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Frequent Transmission of Immunodeficiency Viruses among Bobcats and Pumas▽

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With the exception of human immunodeficiency virus (HIV), which emerged in humans after cross-species transmissions of simian immunodeficiency viruses from nonhuman primates, immunodeficiency viruses of the family *Lentiviridae* represent species-specific viruses that rarely cross species barriers to infect new hosts. Among the *Felidae*, numerous immunodeficiency-like lentiviruses have been documented, but only a few cross-species transmissions have been recorded, and these have not been perpetuated in the recipient species. Lentivirus seroprevalence was determined for 79 bobcats (*Lynx rufus*) and 31 pumas (*Puma concolor*) from well-defined populations in Southern California. Partial genomic sequences were subsequently obtained from 18 and 12 seropositive bobcats and pumas, respectively. Genotypes were analyzed for phylogenic relatedness and genotypic composition among the study set and archived feline lentivirus sequences. This investigation of feline immunodeficiency virus infection in bobcats and pumas of Southern California provides evidence that cross-species infection has occurred frequently among these animals. The data suggest that transmission has occurred in multiple locations and are most consistent with the spread of the virus from bobcats to pumas. Although the ultimate causes remain unknown, these transmission events may occur as a result of puma predation on bobcats, a situation similar to that which fostered transmission of HIV to humans, and likely represent the emergence of a lentivirus with relaxed barriers to cross-species transmission. This unusual observation provides a valuable opportunity to evaluate the ecological, behavioral, and molecular conditions that favor repeated transmissions and persistence of lentivirus between species.

Human immunodeficiency virus (HIV; order *Retroviridae*, family *Lentiviridae*) recently emerged after multiple independent zoonotic events involving transmission from nonhuman primates to humans (16). While HIV infection in humans has resulted in an epidemic of devastating proportions, the originating nonhuman primate lentiviruses (simian immunodeficiency viruses [SIVs]) are prevalent but apparently harmless to their natural hosts. Many felid species harbor lentivirus infections with a pattern of species specificity and phylogenetic divergence remarkably similar to that of primate lentivirus infections (49). The seroprevalence of these infections in natural settings ranges from nearly 100%, as, for example, among lion prides in Africa and pumas in Wyoming and Montana, to rare or absent among small wild felids of the neotropics and Asian lion prides (4, 8, 28, 46, 47). While no noticeable changes in survival rates or fecundity have been observed in populations with high seroprevalence (5, 17), individual animals in captive populations have been diagnosed with an immunodeficiency-like disease (7, 9; S. Kennedy-Stoskopf, L. H. Spelman, and M. Briggs, presented at the American Association of Zoo Veterinarians Annual Meeting, 1994), and others have suggested that feline immunodeficiency virus (FIV) infection of lions may have subclinical (32, 39) or even overt (http://www.lionaid.org/science/results.htm) effects on animal health.

Domestic cats (*Felis catus*) harbor a species-specific variant of feline lentivirus first isolated from a multicat household experiencing numerous fatalities associated with immune system dysfunction (31). Because of its many similarities in biology to HIV, this lentivirus was designated FIV. In addition to being more pathogenic than FIVs occurring in wild felids, domestic cat FIV (FIV*fca*) is significantly more genetically homogeneous than wild-cat FIVs (4, 8, 10, 47). Further, FIV*fca* replicates and accumulates mutations at a higher rate than puma immunodeficiency virus (FIV*pco*) isolated from pumas of the northern midwestern United States (4). The lower genetic diversity, increased replication rate, and clinical immunopathology associated with FIV*fca* infection suggest that FIV*fca*, like HIV, has emerged relatively recently and has not yet achieved a state of host-virus equilibrium (49). Conversely, the high viral divergence and relatively apathogenic infections in wild felids are consistent with ancient infections in which host-virus adaptation has occurred—again, a relationship similar to that of African monkey SIVs with their various hosts (49).

Although all FIV strains are more closely related to each other than to lentiviruses infecting other families, each of the

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eight species of nondomestic cats for which viral sequences are available harbors a unique FIV strain (47). Moreover, each species-specific FIV demonstrates substantial genetic divergence from other FIVs (8, 10, 46), even in the conserved *pol* region (47). Exceptions to the observation that all FIVs isolated from a single felid species form a monophyletic cluster consist of FIV*fca* infection of a captive puma in an Argentinian zoo (10), African lion (*Panthera leo*) FIV infection (FIV*ple*) of a captive tiger and snow leopard in Asian zoos (47), and FIV*fca* infection of a Tsushima leopard cat (27). These events represent the only cross-species transmissions noted among felids, none of which apparently was perpetuated in the new host species. Again, this is similar to the monophyly of SIV species; though transmission between primate species has been noted, interspecies transmission of lentiviruses is the exception rather than the rule (49).

The objectives of this study were to determine FIV prevalence in bobcats with home ranges in urban Southern California and to characterize the genetic characteristics of such viruses. In light of the initial results, which were unexpectedly suggestive of FIV*pco* infection in the study, we expanded our data set to include samples from contemporaneously captured Southern California pumas and one Florida bobcat sequence isolated in the 1990s but never reported in the literature. The purpose of including these samples was to more closely evaluate evidence of cross-species FIV transmissions between these two sympatric species. Further, we sought to assess the genetic signatures of recent cross-species transmissions by characterizing the genomic mutations and amino acid changes in FIV*pco* isolates from bobcats and pumas.

The incidence and phylogeny of FIV infection in bobcats and pumas in Southern California and Florida reported in this study depart significantly from the observation that lentivirus cross-species transmission is rare. Genetic evidence suggests that this shared FIV strain (FIVpcoA) has crossed species multiple times and can be perpetuated in both species. Further, the relationship between the geographic location of an individual and its FIV isolate is highly correlated for both bobcats and pumas in Southern California, underlining the possible utility of FIV sequences for tracking individual movements and contacts in urban areas, as has been shown previously for pumas in rural Western habitats (3). Although nucleotide analysis of viruses from study bobcats and pumas does not provide definitive evidence of the direction of transmission, the distribution of the two viruses in bobcats and pumas throughout North America suggests that bobcats may be the original host species for this unusual FIV strain that now infects both bobcats and pumas.

MATERIALS AND METHODS

Sample collection and processing. Blood samples were obtained from 79 bobcats and 31 pumas from three study sites in Southern California between autumn 2002 and spring 2005 (Table 1). Animals were captured live, and samples were collected in compliance with guidelines and protocols approved by the collection agency and its associated animal care committees. Blood products (whole blood, plasma samples processed by centrifugation at 10 min at 1,000 rpm after collection in EDTA-treated tubes, or serum and coagulated erythrocyte pellet) were used for subsequent immunoblotting and DNA extraction for provirus amplification and sequencing. Published sequences of FIVs from free-ranging Florida panthers (*Puma concolor coryi*) from both Big Cypress Swamp and Everglades National Park were included in phylogenetic analyses (10, 22, 28). In addition, Ray Langley provided an FIV sequence amplified from a bobcat held captive in Florida. Blood samples were collected from this bobcat by M. Roelke in 1984.

TABLE 1. FIV serology and PCR amplification

Study site	Species (no. tested)	No. (%) seropositive	No. (% of amplified isolates)[a]	
			FIV*pco*A	FIV*pco*B
VC	Bobcat (62)	17 (27)	8 (100)	0 (0)
	Puma (4)	4 (100)	1 (50)	1 (50)
OC	Bobcat (17)	10 (59)	9 (100)	0 (0)
	Puma (3)	1 (33)	1 (100)	0 (0)
SDRC	Puma (24)	11 (46)	3 (33)	6 (67)

[a] FIV isolates were not amplified and sequenced from all seropositive individuals.

Immunoblotting. All 110 individuals were screened for antibodies reactive to puma lentivirus antigen by immunoblotting, since we have found this to be the best available method for screening nondomestic cat sera for anti-FIV antibodies when species-specific tests are not available (15). Antigens were prepared from viral cultures of puma lentivirus (PLV-1695). Stocks were grown in the Mya-1 cell line, originating from a domestic cat (26), and viral proteins were isolated as previously described (43, 50).

Samples of serum, plasma, or whole blood were diluted 1:25 in phosphate buffered saline. Positive-control sera (cat sera from an experimentally FIV infected animal) were used at a 1:100 dilution. Negative cat sera were used as a negative control at a 1:25 dilution. Western blotting was performed as previously described (15). Reaction strength was assessed visually and given one of four scores depending on the affinity of the antibody for the $p24^{gag}$ protein: 0, negative; 1, equivocal; 2, positive; 3, very strongly positive. Statistical analyses of differences in bobcat seroprevalence rates between the Ventura County, CA (VC) and Orange County, CA (OC) populations were conducted using Fisher's exact test.

DNA extraction and PCR amplification. Genomic DNA was isolated from all individuals with positive or equivocal reactions and from several individuals with negative reactions as determined by immunoblotting. All coagulated erythrocytes and some EDTA–whole-blood samples were extracted using standard phenol-chloroform extraction (42). From some samples, DNA was recovered from 200 μl of EDTA–whole blood using a QIAamp DNA blood minikit (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions. DNA was analyzed by visualization following electrophoresis and ethidium bromide staining on a 1.2% SeaKem agarose gel.

Nested PCR was performed using 100 to 500 ng of genomic DNA with degenerate primers and reaction conditions described previously (47). Amplifications were performed on either a Perkin-Elmer 9700 system or on a Bio-Rad iCycler. Five or 10 μl of sample was electrophoresed on 1 or 1.2% SeaKem agarose gels at 100 V and inspected visually following ethidium bromide staining for the presence of a ~550-bp amplicon. Positive and negative controls were run concurrently. The resulting amplicons were purified by gel purification or high-speed centrifugation and were sequenced with BigDye according to the manufacturer's directions on an ABI 7000 sequencer (Macromolecular Services, Colorado State University).

Phylogenetic analysis. Nucleotide sequences were compiled and aligned in ClustalX (44) and verified visually. With the exception of Bayesian analysis, all phylogenetic reconstructions were performed using PAUP (phylogenetic analysis using parsimony, version 4.0; Sinauer Associates, Sunderland, MA) and included minimum evolution estimated by neighbor joining (ME), maximum parsimony (MP), and maximum likelihood (ML) (40). ModelTest (33) was used to estimate the optimal model of sequence evolution, obtained by the general time-reversible model of substitution corrected for among-site rate variation (gamma distribution) and a proportion of invariant sites. ME and ML trees were constructed using this model [Lset Base = (0.4677 0.1058 0.1160); Nst = 6; Rmat = (2.8733 12.0716 0.8769 4.8937 16.8913); Rates = gamma Shape = 0.7495; Pinvar = 0.3419], with starting trees obtained by neighbor joining, followed by a tree bisection-reconnection branch-swapping algorithm during a heuristic search for the optimal tree. MP analysis employed a heuristic search of starting trees obtained by stepwise addition, followed by tree bisection-reconnection. Bootstrap analysis included 1,000 iterations for ME and MP and 100 iterations for

ML. Bayesian posterior probabilities were calculated using a Markov chain Monte Carlo sampling approach in MrBayes, version 3.0b4 (18). Starting trees were random, four simultaneous Markov chains were run for 1 million generations, and burn-in values were set at 45,000 generations based on stabilization of likelihood values and convergence of the two runs. Trees were sampled every 20 generations.

Analysis of selection. The ratio of nonsynonymous to synonymous substitutions (K_a/K_s) was calculated by Sequencer, version 6.1.0 (B. Kessing, personal communication), using the algorithm of Li (24) and Pamilo and Bianchi (29). G-to-A mutations were calculated manually in two ways. First, sequences were aligned, and sites with a consensus G were identified. These sites were interrogated for conversion to A in any individual or in multiple individuals from each species. Second, codons for glutamic acid, glutamine, and lysine (twofold degenerate codons containing either G or A at the third position) were identified in MacClade (Sinauer Associates, Inc.), and the frequency of A and G at third-position sites was determined and compared for each sequence.

The influence of selection on these sequences was investigated using the codeml program implemented in PAML, version 3 · 15 (51). A range of codon models for measuring selection were evaluated, and nested models were compared by the likelihood ratio test statistic. To investigate different selective pressures along specific lineages, a model that allows only a single ω (K_a/K_s) for all branches in the tree (M0) was tested against a two-ratio model that allows an additional ω for specific branches in the tree. Because FIV*pco* clade A (FIV*pco*A) sequences from bobcats and puma were present in the same branch of the tree, these analyses were performed individually for each species, with the FIV*pco* clade B (FIV*pco*B) branch remaining constant. Site models that allow for heterogeneous K_a/K_s ratios among sites (models M1, M2, M3, M7, and M8) were used to test for diversifying selection at individual sites (52). The likelihood ratio test was used to evaluate whether allowing sites to have >1 ratio significantly improved the fit of the model to the data (comparing M1 with M2, M0 with M3, and M7 with M8, where M2, M3, and M7 can accommodate positively selected sites). Fisher's exact test was used to compare the percentage of consensus G sites that had A mutations in bobcats versus pumas.

Nucleotide sequence accession numbers. All novel FIV sequences obtained for this study have been deposited in GenBank under accession numbers EF601127 to EF601156. These include sequences from the following animals (where Pco or Lru at the beginning of the identification number stands for puma or bobcat, respectively): Pco-F29 (EF601127), Pco-F19 (EF601128), Pco-F15 (EF601129), Pco-SM1 (EF601130), Pco-F8 (EF601131), Pco-M9 (EF601132), Pco-F14 (EF601133), Pco-F07 (EF601134), Pco-F20 (EF601135), Pco-SM4 (EF601136), FLbobcat (EF601137), Lru-8 (EF601138), Pco-N1R (EF601139), Lru-12 (EF601140), Lru-5 (EF601141), Lru-11 (EF601142), Lru-7 (EF601143), Lru-10 (EF601144), Pco-M17 (EF601145), Lru-13 (EF601146), Lru-SM114 (EF601147), Lru-16 (EF601148), Lru-6 (EF601149), Lru-SM91 (EF601150), Lru-SM93 (EF601151), Lru-SM83 (EF601152), Lru-SM49 (EF601153), Lru-SM117 (EF601154), Lru-SM59 (EF601155), and Lru-SM142 (EF601156). Other sequences included in the tree have the following accession numbers: U53729 (Pco-245), U53726 (Pco-161), U53738 (Pco-332), U53718 (Pco-117), U53742 (Pco-333), AY120832 (Pco-621), U53742 (Pco-333), AY120826 (Pco-608), U53750 (Pco-549), U53735 (Pco-323), U53756 (Pco-28), U53751 (Pco-590), U53755 (Pco-733), U53723 (Pco-145), M95474 (Pco-80), U53719 (Pco-141), U53752 (Pco-593), U53722 (Pco-144), M95473 (Pco-64), U53730 (Pco-247), U53727 (Pco-163), U53725 (Pco-145), DQ308421 (PLV1695), U53747 (Pco-346), U53736 (Pco-328), AY120843 (Pco-616), AY120837 (Pco-630), DQ308420 (PLVCoLV), AY878236 (Pco-696), U53744 (Pco-336), U53734 (Pco-253), M95470 (Pco-182), U03982 (Pco-61), M95478 (Pco-75), U53748 (Pco-366), M95475 (Pco-12), M95476 (Pco-68), M95477 (Pco-65), M95472 (FIVMD), U11820 (FIV-Fca), and M25381 (FIVpetaluma). In addition, sequences from several domestic-cat FIV isolates were used to assess the G-to-A mutation rate in these viruses. The following sequences obtained from GenBank were used: U53764, AF051802, AF051801, U53762, U53760, U53761, L16938, M59418, M36968, U11820, X57002, AY600517, L16944, S67753, M25381, and U53766.

RESULTS

Samples were obtained from two genetically and geographically isolated bobcat populations in OC ($n = 17$) and VC ($n = 62$), under study to evaluate the effects of urban development and habitat fragmentation on individual movement and gene flow among populations (12, 36, 37, 41, 45). Pumas were sampled from VC ($n = 4$), OC ($n = 3$), and an area encompassing San Diego and Riverside Counties (SDRC) ($n = 24$). Puma ranges overlapped those of study bobcats in the OC and VC study areas; the SDRC pumas were also sympatric with bobcats, and while their ranges did not overlap those of study bobcats in OC, they were proximal (Fig. 1a and b).

Forty-three (39%) of the 110 study animals were determined to be seropositive for anti-FIV antibodies (Table 1). Of particular interest was the geographic discordance in FIV prevalence rates among bobcats: 59% of the 17 OC bobcats were seropositive versus 27% of the 62 VC bobcats ($P = 0.02$). Approximately 50% of the 31 pumas tested (at least 1 animal/population) were FIV seropositive.

Sequencing of the reverse transcriptase portion of *pol* from nine OC and eight VC bobcats revealed two highly supported monophyletic groups from these geographically distinct populations (Fig. 1 and 2). When placed in the context of FIV sequences from other species, the FIVs isolated from bobcats were most closely related to FIV*pco*A, previously found only in Florida panthers (Pco-12, Pco-182, Pco-61, Pco-65, Pco-68, and Pco-75 [Fig. 1a and 2]) and a single puma from Southern California sampled in 1991 (Pco-366 [Fig. 2]) (10, 28). Interestingly, the home range of Pco-366 overlapped the home ranges of the OC study bobcats (10). Evaluation of 12 contemporaneously obtained sequences from Southern California pumas (2 from VC, 1 from OC, and 9 from SDRC) showed that 5 harbored FIV*pco*A (Pco-F07, -M17, and -F20 from SDRC, Pco-1 from OC, and Pco-4 from VC [Fig. 1 and 2]), although 7 were infected with FIV*pco*B (Pco-F29, -F15, -M9, -F8, -F14, and -F19 from SDRC and Pco-1 from VC), the predominant FIV isolated from pumas over their entire geographic range (Fig. 1a). Examination of a *pol* sequence obtained from a Florida bobcat captured in 1996 (R. Langley, presented at the Third International Feline Retrovirus Research Symposium, Fort Collins, CO, 1996) revealed that this sequence also clustered with FIV*pco*A and was most closely related to sequences obtained from Florida panthers (Pco-68, Pco-61, and Pco-182 [Fig. 2]) (10, 28).

Within the cluster of FIV*pco*A isolates, *pol* sequences from a single geographic location were more similar to one another than to viruses from a different location, regardless of whether they were isolated from bobcats or pumas (Fig. 1 and 2). Specifically, Florida bobcat *pol* was more closely related to viruses infecting Florida panthers than to viral *pol* from California bobcats. Likewise, the FIV*pco*A sequence from a VC puma north of Los Angeles (Pco-4) was more closely related to VC bobcat FIV (Lru-93, -59, -91, -114, -117, -83, -49, and -142) than to viruses infecting pumas captured south of Los Angeles (OC or SDRC) or pumas from Florida (Fig. 2). Consistent with these observations, FIV*pco*A isolates from pumas in OC and SDRC (Pco-1 from OC and Pco-F07, -M17, and -F20 from SDRC) were more similar to FIV*pco*A isolates infecting sympatric or nearby OC bobcats than to viruses from VC bobcats.

While all of the bobcats in this study were infected with FIV*pco*A, seven study pumas harbored viruses sharing the greatest sequence homology with previously described FIV*pco*B strains. Six of the pumas residing south of Los Angeles had fairly homogeneous FIV*pco*B sequences (Pco-F29, -F15, -M9, -F8, -F14, and -F19 from SDRC; distance, 0.2 to 2.8 nucleotide differences). The single study puma captured north of Los Angeles with FIV*pco*B *pol* (Pco-1 from VC) had a unique viral sequence that was highly divergent from those of

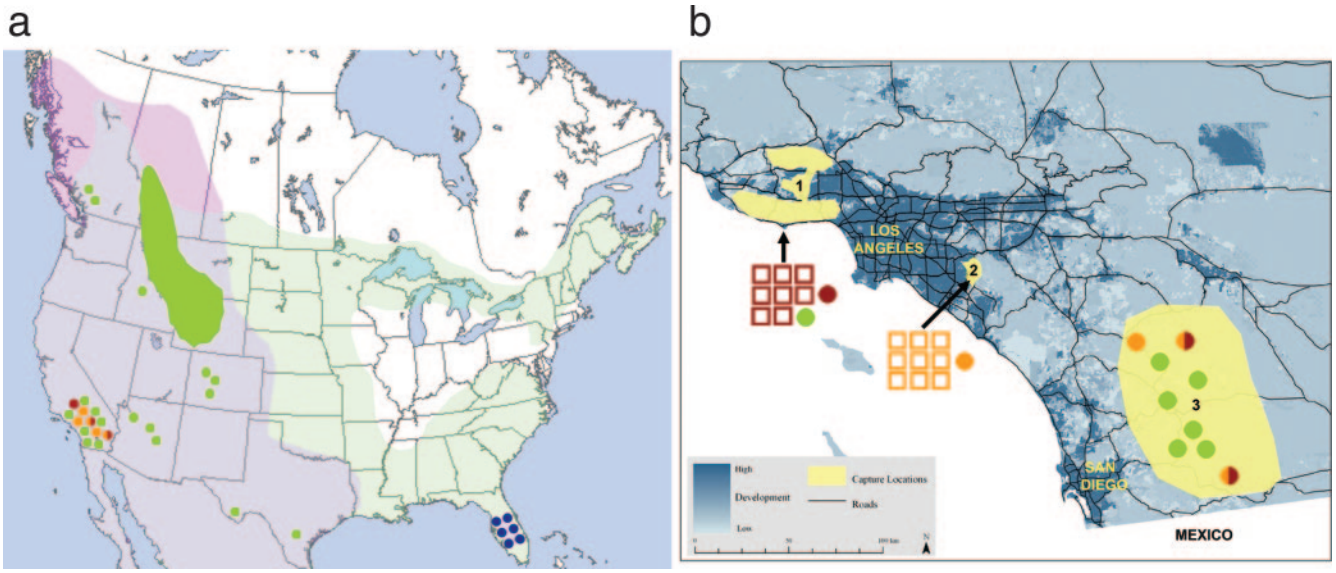

FIG. 1. (a) Map of distribution of FIV*pco*A and FIV*pco*B among pumas in North America. Orange, red, and blue circles represent pumas with FIV*pco*A/FIV*lru* from different populations (OC and SDRC, VC, and Florida panthers, respectively); green circles represent individuals with FIV*pco*B. Locations are approximate. The large green region represents >100 individuals from whom FIV*pco*B isolates were identified (see the text for references). Viruses from Southern California bobcats are shown in an enlarged map (see panel b). Pink and green indicate the geographic ranges of pumas and bobcats, respectively, with areas of overlap in light gray (19). (b) Frequent cross-species FIV transmission between bobcats and pumas in Southern California. Pumas are represented by circles as in panel a, while bobcats are represented by open squares. Circles show approximate locations of pumas in the SDRC capture area only. The colors of the symbols match the phylogram branch on which each sequence is mapped. Green, FIV*pco*B; orange and red, FIV*pco*A/FIV*lru*. Half-orange, half-red circles represent FIV*pco*A/FIV*lru* sequences that cannot be specifically localized to the OC versus VC monophyletic group; see the corresponding phylogram in Fig. 2. The degree of urban development as determined by GIS land use layers is indicated by the blue gradient as shown in the key. Yellow areas represent the three capture locations (1, VC; 2, OC; 3, SDRC).

the SDRC puma viruses (distance, 15.1 to 17.8). The FIV*pco*B isolate found in the puma north of Los Angeles (Pco-4 from VC) is most similar to that found in pumas from the Pacific Northwest (Pco-253 and Pco-1695 from British Columbia), while the FIV*pco*B isolates from pumas south of Los Angeles group with FIV*pco*B isolates found in pumas captured in northern Mexico (Pco-590) and Colorado (Pco-145) and in multiple pumas from Wyoming and British Columbia, with weak bootstrap support (Fig. 2).

Evaluation of amino acid sequences validated the nucleotide analysis. All bobcat FIV isolates and five puma FIV isolates aligned most closely with FIV*pco*A; the remaining seven puma isolates aligned with FIV*pco*B (Fig. 3). There were seven fixed amino acid substitutions between FIV*pco*A and FIV*pco*B. While there were no fixed differences between FIV*pco*A isolates recovered from puma and bobcats, there were substitutions that reflected the geographic isolation of the host. Specifically, the VC puma with FIV*pco*A (Pco-4) had one fixed residue also found in VC bobcats that was not present in bobcats or pumas south of Los Angeles (E in the position of amino acid 243 [Fig. 3]).

In an effort to determine the directionality of cross-species transmission, we searched for evidence of selection in FIV*pco*A strains infecting either bobcats or pumas by a variety of methods. Comparison of nonsynonymous to synonymous nucleotide changes (K_a/K_s ratios) supported a pattern of isolation by geography rather than by species (data not shown). Analysis in PAML (51) indicated that while some residues may be under selection, there was no evidence of differential selection in bobcats versus pumas; both were under strong purifying selection ($\omega = 0.15$ and 0.11, respectively).

Finally, the ratio of G-to-A mutation in puma versus bobcat viral sequences was examined for evidence of in vivo antiviral cytosine deaminase (APOBEC3)-like activity. Interrogation of base composition at twofold degenerate sites containing a G or an A in the third position (the codons for glutamic acid, glutamine, and lysine) demonstrated that the average bias toward an A at these sites was 5-fold for bobcats and 4.3-fold for pumas (Table 2). These biases reflect an overall AT content of 68%. Consensus G sites were also examined for transitions to A in individual animals. There were averages of 2.8 and 2.5 transitions/animal for bobcats and pumas, respectively (Table 2). These transitions occurred at 14% of possible sites in bobcats and at 15% of possible sites in pumas ($P = 0.83$) (Table 2). The bobcat FIV*pco*A isolated from VC Lru-93 had an 11.5-fold increase in the A/G ratio at these degenerate sites and contained A at 13 consensus G sites, 7 of which were different from all other bobcat FIV*pco*A sequences; therefore, it was excluded from these analyses.

DISCUSSION

The phylogenetic analyses described in this report and by others (10, 47) demonstrate that two different clades of lentivirus infect pumas in Southern California, based on a ~500-bp segment of *pol*. FIV*pco*B infects 7 of the 12 pumas from Southern California described here, in addition to >100 pumas throughout ranges from Canada to South America (3–6, 10,

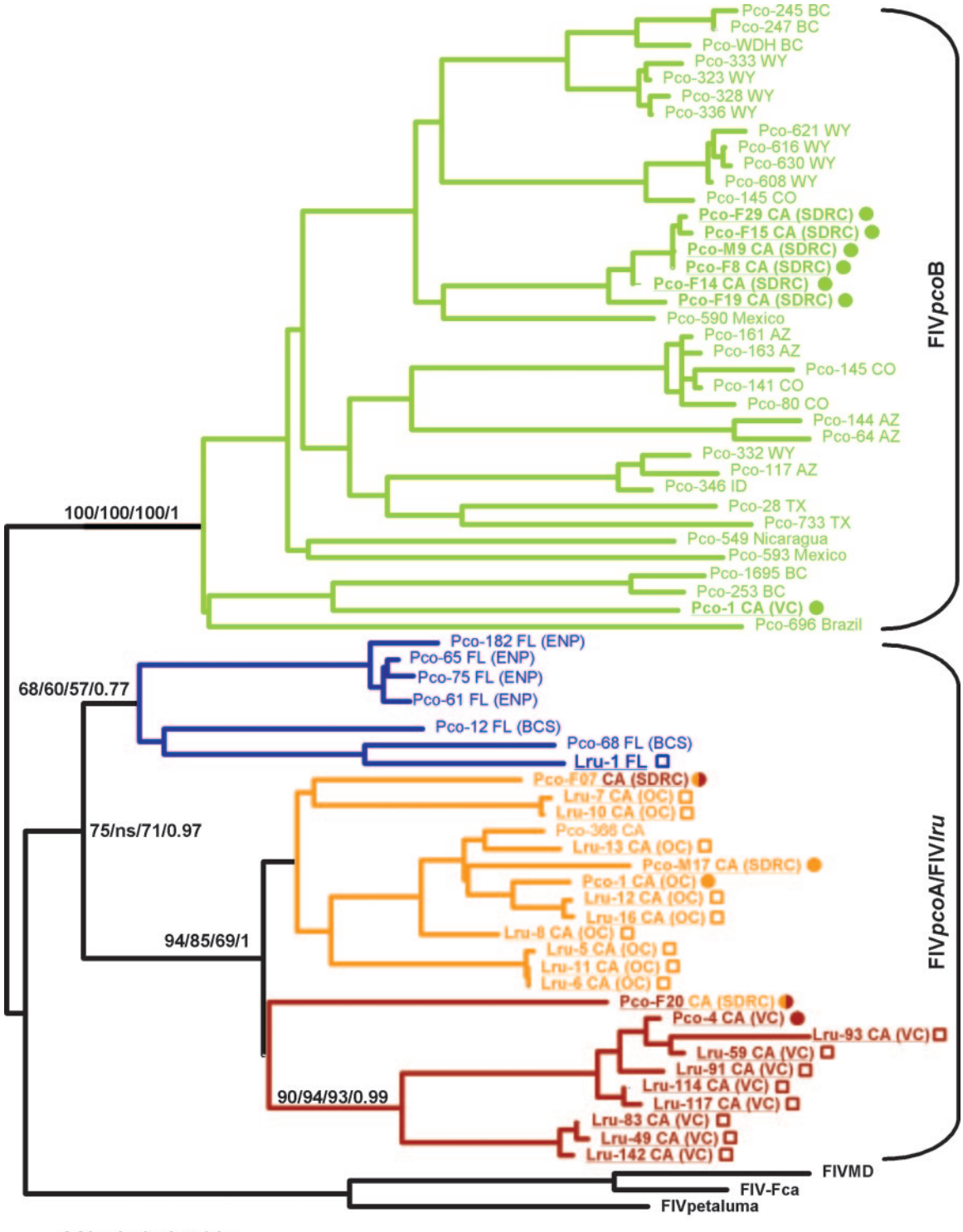

FIG. 2. ML tree demonstrating the relationship between previously and newly characterized FIV sequences from pumas and bobcats. ME, MP, and Bayesian analyses produced trees with similar topologies. Bootstrap values and posterior probabilities are included for nodes discussed in the text (ML/MP/ME/Bayesian). Two primary puma FIV clades are represented: FIV*pco*B (green branches) and FIV*pco*A/FIV*lru* (blue, orange, and red branches, each color indicating a different geographic locale, as explained in the legend to Fig. 1). The half-orange, half-red circles represent two study pumas with FIV*pco*A/FIV*lru* that cannot be specifically placed in the OC versus VC monophyletic group. Domestic-cat FIV (FIV*fca*) (black branches) is clearly a distinct strain not identified in pumas or bobcats. Inclusion of additional taxa (FIVs from other felids) or alternative rooting did not alter the relationship of these viruses to each other (data not shown). Each individual is represented by the species, the identification number, and the location, in that order. Locations consist of a country name or a state/province abbreviation (BC, British Columbia), followed by a specific site in parentheses if available (OC, VC, SDRC, ENP [Everglades National Park], or BCS [Big Cypress Swamp]). The map in Fig. 1b shows the geographic locations of the populations sampled in California. Underlined, boldfaced taxa are unique to this study; all other sequences were obtained from GenBank.

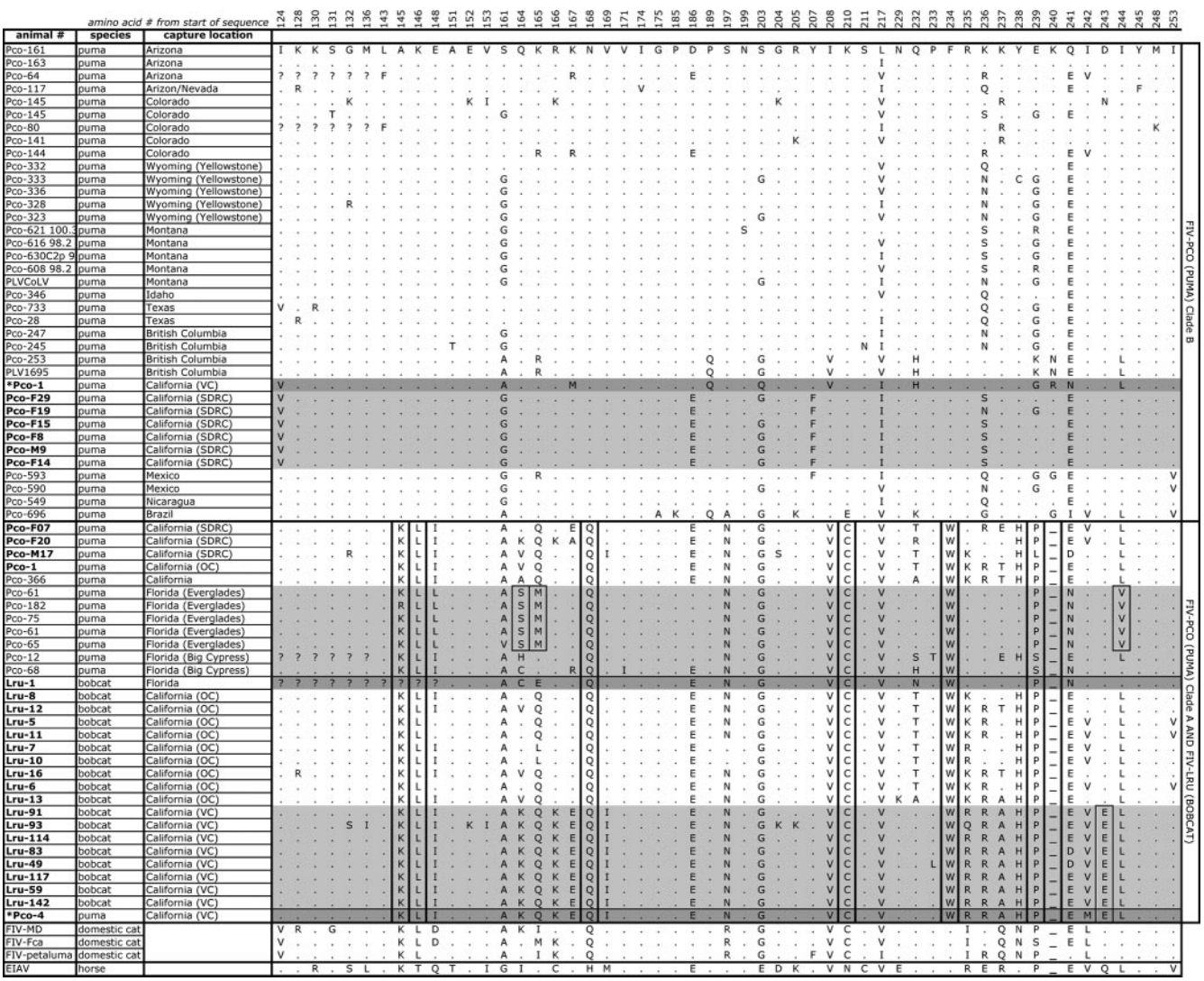

FIG. 3. Alignments of the variable sites from predicted amino acid translation products of FIV*pco*A/FIV*lru* and FIV*pco*B. Single-letter amino acid codes are used. Dots indicate identity with the index sequence; dashes represent gaps introduced to optimize the alignment; question marks indicate missing data. Boldfaced animal identification numbers indicate sequences unique to this study; other sequences were obtained from GenBank. Dark lines separate FIV clades, while light lines are introduced to delineate species differences within a clade. Boxes highlight fixed differences. Light shading sets off specific geographic locations. Dark shading highlights animals of particular interest within these areas: Pco-1 from VC, whose FIV*pco*B sequence is considerably different from other Californian FIV*pco*B sequences; Lru-1, a bobcat from Florida whose FIV*pco*A sequence is more similar to those found in Florida panthers than to those found in other bobcats; and Pco-4 from VC, whose FIV*pco*A sequence groups most closely with those of VC bobcats.

TABLE 2. G-to-A mutations in FIV*pco*A and FIV*pco*B isolates from bobcats and pumas

Virus	Host (*n*)	No. of G-to-A sites (% of consensus G sites)	No. of G-to-A transitions (avg no./ individual animal)	Avg A/G ratio at the 3rd position[a] (range)
FIV*pco*A	Bobcat (18)	18 (22)	61 (3.4)	5.5 (2.9–11.5)
	Bobcat (17)[b]	11 (14)	48 (2.8)	5.0 (2.9–7.8)
	Puma (13)	13 (15)	32 (2.5)	4.3 (2.3–7.8)
FIV*pco*B	Puma (37)	18 (20)	54 (1.5)	4.5 (1.9–6.3)
FIV*fca*	Domestic cat (16)	18 (21)	46 (2.5)	3.2 (1.9–4.2)

[a] Only the twofold degenerate codons for Gln, Lys, and Glu were used.
[b] VC *Lru*-93 was excluded from these analyses.

47). Conversely, FIV*pco*A infects only a small number of pumas located in Southern California and Florida, including five Southern California pumas from this study. Interestingly, lentivirus sequences from 17 bobcats in Southern California and 1 bobcat from Florida were genetically homogeneous with FIV*pco*A in *pol*. FIV*pco*A isolates from pumas in VC, north of Los Angeles, and OC, south of Los Angeles, fall within monophyletic clusters of viruses isolated from bobcats in the same geographic area, while sequences from SDRC pumas were most similar to viruses of the bobcat samples from the most proximal geographic region (OC bobcats). Consistent with this pattern, FIV*pco*A isolates from Florida panthers were most closely related to the Florida bobcat isolate. This geographic rather than species-specific relatedness suggests that cross-species

transmission of FIV*pco*A has taken place in at least three, and probably four, locations (VC, OC, Florida, and possibly SDRC) and that FIV*pco*A has propagated and spread among individuals in both host species. The latter point is best illustrated by the closely related FIV*pco*A sequences found in Florida panthers from the Everglades and in both the VC and OC bobcat populations. Despite endemic lentivirus infection of at least 8 wild species of *Felidae* and 41 African primate species, multiple cross-species transmissions with persistence in the new host to the degree observed here are without precedent in studies conducted to date.

Several pieces of evidence lead us to believe that FIV*pco*A, originally identified as a heterogeneous variant of FIV isolated from pumas in California and Florida, may actually be a bobcat-origin FIV that has been transmitted to pumas in the aforementioned locations. First, in the VC cluster, the FIV*pco*A sequence from one puma clearly clusters within the relatively homogeneous FIV*pco*A sequences found in VC bobcats. Similarly, viral sequences from OC bobcats are basal to the FIV*pco*A sequence from a puma captured at the OC study site. Second, FIV*pco*A and -B do not form a single monophyletic group within the FIV phylogeny, suggesting multiple introductions of FIV in pumas. Third, the acquisition of FIV*pco*A from bobcats in two distinct geographic regions provides a simple explanation for the disjunct distribution of FIV*pco*A in pumas, an otherwise puzzling occurrence given the long dispersal distances, large home ranges, and relative continuity of puma habitat that has allowed for the spread of FIV*pco*B across the Americas. The absence of FIV*pco*B in Florida panthers is puzzling but may be related to the relative, though recent (50 to 100 years), isolation of this population from contiguous puma habitat. It is possible that the transmission of a newly introduced FIV*pco*A strain from puma to puma may have been facilitated among the Florida panthers by the limited ability of this highly inbred population to resist infection (30, 38). While two SDRC pumas (Pco-F07 and -F20) harbored FIV*pco*A isolates that did not align closely with bobcat sequences, we hypothesize that bobcats in the SDRC region harbor an "SDRC bobcat substrain" of FIV*pco*A that would map with the puma isolates from this region.

The behavior of these carnivore species, coupled with the presumed mode of FIV transmission, also supports the supposition of unidirectional transmission from bobcats to pumas. FIVs are labile enveloped viruses that do not persist in the environment and are transmitted primarily by biting and blood-to-blood contact (2, 4, 10, 25, 47). Based on observations of FIV*fca*-infected domestic cats, prolonged direct contact is needed between individuals in order for disease transmission to occur (14). Pumas are typically 5 times larger than bobcats (13, 23), so individual conflicts between pumas and bobcats would likely result in lethal injury to the bobcat while providing an opportunity for virus transmission to the puma. Pumas have been noted to kill bobcats in antagonistic encounters and have also been documented to consume bobcats as prey, a behavior that would enhance the likelihood of FIV exposure (13, 20, 21). We have observed interaction between these two species, primarily at cached prey sites or with carrion, including predation of bobcats by study pumas, supporting this premise (W. M. Boyce et al., unpublished data).

Although bobcats have not been surveyed for FIV seroprevalence as extensively as pumas, previous reports indicate that the cohorts from Southern California evaluated in this study have a higher seroprevalence of FIV than other bobcat populations (1, 28, 35). Since bobcats have smaller home ranges and disperse shorter distances than puma, it is possible that FIV has been more contained or localized in this species than in the puma. It is also possible that restricted habitat in the fragmented landscapes of Los Angeles and South Florida has resulted in higher rates of intra- and interspecies disease transmission. Increased home range "pile-ups" occur at the interface between urban areas and wildland, resulting in greater overlap of home ranges, higher local densities and contact rates, and intensified resource and interference competition (36). These factors may contribute to restricted incidence of FIV*pco*A transmission from bobcats to puma despite the highly overlapping distribution of these two species across much of the United States and Canada (Fig. 1a).

Given our hypothesis that the results we report represent recent lentivirus cross-species transmission events, it is interesting that FIV*pco*A lacks many of the standard genetic signatures of selection predicted during early host-virus adaptation. For example, when a virus enters a new species, there is often a period of rapid adaptation influenced by the host "environment" that is reflected by an increase in the ratio of nonsynonymous to synonymous changes in nucleotide sequences (48). No evidence of such selection pressure was detected in FIV*pco*A sequences from either bobcats or pumas. While *pol* is relatively conserved in lentiviruses from different species, it is a region of the FIV genome under positive selection in natural settings (J. Slattery, personal communication) and in an experimental model of FIV*pco*B cross-species transmission from pumas to laboratory cats (34). In the latter study, FIV*pco*B isolated from experimentally infected domestic cats also contained a significant bias toward G-to-A mutations, substitutions that effectively increase the number of stop codons in the viral nucleic acid sequence, resulting in defective viral progeny genomes. This finding is consistent with viral restriction by host cytosine deaminase, a newly described innate immune mechanism resulting in a bias in G-to-A substitutions during RNA polymerization within a nonadapted host (11). Although we observed an increased rate of G-to-A mutations in FIV*pco*A- versus FIV*pco*B-infected animals, we did not observe a difference in the mutation rate between bobcats and pumas (Table 2).

The absence of genetic markers of recent viral emergence suggests that FIV*pcoA* has experienced a relaxation of barriers to lentivirus cross-species transmission that has resulted in a broader host tropism of this particular lentivirus. Therefore, our observations could also reflect a more ancient host expansion followed by ongoing cross-species transmissions in one or both directions. This premise is indirectly supported by the absence of domestic cat FIV (FIV*fca*) transmission to either bobcats or pumas. The proximity of our study populations to urban areas increases the probability that these pumas and bobcats encounter and consume feral domestic cats (13, 23), populations known to be at risk for infection with FIV*fca* (25). Therefore, we would predict that bobcats and pumas in this study would encounter and consume FIV*fca*-infected domestic cats with at least equal frequency to encounters with FIV*pcoA* cohorts; however, we did not observe FIV*fca* infection in any bobcats or pumas in this study. The nature of the apparent

"promiscuity" of FIV*pcoA* could be ascribed to a number of factors: the circulating viral load, the molecular structure of viral proteins facilitating viral entry and propagation, and the mode of transmission within two different species. Identification of the underlying mechanism for this phenomenon would provide important precedents for features of lentivirus infections that lead to the relaxation of species-specific infections and the emergence of new viral lineages. These findings are relevant in considering that HIV types 1 and 2 arose from inadvertent exposure between humans and nonhuman primates infected with SIV. While the paucity of examples of successful transmission of lentiviruses between species would suggest that such occurrences are rare (49), our data suggest that certain strains of lentiviruses that more easily defy species barriers exist. Determining the mechanisms underlying this phenomenon may provide important lessons about host control of these potentially immunodeficiency inducing agents.

In summary, recently obtained, contemporaneous samples demonstrate multiple instances of cross-species transmission of FIV*pco*A between bobcats and pumas in Southern California. Further, archival analysis reveals a similar historical cross-species transmission of a closely related FIV strain in a relic population of Florida panthers. The persistent and genotypic characteristics of lentivirus infection demonstrate that FIV*pco*A transmission is more geographically restricted than host associated and that this particular clade of lentiviruses demonstrates a capacity to readily infect two distinct species, a highly unusual characteristic compared to the strict tendency for species-specific lentivirus isolates to be monophyletic. We propose that FIV*pco*A may actually be a bobcat-origin lentivirus more appropriately designated FIV*lru*. It is possible that perturbations in home ranges forced by urbanization led to increased FIV*lru* incidence in the bobcat populations in Southern California and/or increased interspecies contacts between bobcats and pumas, resulting in acceleration of cross-species transmission events. Although the ultimate basis for this observation remains unknown, these observations may parallel events involved in the transmission of SIV to humans, which is thought to be associated with increased contact between humans and nonhuman primates associated with changes in trade practices in areas where SIV was endemic (16). The findings reported here present an opportunity for studies of host specificity and the molecular, behavioral, and ecological correlates of the cross-species transmission of lentivirus infection and disease.

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