

Sequence Note

Two Distinct STLV-1 Subtypes Infecting *Mandrillus sphinx* Follow the Geographic Distribution of Their Hosts

M. MAKUWA,¹ S. SOUQUIÈRE,¹ S.L. CLIFFORD,¹ P.T. TELFER,^{1,2} B. SALLÉ,¹
O. BOURRY,¹ R. ONANGA,¹ A. MOUINGA-ONDEME,¹ E.J. WICKINGS,¹ K.A. ABERNETHY,¹
P. ROUQUET,¹ F. SIMON,³ and P. ROQUES^{1,4}

ABSTRACT

The mandrill (*Mandrillus sphinx*) has been shown to be infected with an STLV-1 closely related to HTLV-1. Two distinct STLV-1 subtypes (D and F) infect wild mandrills with high overall prevalence (27.0%) but are different with respect to their phylogenetic relationship and parallel to the mandrills' geographic range. The clustering of these new STLV-1mnd sequences with HTLV-1 subtype D and F suggests first, past simian-to-human transmissions in Central Africa and second, that species barriers are easier to cross over than geographic barriers.

DURING THE PAST 20 YEARS it has been demonstrated that the three simian T lymphotropic viruses (STLVs) naturally infect a number of nonhuman primate species.^{1–3} STLV-1, a retrovirus closely related to human T cell lymphoma/leukemia viruses type 1 (HTLV-1), has been described in primate species from Africa and Asia.^{4,5} In Africa, infection has been detected in both species of great apes (*Pan* and *Gorilla*) as well as in the Old World Monkey family Cercopithecidae.^{6–10} The mandrill (*Mandrillus sphinx*), which has a limited geographic distribution in the tropical forests of Cameroon, south of the Sanaga River, through Equatorial Guinea and Gabon to southern Congo, west of the Congo River,^{11–13} has been shown to be infected with an STLV-1 closely related to HTLV-1.⁸ In addition, a similar STLV-1 virus was found in *Cercopithecus nictitans*¹⁴ sympatric with *Mandrillus sphinx* in the same restricted geographic region in the tropical forests of western Central Africa. Contact between these monkeys is not rare and may account for repeated episodes of interspecies transmission.

The first cases of natural STLV infection in mandrills (STLVmnd) were described after a retrospective serological survey of mandrills at the CIRMF Primate Center in Gabon. Two males, Mnd 7 and Mnd 9, showed STLV-1 seropositivity

on their arrival at CIRMF, which strongly supports the existence of natural STLV infection in the wild. Since the creation of the breeding colony in 1983, the natural transmission of the virus has been followed.⁶ The first STLVmnd genetic studies indicated the presence of two genetically distinct strains (Mnd 7 STLV-1 and Mnd 9 STLV-1) with the intracolony transmission of the Mnd 7 STLV-1 strain occurring predominantly through male-to-male aggressive contacts.⁸ However, two mandrill females (Mnd 12M and Mnd 17D) have become infected with the Mnd 7 strain for which the mode of transmission is currently under study. Further phylogenetic studies of the Mnd 7 STLV-1 strain showed that it clustered in the same monophyletic clade with sequences of HTLV-1 subtype D,^{15,16} while the Mnd 9 STLV-1 strain grouped with a different molecular subtype F, recently described in Gabon.^{3,9,16,17} The hypothesis of cross-species transmission of STLV-1 to humans is supported in this study by the fact that the STLVmnd viruses characterized are genetically similar to HTLV-1 subtype D and F viruses.¹⁶ Moreover, African HTLV-1 and STLV-1 cannot be separated into distinct phylogenetic lineages according to their species of origin, but rather, seem to be related according to the geographic origin of their host.¹⁸ The previous study of the ge-

¹Laboratoire de Virologie, UGENET, CDP, SEGC-Lopé, Centre International de Recherches Médicales, Franceville, Gabon.

²Tulane University, Medical Center, Covington, Louisiana.

³Institut Pasteur, Dakar, Senegal.

⁴Service de Neurologie, CEA, Fontenay aux Roses, France.

TABLE 1. MANDRILLS INCLUDED IN THE STUDY, THEIR STLV SEROLOGIC STATUS AND GEOGRAPHIC ORIGIN^a

Mandrills	Origin: region in Gabon	Number tested	STLV positive	Sex	Age	Sex (age)
	South of Ogooué River					
Wild living	Lopé Reserve	23	18	17M, 6F	Adult	15M, 3F (adult)
Wild-born pets	Bakoumba	10	0	7M, 3F	7 m–3 yrs	
	Lambaréné	10	0	6M, 4F	2–3 yrs	
	Koulamoutou	5	0	3M, 2F	1–3 yrs	
	Mimongo	4	0	1M, 3F	2 yrs	
	North of Ogooué River					
Wild-born pets	Makokou	9	2	4M, 5F	6 m–3 yrs	2M (2–3 yrs)
	Woleu-Ntem	13	0	5M, 4F	8 m–3 yrs	
			0	2M, 2F	Adult	
Total tested/overall prevalence		74	20			27.0%

^aM, male; F, female; m, months; yrs, years.

ographic distribution of two known mandrill simian immunodeficiency viruses (SIVmnd type 1 and 2)¹⁹ and the very recent insights based on the phylogenetic analysis of the mitochondrial cytochrome *b* gene in mandrills throughout Gabon confirm the existence of two phylogeographic groups of mandrills.²⁰

The current phylogeographic studies of STLV strains circulating in wild mandrills and other primates in Gabon aim to verify that the phylogenetic history of these strains is strongly related to the geographic origin of their hosts.

Between 1998 and 2000, blood samples collected from 23 wild mandrills in the Lopé Reserve, Central Gabon (0012S, 1136E)^{13,21} anesthetized for placing radio-collars were tested for the presence of anti-HTLV-1 antibodies (Platelia HTLV-1 New, BioRad, Marnes la Coquette, France; WB HTLV 2.4, Genelabs Diagnostic). In addition, 51 blood samples collected from wild-born pet mandrills throughout Gabon were also tested (Table 1).

Eighteen of 23 (78.3%) wild-living mandrills and 2 of 51 (3.9%) wild-born pet mandrills tested positive in initial screenings and exhibited positive HTLV-1 western blot serological patterns. The majority of positive mandrill samples (14 of 18) exhibited a complete HTLV-1 Western blot serologic pattern with antibody reactivity against the two *gag* antigens (p19 and p24), against the recombinant GD21 protein, and against the HTLV-1 specific recombinant rgp46 or MTA-1 peptide. Two mandrill samples exhibited a nearly complete Western blot pattern, with the absence or very faint presence of the p24 band

(samples MS21 and 22), and the two remaining positive samples lacked or exhibited a very faint MTA-1 band (samples MS19 and 25) (data not shown).

All STLV-positive mandrills from the Lopé population were sexually mature individuals of both sexes. No samples were available from younger individuals. Conversely, the pet mandrills captured before the onset of sexual maturity showed a very low STLV-1 prevalence. These two observations would indicate that low levels of vertical transmission of STLV-1 virus occur in the wild, and suggest that transmission is predominantly between sexually mature individuals. Results from the CIRMf colony confirm these findings, with low infection rates in juvenile individuals and low levels of sexual transmission, but with high transmission through male–male aggression.⁸

To identify the STLV-1 subtype present in the seropositive primates and to examine the evolutionary relationship between these strains in Gabon and the other available HTLV/STLV strains, we analyzed a 422-bp (gp21) and a 459-bp (gp46) fragment of the *env* gene and a 162-bp fragment of the *tax* gene. The different fragments were amplified using previously described primers and polymerase chain reaction (PCR) conditions^{7,15,22} and sequenced directly (Sequentia, Genopole, Evry, France). DNA of mandrills housed at the CIRMf Primate Center were used as STLV-1-positive controls and STLV-1-negative controls. Alignments were carried out for two partial *env* gene regions (gp21 and gp46) and for a partial *tax* gene region using CLUSTAL W (1.7), Se-al V2.0 (<http://evolv.zoo.ox.ac.uk>).²⁰

FIG. 1. Unrooted neighbor-joining tree of a 162-bp *tax* fragment including sequences from reference strains of HTLV/STLV type and subtype, with the bootstrap (1000 bootstrap samples) values (in percent) indicated beside the branches. The GenBank accession numbers for the new strains are STLV-1 subtype D, *M. sphinx*—MSP15G (AJ564759), Mnd 12M (AJ564757); *C. nicotians*—CN01-G (AJ564758); STLV-1 subtype F, *M. sphinx*—MS8, 4, 11, 7, 25, 24, 23, 22, 19, 20 (AJ564747–AJ564756).

FIG. 2. Sequence alignment of 50 amino acids corresponding to a fragment of the p27 rex protein obtained for seven mandrills STLV-1 subtype D strains (Mnd 7—clone 11, Mnd 13—clone 7, Mnd 14—clone 11, Mnd 15—clone 1, Mnd 18, MSP15G, and Mnd 12M, for 11 mandrills STLV-1 subtype F strains (MS4, 7, 8, 11, 19, 20, 22, 23, 24, 25, and Mnd 9—clone 1) and HTLV-1 ATK reference strain.

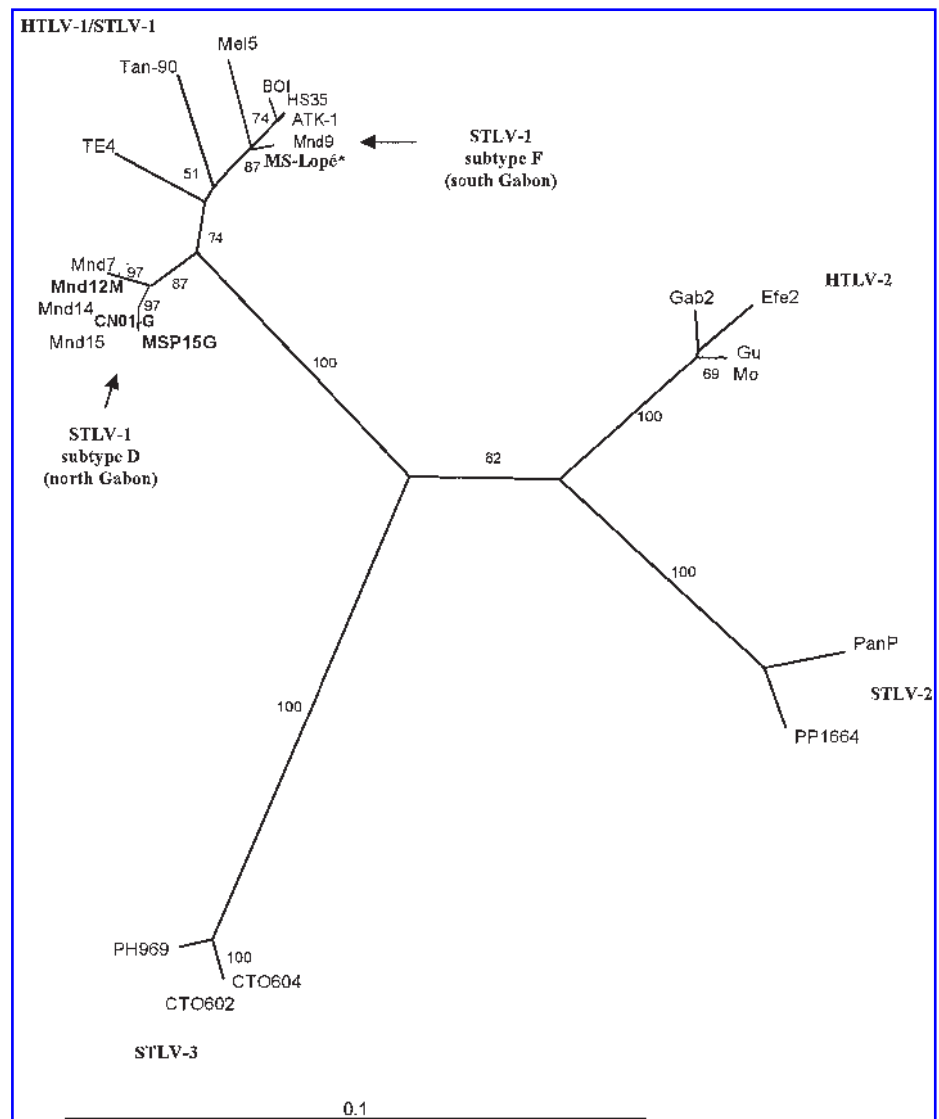
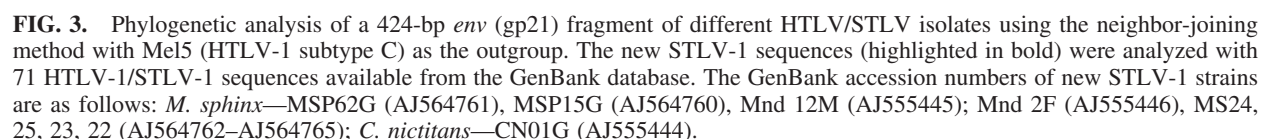


FIG. 1.

	10	20	30	40	50
ATK-1	S	D	T	Q	S
Mnd9-1	T	V	K	A	T
MS4	G	A	P	S	L
MS7	G	D	Y	V	R
MS8	P	A	I	V	I
MS11	T	P	Y	W	P
MS19	P	Q	S	I	R
MS20	S	P	G	T	P
MS22	S	M	D	A	
MS23					
MS24					
MS25					
Mnd12M					
Mnd14-11					
Mnd7-11					
Mnd15-1					
MSP15G					
CN01-G					

FIG. 2.



Kimura two-parameter model (transition/transversion ratio = 2). The appropriate nucleotide substitution model and gamma shape parameter were estimated using the program ModelTest²³ with the AIC criterion: the Hasegawa, Kishino, and Yano (HKY

85) model²⁴ was selected as the optimal model of evolution method. Phylogenetic analyses were carried using PAUP 4.0b8²⁵ (Fig. 4).

Comparative sequence analysis of a 162-bp fragment of the highly conserved *tax* region demonstrated that STLV-1mnd strains from Gabon formed two separated clusters paralleling the north-south geographic origin of their hosts (Fig. 1). The new STLV-1mnd subtype D strains originated from two wild-

born pet mandrills (MSP15G and MSP62G) from the Makokou cluster with previously characterized STLV-1 subtype D strains from CIRMF.¹⁶ The second cluster comprises the STLV-1mnd subtype F strain from Mnd 9 (CIRMF) and strains originating from wild mandrills (MS7, 8, 11, 14, 19, 20, 22, 23, 24, and 25) from the Lopé Reserve. The sequence alignment of 50 amino acids corresponding to a fragment of the p27/rex protein obtained for STLV-1mnd strains and the STLV-1_{CN01G} strain,

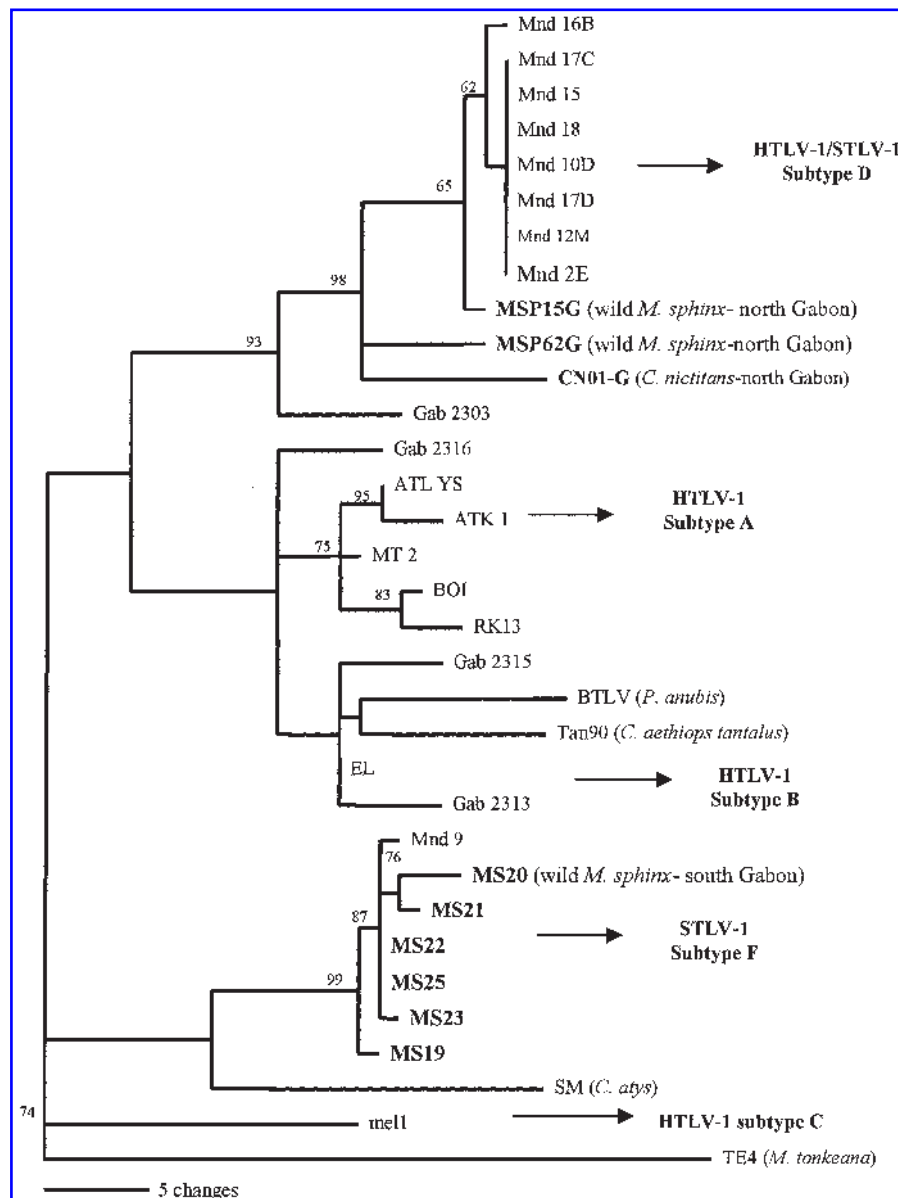


FIG. 4. Phylogenetic analysis of a 459-bp *env* (gp46) fragment of different HTLV/STLV isolates using the neighbor-joining method with TE4 (*M. tonkeana*) as the outgroup. Sequences used were as follows: for STLV-1: *C. nictitans* CN01G (AJ517395), *M. sphinx* (AJ517385–AJ517394) [wild-born mandrills (18 and 15) and captive born (16B, 17C, 17D, 10D, 2E, 12M) were all infected in captivity with STLV-1mnd 7 strain], Mnd 9 (AJ536080), *P. anubis* BTLV (U56855), *C. a. tentalus* Tan90 (AF074966), *C. atys* Sm (U94516), *M. tonkeana* TE4 (Z46900); for HTLV-1: ATK-1 (J02029), MT-2 (L03561), ATL-YS (U19949), EL (S74562), mel1 (L02533), Gab2303 (L26585), Gab2313 (L26586), Gab2315 (L33265), Gab2316 (L33266); BOI (L36905), RK13 (AF042071).

including ATK as a reference strain, confirms these findings.^{8,16} The five amino acid substitutions (Q/P, T/I, S/F, R/Q, and R/K) are specific for the group of STLV-1 subtype D-positive mandrills and *C. nictitans*. Interestingly, the amino acid substitution (M/T) is specific only to the Mnd 9 strain (clone 1) and is not seen in the other mandrill strains belonging to the same STLV-1 subtype F group (Fig. 2).

Phylogenetic analysis of the two fragments (gp21, 422 bp and gp46, 459 bp) of the *env* gene confirms the existence of two separate STLV-1mnd clusters. Employing a neighbor-joining method led to equivalent tree configurations with five distinct and phylogenetically supported HTLV-1/STLV-1 genomic subtypes (A, B, C, D, and F) (Figs. 3 and 4). Both of these analyses allow us to compare our sequences with the GenBank available gp21 and gp46 sequences. The STLV-1mnd strains representative of the two STLV-1 subtypes, D and F, are well supported with bootstrap values of 59% (gp21) and 98% (gp46) for subtype D and 69% (gp21) and 99% (gp46) for subtype F.

As previously published,^{4,6,16} these STLV-1mnd subtype D strains from Gabon, together with two recently analyzed sequences from mandrills from Cameroon,⁹ fall into the same monophyletic clade with the sequences of the previously described human HTLV-1 subtype D divergent strains from Gabon (Gab2303, Gab2318)²² and Cameroon (CMR229, H2-3)¹⁵ (Fig. 3). The HTLV-1/STLV-1 subtype F group includes new strains originating from wild mandrills from the Lopé Reserve, and the human Gabonese strain (Lib2) supported by bootstrap values of 69%. Until this study, Mnd 9, one of the original STLV-1-positive members of the semi-free ranging colony of mandrills of the CIRMF, was the sole representative of an STLV-1mnd subtype F-infected mandrill.^{8,16} In addition, mandrills share the same habitat with humans, and the independent clustering of the different STLVmnds with different HTLVs strongly suggests interspecies transmission of the virus.

In conclusion, our results show that two distinct STLV-1 subtypes infect wild mandrills. They are different with respect to their phylogenetic relationship and parallel the mandrills' north-south distribution.

Moreover, as described for primate lentiviruses (PLVs) and their hosts²⁶ and more specifically mandrills and SIVmnd strains,¹⁹ these two STLV-1 subtypes naturally infect mandrills in the wild, each having a distinct geographic distribution. Both STLV-1 subtype D-infected mandrills originated from northern Gabon, north of the Ogooué river. Conversely, the STLV-1 subtype F-infected mandrills were found south of the Ogooué River. In addition, the distribution of the cytochrome *b* haplotypes suggests that the Ogooué River, which bisects the mandrills range, separates populations in Cameroon and northern Gabon from those in southern Gabon, suggesting that these two mandrill phylogroups have followed different evolutionary trajectories since separation.²⁰ The clustering of these new STLV-1mnd sequences with HTLV-1 subtype D and F suggests first, independent past simian-to-human transmissions in Central Africa^{16,27} and second, that species barriers to transmission of STLV-1 may sometimes be easier to cross than the geographic barriers.

The GenBank accession numbers of the reported sequences are *Tax* region: STLV-1 subtype D: *M. sphinx*—MSP15G

(AJ564759), Mnd 12M (AJ564757); *C. nictitans*—CN01-G (AJ564758); STLV-1 subtype F: *M. sphinx*—MS8, 4, 11, 7, 25, 24, 23, 22, 19, 20 (AJ564747–AJ564756). *env* (gp46) region: STLV-1 subtype D: MSP15G (AJ517386), MSP62G (AJ564605); STLV-1 subtype F: *M. sphinx*—MS23, 25, 20, 21, 19, 22 (AJ536074–AJ536079). *env* (gp21) region: STLV-1 subtype D: *M. sphinx*—MSP62G (AJ564761), MSP15G (AJ564760); STLV-1 subtype F: *M. sphinx*—MS24, 25, 23, 22 (AJ564762–AJ564765).

ACKNOWLEDGMENTS

We thank Michael Bruford for critical review of the manuscript and Marie-Thérèse Niangui for excellent technical help. This work was supported by funds from the Centre International de Recherche Médicales Franceville (CIRMF), Franceville, Gabon, Total-Fina-Elf Gabon and Ministère de la Coopération Française, and by NIH Grant R01 AI44596 (PAM).

REFERENCES

1. Koralnik IJ, Boeri E, Saxinger WC, Lo Monaco A, Fullen J, Gessain A, Guo H-G, Gallo RC, Markham P, Kalyanaraman V, Hirsch V, Allan J, Murthy K, Alfort P, Slattery JP, O'Brien SJ, and Franchini G: Phylogenetic associations of human and simian T-cell leukemia/lymphotropic virus type I strains: Evidence for interspecies transmission. *J Virol* 1994;68:2693–2707.
2. Saksena NK, Hervé V, Durand JP, Le Guenno B, Diop OM, Digoutte JP, Mathiot C, Müllet MC, Love JL, Dube S, Sherman MP, Benz PM, Erensoy S, Galat-Louong A, Galat G, Paul B, Dube DK, Barré-Sinoussi F, and Poiesz BJ: Seroepidemiologic, molecular and phylogenetic analyses of simian T-cell leukemia viruses (STLV-I) from various naturally infected monkey species from central and western Africa. *Virology* 1994;198:297–310.
3. Slattery JP, Franchini G, and Gessain A: Genomic evolution, patterns of global dissemination, and interspecies transmission of human and simian T-cell leukemia/lymphotropic viruses. *Genome Res* 1999;9:525–540.
4. Gessain A, Mahieux R, and de Thé G: Genetic variability and molecular epidemiology of human and simian T cell leukemia/lymphoma virus type I. *AIDS Res Hum Retroviruses* 1996;13(Suppl.):S132–S154.
5. Liu HF, Goubau P, Van Brussel M, Van Laethem K, Chen YC, Desmyter J, and Vandamme A-M: The three human T-lymphotropic virus type 1 subtypes arose from three geographically distinct simian reservoirs. *J Gen Virol* 1996;77:359–368.
6. Georges-Courbot MC, Moisson P, Leroy E, Pingard AM, Nerrienet E, Dubreuil G, Wickings EJ, Debels F, Bedjabaga I, Poaty-Mavoungou V, Hanh NT, and Georges AJ: Occurrence and frequency of transmission of naturally occurring simian retroviral infections (SIV, STLV, and SRV) at the CIRMF Primate Center, Gabon. *J Med Primatol* 1996;25:313–326.
7. Mahieux R, Slattery JP, and Gessain A: Molecular characterization and phylogenetic analyses of a new highly divergent simian T-cell lymphotropic virus type 1, STLV-1marc1 in *Macaca arctoides*. *J Virol* 1997;71:6253–6258.
8. Nerrienet E, Amouretti X, Müller-Trüwin MC, Poaty-Mavoungou V, Bedjabaga I, Thi Nguyen H, Dubreuil G, Corbet S, Wickings EJ, Barre-Sinoussi F, Georges AJ, and Georges-Courbot MC: Phylogenetic analysis of SIV and STLV type I in mandrills (*Mandrillus sphinx*): Indications that intracolony transmissions are predomi-

- nantly the result of male-to-male aggressive contacts. *AIDS Res Hum Retroviruses* 1998;14:785–796.
9. Nerrienet E, Meertens L, Kfutwah A, Foupouapouognigni Y, and Gessain A: Molecular epidemiology of simian T-lymphotropic virus (STLV) in wild-caught monkeys and apes from Cameroon: A new STLV-1, related to human T-lymphotropic virus subtype F, in a *Cercocebus agilis*. *J Gen Virol* 2001;82:2973–2977.
 10. Voevodin AF, Johnson BK, Samilchuk EI, Stone GA, Drouilhet R, Greer WJ, and Gibbs CJ Jr: Phylogenetic analysis of simian T-lymphotropic virus type I (STLV-I) in common chimpanzees (*Pan troglodytes*): Evidence for interspecies transmission of the virus between chimpanzees and humans in Central Africa. *Virology* 1997;238:212–220.
 11. Harisson MJS: The mandrill in Gabon's rain forest—ecology, distribution and status. *Oryx* 1988;22:218–230.
 12. Kingdon J: *The Kingdon Field Guide to African Mammals*. Academic Press, London, 1997.
 13. Rogers ME, Abernethy KA, Fontaine B, Wickings EJ, White LJT, and Tutin C: Ten days in the life of a mandrill horde in the Lopé Reserve, Gabon. *Am J Primatol* 1996;40:297–313.
 14. Makuwa M, Souquière S, Telfer P, Mouinga-Ondeme A, Bourry O, and Roques P: A new STLV-1 in a household pet *Cercopithecus nictitans* from Gabon. *AIDS Res Hum Retroviruses* 2004;20:679–683.
 15. Mahieux R, Ibrahim F, Mauciere P, Herve V, Michel P, Tekaia F, Chappey C, Garin B, Van der Ryst E, Guillemain B, Ledru E, Delaporte E, de The G, and Gessain A: Molecular epidemiology of 58 new African human T-cell leukemia virus type I (HTLV-I) strains: Identification of a new distinct HTLV-I molecular subtype in Central Africa and Pygmies. *J Virol* 1997;71:1317–1333.
 16. Mahieux R, Chappey C, Georges-Courbot MC, Dubreuil G, Mauciere P, Georges A, and Gessain A: Simian T-cell lymphotropic virus type 1 from *Mandrillus sphinx* as a simian counterpart of human T-cell lymphotropic virus type 1 subtype D. *J Virol* 1998;72:10316–10322.
 17. Vandamme A-M, Salemi M, and Desmyter J: The simian origins of the pathogenic human T-cell lymphotropic virus type I. *Trends Microbiol* 1998;6:477–483.
 18. Thompson JD, Higgins DG, and Gibson TJ: CLUSTAL W—improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994;22:4673–4680.
 19. Souquière S, Bibollet-Ruche F, Robertson DL, Makuwa M, Aptei C, Onanga R, Kornfeld C, Plantier JC, Gao F, Abernethy K, White LJT, Karesh W, Telfer P, Wickings EJ, Maucière P, Marx PA, Barré-Sinoussi F, Hanh BH, Muller-Trutwin MC, and Simon F: Wild *Mandrillus sphinx* are carriers of two types of lentivirus. *J Virol* 2001;75:7086–7096.
 20. Telfer PT, Souquière S, Clifford SL, Abernethy KA, Bruford MW, Disotell TR, Sterner KN, Roques P, Marx PA and Wickings EJ: Molecular evidence for deep phylogenetic divergence in *Mandrillus sphinx*. *Mol Ecol* 2003;12(7):2019–2024.
 21. Abernethy KA, White LJT, and Wickings EJ: Hordes of mandrills (*Mandrillus sphinx*): Extreme group size and seasonal male presence. *J Zool Lond* 2002;258:131–137.
 22. Moynot D, Cosnefroy J-Y, Bedjabaga I, Roelants G, Georges-Courbot MC, and Guillemain B: Identification of a new genetic subtypes of human T cell leukemia virus type I in Gabon from encoding sequence of surface envelope glycoprotein. *AIDS Res Hum Retroviruses* 1995;11:1407–1411.
 23. Possada D and Crandall KA: MODELTEST: Testing the model of DNA substitution. *Bioinformatics* 1998;14:817–818.
 24. Hasegawa M, Kishino K, and Yano T: Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *J Mol Evol* 1985;22:160–174.
 25. Swofford DL: PAUP: Phylogenetic Analysis Using Parsimony (and other methods) Version 4.0. Sinauer Associates, Sunderland, MA, 1999.
 26. Charleston MA and Robertson DL: Preferential host switching by primate lentiviruses can account for phylogenetic similarity with the primate phylogeny. *Syst Biol* 2002;51:528–535.
 27. Salemi M, Van Dooren S, Audenaert E, Delaporte E, Goubau P, Desmyter J, and Vandamme A-M: Two new human T-lymphotropic virus type I phylogenetic subtypes in seroindeterminates, a Mbuti Pygmy and Gabonese, have closest relatives among African STLV-I strains. *Virology* 1998;246:277–287.

Address reprint requests to:

M. Makuwa

Centre International de Recherches Médicales (CIRMF)

B.P. 769

Franceville, Gabon

E-mail: mmakuwa@yahoo.com