JOURNAL OF VIROLOGY, Oct. 1992, p. 6008–6018
0022-538X/92/106008-11$02.00/0

Vol. 66, No. 10

Worldwide Prevalence of Lentivirus Infection in Wild Feline Species: Epidemiologic and Phylogenetic Aspects

ROBERT A. OLMSTED,[1,2]† RAYMOND LANGLEY,[2] MELODY E. ROELKE,[3] ROBERT M. GOEKEN,[2] DIANE ADGER-JOHNSON,[2] JULIE P. GOFF,[1] JOHN P. ALBERT,[1] CRAIG PACKER,[4] M. KAREN LAURENSON,[5] TIM M. CARO,[6] LUE SCHEEPERS,[7] DAVID E. WILDT,[8] MITCHELL BUSH,[8] JANICE S. MARTENSON,[9] AND STEPHEN J. O'BRIEN[9]*

Division of Molecular Virology and Immunology, Department of Microbiology, Georgetown University,[1] and Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases,[2] Rockville, Maryland 20852; Wildlife Research Laboratory, Florida Game and Freshwater Fish Commission, Gainesville, Florida 32601[3]; Department of Ecology and Behavior Biology, University of Minnesota, Minneapolis, Minnesota 55455[4]; Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, United Kingdom[5]; Department of Wildlife and Fisheries Biology, University of California, Davis, California 95616[6]; Etosha Ecological Institute, P.O. Okaukuejo via Outjo, Namibia[7]; Department of Animal Health, National Zoological Park, Smithsonian Institution, Washington, D.C. 20008[8]; and Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, Maryland 21702[9]

Received 8 April 1992/Accepted 24 June 1992

The natural occurrence of lentiviruses closely related to feline immunodeficiency virus (FIV) in nondomestic felid species is shown here to be worldwide. Cross-reactive antibodies to FIV were common in several free-ranging populations of large cats, including East African lions and cheetahs of the Serengeti ecosystem and in puma (also called cougar or mountain lion) populations throughout North America. Infectious puma lentivirus (PLV) was isolated from several Florida panthers, a severely endangered relict puma subspecies inhabiting the Big Cypress Swamp and Everglades ecosystems in southern Florida. Phylogenetic analysis of PLV genomic sequences from disparate geographic isolates revealed appreciable divergence from domestic cat FIV sequences as well as between PLV sequences found in different North American locales. The level of sequence divergence between PLV and FIV was greater than the level of divergence between human and certain simian immunodeficiency viruses, suggesting that the transmission of FIV between feline species is infrequent and parallels in time the emergence of HIV from simian ancestors.

The rapid emergence of AIDS during the past decade has brought about extensive efforts to determine the origin and natural history of human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2), the etiologic agents of the disease. Lentiviruses that have gene sequence homology with HIVs have been found in several mammalian species (including sheep, goats, horses, cattle, cats, and several Old World monkey species), indicating that humans are merely the latest unfortunate species to have become infected via another mammalian species (7, 9, 10, 16, 17, 26, 38). The evolutionary relationship between various viral groups has been approached by using genome organization, tissue tropism, pathological sequelae, and viral gene sequence similarity as characters upon which phylogenetic inference is based (7, 9, 16, 17, 26).

The closest relatives of HIV have been isolated from several species of African and Asian primates. The African primates (notably sooty mangabeys, mandrills, green monkeys, grivets, and Sykes' monkeys) appear to serve as natural reservoirs for simian immunodeficiency virus (SIV), although infected native African species do not develop clinical symptoms (25, 35). In contrast, Asian macaques manifest an AIDS-like illness when infected with certain strains of SIV in captive settings but do not appear to have been exposed to the virus in their natural habitat (19, 25, 27, 35). These observations, combined with findings of extensive genetic variation within SIV isolates, have prompted the hypothesis that the human species acquired HIV rather recently by a transspecies infection from African primates that had harbored the ancestral lentiviruses for a long period, perhaps before the radiation of the African primate species (3, 7, 19, 21, 25, 27, 35).

Interpretations of natural histories of retroviral origins are contingent upon virus isolation properly reflecting geographic origins of host species (9, 17, 26). However, most SIV isolates have been recovered from captive monkeys, raising the possibility that the virus was acquired in an unnatural setting where exposure via other captive species can occur (we think that macaques acquired SIV in this way in primate research centers). For example, a recent isolate of simian immunodeficiency virus (SIV-CPZ) from two captive chimpanzees in Gabon is the closest relative to HIV-1 yet described (20, 39), thereby making SIV-CPZ a strong candidate for a precursor of HIV-1. However, the virus is extremely rare in captive chimpanzees (infecting ≤1 of 250 animals), and there is no evidence for its occurrence in nature. Thus, it is possible that the SIV-CPZ was transmitted from humans to chimpanzees and not the other way around. Clearly, to interpret these data in a historical context, we need more information on the prevalence of lentiviruses in nature, specifically in other wild animal species.

* Corresponding author.
† Present address: Cancer and Infectious Diseases, Upjohn Laboratories, Kalamazoo, MI 49001.

Our laboratories have been studying the molecular characterization of feline immunodeficiency virus (FIV) as well as the genetic statuses of several populations of nondomestic cat species. FIV is a recently discovered lentivirus isolated from domestic cats (*Felis catus*) that is distantly related to the primate lentiviruses. Like HIV, FIV is T-cell tropic and associated with immunodeficiency syndrome in infected hosts (38, 47). FIV is widely prevalent in domestic cats and displays abundant inter se genetic diversity, suggesting that it has infected domestic cats for a long period. Recent serological studies have also detected antibodies against FIV in isolated individuals of nondomestic felid species, primarily from zoo collections (5, 24). We extend these studies by a survey of serum samples from 12 species of the family Felidae, including serum samples from free-ranging populations of lions, cheetahs, and pumas collected in their native habitats. We report a high prevalence of cross-reactive antibodies to an FIV-like lentivirus in free-ranging African lions and cheetahs as well as in North American pumas. Multiple infectious isolates were obtained from Florida panthers (*Felis concolor coryi*, a puma subspecies). Genomic sequences of viral *pol* genes permit phylogenetic analysis of puma lentivirus (PLV) genomes that are interpreted in a phylogeographic context. The wide prevalence of lentiviral infections in natural populations of large felids provides a rare opportunity to track retrospectively the pattern and consequences of an ongoing epizootic.

MATERIALS AND METHODS

Western blots (immunoblots) for antibodies against FIV. Plasma samples were incubated with immunoblot strips prepared with FIV-Petaluma antigens, and Western blot assays were performed as previously described (36, 47).

Virus isolation. Peripheral blood mononuclear cells (PBMCs) were purified by density gradient centrifugation of heparinized whole blood from seropositive Florida panthers within 24 h following peripheral venous bleeding. PBMCs (10^7 cells) from seropositive animals were cocultured with donor PBMCs (10^7) from seronegative specific-pathogen-free domestic cats or from seronegative pumas. Cultures were maintained in RPMI 1640 containing 10% fetal calf serum and 10% interleukin-2 after mitogen stimulation (concanavalin A; 5 μg/ml for 72 h). The presence of replicating virus was monitored by measuring Mg^{2+}-dependent reverse transcriptase (RT) activity in the culture supernatant fluids. At the peak of RT activity, cells were processed for electron microscopy.

The Maryland isolate of FIV, FIV-MD, was obtained from a Mt. Airy, Md., domestic male cat, approximately 2 years old, that was suffering from respiratory tract infection, gingivitis, and weight loss. This cat was FIV positive and feline leukemia virus negative as determined by enzyme-linked immunosorbent assay (CITE Combo test kit; Agritech Systems, Portland, Maine). The virus was isolated by cell coculture as described above.

Samples were collected in full compliance with specific federal fish and wildlife permits (CITES: Endangered and Threatened Species; Captive Bred) issued to the National Cancer Institute, principal officer S. J. O'Brien, by the U.S. Fish and Wildlife Service of the Department of the Interior.

Amplification and sequence determination of PLV *pol* gene in virus-infected cells. Genomic DNAs containing the PLV or FIV proviral sequences were isolated from RT-positive PBMC cocultures (isolate PLV-14, PLV-16, PLV-21, PLV-42, or FIV-MD), or from uncultured PBMCs isolated from whole blood (isolate PLV-8, PLV-18, PLV-64, or PLV-80). The RT region was amplified by polymerase chain reaction (PCR) from 0.5 μg of genomic DNA during 30 heating-cooling-extension cycles (94°C for 1 min, 37 or 45°C for 1.5 min, and 72°C for 1 min). Reaction volumes, reagents, equipment, and oligonucleotide syntheses were as previously described (21). A second round of 30 cycles using 10 μl from the first-round reaction products and a pair of oligonucleotides located within the boundaries of the first pair was necessary for the amplification of RT sequences of PLV-8, PLV-18, PLV-64, and PLV-80. PBMC DNA from seronegative pumas and no template controls were used routinely to detect PCR contamination. The amplified products were gel purified, prepared for blunt-end ligation, and cloned into a plasmid vector by conventional methods. Plasmid clones were isolated and sequenced by the chain termination method with T7 DNA polymerase (United States Biochemical). Oligonucleotide primer sequences and the nucleotide positions of the FIV-14 proviral sequence (37) (in parentheses) were as follows: 669F (2403), 5'CAATGGCCATTAA CAAATG3'; 1217R (3118), 5'CCTGCTAATTTTTGCAAC TCATT3'; 1258F (2430), 5'GAAGCATTAACAGAAATAG TAG3'; 1260R (3007), 5'GGTTCTTGTTGTAATTTATC TTC3'; 1259F (2466), 5'GAAGGAAAGGTAAAAAGAGCA GATC3'; 1261R (2990), 5'ATCTTCAGGAGTTTCAAATC CCCA3'; 1152F (2544), 5'TGGAGAATGCTCATAGATTT TAGAGAATT3'; 1314R (2905), 5'GATCCTATATATATAT CATCCATATATTG3'; 1086F (2559), 5'GATTTTAGAGA ATTAAACAA3'; and 1068R (2902), 5'CCTATATAAATG TCATCCAT3'. Primers 669F and 1217R were used for amplification of the PLV-14 and FIV-MD sequences; 1258F and 1260R were used for PLV-16, PLV-21, and PLV-42. A second round of 30 cycles using 10 μl from the first-round reaction products and a pair of oligonucleotides located within the boundaries of the first pair was necessary for the amplification of RT sequences of PLV-8, PLV-18, PLV-64, and PLV-80: 1258F and 1260R (outer pair) and 1259F and 1261R (inner pair) were used for PLV-18; 1152F and 1314R (outer pair) and 1086F and 1068R (inner pair) were used for PLV-8, PLV-64, and PLV-80. Primers 669F, 1217R, 1152F, 1086F, and 1068R are FIV-14 sequence specific and were derived from conserved regions detected in alignments of several lentiviral *pol* gene sequences with FIV (data not shown). Primers 1258F, 1259F, 1260R, 1261R, and 1314R are PLV-14 sequence specific and represent conserved regions shared by PLV-14 and FIV-14 RT sequences.

Phylogenetic analyses. All sequences except PLV-8, PLV-18, PLV-64, and PLV-80 were subjected to phylogenetic analysis by using a total of 576 bp of nucleotide sequence representing 192 amino acid residues upon translation. Shorter sequences (318 bp, 106 amino acids) were also analyzed for all PLV isolates and all control lentiviruses. Each full-length sequence was separately aligned with every other sequence by the GAP program of the Genetics Computer Group (University of Wisconsin) computer software package (8), which uses the algorithm of Needleman and Wunsch (28). Distances are expressed on the basis of percent difference in amino acid sequence identity; gaps are given a weight of a single residue substitution regardless of their length (41).

Nucleotide sequence accession numbers. The nucleotide sequence data reported in this article for PLV-8, PLV-14, PLV-16, PLV-18, PLV-21, PLV-42, PLV-64, PLV-80, and FIV-MD have been deposited in the GenBank sequence library under accession numbers M95475, M95471, M95477,

TABLE 1. Worldwide prevalence of feline lentivirus exposure in nondomestic felids[a]

Felid species	Source(s)	No. positive	No. tested	% Positive
Free-ranging				
Lion (*Panthera leo*)	East Africa (Tanzania and Kenya)	84	106	79
	South Africa (Kruger National Park)	10	12	83
	Southwest Africa (Namibia)	0	22	0
	Total	94	140	67
Cheetah (*Acinonyx jubatus*)	East Africa (Tanzania and Kenya)	10	46	22
Puma (*Felis concolor*)	Florida (Big Cypress Swamp)	9	37	24
	Florida (Everglades National Park)	4	9	44
	Arizona	8	10	80
	California	9	16	56
	Colorado	6	9	67
	New Mexico	1	2	50
	Oregon	1	11	9
	Texas	6	18	33
	Utah	1	2	50
	Wyoming (Yellowstone National Park)	5	25	20
	Idaho	0	3	0
	Canada and Alaska	3	7	43
	South America (Chile)	0	2	0
	Total	53	151	35
Bobcat (*Lynx rufus*)	Florida	2	23	9
Captive				
Lion				
Unknown subspecies	U.S. zoos	0	29	0
	Circuses	2	3	67
	Johannesburg Zoo, Johannesburg, South Africa	6	9[b]	67
Asiatic	U.S. zoos	23	35	66
	Sakkarbaug Zoo, Sakkarbaug, India	0	28[c]	0
	Negara Zoo, Kuala Lumpur, Malaysia	0	8	0
	Total	31	112	28
Cheetah	Zoos (United States, Australia, England)	1	64	1.6
	De Wildt Breeding Center, Pretoria, South Africa	0	45	0
	Total	1	109	1
Puma	Canada	2[d]	8	25
	United States	0	58[e]	0
	Central and South America (Belize, Chile, and Brazil)	0	16[f]	0
	Total	2	82	2
Other	U.S. European and South African zoos			
Tiger (*Panthera tigris*)		0	20	0
Snow leopard (*P. uncia*)		0	11	0
Jaguar (*P. onca*)		0	7	0
Leopard (*P. pardus*)		0	11	0
Serval (*Leptailurus serval*)		0	5	0
Sand cat (*F. margarita*)		0	4	0
Marbled cat (*Pardofelis marmorata*)		0	1	0
Bobcat		0	1	0
Flat-headed cat (*Ictailurus planiceps*)		1	3	33
	Total	1	63	1.6

[a] All serum and plasma samples (1:100 dilution) were tested by immunoblot assay for antibodies to FIV-Petaluma antigens.
[b] All were probably born in the wild.
[c] Four of these lions were born in the wild and had been in captivity for 1 to 12 years before sampling.
[d] Both positive animals were born in the wild; they came into captivity at 6 months and 2 years of age.
[e] Eight of these pumas were born in the wild and came into captivity as juveniles or young adults.
[f] At least 10 of these pumas were born in the wild and came into captivity as juveniles or young adults.

M95476, M95478, M95470, M95473, M95474, and M95472, respectively.

RESULTS

Prevalence of FIV antibodies in wild felid species. A total of 726 serum or plasma samples, representing 12 nondomestic felid species, were screened for antibodies that recognized FIV; 360 of the specimens were from free-ranging cats, and the rest were from captive-held animals. The samples were collected between 1978 and 1991, but the earliest specimens from free-ranging animals were collected in 1983 from Serengeti lions and cheetahs. The sera were typed by Western immunoblotting (Fig. 1), and the results of all the typings are presented in Table 1.

Several important observations are revealed by these data. First, there has been widespread exposure to lentiviruses related to FIV in sampled free-ranging populations of four species (lions, pumas, cheetahs, and bobcats). The incidence of seropositive lions was 79% in the Serengeti (Tanzania and Kenya) and 83% in Kruger National Park in South Africa; 22% of the Serengeti cheetahs sampled were

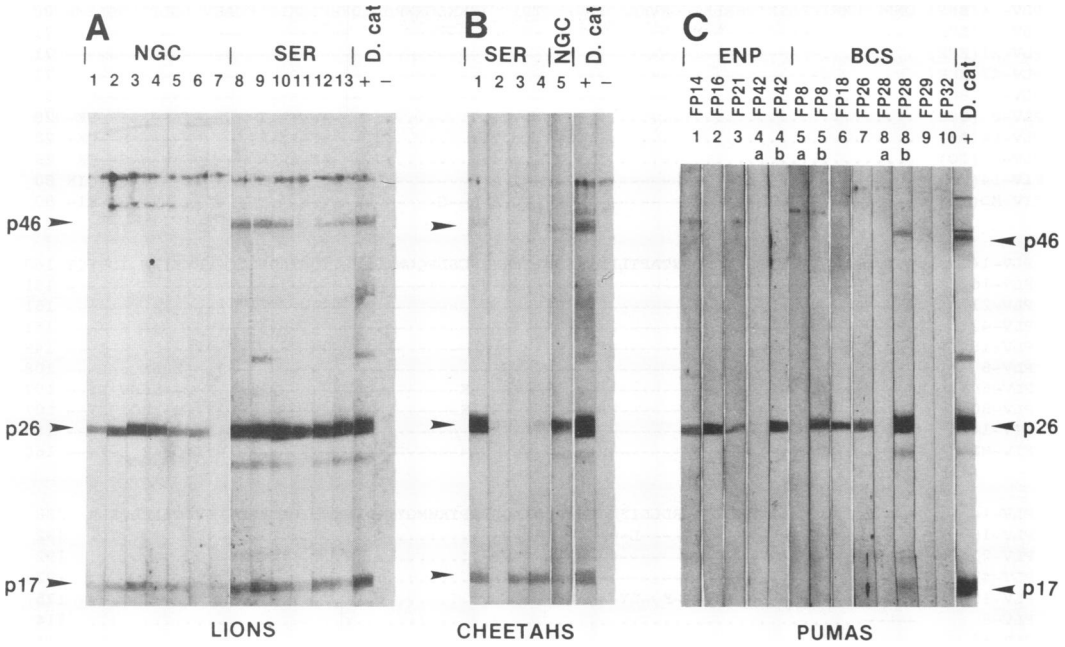
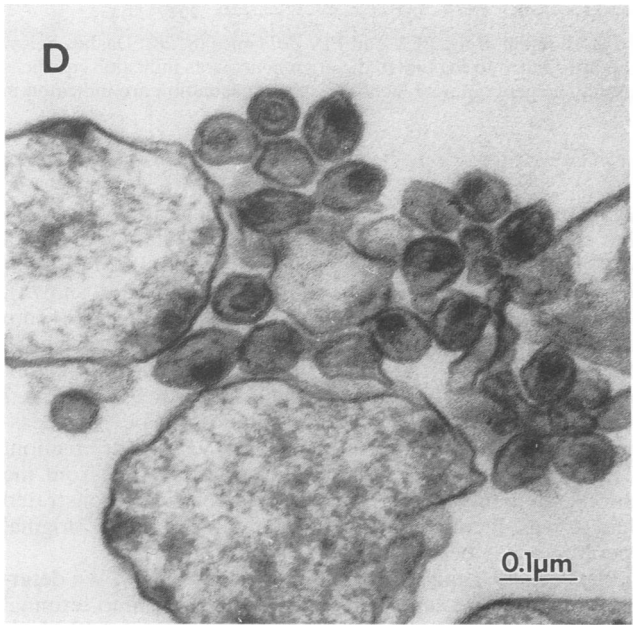


FIG. 1. Western blot analysis of selected serum and plasma samples from free-ranging East African lions and cheetahs and Florida panthers. (A) Samples from East African lions (*P. leo*) from Ngorongoro Crater (NGC) and Serengeti National Park (SER), Tanzania. Lanes 1 to 7, lions Ple-314 to Ple-320 (Ple-320 was seronegative), respectively; lanes 8 to 13, lions Ple-331 to Ple-336, respectively; lanes + and −, positive (FIV-infected) and negative control domestic cat sera. (B) Samples from East African cheetahs (*Acinonyx jubatus*). Lanes 1 to 5, cheetahs Aju-201, Aju-202 (seronegative), Aju-203, Aju-204, and Aju-213, respectively. (C) Samples from Florida panthers (*F. concolor coryi*) inhabiting the Everglades National Park (ENP) and Big Cypress Swamp (BCS) ecosystems. Panther designations are given above the lanes. Lanes 4a and b, samples obtained in March 1990 and May 1991, respectively; lanes 5a and b, samples obtained in March 1984 and January 1986, respectively; lanes 8a and b, samples obtained in March 1989 and January 1991, respectively; lanes 9 and 10, samples from seronegative panthers; lane +, FIV-infected domestic cat serum. (D) Electron micrograph of mature lentivirus particles in cultured PBMCs from Florida panther FP-16.



positive. In North America, 11 of 12 wild puma populations sampled had exposed individuals. Second, animals from certain locales were clearly negative, notably African lions from Namibia in southwestern Africa and Asiatic lions from the Sakkarburg Zoo in India that are derived from the Gir Forest population in western India (30). Third, with the exception of the Asiatic lions in U.S. zoos and Johannesburg Zoo lions, other captive animals were nearly always seronegative. The Johannesburg Zoo lions are derived from Kruger National Park, where they likely acquired infection. The U.S. Asiatic lions are a group of captive-bred lions descended from five founder animals, three authentic Asiatic lions (*Panthera leo persica*) derived from the Gir Forest sanctuary and two African lions (*P. leo leo*) (30). It is possible that the captive lion population was infected by exposure during captivity or via their African founders or both.

The immunoblots in Fig. 1 illustrate the seroreactivity patterns of infected lions, cheetahs, and pumas compared with those of domestic cat positive controls. Sera from two cheetahs recognized the FIV p17^{gagMA} core protein but not the p26^{gagCA} polypeptide seen in other animals. There was also a difference in reactivity patterns between two populations of seropositive lions from East Africa; i.e., the Ngorongoro Crater and Serengeti National Park populations (e.g., see p46^{gag} bands in Fig. 1). These pattern differences may reflect reactivities to distinct viruses in the two locales that differ in immunological epitopes shared with FIV. These immunologic differences were an early suggestion that FIV-related viruses have notable genetic divergences in different locations.

Isolation of PLV from Florida panthers. Our next objective was to isolate the suspected lentivirus(es) from one or more seropositive free-ranging animals. We concentrated on the small free-ranging Florida panther population (<50 individ-

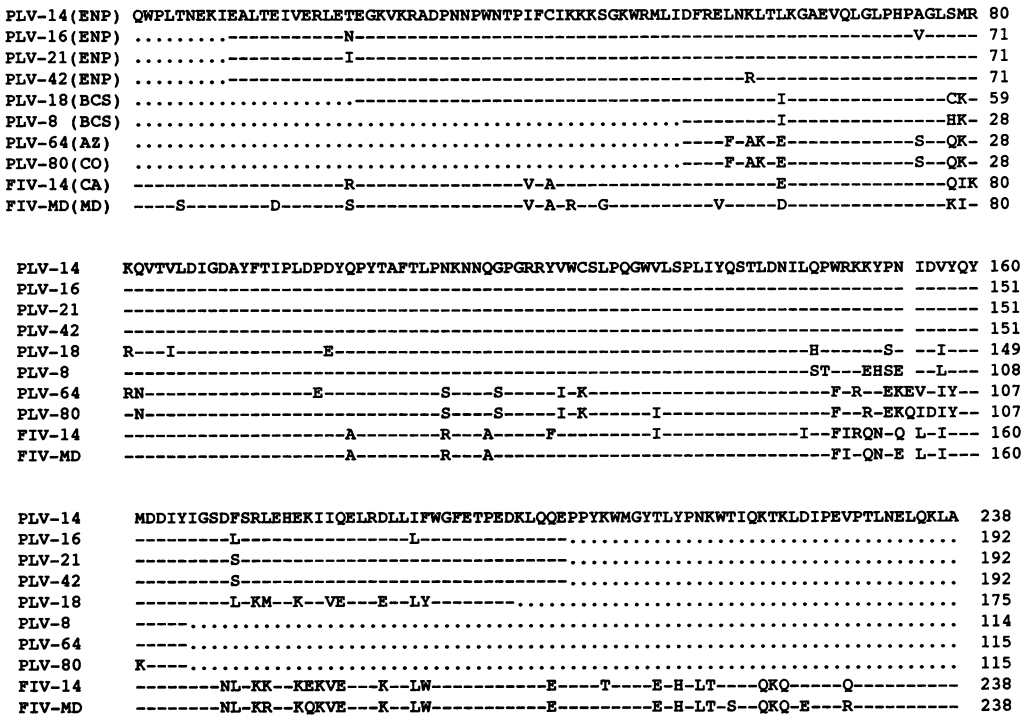


FIG. 2. Alignment of the predicted amino acid sequences of a conserved RT domain in the PLV and FIV *pol* genes (8, 28). Dashes below the PLV-14 sequence indicate identical amino acids. The open space is a gap introduced to maximize the alignment. Dots indicate sequences not obtainable with the available oligonucleotides used for PCR amplification. The geographical locations of virus isolation are indicated in parentheses. ENP, Everglades National Park; BCS, Big Cypress Swamp.

uals remaining) in the Everglades National Park and Big Cypress Swamp ecosystems in southern Florida (31, 33). The Florida panther is an endangered subspecies of *F. concolor* (other common names are puma, cougar, and mountain lion). Through efforts of the Florida Game and Freshwater Fish Commission, the Florida Panther Recovery Program was initiated in the early 1980s to try to prevent the extinction of this puma subspecies (13). Thus, a well-defined and closely monitored free-ranging population was available for both serological and virological studies.

A representative immunoblot analysis of plasma samples from 10 Florida panthers is shown in Fig. 1 (also see Table 1). Twenty-eight percent of the samples contained antibodies to FIV, extending the results presented in an earlier preliminary report (5). Analysis of sequential samples from three different animals revealed the development of cross-reactive antibodies to FIV over time. Notably, Florida panther FP-42, an offspring of FP-14 and FP-16, tested negative at 10 months of age in March 1990 but had seroconverted by 14 months later.

PBMCs collected from four seropositive Florida panthers (FP-14, FP-16, FP-21, and FP-42) were placed in culture and monitored for magnesium-dependent RT activity. RT activity was detected in each culture within 17 to 22 days after coculture. At the peak of RT activity, electron microscopic examination of the FP-16 PBMC coculture demonstrated the presence of virion particles with typical lentivirus morphology (Fig. 1D). The particles appeared to be similar in size to FIV particles but were slightly smaller than primate lentiviruses (38). We designated these isolates PLV.

Primary cell cultures of fresh PBMCs from a seronegative puma were readily infected with cell-free culture fluids from all four RT-positive cultures, thus demonstrating the infective capability of the lentiviruses. PLV isolates displayed a notable preference for growth in fresh puma PBMCs compared with fresh domestic cat PBMCs (data not shown). Transfusion of mitogen-stimulated PBMCs from FP-14 into an uninfected specific-pathogen-free domestic cat resulted in the establishment of a persistent infection. This cat developed humoral antibodies cross-reactive with FIV within 7 weeks posttransfusion, and virus isolation has been successful at each attempt during the first year postinfection. Genomic sequence analysis of the virus isolated from the chronically infected specific-pathogen-free cat demonstrated that it was identical to PLV-14 obtained in the original cocultures (see below).

Phylogenetic analysis of PLV *pol* gene sequences. We determined the nucleic acid sequence of a 714-bp amino-terminal segment of the *pol* gene by direct amplification of PLV-14-infected cellular DNA by the PCR. The PLV genome is 9,165 bp long. This *pol* region of the reverse transcriptase-encoding gene is the most slowly evolving portion of retroviral genomes and, as such, is particularly useful in reconstructing distinct ancestral relationships (3, 9, 21). For sequence analysis, we examined viral *pol* genes from PBMC genomic DNA of six PLV-infected Florida panthers (PLV-14, PLV-16, PLV-21, and PLV-42 from Everglades National Park and PLV-8 and PLV-18 from Big Cypress Swamp) and two western cougars (PLV-64 from Arizona and PLV-80 from Colorado). The nucleotide and translated amino acid sequences were aligned with each other and with 11 other lentivirus sequences (including those of three FIV isolates). The aligned amino acid sequences of the PLVs and FIVs are presented in Fig. 2. A matrix of pairwise sequence identities

TABLE 2. Sequence comparisons of conserved RT domains from *pol* genes of eight PLV isolates, FIV, and other lentiviruses

Virus[a]	% Identity with indicated virus[b]																		
	Everglades National Park				Big Cypress Swamp		PLV-64 (Arizona)	PLV-80 (Colorado)	California		FIV-MD (Maryland)	EIAV	Visna virus	HIV-1	SIV-CPZ	HIV-2	SIV-SM	BIV	BLV
	PLV-14	PLV-21	PLV-42	PLV-16	PLV-18	PLV-8			FIV-PET	FIV-SD									
PLV-14		100	99	99	92	91	82	83	84	83	88	63	61	61	61	57	61	52	33
PLV-21	100		99	99	92	91	82	83	84	83	88	63	61	61	61	57	61	52	33
PLV-42	98	98		98	91	90	82	83	83	83	87	62	60	61	61	56	60	51	33
PLV-16	99	99	98		91	90	82	83	83	83	87	63	61	61	61	56	60	52	33
PLV-18	88	88	88	87		91	84	81	81	79	84	61	60	60	60	56	61	50	33
PLV-8	88	88	88	88	87		80	81	82	80	86	62	61	61	60	57	61	53	34
PLV-64	76	76	75	75	75	76		93	78	77	79	64	57	57	58	55	63	47	33
PLV-80	79	79	79	79	75	78	86		80	78	79	61	58	60	59	56	60	46	33
FIV-PET	79	79	78	78	77	79	75	75		97	92	59	61	61	61	56	59	52	35
FIV-SD	79	79	79	78	77	79	74	75	96		90	58	59	61	60	56	59	51	35
FIV-MD	79	79	78	78	77	78	73	74	85	84		61	59	63	61	57	61	52	34
EIAV	70	70	69	70	67	70	64	66	66	67	68		54	58	59	55	58	54	35
Visna virus	69	69	68	68	66	68	64	64	65	64	64	61		57	57	57	56	50	35
HIV-1	65	65	65	65	64	65	62	63	66	65	65	60	64		94	72	74	52	30
SIV-CPZ	64	64	65	63	63	65	60	63	64	64	63	61	63	81		69	72	49	29
HIV-2	62	62	62	61	61	61	60	59	61	60	60	61	65	72	69		90	51	37
SIV-SM	64	64	63	63	62	63	61	63	63	63	62	61	63	70	69	83		50	37
BIV	63	63	62	62	61	62	58	61	59	58	59	65	59	56	56	57	58		35
BLV	43	43	43	43	43	43	41	41	45	44	42	47	42	40	39	46	42	45	

[a] Virus abbreviations: BLV, bovine leukemia virus; BIV, bovine immunodeficiency virus, EIAV, equine infectious anemia virus; FIV-SD (40) and FIV-PET (37), FIV isolates from San Diego and Petaluma, Calif., respectively.

[b] Percent amino acid (above diagonal) and nucleotide (below diagonal) sequence identities were determined by pairwise alignments of minimum overlaps of all feline lentivirus *pol* sequences shown in Fig. 2 and homologous nonfeline lentivirus *pol* sequences from GenBank. Geographic origins of feline virus isolates are indicated.

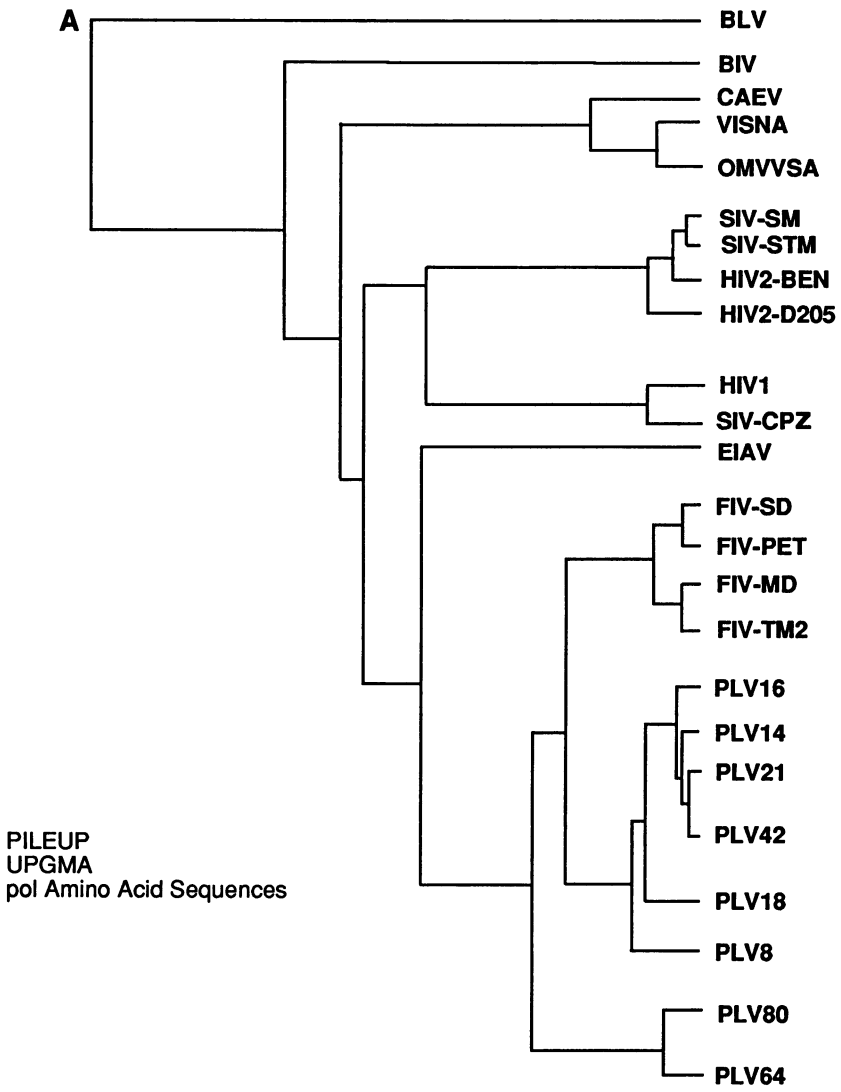


FIG. 3. Evolutionary trees developed from phenetic (A and B) and phylogenetic (C) analysis of *pol* gene sequences from the indicated lentiviruses. Nucleotide sequences were converted to translated amino acid sequences. Each full-length sequence was separately aligned with every other sequence by using the PILEUP program of the Genetics Computer Group software package (8), which uses the algorithm of Needleman and Wunsch (28). Distances are expressed on the basis of percent difference in amino acid sequence; gaps are given a weight of one residue difference (41). (A) UPGMA (Unpaired Group Method Analysis) tree derived by the PILEUP program (8, 44). This program employed a single alignment of all sequences. (B) Phenetic tree derived from amino acid sequence match frequency (Table 2) by using the Fitch-Margoliash algorithm (12), specifically, the KITSCH subroutine of the PHYLIP (Phylogenetic Inference Package) program, version 3.4 (11). This program computes a midpoint-rooted topology based on the least-squares method and the assumption of an evolutionary clock rendering all terminal species as contemporaneous. The numbered leg lengths are the number of amino acid substitution differences of an unrooted tree generated by the FITCH algorithm in the absence of these assumptions. The scale is based on the fraction of substitution differences between species sequences. (C) Phylogenetic tree derived by the PAUP (Phylogenetic Analysis Using Parsimony) program, version 2.4 (45). A strict consensus tree based on midpoint rooting is presented. Topologically equivalent trees were produced when BIV and BLV were designated as an outgroup for rooting. The scale and leg lengths are in amino acid substitutions (aa subs.). The tree shown has a length of 305 changes and an overall consistence index of 0.79, indicating a 21% convergence level. Virus abbreviations not introduced in the text are given in Table 2, footnote *a*, except for CAEV (caprine arthritis-encephalitis virus) and OMVVSA (ovine maedi-visna virus isolate SA).

between each *pol* region sequence is presented in Table 2.

The extent and character of sequence divergence (Fig. 2) allowed the construction of both phenetic (distance matrix-based) and phylogenetic (parsimony based on minimum length) evolutionary trees. We used three different phylogenetic methods to increase the reliability of the derived topologies, since tree-building algorithms depend on different assumptions. The derived trees and a description of the analytical methods are presented in Fig. 3 and the legend thereto.

The phylogenetic analysis of the PLV sequences (Table 2 and Fig. 3) revealed several important relationships between PLV, FIV, and other lentiviruses. First, in almost all cases the *pol* sequence-based trees were topologically equivalent,

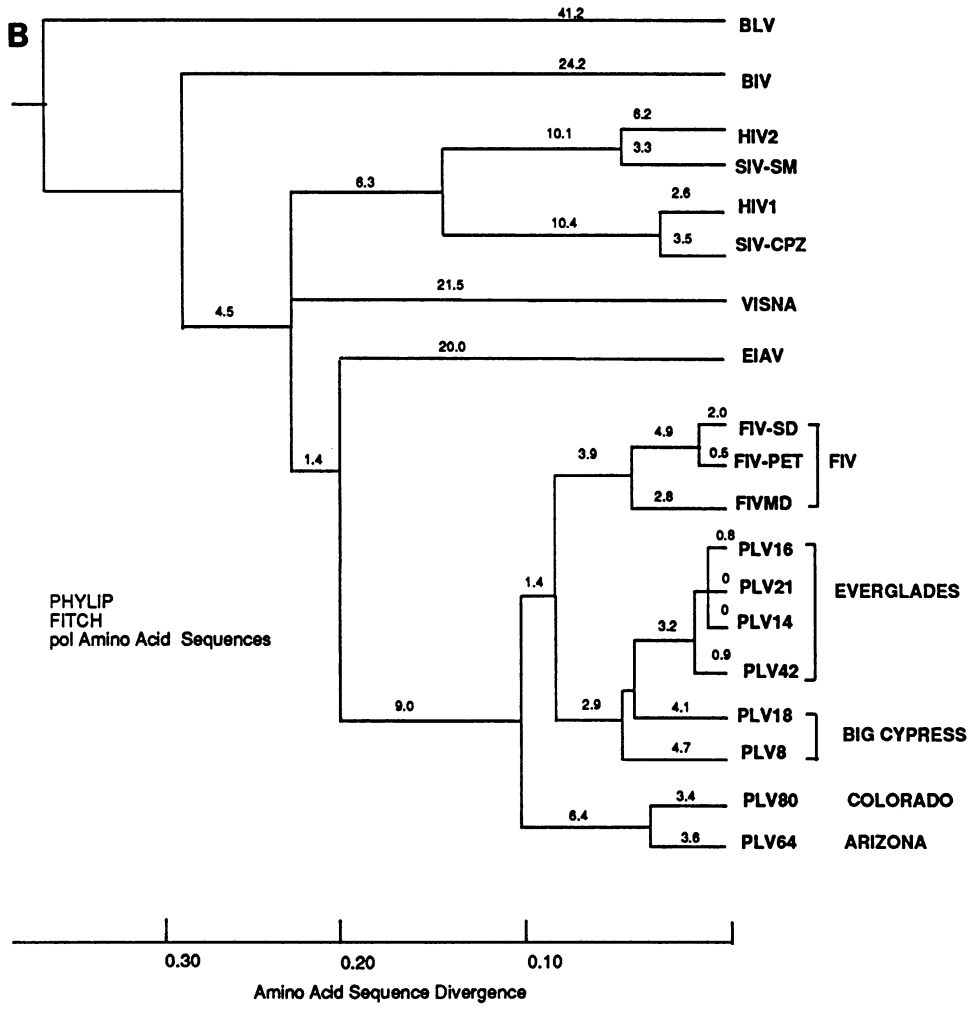


FIG. 3—*Continued.*

with the only uncertainty being the divergence positions of visna virus and equine infectious anemia virus relative to primate and feline lentiviruses. Second, the derived trees recapitulated the conclusions of previous phylogenetic studies of lentivirus evolution (7, 9, 16, 17, 20, 21, 26). Bovine leukemia virus and bovine immunodeficiency virus are more distant outgroups of four major evolutionary lineages, namely, the visna virus, equine infectious anemia virus, SIV-HIV, and feline lentivirus groups. Within the feline lentivirus group, the FIV isolates formed a monophyletic cluster, i.e., each FIV sequence was more closely related to other FIV sequences than they were to any other lentivirus type. As might be predicted from the immunological relatedness, the PLV isolates were more closely related to FIV than to other lentiviruses.

As a group, the PLV isolates assorted according to their geographic origins (Fig. 3A). Thus, the most similar isolates, PLV-14, PLV-16, PLV-21, and PLV-42 (98 to 100% amino acid sequence identity) were from a family group (mother PLV-14 and three offspring) living in Everglades National Park. PLV-8 and PLV-18, derived from animals that reside in the adjacent Big Cypress Swamp, showed 90 to 92% sequence identity to the Everglades group. Two PLV sequences from the western United States, PLV-64 and PLV-80, were as divergent from the Florida PLV sequences (80 to 84% identity) as any PLV isolates were from FIV (77 to 88% identity).

DISCUSSION

The serological survey and the genetic characterization of isolated PLV reported here demonstrate the widespread natural lentivirus prevalence in four genera of the cat family, Felidae. Feline lentiviruses appear to be endemic in lions of the Serengeti and Ngorongoro Crater in eastern Africa and in Kruger National Park in southern Africa, with infection rates surpassing the rates reported for SIV infection in African green monkeys (3, 7, 14, 15, 19, 21, 27). In contrast, free-ranging lions from Namibia (southwestern Africa) appear free of infection, at least by the limits of our sampling and assay criteria. Similarly, East African cheetahs were infected (22% incidence), while captive cheetahs in South Africa, despite being descended from cheetahs caught in the wild (Kruger National Park and Namibia) in the 1970s (32, 34), were negative. These observations suggest that FIV-like infection may be restricted geographically between African locales.

When the pattern of genomic variation among the feline

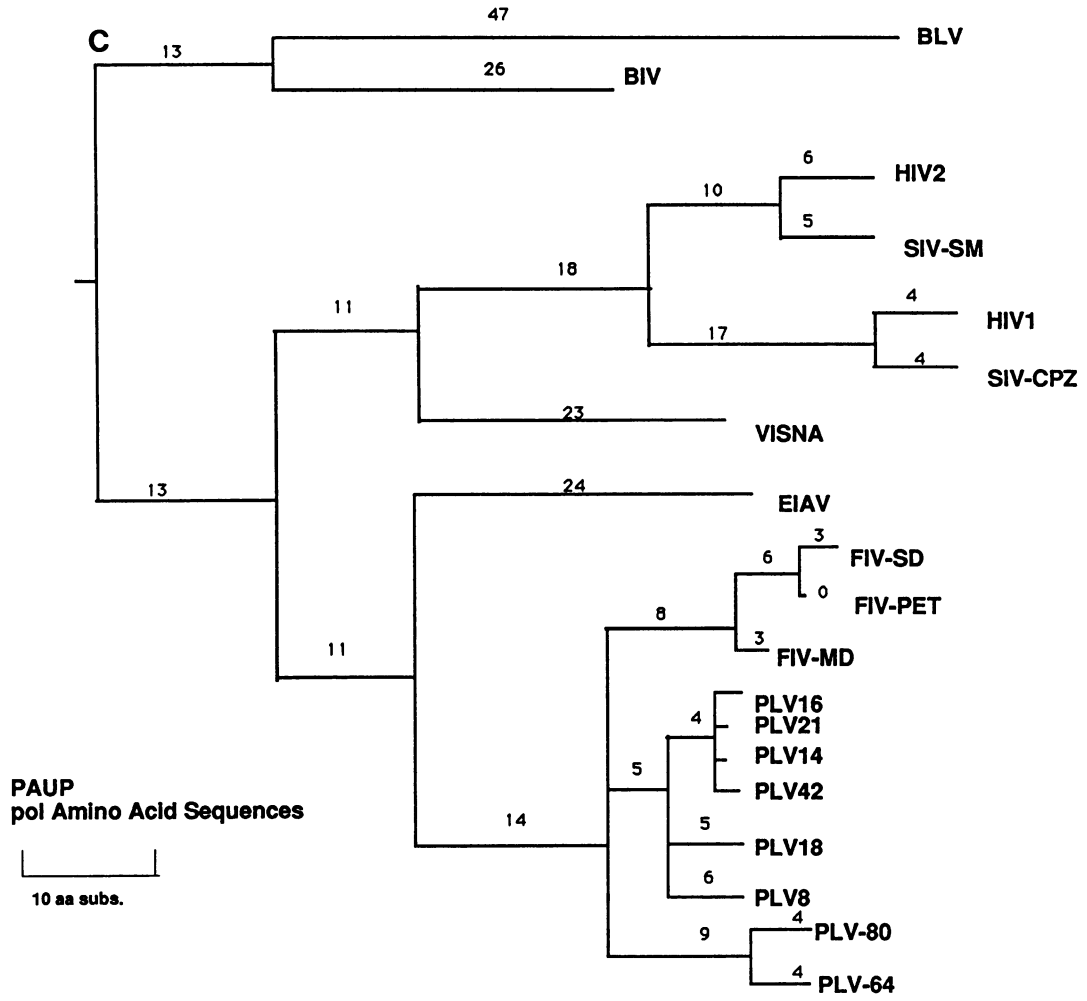


FIG. 3—*Continued.*

lentiviruses is compared with HIV-SIV divergence in primates, some important parallels become apparent. The closest simian virus to HIV-2 is SIV-SM, which was recently isolated from sooty mangabeys and is likely responsible for infection of Asian macaque species in U.S. primate research centers (15, 19, 27). The divergence between HIV-2 isolates and SIV-SM is greater than the amount of variation observed between most HIV-2 variants (19). The closest simian isolate to HIV-1 is SIV-CPZ, which was isolated from two chimpanzees at a primate facility in Gabon (20, 39). The genetic distance between HIV-1 isolates and SIV-CPZ is similarly outside the range of variation observed between most HIV-1 variants, making SIV-CPZ an attractive candidate for a recent ancestor of HIV-1. HIV-1 apparently diverged from HIV-2 much earlier than either split from the simian counterpart (Fig. 3), leading to speculation that HIV-1 and HIV-2 evolved separately in simian ancestors, probably species of the genus *Cercopithecus* (guenons and African green monkeys) (3, 10, 14, 21). Because *Cercopithecus* species display a relatively high degree of SIV sequence variation, their common simian ancestors were likely hosts of the primordial HIVs that have diverged into HIV-1 and HIV-2.

The divergence between PLV and FIV reported here (12 to 33% for amino acid sequences and 21 to 27% for nucleotide sequences [Table 2]) is greater than the divergence in the homologous *pol* region between HIV-2 and SIV-SM (10% amino acid sequence and 17% nucleotide sequence divergence) or between HIV-1 and SIV-CPZ (6% amino acid sequence and 19% nucleotide sequence divergence). Furthermore, the most extreme divergence observed between PLV isolates (25% nucleotide sequence difference [Table 2]) approaches the difference observed between HIV-1 and HIV-2 (28% nucleotide sequence difference). If these conserved *pol* sequences are changing at about the same rate in felids as in primates, it is likely that PLV and FIV are rather old felid viruses whose genomic divergence has proceeded primarily within separate species with interspecies exchange being rare. It is even conceivable that FIV and PLV have been isolated from each other since the species divergence estimated to have occurred 3 million to 6 million years ago (6, 23, 43, 46). Although it is not possible with available data to determine the direction in which an ancient FIV transfer between species occurred, the pattern of genetic divergence indicates that the FIV-PLV split occurred long ago and cannot be considered a frequent event.

To date, there have been no apparent immunological or pathological symptoms observed in infected free-ranging large cats. Since T-lymphocyte depletion has been observed

in FIV-infected domestic cats (2), it seems important to monitor certain infected free-living populations for possible disease or T-cell subset depletions by using newly available felid-specific monoclonal antibody reagents (1, 22, 42). Long-term clinical tracking may prove particularly informative in establishing either pathological symptoms or virus-to-host synergism that may have developed during the recent natural history of the populations. Ongoing field studies with lions, cheetahs, and pumas led by authors of this report (C.P., T.M.C., and M.E.R.) offer a rare opportunity to track virus and/or disease progression in a natural setting. The apparent asymptomatic character of SIV infection in African green monkeys may also parallel the feline situation, in which historic selective episodes may have led to genomic adaptations of both the virus and the host, leading to a modern symbiosis. The critical role of the dynamic balance between pathogen and host genomes in epidemics has been discussed in detail elsewhere (4, 18, 29). The natural history of feline lentivirus infection and disease processes in free-ranging felids would provide a new model system for empirically describing this still poorly understood natural process.

ACKNOWLEDGMENTS

We are grateful to R. Chanock, P. Johnson, V. Hirsch, R. Benveniste, D. Derse, E. Brown, and N. Yuhki for comments and to A. Burke for excellent assistance in phylogenetic analyses.

This work was supported in part by grants from the National Geographic Society and from the NOAHS Center, Smithsonian Institution, and by contract NO1-AI-72623 between the National Institutes of Health and Georgetown University.

REFERENCES

1. **Ackley, C. D., E. A. Hoover, and M. D. Cooper.** 1990. Identification of a CD4 homologue in the cat. Tissue Antigens **35**:92–98.
2. **Ackley, C. D., J. K. Yamamoto, N. Levy, N. C. Pedersen, and M. D. Cooper.** 1990. Immunologic abnormalities in pathogen-free cats experimentally infected with feline immunodeficiency virus. J. Virol. **64**:5652–5655.
3. **Allan, J. S., M. Short, M. E. Taylor, S. Su, V. M. Hirsch, P. R. Johnson, G. M. Shaw, and B. H. Hahn.** 1991. Species-specific diversity among simian immunodeficiency viruses from African green monkeys. J. Virol. **65**:2816–2828.
4. **Anderson, R. C., and R. M. May (ed.).** 1982. Population biology of infectious diseases. Springer-Verlag KG, Berlin.
5. **Barr, M. C., P. P. Calle, M. E. Roelke, and F. W. Scott.** 1989. Feline immunodeficiency virus infection in nondomestic felids. J. Zoo Wildl. Med. **20**:265–272.
6. **Collier, G. E., and S. J. O'Brien.** 1985. A molecular phylogeny of the Felidae: immunological distance. Evolution **39**:473–487.
7. **Desrosiers, R. C.** 1990. HIV-1 origins. A finger on the missing link. Nature (London) **345**:288–289.
8. **Devereux, J.** 1987. Sequence Analysis Software Package program manual, version 7.0. University of Wisconsin Genetics Computer Group, Madison.
9. **Doolittle, R. F., D. F. Feng, M. S. Johnson, and M. A. McClure.** 1989. Origins and evolutionary relationships of retroviruses. Q. Rev. Biol. **64**:1–30.
10. **Emau, P., H. M. McClure, M. Isahakia, J. G. Else, and P. N. Fultz.** 1991. Isolation from African Sykes' monkeys (*Cercopithecus mitis*) of a lentivirus related to human and simian immunodeficiency viruses. J. Virol. **65**:2135–2140.
11. **Felsenstein, J.** 1991. PHYLIP: Phylogenetic inference package, version 3.4. University of Washington, Seattle.
12. **Fitch, W. M., and E. Margoliash.** 1967. Construction of phylogenetic trees. Science **155**:279–284.
13. **Florida Panther Interagency Committee.** 1987. Florida panther (*Felis concolor coryi*) recovery plan. U.S. Fish and Wildlife Service, Atlanta.
14. **Fomsgaard, A., V. M. Hirsch, J. S. Allan, and P. R. Johnson.** 1991. A highly divergent proviral DNA clone of SIV from a distinct species of African green monkey. Virology **182**:397–402.
15. **Fultz, P. N., H. M. McClure, D. C. Anderson, R. B. Swenson, R. Anand, and A. Srinivasan.** 1986. Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (*Cercocebus atys*). Proc. Natl. Acad. Sci. USA **83**:5286–5290.
16. **Garvey, K. J., M. S. Oberste, J. E. Elser, M. J. Braun, and M. A. Gonda.** 1990. Nucleotide sequence and genome organization of biologically active proviruses of the bovine immunodeficiency-like virus. Virology **175**:391–409.
17. **Gojobori, T., E. N. Moriyama, Y. Ina, K. Ikeo, T. Miura, H. Tsujimoto, M. Hayami, and S. Yokoyama.** 1990. Evolutionary origin of human and simian immunodeficiency viruses. Proc. Natl. Acad. Sci. USA **87**:4108–4111.
18. **Haldane, J. B. S.** 1949. Disease and evolution. Ric. Sci. **19**(Suppl.):68–76.
19. **Hirsch, V. M., R. A. Olmsted, M. Murphey-Corb, R. H. Purcell, and P. R. Johnson.** 1989. An African primate lentivirus (SIVsm) closely related to HIV-2. Nature (London) **339**:389–392.
20. **Huet, T., R. Cheynier, A. Meyerhans, G. Roelants, and S. Wain-Hobson.** 1990. Genetic organization of a chimpanzee lentivirus related to HIV-1. Nature (London) **345**:356–359.
21. **Johnson, P. R., A. Fomsgaard, J. Allan, M. Gravell, W. T. London, R. A. Olmsted, and V. M. Hirsch.** 1990. Simian immunodeficiency viruses from African green monkeys display unusual genetic diversity. J. Virol. **64**:1086–1092.
22. **Klotz, F. W., and M. D. Cooper.** 1986. A feline thymocyte antigen defined by a monoclonal antibody (FT2) identifies a subpopulation of non-helper cells capable of specific cytotoxicity. J. Immunol. **136**:2510–2514.
23. **Kurten, B.** 1976. Fossil puma (Mammalia:Felidae) in North America. Neth. J. Zool. **26**:502–534.
24. **Letcher, J. D., and T. P. O'Connor.** 1991. Incidence of antibodies reacting to FIV in a population of Asian lions. J. Zoo Wildl. Med. **22**:324–329.
25. **Lowenstine, L. J., N. C. Pedersen, J. Higgins, K. C. Pallis, A. Uyeda, P. Marx, N. W. Lerche, R. J. Munn, and M. B. Gardner.** 1986. Seroepidemiologic survey of captive Old-World primates for antibodies to human and simian retroviruses and isolation of a lentivirus from sooty mangabeys (*Cercocebus atys*). Int. J. Cancer **38**:563–574.
26. **McClure, M. A., M. S. Johnson, D.-F. Feng, and R. F. Doolittle.** 1988. Sequence comparisons of retroviral proteins: relative rates of change and general phylogeny. Proc. Natl. Acad. Sci. USA **85**:2469–2473.
27. **Murphey-Corb, M., L. N. Martin, S. R. Rangan, G. B. Baskin, B. J. Gormus, R. H. Wolf, W. A. Andes, M. West, and R. C. Montelaro.** 1986. Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. Nature (London) **321**:435–437.
28. **Needleman, S. B., and C. D. Wunsch.** 1970. A general method applicable to the search for similarities in the amino acid sequence of two proteins. J. Mol. Biol. **48**:443–453.
29. **O'Brien, S. J., and J. F. Evermann.** 1988. Interactive influence of infectious disease and genetic diversity in natural populations. Trends Ecol. Evol. **3**:254–259.
30. **O'Brien, S. J., P. Joslin, G. L. Smith III, R. Wolfe, N. Schaffer, E. Heath, J. Ott-Joslin, P. P. Rawal, K. K. Bhattacherjee, and J. S. Martenson.** 1987. Evidence for African origins of founders of the Asiatic lion species survival plan. Zoo Biol. **6**:99–116.
31. **O'Brien, S. J., and E. Mayr.** 1991. Bureaucratic mischief: recognizing endangered species and subspecies. Science **251**: 1187–1188.
32. **O'Brien, S. J., M. E. Roelke, L. Marker, A. Newman, C. A. Winkler, D. Meltzer, L. Colly, J. F. Evermann, M. Bush, and D. E. Wildt.** 1985. Genetic basis for species vulnerability in the cheetah. Science **227**:1428–1434.
33. **O'Brien, S. J., M. E. Roelke, N. Yuhki, K. W. Richards, W. E. Johnson, W. L. Franklin, A. E. Anderson, O. L. Bass, Jr., R. C. Belden, and J. S. Martenson.** 1990. Genetic introgression within the Florida panther *Felis concolor coryi*. Natl. Geogr. Res. **6**:485–494.
34. **O'Brien, S. J., D. E. Wildt, D. Goldman, C. R. Merril, and M. Bush.** 1983. The cheetah is depauperate in genetic variation.

Science 221:459–462.
35. **Ohta, Y., T. Masuda, H. Tsujimoto, K. Ishikawa, T. Kodama, S. Morikawa, M. Nakai, S. Honjo, and M. Hayami.** 1988. Isolation of simian immunodeficiency virus from African green monkeys and seroepidemiologic survey of the virus in various non-human primates. Int. J. Cancer **41**:115–122.
36. **Olmsted, R. A., A. K. Barnes, J. K. Yamamoto, V. M. Hirsch, R. H. Purcell, and P. R. Johnson.** 1989. Molecular cloning of feline immunodeficiency virus. Proc. Natl. Acad. Sci. USA **86**:2448–2452.
37. **Olmsted, R. A., V. M. Hirsch, R. H. Purcell, and P. R. Johnson.** 1989. Nucleotide sequence analysis of feline immunodeficiency virus: genome organization and relationship to other lentiviruses. Proc. Natl. Acad. Sci. USA **86**:8088–8092.
38. **Pedersen, N. C., E. N. Ho, M. L. Brown, and J. K. Yamamoto.** 1987. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. Science **235**:790–793.
39. **Peeters, M., C. Honore, T. Huet, L. Bedjabaga, S. Ossari, P. Bussi, R. W. Cooper, and E. Delaporte.** 1989. Isolation and partial characterization of an HIV-related virus occurring naturally in chimpanzees in Gabon. AIDS **3**:625–630.
40. **Phillips, T. R., R. L. Talbott, C. Lamont, S. Muir, K. Lovelace, and J. H. Elder.** 1990. Comparison of two host cell range variants of feline immunodeficiency virus. J. Virol. **64**:4605–4613.
41. **Reitz, M. S., Jr., H. Z. Streicher, and R. C. Gallo.** 1991. Gallo's virus sequence. Nature (London) **351**:358.
42. **Rojko, J. L., G. J. Kociba, J. L. Abkowitz, K. L. Hamilton, W. D. Hardy, Jr., J. N. Ihle, and S. J. O'Brien.** 1988. Feline lymphomas: immunological and cytochemical characterization. Cancer Res. **49**:345–351.
43. **Seidensticker, J., and S. Lumpkin (ed.).** 1991. Great cats: majestic creatures of the wild. Weldon Owen Pty. Ltd., Sydney, Australia.
44. **Sneath, P. H. A., and R. R. Sokal.** 1973. Numerical taxonomy: the principles and practice of numerical classification. W. H. Freeman & Co., San Francisco.
45. **Swofford, D. L.** 1985. Phylogenetic analysis using parsimony (PAUP), version 2.4. Illinois Natural History Survey, Champaign.
46. **Wayne, R. K., R. E. Benveniste, D. N. Janczewski, and S. J. O'Brien.** 1989. Molecular and biochemical evolution of the Carnivora, p. 465–494. *In* J. L. Gittleman (ed.), Carnivore behavior, ecology and evolution. Cornell University Press, New York.
47. **Yamamoto, J. K., E. Sparger, E. W. Ho, P. R. Anderson, T. P. O'Connor, C. P. Mandell, L. Lowenstine, R. Munn, and N. C. Pederson.** 1988. Pathogenesis of experimentally induced feline immunodeficiency virus infection in cats. Am. J. Vet. Res. **8**:1246–1258.