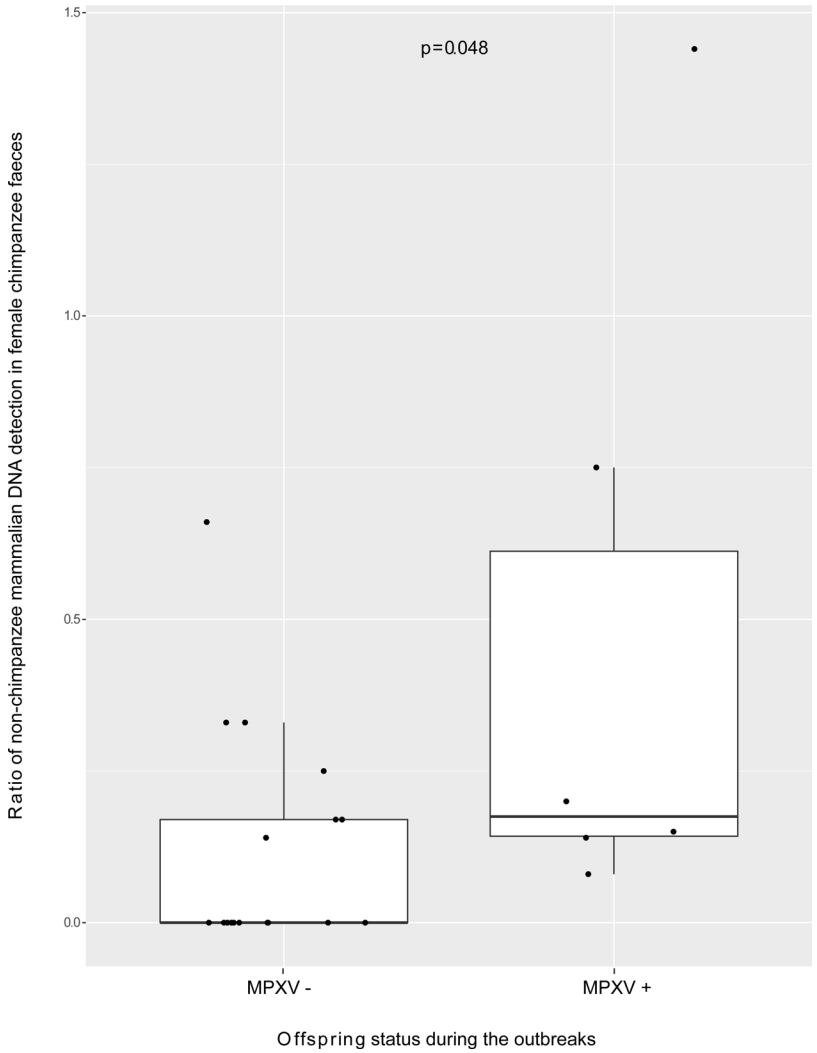


Extended Data Fig. 7 | See next page for caption.

Extended Data Fig. 7 | Social network metrics. Individual social network metrics in-strength **a**, out-strength **b**, in-degree **c**, and out-degree **d**, in the North community. Individuals (n=9) are classified as MPXV negative and positive according to PCR results from faecal samples. Timeframes before, during and after the outbreak are based on first and last positive PCR result. The network metrics are also shown for the year 2015 as control. The horizontal line in the whisker plots represents the mean. The lower and upper bounds of the boxes indicate the first and third quartile, respectively. Vertical lines are the upper and lower whisker representing a maximum of the largest/smallest value, but not over 1.5 times the interquartile distance. P values were calculated with a two-sided Mann-Whitney test. (●female ●male).



Extended Data Fig. 8 | Ratio of non-chimpanzee mammalian DNA detection in chimpanzee faecal samples from the three communities. Individuals (n=46) are divided into MPXV positive and negative based on PCR results from faecal samples. The horizontal line in the whisker plots represents the mean. The lower and upper bounds of the boxes indicate the first and third quartile, respectively. Vertical lines are the upper and lower whisker representing a maximum of the largest/smallest value but not over 1.5 times the inter quartile distance. P values were calculated with a two-sided Mann-Whitney test. (●female ●male).



Extended Data Fig. 9 | Ratio of non-chimpanzee mammalian DNA detection in faecal samples of female chimpanzees with offspring from the three communities. Individuals (n=23) are divided into MPXV positive and negative based on the status of their offspring during the outbreaks. The horizontal line in the whisker plots represents the mean. The lower and upper bounds of the boxes indicate the first and third quartile, respectively. Vertical lines are the upper and lower whisker representing a maximum of the largest/smallest value but not over 1.5 times the inter quartile distance. P values were calculated with a two-sided Mann-Whitney test.

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used
Data analysis	For the social network analyses the R-package igraph was implemented using R-Studio v1.2.1335. For taxonomic assignement of sequences resulting from a metabarcoding experiment a custom pipeline using OBITools v1.1 was used (described in detail in the methods section of the paper). For processing of raw NGS reads we used a custum script which implements the following tools: Trimmomatic v0.36, BWA-MEM v0.7.15-r1140, and the SortSam and MarkDuplicates tools from Picard v1.113 (http://broadinstitute.github.io/picard). The software Geneious v10.0.5 was used for visualization of NGS data. For de novo assembly we used metaSPAdes v3.12.0. Genome colinearity was checked using ProgressiveMauve v2.4.0 as implemented in Geneious.For phylogenetic analyses the following softwares or packages were used: MAFFT v7, Gblocks v0.91b as implemented in Seaview v4, FaBox v1.4, jModeltest v2.1.10, PhyML v3, TemEst v1.5, BEAST v1.10.4, logCombiner v1.10.4, TreeAnnotator v1.10.4, Figtree v1.4.0 and ITOL v5.5.

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Raw reads of 16S amplicons are available in the European Nucleotide Archive (ENA) under project accession number PRJEB34040, sample accession numbers ERS3658904-ERS3659288. Raw reads resulting from MPXV target enrichment in non-invasive samples are available in the ENA under project accession number PRJEB34056, sample accession numbers ERS3659494-ERS3659510. Consensus sequences are available in GenBank, accession numbers MN346690-MN3466703.

Annotated MPXV genomes are available in Zenodo (record nr 3373886). Sequences derived from the fly species identification are also available in Zenodo (record nr3606577).

Field-specific reporting

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Here we describe three outbreaks of monkeypox virus in human-habituated wild-living chimpanzees in Tai National Park, Ivory Coast. This work falls within the long term behavioural and health monitoring program of the Tai Chimpanzee Project. We preformed outbreak investigations by monitoring the chimpanzees on a daily basis and collecting non-invasive samples from any individual whenever we had the opportunity. This type of study is observational and involves wild animals, therefore no statistical calculations on sample size can be applied.
Research sample	The research sample is represented by three neighboring communities of western wild chimpanzees (Pan troglodytes verus). A total of 93 individuals (40 males and 53 females) were present at the time of this study and the age ranged from 1 month to 52 years. The three communities are monitored daily by trained staff who is able to recognize them individually.
Sampling strategy	We performed non-invasive sampling through collection of faeces, urine, fruit wedges and flies from symptomatic and asymptomatic chimpanzees. Sampling from wild chimpanzees is opportunistic and cannot be calculated beforehand. Samples were taken in any occasion possible without disturbing the natural beahviour of the chimpanzees. Necropsies on dead wildlife were performed by trained veterinarians whenever a carcass was encountered in the research area.
Data collection	Data collection was performed by local field assistants, researchers and veterinarians working for the Tai Chimpanzee project. Behavioral data were collected on apposite data sheets. Clinical data was collected by a veterinarian and documented via pictures and videos.
Timing and spatial scale	Sample collection for the project started in 1994 and has been routinely carried out ever since in the research area (170km2). Over 25 years we have accumulated a collection of chimpanzee faecal and urine samples and necropsy samples from all wildlife found dead in the area.
Data exclusions	No specifica data were excluded from the study.
Reproducibility	All real-time PCRs were performed at least in duplicate. In presence of doubtful results all tests were repeated from the first step. All results presented were reproducible.
Randomization	Randomization is not relevant for this type of study, which is based on investigating infectious causes of illness in wildlife. To maximize our chances of pathogen detection we sampled all individuals, whenever possible.
Blinding	Not applicable due to the nature of the study (outbreak investigation in wild animals).

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Primary tropical rainforest
Location	Tai National Park, Ivory Coast
Access and import/export	All research is conducted under the umbrella of a collaboration with Ivorian partners and health authorities. Samples are routinely exported to Germany for diagnostic purposes following international guidelines and prior official authorization through CITES permits yearly issued from the Bundesamt für Naturschutz, where necessary. Such permits allow for sample import at the Robert Koch Insitute from the Centre Suisse de Recherches Scientifiques (partner in Ivory Coast). The latest was issued on 12.07.2019 (Nr. DE E-04972/19).
Disturbance	All activities conducted for this study were carried out as part of the Tai Chimpanzee Project. This project is aimed at collecting behavioural observations from wild chimpanzees through an habituation process which has been running since 1979. All samples and observations collected are done with the minimum disturbance to wildlife and the environment.

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☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	This study did not involve laboratory animals.
Wild animals	These western wild chimpanzee (Pan troglodytes verus) communities have been under human habituation since 1979. A total of 93 individuals (40 males and 53 females) were present at the time of this study and the age ranged from 1 month to 52 years. A team of field assistants and researchers is following the animals on a dialy basis from a 7-meter distance, recording behavioural data and collecting faeces and urine samples whenever possible. In normal situations, each assistant or researcher has one focal individual per day to collect data and samples from. In outbreak situations, monitoring efforts are reinforced and sampling is attempted from all symptomatic and asymptomatic individuals.
Field-collected samples	Samples are collected upon defecation or urination of the chimpanzees and stored in 2ml cryotubes. The research camps of the Tai Chimpanzee Project are equipped with liquid nitrogen tanks for storage of samples. Samples are then transported to Abidjan for temporary storage at the Centre Suisse de Recherches Scientifiques and subsequently shipped to Germany on dry ice whenever someone is traveling.
Ethics oversight	No ethical approval was required for this study since it is based on collecting observational data from wild chimpanzees. Sampling was performed non-invasivaly without disturbing chimpanzees' natural behaviour. Tissue samples were collected from natually dead animals found in the research area. The work was done in collaboration and under the permission of local park authorities and local partners.

Note that full information on the approval of the study protocol must also be provided in the manuscript.