MBE Advance Access published May 13, 2015

Multiple Cross-Species Transmission Events of Human Adenoviruses (HAdV) during Hominine Evolution

Eileen Hoppe,[1] Maude Pauly,[1,2] Thomas R. Gillespie,[3,4] Chantal Akoua-Koffi,[5] Gottfried Hohmann,[6] Barbara Fruth,[7,8] Stomy Karhemere,[9] Nadège F. Madinda,[2,6,10] Lawrence Mugisha,[11,12] Jean-Jacques Muyembe,[9] Angelique Todd,[13] Klara J. Petrzelkova,[14,15,16,17] Maryke Gray,[18] Martha Robbins,[6] Richard A. Bergl,[19] Roman M. Wittig,[6,20] Klaus Zuberbühler,[21,22,23] Christophe Boesch,[6] Grit Schubert,[2] Fabian H. Leendertz,[2] Bernhard Ehlers,*[,†,1] and Sébastien Calvignac-Spencer*[,†,2]

[1]Division 12 "Measles, Mumps, Rubella and Viruses affecting immunocompromised patients", Robert Koch Institute, Berlin, Germany

[2]Epidemiology of highly pathogenic microorganisms, Robert Koch Institute, Berlin, Germany

[3]Department of Environmental Sciences and Program in Population Biology, Ecology and Evolution, Emory University

[4]Department of Environmental Health, Rollins School of Public Health, Emory University

[5]Centre de Recherche pour le Développement, Université Alassane Ouattara de Bouake, Bouake, Côte d'Ivoire

[6]Department of Primatology, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

[7]Division of Neurobiology, Ludwig-Maximilians-University, Munich, Germany

[8]Centre for Research and Conservation, Royal Zooological Society of Antwerp, Antwerp, Belgium

[9]Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of Congo

[10]Institut de Recherche en Ecologie Tropicale, Libreville, Gabon

[11]College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda

[12]Conservation & Ecosystem Health Alliance (CEHA), Kampala, Uganda

[13]World Wildlife Foundation (WWF), Dzanga Sangha Protected Areas, Bangui, Central African Republic

[14]Institute of Vertebrate Biology, Academy of Sciences, Brno, Czech Republic

[15]Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

[16]Biology Centre, Institute of Parasitology, Academy of Sciences of the Czech Republic, Ceske Budejovice, Czech Republic

[17]Liberec Zoo, Liberec, Czech Republic

[18]International Gorilla Conservation Program, Kigali, Rwanda

[19]North Carolina Zoological Park, Asheboro, NC

[20]Taï Chimpanzee Project, Centre Suisse de Recherches Scientifiques, Abidjan, Côte d'Ivoire

[21]Institute of Biology, University of Neuchatel, Neuchatel, Switzerland

[22]Budongo Conservation Field Station, Masindi, Uganda

[23]School of Psychology, University of St. Andrews, St. Andrews, Scotland, United Kingdom

[†]These authors contributed equally to this work.

***Corresponding author:** E-mail: calvignacs@rki.de; ehlersb@rki.de.

Article

Abstract

Human adenoviruses (HAdV; species HAdV-A to -G) are highly prevalent in the human population, and represent an important cause of morbidity and, to a lesser extent, mortality. Recent studies have identified close relatives of these viruses in African great apes, suggesting that some HAdV may be of zoonotic origin. We analyzed more than 800 fecal samples from wild African great apes and humans to further investigate the evolutionary history and zoonotic potential of hominine HAdV. HAdV-B and -E were frequently detected in wild gorillas (55%) and chimpanzees (25%), respectively. Bayesian ancestral host reconstruction under discrete diffusion models supported a gorilla and chimpanzee origin for these viral species. Host switches were relatively rare along HAdV evolution, with about ten events recorded in 4.5 My. Despite presumably rare direct contact between sympatric populations of the two species, transmission events from gorillas to chimpanzees were observed, suggesting that habitat and dietary overlap may lead to fecal-oral cross-hominine transmission of HAdV. Finally, we determined that two independent HAdV-B transmission events to humans occurred more than 100,000 years ago. We conclude that HAdV-B circulating in humans are of zoonotic origin and have probably affected global human health for most of our species lifetime.

Key words: **adenovirus, African great apes, zoonosis.**

Introduction

African great apes (hereafter great apes) are commonly infected with close relatives of human pathogens (recently reviewed in Calvignac-Spencer et al. [2012]). This phylogenetic proximity can be explained by host–parasite codivergence, whereby host divergence drives their parasite divergence, and/or host switch, whereby human or great ape infections result from the transfer of parasites in either direction (here and in the following we use the term parasite in its ecological sense). Striking examples of great ape-borne human infections have been revealed over the last decade. HIV-1 was shown to have a recent origin in chimpanzees and gorillas (depending on the HIV-1 group; Keele et al. 2006; Van Heuverswyn et al. 2006). *Plasmodium falciparum* was identified as having emerged in a more distant past from malaria parasites infecting gorillas (Liu et al. 2010). Host switches from great apes to humans have therefore driven the emergence of some of the most devastating human infectious diseases. Host switches between great ape species have also been documented (e.g., simian immunodeficiency viruses from gorillas—SIVgor—originate in chimpanzees; Van Heuverswyn et al. 2006; Neel et al. 2010) but their significance for great ape health remains unknown.

Cross-species transmission may result from direct contact between species, species bridging through a vector or contact with contaminated environmental items. Great apes are a valued bushmeat in Central and West Africa, and direct exposure of humans to great ape tissues can occur through hunting (which sometimes results in hunters being severely bitten), or during preparation and consumption of great ape bushmeat (Sharp and Hahn 2011). Similarly, sympatric chimpanzees and gorillas have been observed to co-occupy fruit trees (Walsh et al. 2007), which may result in conflict and direct physical challenge. Transmissions of foamy viruses from great apes to hunters and of SIV from chimpanzees to gorillas most likely stemmed from such direct contacts (Van Heuverswyn et al. 2006; Neel et al. 2010; Betsem et al. 2011).

Vector-borne diseases and environmental contamination only require the effective sharing of habitats. Some arthropod vectors feed on multiple host species and this paves the way to parasite transmission (Kent 2009). Transmission of malaria parasites was demonstrated in a number of cases—from gorillas to humans (*P. praefalciparum*; Liu et al. 2010), from gorillas or chimpanzees to humans (*P. vivax*; Prugnolle et al. 2013), and from humans to bonobos, chimpanzees and gorillas (*P. falciparum*; Duval et al. 2010; Krief et al. 2010; Prugnolle et al. 2010). In the latter case, transmission often occurred in the context of great ape sanctuaries (Duval et al. 2010; Krief et al. 2010), which is an extreme example of habitat sharing with humans.

Exposure to contaminated environmental items can frequently be equated with exposure to fecal contamination. Great ape and human habitats broadly overlap, including around and sometimes within protected areas (Rayner et al. 2011). Similarly, chimpanzees and gorillas share habitats and have even been observed to simultaneously forage the same fruit trees (Walsh et al. 2007). The direct and vector-borne routes are presumably constrained by high fitness costs (e.g., risk of injury) and the actual presence of a bridge vector (Verhulst et al. 2012) but the fecal-oral route is not: cross-species transmission is possible irrespective of behavioral hurdles and in any shared habitat. It is also expected that individuals will be exposed to fecal contamination in a relatively blind manner: any individual might serve as an entry point into the new host population. This suggests that parasites that use the fecal-oral route may have more opportunities to cross species barriers. Transmission of such parasites between great apes or between great apes and humans has, however, only rarely been demonstrated. *Escherichia coli* is regularly transmitted from humans to gorillas (possibly through livestock; Rwego et al. 2008), and hookworms (*Necator* spp.) simultaneously infect sympatric chimpanzees, gorillas, and humans (Hasegawa et al. 2014). Whether viruses that use the fecal-oral route are transmitted between hominine species is currently unclear (Calvignac-Spencer et al. 2012).

Adenoviruses (AdV; family *Adenoviridae*) are nonenveloped icosahedral viruses with a double-stranded DNA genome. They naturally infect many vertebrates, including primates and among them humans, and are primarily transmitted through the fecal-oral and/or respiratory routes. AdV can cause a variety of pathologies, especially when hosts are immunocompromised. In humans, these viruses have been associated with outbreaks of acute respiratory illnesses, gastroenteritis, conjunctivitis, and cystitis (Lion 2014). Non-human primate AdV have occasionally been suspected/shown to be transmitted between species (Mwenda et al. 2005), including in direction of humans (Ersching et al. 2010; Chen et al. 2011; Chiu et al. 2013), demonstrating their ability to switch between primate hosts. Recent studies identified a variety of AdV in captive and wild great apes (Roy et al. 2004, 2009; Wevers et al. 2010, 2011): nearly all of them could be related to four of the seven AdV species initially thought to be human-restricted (*Human mastadenovirus A* to *G*; human adenoviruses (HAdV)-A to -G). Phylogenetic analyses of their sequences either revealed sistership to (HAdV-C) or interspersion with those of HAdV responsible of human outbreaks (HAdV-B, -E, and -F). This, together with a serological study that reported reactivity to a chimpanzee AdV in human sera collected in sub-Saharan Africa (Xiang et al. 2006), prompted some authors to speculate that some human infections and maybe some human HAdV may originate in great apes (Purkayastha et al. 2005; Roy et al. 2009; Wevers et al. 2011). However, information acquired on HAdV circulating in wild great apes remains limited which prevents drawing any robust conclusion.

To overcome this limitation, we tested 860 great ape and human fecal samples collected throughout sub-Saharan Africa to investigate the molecular epidemiology and the cross-species transmission frequency of HAdV infecting great apes and humans. Our analyses support the notion that HAdV-B originates in gorillas and was transmitted to both humans and chimpanzees (most likely to some of

their ancestors), presumably through the fecal-oral route. While successful HAdV transmission events were rare, association with new hosts persisted over considerable timescales.

Results

Prevalence of HAdV Species in Great Apes and Humans from Sub-Saharan Africa

We used four polymerase chain reaction (PCR) systems, respectively generic for primate AdV and specific for HAdV-B, -D, and -E to screen 568 fecal samples collected in the wild from eight great ape species and subspecies at nine forest sites, and 292 samples collected from people living in neighboring villages at four of these sites (fig. 1 and table 1). Due to the high similarity of HAdV-B, -D, and -E sequences, these PCR were not completely specific; in the absence of the target HAdV species others were occasionally amplified, as revealed by sequencing of all PCR products. For all sequences, BLAST search returned primate AdV sequences as first hits. Cumulative primate AdV prevalence was 51% (75/146) in chimpanzees (*Pan troglodytes*), 94% (79/84) in bonobos (*Pa. paniscus*), 74% (251/338) in gorillas (*Gorilla* spp.), and 69% (201/292) in humans (*Homo sapiens*; table 1). Further sequence assignment was performed based on BLAST search hit lists.

Western, Central, and Eastern chimpanzees (*Pa. troglodytes verus*, *Pa. t. troglodytes*, and *Pa. t. schweinfurthii*) were infected with HAdV-B, -C, and -E with mean detection rates of 11%, 21%, and 25%, respectively. In bonobos, we detected HAdV-C and -E in 55% and 67% of the samples, respectively. Western lowland gorillas (*Gorilla gorilla gorilla*), Cross River gorillas (*G. g. diehli*), Eastern lowland gorillas (*G. beringei graueri*), and mountain gorillas (*G. b. beringei*) were infected with HAdV-B and -C with mean detection rates of 55% and 37%, respectively. In a single Central chimpanzee and three Western gorillas HAdV-F could be found. Coinfection of great apes with different HAdV species was often observed, for example, 17% of the gorilla samples showed coinfection with HAdV-B and -C. Coinfection of gorillas with different HAdV-B types was also detected. HAdV-A or HAdV-D were not detected in any of the great ape samples. In humans, HAdV-C, -D, and -F were identified with detection rates of 9%, 43%, and 3%, respectively. HAdV-B and -E were not detected in human samples. Collectively, these results clearly identify great apes as the major hosts of HAdV-B and -E in sub-Saharan Africa.

Genetic Diversity and Species Delineation of HAdV Species

Additional sequence information was acquired to examine the evolutionary relationships of great ape and human AdV and possibly unravel and date transmission events between these hosts. All HAdV-positive samples were subjected to long-distance PCR using four nested primer sets, targeting either HAdV-B, -C, -D, or -E sequences, which amplified a gene block (5.6 kb) that comprises the V, pX, pVI, and hexon genes. Using this approach we were able to amplify 37 HAdV-B and 3 HAdV-C from gorillas, 2 HAdV-B and 2 HAdV-E from chimpanzees, and 14 HAdV-D from humans ($N = 58$; supplementary material S1, p. 2, Supplementary Material online). These sequences were then aligned with a set of chimpanzee, bonobo, gorilla, and human AdV sequences comprising all known serotypes and genotypes ($N = 119$). As HAdV are prone to recombination and recombination can mislead phylogenetic inference, sequences derived from long-distance PCR were scanned for recombination cold-spots. The largest recombination cold-spot was identified in the V gene and comprised 429 bp (supplementary material S1, p. 3, Supplementary Material online). In the following, we primarily report results obtained from analyses focused on this fragment. Most of these analyses were however also performed on a data set comprising all DNA polymerase sequences generated during the initial screening (see Materials and Methods); those generally yielded similar results which will not be discussed below and are summarized in supplementary material S3, p. 5–9, Supplementary Material online.

We first explored the genetic diversity comprised in this data set by performing a maximum likelihood (ML) analysis using PhyML (Guindon et al. 2010). The recognized HAdV species could be identified from the corresponding phylogenetic tree and all received high bootstrap support (Bp = 100) except HAdV-E (Bp = 52; fig. 2). Maximum patristic distances within these clades varied substantially: 0.49 substitutions.site^{-1} (sub.s^{-1}; HAdV-B), 0.85 sub.s^{-1} (HAdV-C), 0.10 sub.s^{-1} (HAdV-D), and 0.12 sub.s^{-1} (HAdV-E). In addition, tree topology differed markedly among the four species: while HAdV-B and -C exhibited a number of long deep internal branches, any comparable deep structure was absent in HAdV-D and -E. Altogether this suggested that the genetic diversity of these species may have been shaped by different processes. To further investigate this question, we used a method based on the analysis of branching patterns, the general mixed Yule-coalescent method (GMYC). GMYC compares models combining species-level and population-level diversification processes (GMYC models) and a null model assuming only population-level processes were at play (Pons et al. 2006; Fujisawa and Barraclough 2013). GMYC requires providing an ultrametric tree, which we produced using BEAST (Drummond et al. 2012). Rate variation was already apparent from the ML tree (coefficient of variation of the root-to-tip distances when best-rooting with Path-O-Gen = 0.24). Therefore, this analysis was run, as all the following, under the assumption of a relaxed clock. The resulting maximum clade credibility (MCC) tree was used for a GMYC analysis. The null model was clearly rejected by a likelihood ratio test ($P = 2.6E^{-5}$). The best GMYC model identified 12 evolutionary entities (later referred to as operational taxonomic unit or OTU; fig. 2 and supplementary material S1, p. 4–5, Supplementary Material online).

Species HAdV-D and -E were identified as distinct OTU (OTU 5 and 12); species HAdV-B and -C were composites of, respectively, six and four OTU (OTU 6–11 and 1–4). Five HAdV-B OTU and all four HAdV-C OTU were supported by moderate to high Bp values in the ML tree and posterior probabilities (pp) in the MCC tree ($56 < Bp < 100$,

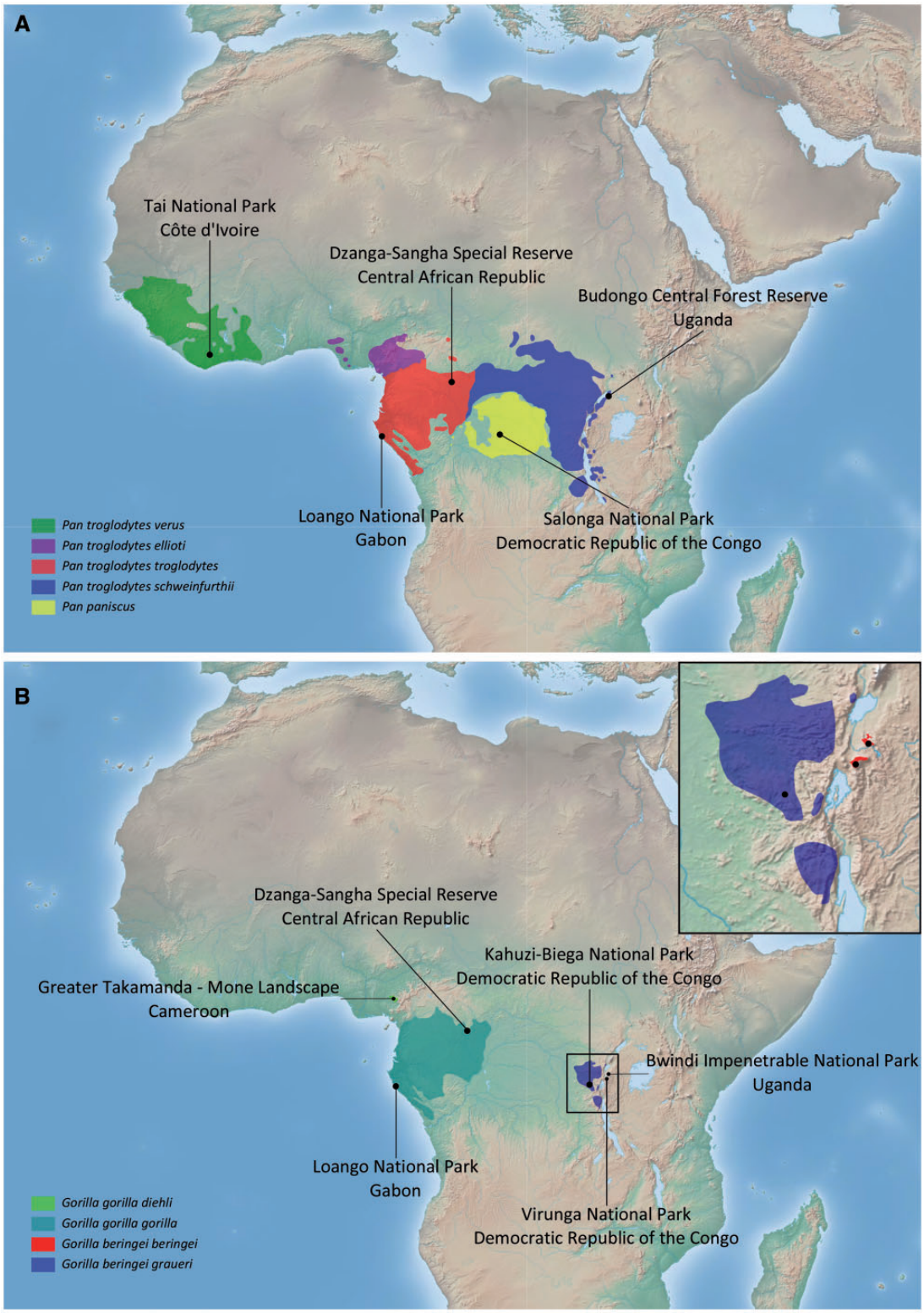

Fig. 1. Distribution areas of bonobo, chimpanzee, and gorilla subspecies and study site locations. (Panel *A*) Bonobo and chimpanzee subspecies. (Panel *B*) Gorilla subspecies, the top-right inset is a zoom-in of the African Great Lakes region.

pp $\geq$ 0.97). The monophyly of HAdV-B OTU 11 was only weakly supported (Bp = 38, pp = 0.94). As OTU 11 formed a well supported clade with OTU 10, we considered that OTU 10 and OTU 11 may actually correspond to a single OTU (hereafter referred to as OTU 10). In summary, HAdV-B comprised five OTU of which three infect multiple host species (OTU 8–10). The two remaining HAdV-B OTU only infect a single host, respectively gorillas (OTU 6) and humans (OTU 7). In contrast, all four HAdV-C OTU are host-specific (OTU 1–4).

Table 1. Primate Adenovirus Screening.

Species/Subspecies	Country	Site	Number of Samples	HAdVn (%)	HAdV-B n (%)	HAdV-C n (%)	HAdV-D n (%)	HAdV-E n (%)	HAdV-F n (%)
Pan troglodytes spp.	—	—	146	75 (51)	16 (11)	31 (21)		37 (25)	1 (1)
Pa. troglodytes verus	Côte d'Ivoire	Taï National Park	41	24 (58)	2 (5)	12 (29)		12 (29)	
Pa. troglodytes troglodytes	Gabon	Loango National Park	62	22 (35)	10 (16)	6 (10)		6 (10)	
	Central African Republic	Dzanga-Sangha Protected Areas	12	4 (33)				4 (33)	
Pa. troglodytes schweinfurthii	Uganda	Budongo Central Forest Reserve	31	25 (81)	4 (13)	13 (42)		15 (48)	1 (3)
Pa. paniscus	Democratic Republic of the Congo	Salonga National Park	84	79 (94)		46 (55)		56 (67)	
Gorilla spp.	—	—	338	251 (74)	185 (55)	125 (37)			3 (1)
Gorilla gorilla gorilla	Gabon	Loango National Park	80	38 (48)	21 (26)	15 (19)			2 (3)
	Central African Republic	Dzanga-Sangha Protected Areas	50	31(62)	22 (44)	10 (20)			
G. gorilla diehli	Cameroon	Greater Takamanda – Mone Landscape	58	44 (76)	38 (66)	11 (19)			1 (2)
G. beringei beringei	Uganda	Bwindi Impenetrable National Park	50	48 (96)	24 (48)	39 (78)			
	Democratic Republic of the Congo	Virunga National Park	50	50 (100)	49 (98)	22 (44)			
G. beringei graueri	Democratic Republic of the Congo	Kahuzi-Biega National Park	50	40 (80)	31 (62)	28 (56)			
Homo sapiens	—	—	292	132 (45)		26 (9)	126 (43)		8 (3)
H. sapiens	Democratic Republic of the Congo	Salonga National Park	105	69 (66)		14 (13)	52 (50)		8 (8)
	Uganda	Bwindi Impenetrable National Park	69	53 (77)		3 (4)	51 (74)		
	Côte d'Ivoire	Taï National Park	100	71 (71)		9 (9)	68 (68)		
	Central African Republic	Dzanga-Sangha Protected Areas	18	8 (44)		1 (6)	7 (39)		

To determine the relative merit of the OTU delineation and the recognized species demarcation, we performed a Bayes factor (BF) comparison of four models (table 2): 1) a model that assumes all sequences were derived from a single species (equivalent to the null model of the GMYC analysis), 2) a model that assumes all sequences represent distinct evolutionary entities (Yule model), and 3) and 4) two multispecies models that delineate 4 (HAdV-B, -C, -D, and -E) and 11 species (GMYC OTU), respectively [note that the term species in this sentence only reflects the terminology of multispecies models, that is, it does not have taxonomical implications per se]. Models incorporating species delineations were clearly favored and among these the 11-OTU model had a much better marginal likelihood (2 ln(BF) = 140.2 and 140.4 using path and stepping stone sampling, respectively). Hereafter, we report only results obtained using the 11-OTU model but all analyses were performed under the four abovementioned models, with similar results across models.

Ancestral Host Reconstruction and Host Change Counts

Ancestral host reconstruction was performed in a Bayesian framework. Host assignment was considered a discrete geographical location and used to feed an asymmetric diffusion model (Lemey et al. 2009; Weinert et al. 2012). Gorillas were identified as the best supported ancestral host of the entire HAdV-B group (pp = 0.98; table 3 and supplementary material S1, p. 6–7, Supplementary Material online). Because rate heterogeneity was likely within this group (fig. 2) and this could lead to long-branch attraction when using the complete data set, we also performed ancestral host reconstruction on a data set only comprising HAdV-B sequences. Gorillas were still the best supported ancestral host (pp = 0.71; humans pp = 0.20; and chimpanzees pp = 0.09), the reduced support being explained by uncertainty so as to the earliest offshoot of this radiation (HAdV-B OTU 6 or 7). Three HAdV-B OTU comprised gorilla sequences (OTU 6, 9, and 10) and gorilla was the best supported ancestral host for these OTU (pp = 1.00). The ancestral hosts of HAdV-B OTU 7 (only comprising human-derived sequences) and 8 were respectively humans and chimpanzees (pp = 1.00). Similar results were obtained for these five OTU using the HAdV-B only data set. The HAdV-C group consisted of four OTU which all comprised sequences derived from a single host (human, bonobo, chimpanzee, or gorilla); the expected ancestral host was retrieved in all cases (pp $\geq$ 0.99). Similarly, humans were identified as the best supported ancestral host (pp = 1.00) for species HAdV-D, which only comprised human-derived sequences. For species HAdV-E, which comprised sequences derived from chimpanzees, bonobos, and humans, chimpanzees were the best supported ancestral host (pp = 0.99).

The pattern observed within species HAdV-B was suggestive of an origin in gorillas followed by transmission events to humans and chimpanzees. Therefore, we performed targeted analyses aimed at determining the number of transitions

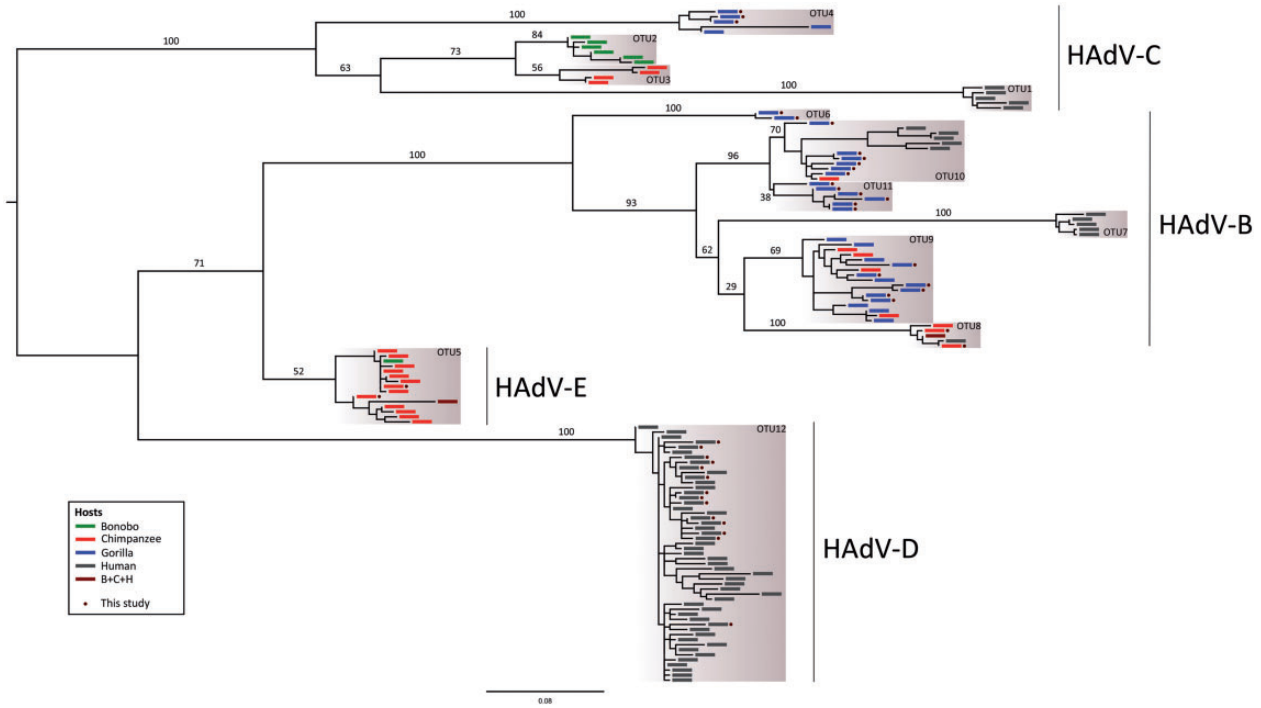

Fig. 2. ML tree of a partial fragment of the V gene. Gray rectangles highlight species delineated through GMYC. Bootstrap values are shown above all internal branches discussed in this article. Scale is in substitution per site. This tree was rooted by minimizing the variance of root-to-tip distance using Path-O-Gen (http://tree.bio.ed.ac.uk/software/pathogen/, last accessed April 29, 2015). Note that sequences identified from more than one host (brown labels) were all derived from captive individuals; dots point at sequences identified from samples analyzed in this study, which does not necessarily mean that these sequences were never detected elsewhere (e.g., in captive great apes). An alternative version of this tree in which tips wear explicit strain names is also presented in supplementary material S1, p. 4, Supplementary Material online.

Table 2. Log Marginal Likelihood Values for Different Species Delineation Schemes.

	Path Sampling	Stepping Stone Sampling
Coalescent: constant population size	−6323.5	−6324.4
Multispecies: 4 species	−6153.2	−6153.6
Multispecies: 11 species	−6083.1	−6083.4
Speciation: Yule	−6331.8	−6332.1

NOTE.—The best delineation scheme model is the model assuming 11 species (underlined).

Table 3. Ancestral Host Posterior Probabilities.

	Host			
	Bonobo	Chimpanzee	Gorilla	Human
B	0	0.01	0.98	0.01
OTU 6	0	0	1.00	0
OTU 7	0	0	0	1.00
OTU 8	0	1.00	0	0
OTU 9	0	0	1.00	0
OTU 10	0	0	1.00	0
C	0.02	0.15	0.75	0.08
OTU 1	0	0	0	1.00
OTU 2	1.00	0	0	0
OTU 3	0	0.99	0.01	0
OTU 4	0	0	1.00	0
D	0	0	0	1.00
E	0	0.99	0	0

NOTE.—Best values are underlined. Values on a line do not always sum up to 1.00 as values <0.01 were rounded to 0. These values were obtained under the 11-species model, assuming an asymmetric diffusion process. Other models yielded similar values (supplementary material S1, p. 6–7, Supplementary Material online).

from one to another host (fig. 3). Gorilla was clearly identified as the major source of HAdV-B with a mean of 8.0 inferred transitions (95% highest posterior density [HPD]: 5.3–10.2), a number markedly higher than the 1.7 (95% HPD: 1.0–4.3) and 0.5 (95% HPD: 0.0–1.8) for chimpanzees and humans, respectively. Gorilla-borne HAdV-B transmissions occurred more frequently in the direction of chimpanzees (mean: 6.5, 95% HPD: 5.0–7.8) than toward humans (mean: 1.5, 95% HPD: 0.3–2.4). Evidence for chimpanzee to human HAdV-B transmission was also observed (mean: 1.3, 95% HPD: 1.0–2.3).

Timing of HAdV Evolution

As previously reported (Roy et al. 2009; Wevers et al. 2011), the pattern observed within species HAdV-C was compatible with host/parasite codivergence (fig. 2), although in our analysis the branch supporting the human/panine clade was relatively weakly supported (Bp = 63, pp = 0.65). We therefore used the divergence of all hominines to calibrate our analyses and derive divergence date estimates across the entire HAdV phylogeny. Under the assumption of HAdV-C virus/hominine codivergence, an average mean rate of

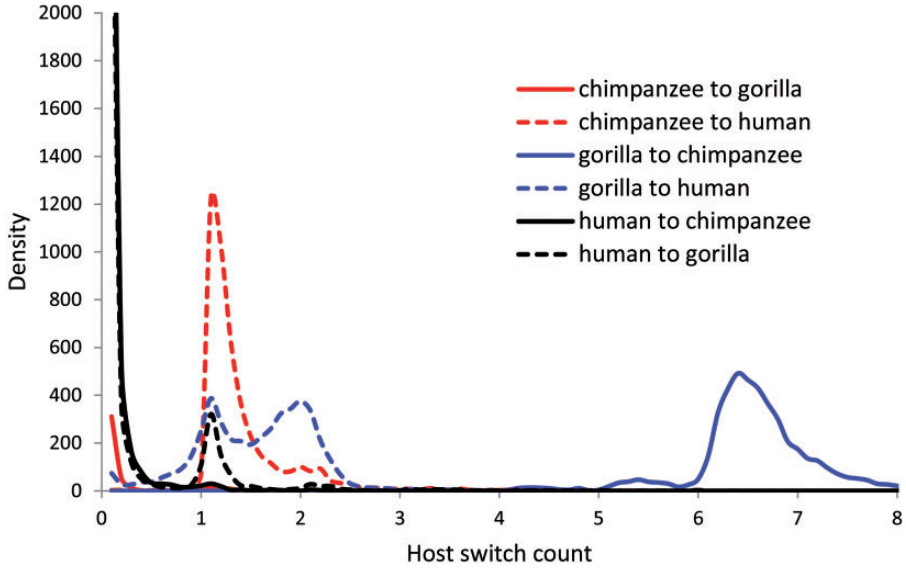

Fig. 3. HAdV-B host switch counts.

substitution of $2.8E^{-8}$ sub.s^{-1}.y-1 (95% HPD: $1.2E^{-8}$–$4.6E^{-8}$) was observed, consistent with expectations for double stranded DNA (dsDNA) viruses (but note that for dsDNA viruses most estimates are derived from assumed codivergence events; Duffy et al. 2008). Significant rate heterogeneity across lineages was confirmed, with an average coefficient of variation of 0.62 (95% HPD: 0.43–0.82).

In the MCC tree, the root was positioned on the branch separating HAdV-C from HAdV-B, -D, and -E, defining two lineages comprising host-associated clades of viruses infecting humans (HAdV-C OTU 1 and HAdV-D, respectively), chimpanzees (HAdV-C OTU 3 and HAdV-E), and gorillas (HAdV-C OTU 4 and HAdV-B). This root location was the most frequent in the posterior sample of trees (pp = 0.78) and possibly indicates an ancestral viral lineage duplication predating the divergence of African hominids. The branching order of HAdV-B, -D, and -E did not suggest the existence of a single lineage codiverging with their hominine hosts (figs. 2 and 4). As support values for the according tree topology were relatively low, we investigated this question further by reanalyzing eight complete gene sequences previously examined by Roy et al. (2009). Bayesian Markov chain Monte Carlo (BMCMC) analyses showed that all individual gene trees and the species tree favored the topology (HAdV-C,(HAdV-D,(HAdV-B, HAdV-E))) (supplementary material S2, p. 3, Supplementary Material online).

The estimated dates of the divergence events within HAdV-C (OTU 2/OTU 3 and OTU 2 and 3/OTU 4) were also compatible with host/parasite codivergence; 95% HPD for parasite divergence events comprised estimates of host divergence dates derived from genomic analyses (supplementary material S2, p. 2, Supplementary Material online). The time to the most recent common ancestor (tMRCA) of HAdV-B, -D, and -E was 7.7 My (95% HPD: 3.4–14.4), which was close to the estimated 6.8 My observed for the divergence of all HAdV-C (95% HPD: 4.8–10.8; fig. 4 and supplementary material S1, p. 8–9, Supplementary Material online). The tMRCA of species HAdV-B group and species HAdV-D and HAdV-E were 4.5, 0.8, and 1.1 My (95% HPD: 1.9–9.4, 0.3–1.6, and 0.3–2.5, respectively). These tMRCA antedate the divergence of all modern gorillas (Prado-Martinez et al. 2013), the first appearance of *H. sapiens* in the fossil record (McDougall et al. 2005) and the divergence of all modern panines (Prado-Martinez et al. 2013), respectively. Consistent with these estimates, the inferred rate of gorilla-derived HAdV-B transmission events was low, with a mean of 2.2 transitions to chimpanzees per My (95% HPD: 0.4–4.) and 0.7 transitions to humans per My (95% HPD: 0.0–2.0).

Four independent transmission events of great ape HAdV to humans were apparent from figure 4. Three of these involved viruses belonging to the HAdV-B lineage, with their putative reservoirs being either gorillas (two events: species 7 and 10) or chimpanzees (one event: species 8), which is consistent with HAdV-B host switch counts (fig. 3). A single HAdV-E transmission event was also identified, most likely originating in chimpanzees. Median estimates of the transmission windows supported transmissions predating the emergence of anatomically modern humans for the two gorilla-borne events; the two chimpanzee-borne events occurred on terminal branches, thereby preventing the identification of an upper bound for the date of transmission. Only three transmission events of HAdV-B from gorillas to chimpanzees could be bound to a unique branch; up to three other may have occurred but phylogenetic uncertainty in the according zone of the tree (OTU 9) prevented any unambiguous positioning. According to median estimates, a single firmly established event predated the divergence of all modern panines; the two other occurred on terminal branches.

Phylodynamics of HAdV OTU and Species

Finally, we estimated viral population dynamics for three species that primarily infected great apes: HAdV-B OTU 9 and 10

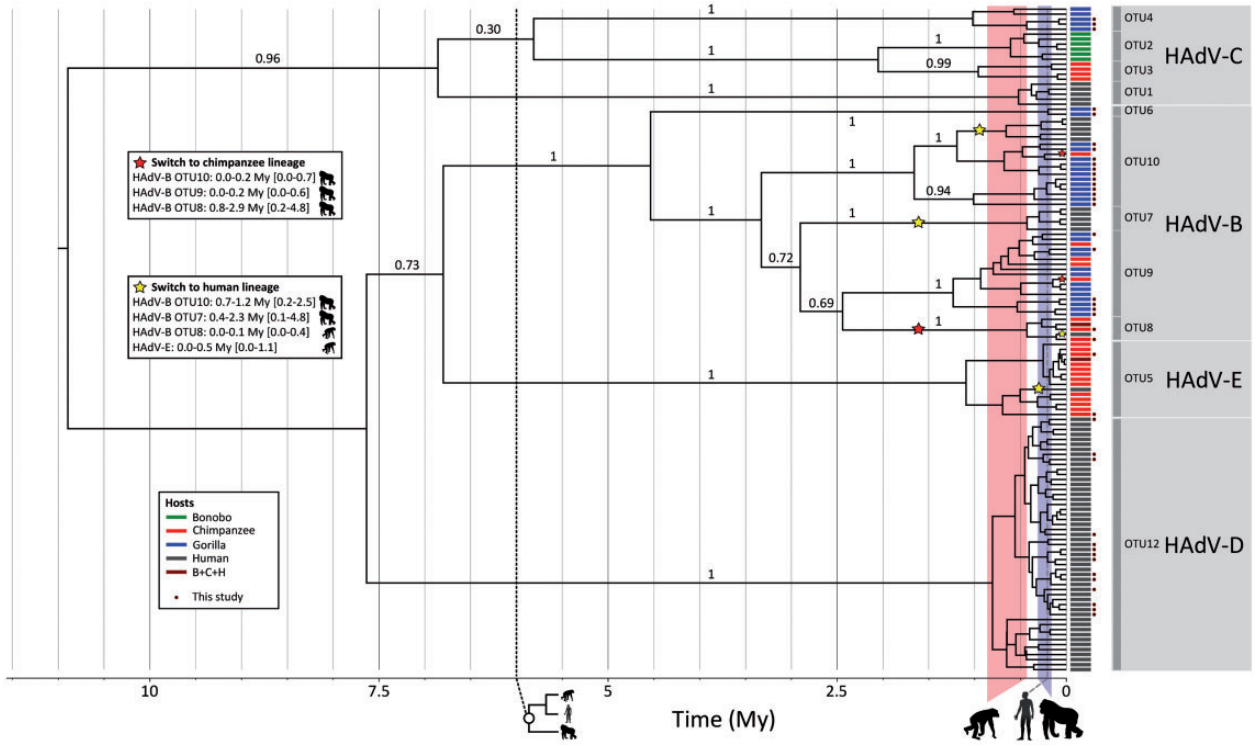

Fig. 4. Bayesian chronogram of a partial fragment of the V gene. Stars point at transmission events that could be bound to single branches (no phylogenetic uncertainty). The human silhouette points at the oldest fossils of anatomically modern humans (McDougall et al. 2005). Great ape silhouettes highlight divergence times of all *P. troglodytes* and of all *Gorilla* sp., following Prado-Martinez et al. (2013). The symbolic hominine phylogeny refers to the time to the last common ancestor of this lineage as set up for these analyses, that is, 6 My [5–7 My]. Posterior probability values are shown above all internal branches discussed in this article. Note that in this maximum credibility tree, the branching order of the different HAdV-C OTU differs from the most frequently observed branching order in the entire posterior sample of trees (which complies with the codivergence hypothesis). This chronogram was obtained using the 11-species model (in which OTU 10 and OTU11 highlighted in figure 2 were merged in a single OTU, named OTU 10); tree priors affected date estimates (supplementary table S1, p. 8–9, Supplementary Material online) but not in a way that would modify our conclusions. This tree was built assuming a relaxed molecular clock and therefore is rooted; the root location received a posterior probability of 0.78. Note that sequences identified from more than one host (brown labels) were all derived from captive individuals; dots point at sequences identified from samples analyzed in this study, which does not necessarily mean that these sequences were never detected elsewhere (e.g., in captive great apes).

Table 4. Log Marginal Likelihood Values for Different Models of Population Growth.

	Constant Population Size	Bayesian SkyGrid	2 ln BF
B OTU 9	−1180.3/−1180.3	−1179.8/−1179.9	1.0/0.8
B OTU 10	−1318.5/−1318.4	−1318.2/−1318.0	0.6/0.8
E	−883.3/−883.3	−885.8/−885.9	−5.0/−7.6

NOTE.—Marginal likelihoods are as derived from path sampling/stepping stone sampling. For BF calculations the constant population size model was considered the null hypothesis. 2 ln BF $>$ 0 indicates a better performance of the Bayesian SkyGrid model but only values $>$ 10 are considered decisive.

and HAdV-E. Other HAdV species did not comprise enough sequences to explore their demographic history. For this analysis, we performed a BF comparison of two coalescent models (table 4): a model that assumes a constant population size through time and a second that allows for population size variation through time (Bayesian SkyGrid). The constant population size model could not be rejected or was slightly favored for the three species.

Discussion

We investigated the evolutionary history and zoonotic potential of HAdV using fecal samples from wild African great apes and sympatric human populations. We found high prevalence of HAdV-B and -E in gorillas and chimpanzees, respectively. HAdV-C reached relatively high prevalence in most great ape populations and was also detected in the four human populations. HAdV-D was the predominant species in humans and was never detected in great apes. Using a GMYC approach, we showed that the genetic diversity of the HAdV species has likely been shaped by distinct processes; a combination of typical between-species and within-species processes for HAdV-B and -C versus pure within-species processes for HAdV-D and -E. Although this delineation identified six host-specific OTU (out of a total of 9 HAdV-B and -C OTU), and this may reinforce their biological relevance, our findings presently have no direct taxonomical impact, as species demarcation would require accumulating much more information (Harrach et al. 2011).

Our findings add to the growing body of literature (e.g., Roy et al. 2009; Wevers et al. 2011) suggesting that HAdV evolution has been mainly governed by strong, long-term association with their hominine hosts. This is notably evidenced by a marked overall pattern of host specificity, even after we considerably enlarged the pool of AdV sequences available from wild great apes. Interestingly, the host-HAdV association seems strong enough to have resulted in the codivergence of HAdV-C with their hosts. On the contrary, BMCMC analyses support the notion that the branching order of HAdV-B, -D, and -E contradicts a pure codivergence pattern (irrespective of the gene considered). This may suggest a more complex evolutionary history, possibly involving HAdV extinction events (Roy et al. 2009).

Confirming earlier speculations (Purkayastha et al. 2005; Roy et al. 2009; Wevers et al. 2011), we demonstrate here that HAdV evolution was also marked by natural host switches that resulted in perennial associations with new hominine hosts, which is particularly obvious in the case of HAdV-B viruses. Several lines of evidence support the notion that members of this very diverse lineage, which in our analyses comprised five OTU, have parasitized members of the gorilla lineage for several million years. First, all species and subspecies of gorillas are infected at high prevalence with these viruses. Second, three of the five HAdV-B OTU infect gorillas and their reconstructed ancestral hosts are gorillas. Third, population sizes of two of these OTU remained stable over hundreds of thousands of years, possibly suggesting high prevalence over the same timescales. Finally, the reconstructed ancestral host of the last common ancestor of the entire HAdV-B lineage is gorillas.

We also show that HAdV-B viruses were transmitted to chimpanzees and humans, resulting in their long-term establishment in these new host species. Two key questions are the frequency and the mode of such transmission events. Assuming the calibration of our phylogenetic analyses is correct, it appears that cross-hominine transmission of HAdV has occurred at very low frequency. Within the HAdV-B lineage, we inferred about ten transmission events over a period spanning about 4.5 My, of which only three were in direction of the human lineage. While this does not account for (the possibly many) dead-end transmission events, the fact that no HAdV-B infection was detected in human populations living in the vicinity of great ape habitat is also suggestive of a low frequency of instantaneous transmission. This situation is very comparable to what has been reported for Laveranian malaria parasites, the complex of species from which *P. falciparum* emerged, most likely as the result of a single gorilla-to-human transmission event (Liu et al. 2010; Rayner et al. 2011). While wild great apes are frequently infected with these parasites (Kaiser et al. 2010; Liu et al. 2010), they have not yet been detected in any human population sharing great ape habitats (Sundararaman et al. 2013). In contrast, SIVcpz and SIVgor, which were transmitted at least four times to human populations over the last 100 years, present a very heterogeneous distribution in chimpanzee and gorilla populations, with overall fecal detection rate much lower than those observed for HAdV or Laveranian malaria parasites (Sharp and Hahn 2011). Taken together, these three examples further highlight that prevalence and host-specificity are poor predictors of the zoonotic potential of great ape parasites (Calvignac-Spencer et al. 2012).

Barriers to cross-species transmission of great ape and human parasites (in either direction) may reside in parasite–host incompatibility and/or a paucity of transmission opportunities for the pathogens, that is, a lack of effective exposure (Rayner et al. 2011). Exposure in turn depends on the mode of transmission of the parasite, as well as on host species geographical overlap and ecological interactions. In Central Africa, the ranges and diets of chimpanzees and gorillas overlap considerably (Yamagiwa and Basabose 2006, 2009; Head et al. 2011; Junker et al. 2012; Oelze et al. 2014). Chimpanzee and gorilla groups have been observed foraging the same fruit tree on the same day or even feeding simultaneously in the very same tree (Walsh et al. 2007). Physical challenge involving chimpanzees and gorillas in the wild is possible but, to our knowledge, has never been reported. There appears to be ample opportunity for parasite transmission from gorillas to chimpanzees through the environment, but much less so through direct contact. It is well-established that HAdV are readily transmitted from human to human through the environment (primarily via fecal-oral and respiratory routes). It therefore seems likely that at least part of the six HAdV-B transmission events from gorillas to chimpanzees detected here similarly occurred through the environment. Habitat sharing may be the only necessary condition to cross-hominine transmission of HAdV and the "intensity" of habitat sharing an important risk factor. This does not necessarily mean that HAdV-B and HAdV-E transmission from gorillas and chimpanzees to humans followed the same route. Human populations living in or close to rainforest areas have probably always relied (and still rely) on bushmeat, including great ape bushmeat (Malhi et al. 2013), and its acquisition results in substantial direct physical contact with animals. Contamination with HAdV infecting great apes during hunting or butchering therefore also appears as a plausible scenario.

Finally, it is interesting to note that two HAdV-B emergence events took place in a relatively distant past, maybe even predating the appearance of *H. sapiens*. Nowadays, HAdV-B infections are associated with acute respiratory illnesses in humans, which incur a significant morbidity burden and can sometimes be lethal (Kajon et al. 2010). Assuming that the outcome of HAdV-B infections was no better in the past, these viruses of zoonotic origin may have had similar detrimental effects on human health over much of our species lifetime.

Materials and Methods

Sample Collection and Processing

Fecal samples were collected using single-use gloves and preserved in RNAlater (Qiagen, Hilden, Germany), in liquid nitrogen, or by drying over silica. Great ape sample importations occurred according to German veterinary regulations for import of organic materials. Human samples were collected

after written informed consent was obtained from every study participant. The collection was approved by the responsible ethic commission of each country and performed according to the declaration of Helsinki.

DNA was extracted from fecal samples with a GeneMATRIX stool DNA purification kit (Roboklon, Berlin, Germany), according to manufacturer's instructions.

PCR and Sequencing

The presence of primate AdV was first tested for using a generic nested PCR system targeting of 650 base pair (bp) fragment of the DNA polymerase gene. In addition, three specific nested PCR systems targeting a conserved region in the C-terminal region of the hexon gene were designed so as to preferentially amplify HAdV-B, -D, or -E fragments (324, 322, and 309 bp, respectively). For these four short PCRs, conditions were as described in Wevers et al. (2010). Three nested long distance (LD) primer sets were also designed on the basis of HAdV genome sequences available in Genbank and used on positive samples. The amplified fragments (~5.5 kb) span the genes pVII, V, pX, pVI, and hexon. LD PCR were performed with the TaKaRa-EX PCR kit according to the instructions of the manufacturer (Takara Bio Inc., Otsu, Japan). Primer sequences and cycling conditions can be found in supplementary table S1, Supplementary Material online.

All PCR products were purified using the Invisorb DNA clean up kit according to manufacturer's instructions (Invitek, Berlin, Germany). Sequencing reactions were performed with the Big Dye terminator cycle sequencing kit (Applied Biosystems, Warrington, UK) and products analyzed on a 377 automated DNA sequencer (Applied Biosystems). All DNA polymerase sequences and all unique sequences generated by LD PCR were deposited in GenBank (accession numbers LN828977–LN829111 and KM659129–KM659172). A fasta file comprising all hexon sequences was deposited on DRYAD (doi: 10.5061/dryad.rs5g0).

Phylogenetic Analyses

All HAdV

Analyses were first performed on a data set that comprised all sequences obtained from LD PCR products ($N = 58$) as well as representative sequences of all known HAdV-B, -C, -D, and -E serotypes and types ($N = 119$). Nucleotide sequences were aligned with MUSCLE (Edgar 2004) and conserved nucleotide blocks selected with Gblocks (Talavera and Castresana 2007); both softwares were run in SeaView (Gouy et al. 2010). Because HAdV are prone to recombination, we then identified recombination hot/coldspots using RDP v4.9 (Martin et al. 2010). The largest coldspot, a 429 bp sequence block exhibiting very little evidence for recombination (supplementary material S1, p. 3, Supplementary Material online), was selected for further analyses. This alignment was reduced to only comprise unique sequences ($N = 133$) using Fabox v1.41 (Villesen 2007). Model selection was performed in an ML framework using jModelTest v2.1.3 (Darriba et al. 2012) and the Bayesian information criterion (model selected: HKY + I + G). An ML analysis was performed under this nucleotide substitution model using PhyML v3.1 (Guindon et al. 2010), as implemented on the PhyML webserver (Guindon et al. 2005). Tree search combined the nearest-neighbor interchange and subtree pruning and regrafting approaches. Branch support was estimated by bootstrapping the data set (250 pseudo-replicates).

Patristic distances were derived from the resulting tree using Patristic (Fourment and Gibbs 2006). Together with topological features, within-species maximum patristic distances suggested that the diversification process may have differed between HAdV species. To investigate this hypothesis, we used an approach based on general-mixed Yule/coalescent models (GMYC; Pons et al. 2006; Fujisawa and Barraclough 2013). For this, we generated an ultrametric tree which we derived from BMCMC analyses with BEAST v1.8.0 (Drummond et al. 2012). These first BMCMC analyses were run under the assumption of a relaxed molecular clock (uncorrelated lognormal) and a coalescent tree prior (constant population size). The resulting MCC tree was used as the input of a GMYC analysis using the R package splits (single threshold; Fujisawa and Barraclough 2013).

We then performed a series of BMCMC analyses (all with BEAST) to reconstruct ancestral hosts at all nodes of the HAdV phylogeny and estimate the timescale of HAdV evolution. These analyses were performed according to four distinct speciation scenarios that implied the existence of: 1) a single species (coalescent model: constant population size), 2) 4 species (*BEAST with a Yule process as species tree prior and a piecewise linear and constant root population size model; Heled and Drummond 2010), 3) 11 species (*BEAST, same priors), and 4) as many species as sequences (speciation model: Yule process). It should be noted that the term species in the previous sentence only reflects the terminology of multispecies models, that is, it does not have taxonomical implications per se. Host assignment (bonobo, chimpanzee, human, and gorilla) was considered a discrete geographical location and used to inform a discrete asymmetric diffusion model (preliminary analyses evidenced a better performance of asymmetric models over symmetric ones; Lemey et al. 2009; Weinert et al. 2012). All analyses assumed a strict clock for the host model and a relaxed clock (uncorrelated lognormal) for the nucleotide substitution model. For all but the four species analysis, we attempted to calibrate the molecular clock by using a prior on the divergence of GMYC-delimited species forming HAdV-C (OTU 1–4). Within this species, the viral topology indeed seemed to recapitulate host divergence events [(gorilla, (human, (chimpanzee, bonobo)))]. We therefore chose the divergence of all hominines as a calibration point. We modeled the latter using a normal distribution of mean 6.0 and SD 0.51 so that 95% of the values be comprised between 5 and 7 My. Recent genome-wide molecular estimates of the hominine split fall within this interval [assuming a substitution rate of 10^{-9} subs./site/year] (Scally et al. 2012; Prado-Martinez et al. 2013). We also placed a poorly informative prior on the mean nucleotide substitution rate by describing it with a uniform distribution with a lower limit of 10^{-3} and an

upper limit of 10 substitutions per site per My. These numbers were derived from the whole range of substitution rates of double-stranded DNA viruses (Duffy et al. 2008). Model marginal likelihoods were estimated using path and stepping stone sampling (Baele et al. 2012). The best model was considered as receiving decisive support when 2 ln BF $>$ 10.

HAdV-B and HAdV-E

We also performed focused analyses aimed at determining the number of transitions from one to another host within the species HAdV-B. Because all models of diversification converged to similar estimates of host switch relative rates on the global data set (data not shown), these analyses were performed assuming the simplest of these models (coalescent process: constant population size). Host switches were modeled using a discrete asymmetric diffusion model, and stochastic mapping was used to assess the number of transitions (Minin and Suchard 2008). To be able to replace this in an absolute timeframe, we calibrated the relaxed molecular clock (uncorrelated lognormal) using the tMRCA of HAdV-B retrieved in the all HAdV analysis. This time was modeled as a lognormal distribution of real mean 5.27 My and log(SD) 0.51 (offset = 0).

Note that all analyses described until this point were also performed using an alignment of DNA polymerase sequences comprising all sequences obtained in the screening phase ($N = 135$) as well as representative sequences of all known HAdV-B, -C, -D, and -E serotypes and types ($N = 77$). This data set was reduced to a total of 144 unique sequences before the analyses were performed. All the according results were in broad agreement with those obtained from the analyses of the V gene fragment; they are summarized in supplementary material S3, Supplementary Material online.

Finally, we estimated viral population dynamics for GMYC OTU 9 and 10 (HAdV-B) and HAdV-E. For these analyses, alignments of the V gene fragment were not reduced to unique sequences. This resulted in data sets respectively comprising 21, 39, and 18 sequences. For each data set, analyses were performed assuming a relaxed clock (uncorrelated lognormal) and under two coalescent models: constant population size and a Bayesian SkyGrid. To be able to replace population dynamics in an absolute timeframe, we calibrated the molecular clocks with the times to the most recent common ancestor of these lineages as retrieved in the all HAdV analysis. These were modeled using the following lognormal distributions (all offsets = 0): HAdV-B OTU 9: mean- = 1.40, SD = 0.49; HAdV-B OTU 10: mean = 1.89, SD = 0.47; HAdV-E: mean = 1.26, SD = 0.54. Model marginal likelihoods were estimated and compared as abovementioned.

Supplementary Material

Supplementary materials S1–S3 and table S1 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

This study was only possible with the international cooperation of multiple countries, organizations, and individuals. Research on Cross-River gorillas in Cameroon was conducted with permission from the Ministry of Forests and Wildlife; received support from Working Dogs for Conservation, the Wildlife Conservation Society (WCS), the United States Fish and Wildlife Service Great Ape Conservation Fund, and the Association of Zoos and Aquariums Conservation Endowment Fund; and benefited from the assistance of R. Ikfuingei in the field and K. Cross in the lab. Research on Central chimpanzees and Western lowland gorillas in the Central African Republic was conducted with permission from the Ministère de l'Education Nationale, de l'Alphabetisation, de l'Enseignement Supérieur et de la Recherche; received support from the Primate Habituation Program, the World Wildlife Fund, the Grant Agency of the Czech Republic (#206/09/0927), the Institute of Vertebrate Biology of the Academy of Sciences of the Czech Republic (#RVO68081766), the European Social Fund, the State Budget of the Czech Republic, and a Praemium Academiae award to J. Lukes; and benefited from the assistance of the staff of Dzanga-Sangha Protected Areas and local trackers and assistants. Research on Western chimpanzees in Côte d'Ivoire was conducted with permission from the Ministère de la Recherche Scientifique, the Ministère de l'Environnement et des Eaux et Forêts, the Direction Générale de la Recherche Scientifique et de l'Innovation Technique, and the Office Ivoirien des Parcs et Réserves; received support from the Max Planck Society (MPS); and benefited from the assistance of all students and field assistants of the Taï Chimpanzee Project. Research on bonobos in the Democratic Republic of the Congo (DRC) was conducted with permission of the Institut Congolais pour la Conservation de la Nature (ICCN) and benefited from the assistance of students and field assistants of the Salonga Bonobo Project. Research on Central chimpanzees and Western lowland gorillas in Gabon was conducted with permission from the Agence Nationale des Parcs Nationaux (ANPN) and the Centre de la Recherche Scientifique et Technologique; received support from WCS and the Société pour la Conservation et le Développement; benefited from the assistance of WCS and ANPN staff as well as of J. Head, L. Rabanal, L. Makaga, U. Bora, I. Bollini, Y. Nkombe, and other field assistants of the Loango Ape Project. Research on Eastern chimpanzees in Uganda was conducted with permission from the Uganda Wildlife Authority (UWA) and the Uganda National Council for Science and Technology; received support from the Royal Zoological Society of Scotland (core funding of the Budongo Conservation Field Station); and benefited from the assistance of many students and assistants at the Budongo Conservation Field Station. Research on Eastern lowland and mountain gorillas in DRC, Rwanda, and Uganda was conducted with permission from ICCN, the Rwandan Development Board, and UWA; received support from WWF Sweden, the Fair Play Foundation, the Netherlands Directorate General for International

Cooperation through the Greater Virunga Transboundary Collaboration, the Berggorilla & Regenwald Direkthilfe e.V., MPS, and WCS; benefited from the assistance of the Mountain Gorilla Veterinary Project, the Dian Fossey Gorilla Fund International, and Conservation Through Public Health for the organization of the censuses. The authors are finally thankful to Michael Jarvis for constructive comments on an earlier version of this manuscript, to Franzi Zickmann for running some of the analyses on the cluster of the Renard's group at RKI, and to Maja Kovacev-Wegener for her efficient assistance with administrative tasks.

References

Baele G, Lemey P, Bedford T, Rambaut A, Suchard MA, Alekseyenko AV. 2012. Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Mol Biol Evol.* 29:2157–2167.

Betsem E, Rua R, Tortevoye P, Froment A, Gessain A. 2011. Frequent and recent human acquisition of simian foamy viruses through apes' bites in central Africa. *PLoS Pathog.* 7:e1002306.

Calvignac-Spencer S, Leendertz SA, Gillespie TR, Leendertz FH. 2012. Wild great apes as sentinels and sources of infectious disease. *Clin Microbiol Infect.* 18:521–527.

Chen EC, Yagi S, Kelly KR, Mendoza SP, Tarara RP, Canfield DR, Maninger N, Rosenthal A, Spinner A, Bales KL, et al. 2011. Cross-species transmission of a novel adenovirus associated with a fulminant pneumonia outbreak in a new world monkey colony. *PLoS Pathog.* 7:e1002155.

Chiu CY, Yagi S, Lu X, Yu G, Chen EC, Liu M, Dick EJ Jr, Carey KD, Erdman DD, Leland MM, et al. 2013. A novel adenovirus species associated with an acute respiratory outbreak in a baboon colony and evidence of coincident human infection. *MBio* 4:e00084.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods.* 9: 772–772.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol.* 29: 1969–1973.

Duffy S, Shackelton LA, Holmes EC. 2008. Rates of evolutionary change in viruses: patterns and determinants. *Nat Rev Genet.* 9:267–276.

Duval L, Fourment M, Nerrienet E, Rousset D, Sadeuh SA, Goodman SM, Andriaholinirina NV, Randrianarivelojosia M, Paul RE, Robert V, et al. 2010. African apes as reservoirs of *Plasmodium falciparum* and the origin and diversification of the Laverania subgenus. *Proc Natl Acad Sci U S A.* 107:10561–10566.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32:1792–1797.

Ersching J, Hernandez MI, Cezarotto FS, Ferreira JD, Martins AB, Switzer WM, Xiang Z, Ertl HC, Zanetti CR, Pinto AR. 2010. Neutralizing antibodies to human and simian adenoviruses in humans and New-World monkeys. *Virology* 407:1–6.

Fourment M, Gibbs MJ. 2006. PATRISTIC: a program for calculating patristic distances and graphically comparing the components of genetic change. *BMC Evol Biol.* 6:1.

Fujisawa T, Barraclough TG. 2013. Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent approach: a revised method and evaluation on simulated data sets. *Syst Biol.* 62:707–724.

Gouy M, Guindon S, Gascuel O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol.* 27:221–224.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol.* 59:307–321.

Guindon S, Lethiec F, Duroux P, Gascuel O. 2005. PHYML Online-a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Res.* 33:W557–W559.

Harrach B, Benkö M, Both GW, Brown M, Davison AJ, Echavarria M, Hess M, Jones MS, Kajon A, Lehmkuhl HD, et al.. 2011. Family *Adenoviridae*. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus taxonomy: classification and nomenclature of viruses. Ninth Report of the International Committee on Taxonomy of Viruses. San Diego: Elsevier. p. 125–141.

Hasegawa H, Modry D, Kitagawa M, Shutt KA, Todd A, Kalousova B, Profousova I, Petrzelkova KJ. 2014. Humans and great apes cohabiting the forest ecosystem in central african republic harbour the same hookworms. *PLoS Negl Trop Dis.* 8:e2715.

Head J, Boesch C, Makaga L, Robbins M. 2011. Sympatric chimpanzees (*Pan troglodytes troglodytes*) and gorillas (*Gorilla gorilla gorilla*) in Loango National Park, Gabon: dietary composition, seasonality, and intersite comparisons. *Int J Primatol.* 32:755–775.

Heled J, Drummond AJ. 2010. Bayesian inference of species trees from multilocus data. *Mol Biol Evol.* 27:570–580.

Junker J, Blake S, Boesch C, Campbell G, Toit LD, Duvall C, Ekobo A, Etoga G, Galat-Luong A, Gamys J, et al. 2012. Recent decline in suitable environmental conditions for African great apes. *Divers Distrib.* 18:1077–1091.

Kaiser M, Lowa A, Ulrich M, Ellerbrok H, Goffe AS, Blasse A, Zommers Z, Couacy-Hymann E, Babweteera F, Zuberbuhler K, et al. 2010. Wild chimpanzees infected with 5 *Plasmodium* species. *Emerg Infect Dis.* 16:1956–1959.

Kajon AE, Lu X, Erdman DD, Louie J, Schnurr D, George KS, Koopmans MP, Allibhai T, Metzgar D. 2010. Molecular epidemiology and brief history of emerging adenovirus 14-associated respiratory disease in the United States. *J Infect Dis.* 202:93–103.

Keele BF, Van Heuverswyn F, Li Y, Bailes E, Takehisa J, Santiago ML, Bibollet-Ruche F, Chen Y, Wain LV, Liegeois F, et al. 2006. Chimpanzee reservoirs of pandemic and nonpandemic HIV-1. *Science* 313:523–526.

Kent RJ. 2009. Molecular methods for arthropod bloodmeal identification and applications to ecological and vector-borne disease studies. *Mol Ecol Resour.* 9:4–18.

Krief S, Escalante AA, Pacheco MA, Mugisha L, Andre C, Halbwax M, Fischer A, Krief JM, Kasenene JM, Crandfield M, et al. 2010. On the diversity of malaria parasites in African apes and the origin of *Plasmodium falciparum* from Bonobos. *PLoS Pathog.* 6:e1000765.

Lemey P, Rambaut A, Drummond AJ, Suchard MA. 2009. Bayesian phylogeography finds its roots. *PLoS Comput Biol.* 5:e1000520.

Lion T. 2014. Adenovirus Infections in Immunocompetent and Immunocompromised Patients. *Clin Microbiol Rev.* 27:441–462.

Liu W, Li Y, Learn GH, Rudicell RS, Robertson JD, Keele BF, Ndjango JB, Sanz CM, Morgan DB, Locatelli S, et al. 2010. Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. *Nature* 467: 420–425.

Malhi Y, Adu-Bredu S, Asare RA, Lewis SL, Mayaux P. 2013. African rainforests: past, present and future. *Philos Trans R Soc Lond B Biol Sci.* 368:20120312.

Martin DP, Lemey P, Lott M, Moulton V, Posada D, Lefeuvre P. 2010. RDP3: a flexible and fast computer program for analyzing recombination. *Bioinformatics* 26:2462–2463.

McDougall I, Brown FH, Fleagle JG. 2005. Stratigraphic placement and age of modern humans from Kibish, Ethiopia. *Nature* 433:733–736.

Minin VN, Suchard MA. 2008. Fast, accurate and simulation-free stochastic mapping. *Philos Trans R Soc Lond B Biol Sci.* 363:3985–3995.

Mwenda JM, Nyachieo A, Langat DK, Steele DA. 2005. Serological detection of adenoviruses in non-human primates maintained in a colony in Kenya. *East Afr Med J.* 82:371–375.

Neel C, Etienne L, Li Y, Takehisa J, Rudicell RS, Bass IN, Moudindo J, Mebenga A, Esteban A, Van Heuverswyn F, et al. 2010. Molecular epidemiology of simian immunodeficiency virus infection in wild-living gorillas. *J Virol.* 84:1464–1476.

Oelze VM, Head JS, Robbins MM, Richards M, Boesch C. 2014. Niche differentiation and dietary seasonality among sympatric gorillas and

chimpanzees in Loango National Park (Gabon) revealed by stable isotope analysis. *J Hum Evol.* 66:95–106.

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Syst Biol.* 55:595–609.

Prado-Martinez J, Sudmant PH, Kidd JM, Li H, Kelley JL, Lorente-Galdos B, Veeramah KR, Woerner AE, O'Connor TD, Santpere G, et al. 2013. Great ape genetic diversity and population history. *Nature* 499: 471–475.

Prugnolle F, Durand P, Neel C, Ollomo B, Ayala FJ, Arnathau C, Etienne L, Mpoudi-Ngole E, Nkoghe D, Leroy E, et al. 2010. African great apes are natural hosts of multiple related malaria species, including *Plasmodium falciparum*. *Proc Natl Acad Sci U S A.* 107: 1458–1463.

Prugnolle F, Rougeron V, Becquart P, Berry A, Makanga B, Rahola N, Arnathau C, Ngoubangoye B, Menard S, Willaume E, et al. 2013. Diversity, host switching and evolution of *Plasmodium vivax* infecting African great apes. *Proc Natl Acad Sci U S A.* 110:8123–8128.

Purkayastha A, Ditty SE, Su J, McGraw J, Hadfield TL, Tibbetts C, Seto D. 2005. Genomic and bioinformatics analysis of HAdV-4, a human adenovirus causing acute respiratory disease: implications for gene therapy and vaccine vector development. *J Virol.* 79:2559–2572.

Rayner JC, Liu W, Peeters M, Sharp PM, Hahn BH. 2011. A plethora of *Plasmodium* species in wild apes: a source of human infection? *Trends Parasitol.* 27:222–229.

Roy S, Gao G, Clawson DS, Vandenberghe LH, Farina SF, Wilson JM. 2004. Complete nucleotide sequences and genome organization of four chimpanzee adenoviruses. *Virology* 324:361–372.

Roy S, Vandenberghe LH, Kryazhimskiy S, Grant R, Calcedo R, Yuan X, Keough M, Sandhu A, Wang Q, Medina-Jaszek CA, et al. 2009. Isolation and characterization of adenoviruses persistently shed from the gastrointestinal tract of non-human primates. *PLoS Pathog.* 5:e1000503.

Rwego IB, Isabirye-Basuta G, Gillespie TR, Goldberg TL. 2008. Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. *Conserv Biol.* 22:1600–1607.

Scally A, Dutheil JY, Hillier LW, Jordan GE, Goodhead I, Herrero J, Hobolth A, Lappalainen T, Mailund T, Marques-Bonet T, et al. 2012. Insights into hominid evolution from the gorilla genome sequence. *Nature* 483:169–175.

Sharp PM, Hahn BH. 2011. Origins of HIV and the AIDS pandemic. *Cold Spring Harb Perspect Med.* 1:a006841.

Sundararaman SA, Liu W, Keele BF, Learn GH, Bittinger K, Mouacha F, Ahuka-Mundeke S, Manske M, Sherrill-Mix S, Li Y, et al. 2013. *Plasmodium falciparum*-like parasites infecting wild apes in southern Cameroon do not represent a recurrent source of human malaria. *Proc Natl Acad Sci U S A.* 110:7020–7025.

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst Biol.* 56:564–577.

Van Heuverswyn F, Li Y, Neel C, Bailes E, Keele BF, Liu W, Loul S, Butel C, Liegeois F, Bienvenue Y, et al. 2006. Human immunodeficiency viruses: SIV infection in wild gorillas. *Nature* 444:164.

Verhulst NO, Smallegange RC, Takken W. 2012. Mosquitoes as potential bridge vectors of malaria parasites from non-human primates to humans. *Front Physiol.* 3:197.

Villesen P. 2007. FaBox: an online toolbox for fasta sequences. *Mol Ecol Notes.* 7:965–968.

Walsh PD, Breuer T, Sanz C, Morgan D, Doran-Sheehy D. 2007. Potential for Ebola transmission between gorilla and chimpanzee social groups. *Am Nat.* 169:684–689.

Weinert LA, Welch JJ, Suchard MA, Lemey P, Rambaut A, Fitzgerald JR. 2012. Molecular dating of human-to-bovid host jumps by *Staphylococcus aureus* reveals an association with the spread of domestication. *Biol Lett.* 8: 829–832.

Wevers D, Leendertz FH, Scuda N, Boesch C, Robbins MM, Head J, Ludwig C, Kuhn J, Ehlers B. 2010. A novel adenovirus of Western lowland gorillas (*Gorilla gorilla gorilla*). *Virol J.* 7:303.

Wevers D, Metzger S, Babweteera F, Bieberbach M, Boesch C, Cameron K, Couacy-Hymann E, Cranfield M, Gray M, Harris LA, et al. 2011. Novel adenoviruses in wild primates: a high level of genetic diversity and evidence of zoonotic transmissions. *J Virol.* 85:10774–10784.

Xiang Z, Li Y, Cun A, Yang W, Ellenberg S, Switzer WM, Kalish ML, Ertl HC. 2006. Chimpanzee adenovirus antibodies in humans, sub-Saharan Africa. *Emerg Infect Dis.* 12:1596–1599.

Yamagiwa J, Basabose AK. 2006. Diet and seasonal changes in sympatric gorillas and chimpanzees at Kahuzi-Biega National Park. *Primates* 47: 74–90.

Yamagiwa J, Basabose AK. 2009. Fallback foods and dietary partitioning among *Pan* and *Gorilla*. *Am J Phys Anthropol.* 140:739–750.