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Demography, life-history trade-offs, and the gastrointestinal virome of wild chimpanzees

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In humans, senescence increases susceptibility to viral infection. However, comparative data on viral infection in free-living non-human primates—even in our closest living relatives, chimpanzees and bonobos (*Pan troglodytes* and *P. paniscus*)—are relatively scarce, thereby constraining an evolutionary understanding of age-related patterns of viral infection. We investigated a population of wild eastern chimpanzees (*P. t. schweinfurthii*), using metagenomics to characterize viromes (full viral communities) in the faeces of 42 sexually mature chimpanzees (22 males, 20 females) from the Kanyawara and Ngogo communities of Kibale National Park, Uganda. We identified 12 viruses from at least four viral families possessing genomes of both single-stranded RNA and single-stranded DNA. Faecal viromes of both sexes varied with chimpanzee age, but viral richness increased with age only in males. This effect was largely due to three viruses, salivirus, porprismacovirus and chimpanzee stool-associated RNA virus (chisavirus), which occurred most frequently in samples from older males. This finding is consistent with the hypothesis that selection on males for early-life reproduction compromises investment in somatic maintenance, which has delayed consequences for health later in life, in this case reflected in viral infection and/or shedding. Faecal viromes are therefore useful for studying processes related to the divergent reproductive strategies of males and females, ageing, and sex differences in longevity.

This article is part of the theme issue 'Evolution of the primate ageing process'.

1. Background

Finite energetic resources catalyse trade-offs between immunity and other biological processes, such as growth and reproduction [1,2]. Immunosenescence, the deterioration of the immune system with advanced age, may occur more rapidly when resources allocated to reproduction outweigh those allocated to somatic investment, with consequent reductions in health and longevity. Immunosenescence involves a suite of changes to innate and adaptive immune function, including increased concentrations of circulating proinflammatory markers [3], decreased thymus size and naive T-cell proliferation [4], and decreased responsiveness of memory T cells [5]. In humans, immunosenescence increases susceptibility to novel viral infection [6] as well as reactivation of latent infections [7]. Common and largely apathogenic viral agents such as

cytomegalovirus may accelerate immunosenescence through chronic antigenic stimulation and inflation of virus-specific $CD8^+$ T cell populations [8,9].

Sex differences in longevity are common across mammalian species with female life expectancy often greater than that of males [10], including in humans [11]. This phenomenon may reflect divergent reproductive strategies. Females are hypothesized to invest more than males in somatic maintenance across the lifespan because, due to constraints on female reproductive rates, longevity is more important for female reproductive success [12]. The importance of early life fertility to male reproductive success, as reflected in the more rapid decline of males' age-specific fertility [13], likely disincentivizes investment in somatic maintenance, especially when male–male competition is high and male investment in offspring is low [14]. Studies of immune biomarkers support these ideas, suggesting accelerated immunosenescence in male rhesus macaques (*Macaca mulatta*) [15], brown rats (*Rattus norvegicus*) [16] and roe deer (*Capreolus capreolus*) [17]. Similarly, T and B cell populations (including their proliferative capacity) decline faster in Japanese men than in women [18], and men exhibit higher innate immune activity, indicating a sex bias in immunological investment [19]. Men also experience higher prevalence and load of viral infection across the lifespan than do women [20], mirroring these trends.

Chimpanzees (*Pan troglodytes*), our closest living relatives, provide a valuable comparison for understanding age-related trade-offs between the energetics of reproduction and other physiological functions. Critically, chimpanzees live long lives. In the wild, chimpanzee life expectancy at birth for both sexes combined ranges from approximately 13 to 33 years [21], and the maximum lifespan exceeds 60 years [21,22]. Chimpanzees exhibit a human-like sex-bias in lifespan, with females living longer than males [21]. Previous studies have examined energetic trade-offs related to these patterns. Physiological [23] and observational studies [24] indicate that male chimpanzees exhibit greater total daily energy expenditures than do females, due in part to the greater body mass of males [25] and the physiological costs of male–male mating competition [26]. Total energy expenditure rates are expected to correlate negatively with longevity, as the accumulation of free radicals, produced by mitochondria [27], spurs senescence [28].

Studies of chimpanzee health and immune function in the context of energetic trade-offs have largely focused on gastrointestinal parasites because they can be identified and enumerated microscopically [29]. These studies suggest that male reproductive strategies may impose immunological costs. For instance, at Gombe Stream National Park, Tanzania, male chimpanzees sometimes exhibit higher prevalence of the gastrointestinal parasites *Strongyloides fulleborni* and *Iodamoeba bütschlii* than do females [30]. Furthermore, male chimpanzees who successfully vie for high social status exhibit greater helminthic parasite richness [31]. By contrast, studies of chimpanzee gastrointestinal bacterial communities show that, despite links with season [32] and social behaviour [33], microbiotal enterotypes do not readily cluster by age or sex [34].

Wild chimpanzees are also exposed to viruses, but most of our current knowledge about these viruses comes from studies of highly virulent pathogens [35–39]. Notably, respiratory disease outbreaks in chimpanzees in Kibale National Park, Uganda, have been attributed universally thus far to viral pathogens [35,36]. Even in these systems, however, there is evidence suggesting age and sex-related susceptibility. For instance, in the Kanyawara chimpanzees of Kibale, older individuals of both sexes were more likely to exhibit clinical signs of respiratory disease over a 20-year period than young individuals [40]. Furthermore, clinical signs were more common in males during the years of peak reproductive effort than at other life stages, although there was no sex bias among older chimpanzees [40].

In this study, we employed metagenomic next-generation sequencing to characterize the community of gastrointestinal viruses (the 'virome') in apparently healthy animals from the Kanyawara and Ngogo chimpanzee communities in Kibale National Park, Uganda (Kibale, hereafter). In accord with the expectation that wild chimpanzees, like humans, experience a prolonged immunosenescence, we predicted that the presence and burden (i.e. load) of viral infection, measured as virus shed in faeces, would increase with age in sexually mature chimpanzees. We also predicted that males, who invest more heavily in mating competition early in life, would harbour gastrointestinal viruses at a higher presence and burden than would females, especially later in life.

2. Methods

(a) Study sites, subjects and sample collection

Between July and October 2016, we collected faecal samples from chimpanzees in the Kanyawara and Ngogo chimpanzee communities in Kibale. At the time of sample collection, these communities comprised approximately 55 and 204 individuals, respectively. Chimpanzees have been observed continuously at Kanyawara since 1987 [41] and at Ngogo since 1995 [42]. Faecal samples were collected immediately after an individual was observed to defecate and stored in RNAlater buffer (Thermo Fisher Scientific, Waltham, MA, USA) at a 1 : 1 ratio. Samples were stored at each site at −20°C until transported on ice to the USA. We analysed one sample from each of 42 individuals ranging in age from 9 to 66 years (Kanyawara males = 10; Kanyawara females = 10; Ngogo males = 12; Ngogo females = 10). The ages of most individuals in these communities are known from their dates of birth. The ages of individuals born in each community before the start of long-term study were estimated based on their physical appearance relative to individuals of known age and genealogical relationships [21,43].

(b) Viromics

We used metagenomics to identify viruses in chimpanzee faeces, following previously described methods [44–48]. First, we homogenized 200 µl of sample (faeces + RNAlater) by bead beating in 1 ml Hanks balanced salt solution and then treated the homogenate with nucleases to reduce DNA and RNA not encapsidated within virions [49]. We then extracted nucleic acids using the Qiagen QIamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany), and converted RNA to double-stranded cDNA, which we then purified using Agencourt AmpureXP beads (Beckman Coulter, Brea, CA, USA) as previously described [44–48]. We then prepared libraries for sequencing on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) using 150 × 150 cycle paired-end (V2) chemistry using the Illumina Nextera XT kit. These protocols are the same as previously described methods for sample preparation [44] and bioinformatics analyses [45] and, in these previous studies, have successfully identified communities of viruses in clinical samples, including validation with controls and viral community standards [44–48].

(c) Bioinformatics

We analysed viral sequences using CLC Genomics Workbench v. 11.0.1 (CLC bio, Aarhus, Denmark). We trimmed sequences

of low quality and short length (less than 50 bp) and removed sequences matching known contaminants and host DNA. Remaining reads were then subjected to de novo assembly. We compared the resultant assembled contiguous sequences, or contigs, to viruses in the GenBank database at both the nucleotide and amino acid levels using the BLASTn and BLASTx algorithms, respectively [50,51]. We retained contigs matching mammalian viruses for further analysis and disregarded contigs matching viruses of known non-mammalian hosts (e.g. insects, plants, fungi). To assess viral loads, we mapped reads back to viral contigs and calculated the proportion of reads mapping to each virus (for virus-specific load) or the proportion of reads mapping to any virus (for total viral load). We then normalized this measure to one million reads [46] and to the length of the target sequence (contig) for each virus, such that our final measure of viral load was viral reads per million per kilobase of target (vRPM/kb), which is correlated with results from real-time quantitative polymerase chain reaction [46].

Phylogenetic relationships among viruses were inferred from viral replicase (polymerase) genes when possible and with other viral genes when only these were available in GenBank. We first aligned sequences of newly identified viruses with published sequences of related viruses in the GenBank database using the Prank algorithm [52] in TranslatorX [53], with the Gblocks algorithm [54] applied to remove poorly aligned regions. We then inferred maximum-likelihood phylogenetic trees from the resulting alignments using PhyML 3.0 [55] with 1000 bootstrap replicates to assess statistical confidence in clades. We displayed final bootstrapped trees using FigTree v. 1.4.4 [56].

(d) Inferential statistics

We calculated the prevalence of each virus by sex and study community, with 95% confidence intervals computed using the modified Wald method [57]. We conducted statistical analyses of viral presence (i.e. sequence reads matching a virus), richness (i.e. the number of viral species present in a sample), and load (i.e. vRPM/kb) using R v. 3.5.1 [58]. First, to analyse the presence of individual viruses, we generated a series of generalized linear models with a binomial error structure and logit link function using the 'glm' function. In each model, we included the presence of a virus as the dependent variable and chimpanzee age (as of July 2016), sex, an age by sex interaction, and community as the independent variables. When the inclusion of a predictor caused complete or partial model separation, we removed the term from the final model. We then constructed parallel linear models using the 'lm' function in R for the following dependent variables: viral load (for all viruses detected in 10 or more chimpanzees), viral richness, and total viral load (i.e. reads of all viruses per million reads per combined kilobase of target sequences) [46,59]. As for viral presence, we included chimpanzee age, sex, an age by sex interaction, and study community as independent variables. In all linear models, we evaluated the normality of residuals with Shapiro–Wilk tests [60] using the 'shapiro.test' function in R, as well as inspection of Q-Q plots [61]. No deviance from normality occurred, except in the linear model for total viral load. In this case, we Box-Cox transformed [62] total viral load and ran the model again. We set alpha to 0.05 in all models. To control for multiple testing, we adjusted all p-values for a given predictor (age, sex, age * sex and community) using the Benjamini–Hochberg procedure [63] implemented with the function 'p.adjust' in R, and we report both the original and corrected p-values.

3. Results

We identified 12 viruses in the faeces of chimpanzees (table 1). Amino acid sequence similarity to known viruses ranged from 55.91% to 99.26%. The prevalences of each virus, including prevalence by sex and study community, are shown in electronic supplementary material, table S1. Viruses were detected in all but two of the 42 faecal samples (these two samples were collected from two females aged 23.7 and 50.5 years, respectively). The overall prevalence of each virus ranged from 2.4% (95% CI: 0.0%, 13.4%, representing a single sample) to 45.2% (95% CI: 31.2%, 60.1%, representing 19 samples). A picobirna-like virus exhibited the lowest prevalence, whereas three porprismacoviruses exhibited the highest prevalences. Eight of the 12 viruses were found in both communities. An unclassified circular single-stranded DNA virus and a picobirna-like virus were detected only in faecal samples from Kanyawara chimpanzees, whereas an astrovirus and a salivirus were detected only in faecal samples from Ngogo chimpanzees. Porprismacoviruses 4 and 5 were more commonly detected in the Ngogo community, but tests adjusting for multiple comparisons indicated that their prevalence at Ngogo did not exceed their prevalence at Kanyawara (electronic supplementary material, table S2). There were no other effects of community.

Results of a linear model showed that viral richness increased with age in males but not in females (figure 1; $\beta = 0.100$, s.e. = 0.031, uncorrected $p = 0.003$, corrected $p = 0.031$). To examine the contributions of each virus to this finding, we ran a series of *post hoc* analyses emulating a backward selection procedure (e.g. Rodríguez-Perálvarez *et al*. [64]), in which we removed, one-by-one, the viruses with the largest effect sizes for the age-by-sex interaction. Removing all three viruses with the largest effect sizes (salivirus, chisavirus and proprismacovirus 1; figure 2) reduced the strength of the age-by-sex interaction and made the trend statistically non-significant without adjustments for multiple testing ($\beta = 0.051$, s.e. = 0.028, uncorrected $p = 0.072$). To determine whether the observed change in effect of this size was greater than expected by chance alone, we performed 1000 simulations in which we removed three viruses at random from the richness calculation. Only 0.9% of simulated models yielded a p-value as or more extreme than the observed p-value of 0.072.

The interaction between age and sex exhibited a notable trend for chimpanzee stool-associated RNA virus (chisavirus; figure 2*b*) and porprismacovirus 1 (figure 2*c*), in that males were more likely to harbour these viruses as they aged. However, the effect was not significant for either virus after controlling for multiple comparisons (electronic supplementary material, table S2). Total viral load did not vary with the age-by-sex interaction ($\beta = 0.014$, s.e. = 0.018, uncorrected $p = 0.465$, corrected $p = 0.680$). Of the seven viruses for which viral load could be analysed individually, the interaction between age and sex predicted only bufavirus load ($\beta = -0.082$, s.e. = 0.019, uncorrected $p = 0.003$, corrected $p = 0.031$): bufavirus load increased with age in females and decreased with age in males.

Age did not influence the presence of any virus (electronic supplementary material, table S2), nor did age impact viral richness ($\beta = -0.025$, SE = 0.021, uncorrected $p = 0.249$, corrected $p = 0.725$), total load ($\beta = 0.003$, s.e. = 0.013, uncorrected $p = 0.824$, corrected $p = 0.871$), or load by individual virus (electronic supplementary material, table S3). Salivirus tended to occur more frequently in males than in females ($\beta = 3.072$, s.e. = 1.423, uncorrected $p = 0.031$, corrected $p = 0.31$) and was detected in only one female, the oldest individual sampled (figure 2*a*). However, sex did not affect the presence of any

Table 1. Viruses detected in 42 faecal samples from wild chimpanzees in the Kanyawara and Ngogo communities of Kibale National Park, Uganda.

virus	closest match (accession number[a])	family	host (country, year)	genome	length (nt)[b]	% identity[c]	E-value[d]	accession number[e]
chimpanzee astrovirus	human astrovirus BF34 (YP_009047078)	Astroviridae	human (Burkina Faso, 2010)	ssRNA	963	87.85	0.00	MT076199
chimpanzee bufavirus	human bufavirus (AOR39545)	Parvoviridae	human (Tunisia, 2013)	ssDNA	1500	81.40	0.00	MT076200
chimpanzee unidentified circular ssDNA virus	unidentified circular ssDNA virus (AWU66046)	unclassified	human (Venezuela, 2015)	ssDNA	834	94.96	0.00	MT076201
chimpanzee stool-associated RNA virus (chisavirus)	husavirus sp. (AWU65954)	unclassified Picornavirales	human (Venezuela, 2015)	ssRNA	7122	65.67	0.00	MT076204
chimpanzee picobirna-like virus	Kumba picobirna-like virus (QAA77647)	unclassified Picornavirales	human (Cameroon, 2014)	RNA	1188	93.43	0.00	MT076202
eastern chimpanzee associated porprismacovirus 1	*Macaca mulatta* faeces associated virus 4 (APG55823)	Smacoviridae	Rhesus macaque (USA, 2014)	ssDNA	777	64.00	3.00E-120	MT076205
eastern chimpanzee associated porprismacovirus 2	porcine associated porprismacovirus (QBP37091)	Smacoviridae	pig (Vietnam, 2013)	ssDNA	735	71.91	6.00E-133	MT076206
eastern chimpanzee associated porprismacovirus 3	porcine associated porprismacovirus 8 (YP_009054991)	Smacoviridae	pig (USA, 2011)	ssDNA	786	55.91	3.00E-106	MT076207
eastern chimpanzee associated porprismacovirus 4	chimpanzee stool associated circular ssDNA virus (ADB24816)	Smacoviridae	chimpanzee (Tanzania, 2004)	ssDNA	816	99.26	0.00	MT076208
eastern chimpanzee associated porprismacovirus 5	chimpanzee stool associated circular ssDNA virus (ADB24816)	Smacoviridae	chimpanzee (Tanzania, 2004)	ssDNA	777	91.89	0.00	MT076209
eastern chimpanzee associated porprismacovirus 6	*Macaca mulatta* faeces associated virus 7 (APG55812)	Smacoviridae	Rhesus macaque (USA, 2014)	ssDNA	780	68.34	5.00E-134	MT076210
chimpanzee salivirus	salivirus CH (AEX38455)	Picornaviridae	chimpanzee (China, 2011)	ssRNA	1347	82.54	0.00	MT076203

[a]GenBank accession number of closest match is shown in parentheses.
[b]Length refers to the length of the sequence used for phylogenetic and viral load analyses.
[c]% identity refers to the per cent amino acid identity of the new virus to its closest match in GenBank.
[d]The E-value is the number of hits of similar quality expected to match the sequence simply by chance.
[e]Accession number of new viral sequence deposited in GenBank.

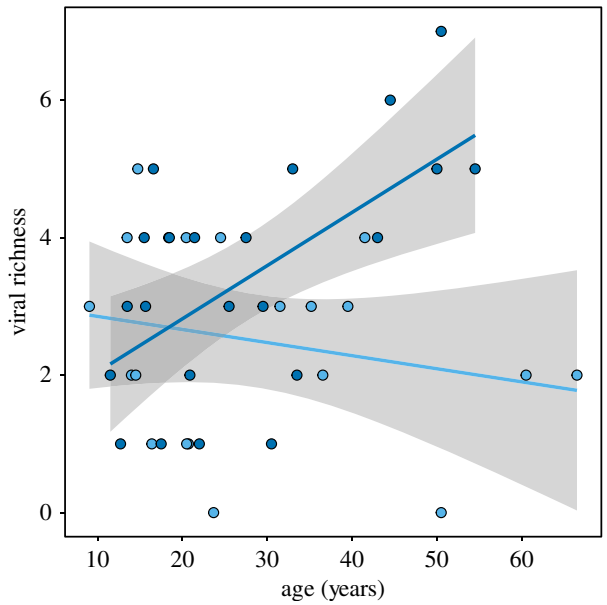

Figure 1. Chimpanzee gastrointestinal viral richness as a function of chimpanzee age and sex (light points, females; dark points, males). Grey shading around regression lines indicates 95% confidence intervals. (Online version in colour.)

virus, nor did it affect viral richness ($\beta = -1.882$, s.e. = 0.975, uncorrected $p = 0.061$, corrected $p = 0.407$), total load ($\beta = -0.578$, s.e. = 0.575, uncorrected $p = 0.322$, corrected $p = 0.644$), or load by individual virus (electronic supplementary material, table S3).

4. Discussion

Although viral infection is thought to be both a cause and consequence of immunosenescence, comparative data from wild nonhuman primates pertaining to this idea are exceedingly rare. To investigate the relationship between viral infection and immunosenescence in our closest living relatives, we assessed faecal viromes in a population of wild eastern chimpanzees. We observed age-related changes in the faecal viromes of both male and female chimpanzees. Most notably, we observed an age-related increase in viral richness in male chimpanzees but not in females. Evidence from other primates connects viromic expansion to immunocompromise and disease. For example, in captive rhesus macaques, increased richness of the gastrointestinal virome is correlated with advanced simian immunodeficiency virus (SIV) infection [65] and SIV-related gastrointestinal disease [66]. Similarly, in humans, immunocompromised patients exhibit expanded skin viromes [67], while patients with inflammatory bowel disease exhibit richer gastrointestinal viromes [68]. It is, therefore, plausible that the increased viral richness observed in older male chimpanzees in our study reflects loss of immunocompetence, supporting the hypothesis that senescence manifests in the gastrointestinal virome.

This finding supports our central hypothesis that selective trade-offs between reproduction and somatic maintenance impact sex differences in immunity, and that this trade-off, in turn, influences viral infection in chimpanzees. Our results, which are consistent with data on sex differences in survival and immune function across animal species [10,69], also accord with the life-histories of chimpanzees. Life expectancy

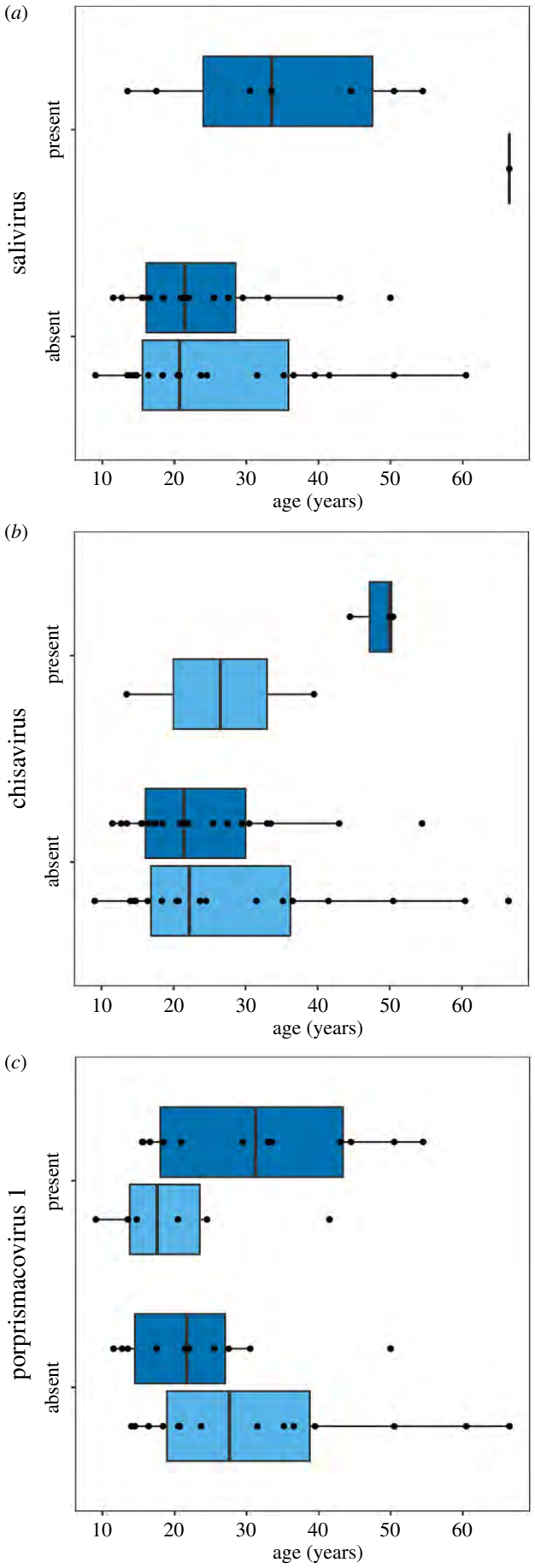

Figure 2. Presence of (*a*) salivirus, (*b*) chisavirus and (*c*) porprismacovirus 1 in chimpanzee faeces as a function of chimpanzee age. Boxes indicate the 25th and 75th percentiles, and thick black vertical lines indicate medians. Light and dark boxes indicate females and males, respectively. (Online version in colour.)

at birth is greater for female chimpanzees than for males [21]. This difference corresponds to differences in reproductive output: male chimpanzee fertility peaks in early-to-mid adulthood, around 20 years at Gombe [70] and 25 years at Kanyawara [71] and Ngogo (KE Langergraber 2020, unpublished data), whereas female chimpanzee fertility shows no distinct age-related peak [72]. During years of heightened reproductive output, male chimpanzees compete for receptive females, an activity that imposes substantial energetic costs [26,73,74] and may necessitate decreased investment in other energetically costly processes, such as somatic maintenance. Notably, we also observed an age-related increase in viral load in females for a single virus (bufavirus), suggesting that not all viruses follow the pattern predicted by life-history theory. Further analyses of the chimpanzee virome promise to elucidate additional consequences of investing in reproduction and immune function in this species. For example, analyses that consider the impact of variation in the social [75,76] and reproductive status (e.g. De Nys *et al*. [77]) of individuals on the chimpanzee virome are likely to be especially informative in this regard.

Although the quantity and taxonomic classifications of viruses we found were consistent with those observed in other primates (e.g. Sawaswong *et al*. [78]), much about the viruses identified in this study remains unknown. For example, three viruses identified in this study cannot yet be classified within families, complicating inferences about their basic biology and pathogenesis. With the possible exception of astrovirus, which can cause diarrhoea [79] and encephalitis [80], the newly identified viruses are not known to cause disease. Bufaviruses [81], husaviruses [82], porprismacoviruses [83] and saliviruses [84] have all been found in human patients with gastroenteritis, but at present, the relationship between infection and disease remains largely speculative. Importantly, most of the viruses we identified did not exhibit clear associations with either chimpanzee age or sex. This result underscores a growing understanding of the virome as a community containing members that range from beneficial to harmful, rather than an assemblage of pathogens [85,86]. For example, in mice (*Mus musculus*), commensal viruses regulate lymphocyte populations and maintain intestinal homeostasis [87]. Similarly, in captive rhesus macaques, some gastrointestinal viruses are associated with diarrhoea while others, including circular single-stranded DNA viruses, are associated with apparent health [88]. Consequently, some of the viruses identified in this study may not impose disease or fitness burdens on chimpanzees. Furthermore, the mode of transmission of most of these viruses remains unknown. For example, male chimpanzees are more gregarious [89], engage in higher levels of aggression [90], may endure higher rates of wounding, as has been observed in baboons (*Papio* spp.) [91], and are more likely to inflict bite wounds risking oral-blood contact [92]. Thus, male chimpanzees may be both more exposed and more susceptible to viral infections. In wild chimpanzees, we suspect that infection with these poorly known viruses likely results from complex interactions among exposure, susceptibility and viral biology.

The direction of effect of the trends we have documented are similarly unclear. For example, our data do not distinguish among direct effects of immune dysfunction on viral infection or shedding, age-related differences in exposure to viruses, or the effects of unmeasured confounds on both ageing and viral infection/shedding. We also note that we did not measure immunity (or reproductive energetic expenditure) directly, nor is it clear how immunity regulates infection or shedding of the viruses we identified. However, because viruses are obligate intracellular molecular parasites [93], viral systems may be particularly suited to examining immunological trade-offs.

Despite these caveats, the demographic patterns we have documented may provide a starting point for assessing the fitness costs of particular viruses, and of interacting viral communities. Notably, we documented an increase in overall viral richness with male age. Three viruses—salivirus, porprismacovirus 1 and chisavirus—drove this trend, in that once they were removed from the calculation of richness, the effect was marginally non-significant. Thus, different viruses and sets of viruses within viral communities contribute differently to community-level trends. Although our sample sizes may have been too small to detect the contributions of individual viruses to this trend, especially given corrections for multiple testing, we propose that those viruses most strongly associated with age and other energetically depleted physiological states (e.g. injury, pregnancy) are likely to be the most harmful agents within viral communities.

In summary, we observed demographic patterns in the faecal viromes of wild chimpanzees that are consistent with life-history theory predicting age- and sex-related energetic trade-offs between reproduction and somatic maintenance. Notably, male and female chimpanzees exhibited divergent age-related patterns, including increased viral richness with age in males but not females. Several mechanisms could drive this relationship, ranging from internal processes such as differential hormone secretion and gene expression [94] to external processes such as disparate exposure to viruses and environmental stressors [95,96]. Elucidating these mechanisms has great potential for expanding our understanding of infection biology, life-history theory, and their intersection.

Ethics. All procedures for this non-invasive study of wild chimpanzees were approved by the Uganda Wildlife Authority, the Uganda National Council for Science and Technology, and by the Institutional Animal Care and Use Committees (IACUCs) of Harvard University (protocol no. 96-03) and the University of New Mexico (protocol no. 14-101186-MCC). The study was exempt from review by the University of Michigan's IACUC.

Data accessibility. Viral nucleotide sequences are available on GenBank under accession nos. MT076199 to MT076210. Additional data and R code are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.w6m905qmk [97].

Authors' contributions. M.E.T., K.E.L., Z.P.M., J.C.M., M.N.M., E.O., R.W.W. and T.L.G. conceived the study; J.D.N. and T.L.G. drafted the manuscript; L.A.O. and J.D.N. performed laboratory work; J.D.N. completed bioinformatics and statistical analyses; M.E.T., K.E.L., Z.P.M., J.C.M., M.N.M., E.O. and R.W.W. coordinated sample collection; all authors made significant intellectual contributions, revised the manuscript and approved the final draft.

Competing interests. We have no competing interests.

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