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Nucleotide sequences of Australian isolates of the feline immunodeficiency virus: comparison with other feline lentiviruses

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Summary. Proviral DNA from four Australian isolates of feline immunodeficiency virus (FIV) was amplified by PCR and the nucleotide sequence determined for two conserved regions within *gag* (p15/p24) and *pol* (RT) genes. Comparison with the nucleotide and deduced amino acid sequence of two previously described U.S. isolates from California (Petaluma and PPR), and a third from Maryland (MD) as well as the Japanese isolate TM2, revealed a close similarity between the Australian and Californian isolates with 95–97% nucleotide and 96–99% amino acid homologies. By contrast, the Maryland and Japanese isolates were more distantly related with only 84–87% nucleotide and 90–94% amino acid homology with either the Australian or Californian isolates. The relationship of the Australian FIV isolates to other domestic isolates as well as eight lentiviral isolates from wild felidae (panthers) published previously, was investigated further by constructing a phylogenetic tree based on the *pol* sequence. This revealed two subgroups of FIV, an Australian/Californian group and a less tightly clustered Maryland/Japanese group. These results suggest that the genomic variability of FIV is reflected by more than simply geographic distance. Furthermore, the relative genetic homogeneity found between Australian isolates suggest a shorter period of evolution of the virus in Australia than in North America.

Introduction

Feline immunodeficiency virus (FIV), a close relative of human immunodeficiency virus (HIV), the causative agent of AIDS, is a T-lymphotrophic lentivirus of cats first reported by Pederson et al. [20]. FIV infection has a worldwide prevalence, as revealed by sero-epidemiological surveys [10, 11, 23, 26], which suggests that this virus has been present in cats for some time. Studies have

also shown that there is a higher prevalence of FIV in certain countries, most notably Australia, with a reported prevalence rate in sick cats of up to 25% [8, 23]. FIV infection is spread horizontally and like HIV, can lead to immunological abnormalities in cats such as a depletion of circulating $CD4^+$ cells [1, 3, 29]. The disease pattern is characterised by generalised lymphadenopathy, anaemia, and increased susceptibility to opportunistic infections [12, 21, 30].

The similarity between FIV and HIV-1 makes FIV infection of cats, its natural host, a useful small animal model for human AIDS, and in particular for the development of vaccines and evaluation of chemotherapeutic agents. One important aspect of lentiviral research is the study of genetic variation among different viral isolates. Such work, in addition to helping elucidate the evolution and origin of different groups of lentiviruses, enables comparisons between genomic structure and biological characteristics. This may provide an insight into the molecular basis of viral virulence and drug resistance, as well as aid in selecting target antigens/epitopes for vaccine development.

For HIV the extent of genetic variation has been relatively well documented [2, 4, 6, 7, 31]. Such work has revealed that the internal structural genes and viral enzymes encoded by *gag* and *pol*, respectively undergo much less variation than *env*, which encodes the surface glycoproteins. In the case of FIV, however, the complete genomic sequence of only three isolates comprising two from the United States, the 34 TF 10 and FIV-14 clones of the Petaluma strain [18, 28] and the PPR clone of the San Diego strain [22] and one (the TM2 isolate) from Japan [15] have been reported to date. A partial *pol* gene sequence has also been reported for a third U.S. isolate (MD) and *env* gene sequences for two other strains comprising one (TM 1) from Japan [16] and one (clones 19K1 and 19K32) from the Netherlands [24].

In this report, we present the first detailed sequence data obtained for Australian isolates of FIV. Partial nucleotide sequence corresponding to nucleotides 948–1370 of *gag* and 2425–3013 of *pol* from four distinct isolates were determined and compared with the corresponding sequences of the previously published U.S. isolates Petaluma, PPR and MD, and the Japanese isolate TM2. A phylogenetic tree based on the nucleotide sequences obtained for the highly conserved *pol* gene was constructed in order to establish their possible ancestral relationship to each other and to isolates obtained from non-domestic felids [19].

Materials and methods

Virus isolation and culture

FIV isolates T91, N91, and DC91 were obtained from local (Perth) cats and S90 was obtained from a domestic cat in Melbourne, Australia. All cats had naturally acquired infection. The peripheral blood mononuclear cells (PBMCs) from the infected cats were purified over Ficoll-Hypaque. Viruses were isolated by co-cultivation of PBMCs from the infected cats with MYA-1 cells, a feline interleukin-2 dependent T-lymphoblastoid cell line [17], kindly provided by Prof. T. Mikami, University of Tokyo, Japan. Cultures were

maintained in RPMI-1640, supplemented with 10% fetal bovine serum, antibiotics, 50 μM 2-mercaptoethanol, 2 μg/ml polybrene, and 100 units/ml recombinant human interleukin-2. Supernatant was collected after 5–7 days of culture and further passaged on MYA-1 cells. Culture supernatant was assessed for the presence of FIV p 24 antigen by antigen capture ELISA (Petchek, Idexx, Portland, ME).

DNA isolation

Genomic DNA containing FIV proviral sequences for use in PCR reactions was isolated from p24-positive cell cultures and extracted as described by Kellogg and Kwok [13] for HIV. Briefly, cells washed in PBS (×2) were lysed in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA buffer containing 0.5% Tween 20 and 1 mg/ml proteinase K (Boehringer Mannheim, Mannheim, Federal Republic of Germany). The cells were incubated at 60 °C for 1 h and the proteinase K subsequently inactivated at 95 °C for 10 min.

Oligonucleotide primers

Two oligonucleotide primer pairs were used to amplify a region of *gag* and *pol*, respectively, within the genome of FIV. The first was L 928-R 1394 which amplified a fragment of predicted size 467 bp. L 928, located at positions 928–946 of the *gag* (MA) sequence according to the numbering of the published Petaluma FIV isolate sequence [28], has the sequence 5′CTACTGCTGCTGCAGCTGA3′ [14]. R 1394 is located at positions 1394–1371 of *gag* (CA) and has the sequence 5′ACACTGCATCCTAGCTGGTGCAAA3′. The second primer pair was L 2402-R 3039 which amplified a fragment of predicted size 638 bp. L 2402 is located at positions 2402–2424 of *pol* (reverse transcriptase, RT) and has the sequence 5′CAATGGCCATTAACAAATGAAAA3′, while R 3039 is located at positions 3039–3017 of *pol* (RT) and has the sequence 5′GGATGTAATTCATAACCCATCCA3′.

PCR

PCR was performed according to the manufacturer's instructions (Biotech International, Perth, WA) using 10 pmol of each primer, 50 ng DNA, 0.2 mM each dNTP, 2 mM $MgCl_2$, and 2U Taq polymerase in a 25 μl total reaction volume. The reaction was overlayed with paraffin oil (30 μl) and cycled in a Hybaid thermoreactor (Hybaid, Teddington, U.K.) for 30 sec at 94 °C, 1 min at 55 °C, and 2 min 72 °C for 35 cycles, with the exception of 5 min at 94 °C on the first cycle and 10 min at 72 °C on the final cycle. PCR products were resolved on a 1.5% agarose gel stained with 0.1 μg/ml ethidium bromide.

DNA sequencing

FIV proviral DNA amplified by PCR using either the L 928-R 1394 or L 2402-R 3039 primer pair was first purified using Prep-a-Gene (BioRad, Richmond, CA) to remove unincorporated deoxynucleotides and primers. Sequencing reactions were performed by the dideoxynucleotide chain termination method using a Taq Dye Deoxy Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The sequence was resolved on an Applied Biosystems model 373 A automated DNA sequencer.

Phylogenetic tree construction

Phylogenetic tree construction was achieved by the PAUP (Phylogenetic Analysis Using Parsimony) program, version 3.0 [27]. Distances were expressed on the basis of amino acid sequence identity with any gaps being given a weight of a single residue substitution.

Results

Comparison of gag *region (948–1370) sequences*

The nucleotide and predicted amino acid sequence homologies of four Australian FIV isolates (T91, N91, DC91 and S90) between bases 948 and 1370 of the FIV proviral genome, corresponding to the 3′ end of the *gag* p15 (MA) gene and the 5′ portion of p24 (CA), are shown in comparison with the previously determined sequences of the Petaluma [28], PPR [22], and TM2 [15] isolates (Table 1, Fig. 1). The two Californian isolates (Petaluma and PPR) were as different to one another as they were to the Australian isolates with between

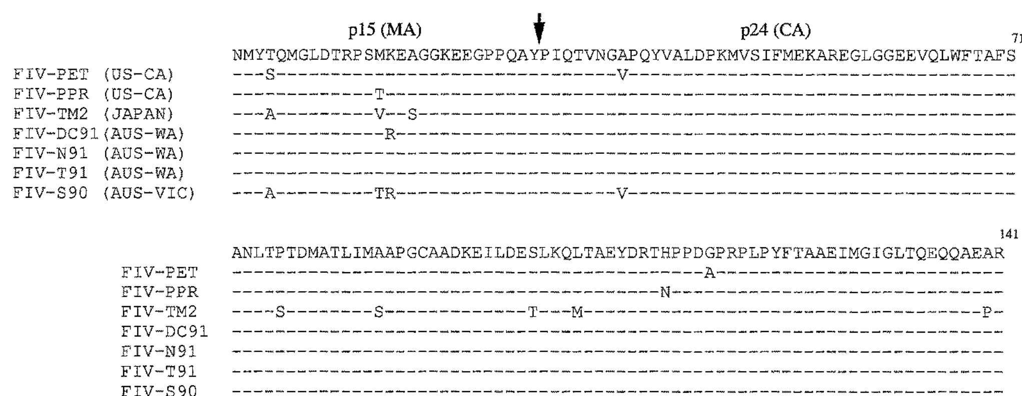

Fig. 1. Predicted amino acid sequence alignment of p15/p24 in FIV *gag* genes. Dashes indicate identity with a consensus sequence. The arrow denotes the protease cleavage site between p15 (*MA*) and p24 (*CA*). The geographic origins of virus isolates are indicated in parentheses: *CA* California; *WA* Western Australia; *VIC* Victoria

Table 1. Sequence comparisons (% identity) of p15/p24 (948–1370) from *gag* genes of FIV isolates

	U.S.A.		Japan	Australia			
	FIV-PET	FIV-PPR	FIV-TM2	Western Australia			Victoria
				FIV-DC91	FIV-N91	FIV-T91	FIV-S90
FIV-PET		95.7	84.2	94.8	96.2	94.8	93.8
FIV-PPR	96		85.1	95.5	96.9	95.3	94.8
FIV-TM2	93	94		84.9	85.3	84.9	84.2
FIV-DC91	97	98	94		97.6	96.4	96.0
FIV-N91	98	99	94	99		97.9	96.4
FIV-T91	98	99	94	99	100		95.3
FIV-S90	97	97	94	98	97	97	

Percent nucleotide (above diagonal) and amino acid (below diagonal) sequence identities were determined by pairwise alignments of minimum overlaps

94–97% nucleotide and 97–98% amino acid sequence identity. Of the Australian isolates, N 91 appeared most closely related to the two Californian isolates with 96.2% and 96.9% nucleotide sequence homology with Petaluma and PPR, respectively. Conversely, PPR showed a slightly higher similarity with all the Australian isolates than did the Petaluma isolate. By contrast, the Japanese isolate TM 2, was quite distinct with 15–16% nucleotide and 6–7% amino acid sequence divergence from both the Californian and Australian isolates. Most nucleotide substitutions between all the FIV isolates occurred in the p 15 gene which was also reflected at the amino acid level (Fig. 1).

Comparison of pol *region (2425-3013) sequences*

The nucleotide and predicted amino acid sequence homologies of T 91, N 91, DC 91 and S 90 between bases 2425 and 3013 of the FIV genome, which corresponds to a 5′ region of the RT gene, are illustrated (Table 2, Fig. 2) in comparison with those previously determined for Petaluma, PPR, and TM 2 as well as a Maryland isolate (MD), and isolates from panthers in the U.S.A. [19]. Overall, the nucleic acid identity was highly conserved, being slightly higher than that found in the *gag* region. The nucleotide and amino acid sequence homologies of all the isolates for the *pol* region revealed the Australian and Californian isolates to be closely related with only 4–5% nucleotide and 2–4% amino acid sequence difference compared with 13–14% and 6–8% respectively, for the Japanese isolate (Table 2). It was noteworthy that the isolate from the east coast of the United States (MD) was even more divergent from the Australian and Californian isolates than TM 2, with 84–85% nucleotide and 90–92% amino acid sequence homologies. TM 2 and MD were more closely releated to each other than to either the Australian or Californian isolates, although they were somewhat divergent with only 93% nucleotide and 95% amino acid sequence homologies.

Phylogenetic analysis of feline lentiviral pol *gene sequences*

To investigate the genetic relationship between all the FIV isolates, a phylogenetic tree was constructed based on the *pol* region using PAUP [27] for the four Australian isolates as well as all other feline lentiviruses for which nucleotide sequences in this region had been previously reported. As previously shown by Olmsted et al. [19], the FIV isolates sequenced to date form a separate group from the isolates so far obtained from wild felidae (Fig. 3). These groups differ nucleotide and amino acid sequence in the *pol* region by approximately 20–25% and 16–20% respectively. Within the FIV group, the Australian and Californian isolates cluster together as one subgroup differing in amino acid sequence by a maximum of 4%. The Japanese and Maryland isolates occupy another, less highly related subgroup, which differ in amino acid sequence from the other FIV isolates by about 6–10% and from each other by 5%.

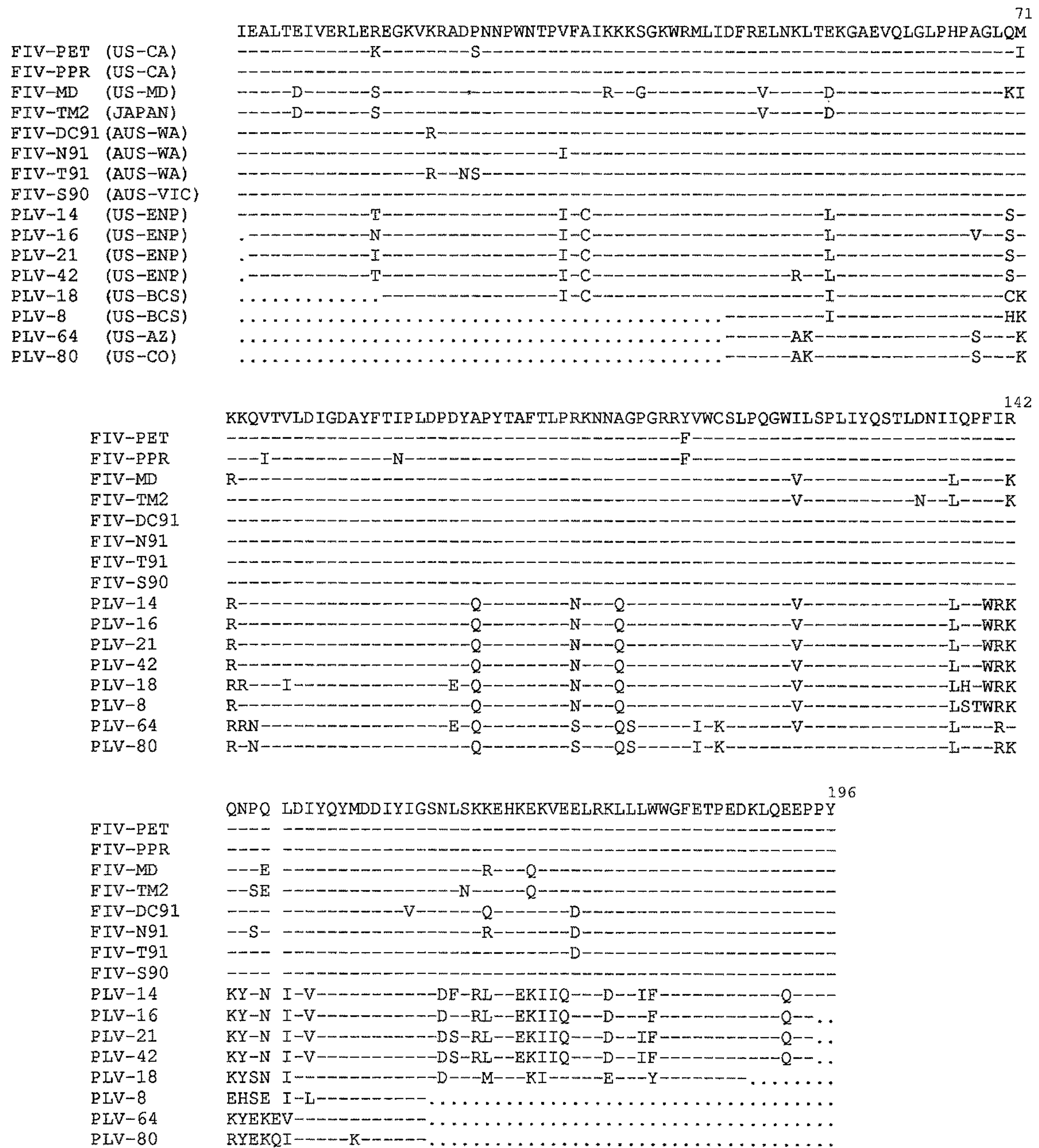

Fig. 2. Predicted amino acid sequence alignment of a conserved N-terminal region of RT in feline lentiviral *pol* genes. Dashes indicate identity with a consensus sequence based on the domestic isolates. Dots indicate regions for which sequence information was not available. The geographic origins of virus isolates are indicated in parentheses: *PLV* panther lentivirus; *ENP* Everglades, National Park; *BCS* B Cypress Swamp

Discussion

The availability of complete nucleotide sequences for three independent isolates of FIV [15, 22, 28], as well as partial sequences for several others [16, 19, 24]

Table 2. Sequence comparisons (% identity) of a conserved RT region (2425-3013) from *pol* genes of feline lentiviruses

	U.S.A.			Japan	Australia				U.S.A.: non-domestic							
	California		Maryland		Western Australia			Victoria	Everglades National Park				Big Cypress Swamp		Arizona	Colorado
	FIV-PET	FIV-PPR	FIV-MD	FIV-TM2	FIV-DC91	FIV-N91	FIV-T91	FIV-S90	PLV-14	PLV-16	PLV-21	PLV-42	PLV-18	PLV-8	PLV-64	PLV-80
FIV-PET		96.6	84.9	87.1	95.4	95.9	96.1	96.3	78.6	78.7	78.9	78.5	78.3	79.4	73.5	74.1
FIV-PPR	97		84.4	86.2	96.1	95.9	96.1	96.3	78.3	78.2	78.5	78.5	78.7	78.8	72.7	74.4
FIV-MD	92	91		93.0	84.0	84.4	83.5	85.0	76.6	76.1	76.8	76.5	77.5	77.6	72.4	72.7
FIV-TM2	92	92	95		85.7	86.2	85.7	87.1	77.4	76.8	77.5	77.2	77.3	76.4	72.4	73.8
FIV-DC91	96	96	91	92		95.4	97.3	96.1	77.9	78.0	78.4	78.0	78.3	77.9	74.1	75.0
FIV-N91	96	96	91	93	97		96.1	96.6	78.3	78.2	78.5	77.8	77.9	78.8	73.2	74.4
FIV-T91	97	96	90	92	98	97		96.4	77.1	77.0	77.3	77.0	77.0	77.6	73.2	73.8
FIV-S90	98	98	92	94	98	98	98		78.9	78.9	79.6	78.9	79.0	79.1	73.2	75.0
PLV-14	82	82	83	83	82	83	82	84		98.4	98.6	97.4	86.3	88.4	75.0	78.8
PLV-16	82	82	83	83	82	83	82	84	98		98.6	96.9	86.8	87.8	74.7	78.2
PLV-21	82	82	83	82	82	83	81	83	99	98		97.4	86.7	88.4	75.0	78.8
PLV-42	81	81	82	82	81	82	81	83	99	97	99		86.1	87.8	74.4	78.8
PLV-18	82	82	84	84	82	84	81	83	90	90	90	89		86.6	73.8	74.1
PLV-8	82	80	86	84	82	83	83	83	91	90	91	90	91		75.0	77.9
PLV-64	79	78	79	78	79	80	80	80	83	83	83	83	84	80		86.0
PLV-80	80	78	80	79	80	81	81	81	84	84	84	84	82	82	93	

Percent nucleotide (above diagonal) and amino acid (below diagonal) sequence identities were determined by pairwise alignments of minimum overlaps.
PLV Panther lentivirus

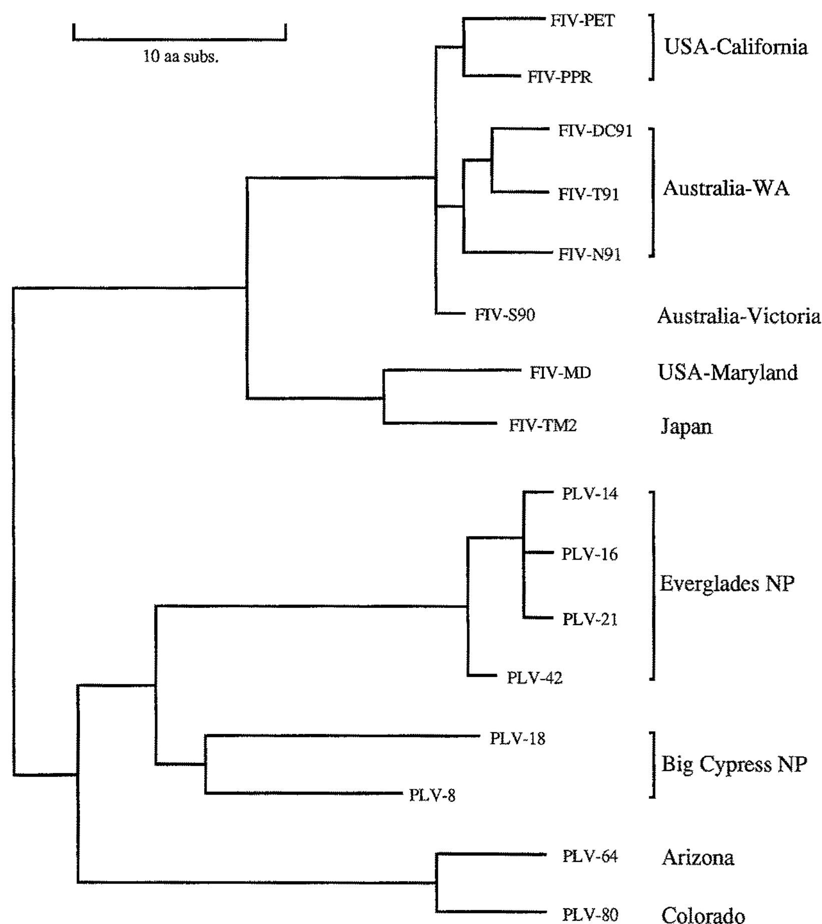

Fig. 3. Evolutionary tree showing the relationship between the RT amino acid sequences from feline lentiviruses shown in Fig. 2 using PAUP (Phylogenetic Analysis Using Parsimony) version 3.0. The scale and leg lengths are in amino acid substitutions. The tree shown has an overall length of 104 changes and a consistency index of 0.875

has begun to shed some light on the extent of genetic variation within this lentivirus. As is the case for HIV, variation between isolates of FIV is characterised by point mutations, which may result in amino acid substitutions, especially in the less highly conserved envelope region. These mutations occur relatively frequently, because like all RNA viruses, retroviruses have very short generation times, and more importantly, because of the infideliy of the reverse transcriptase used for replication, which has limited or no proof-reading function [25].

In this study we report partial nucleotide sequences of two well conserved regions, one in *gag* and one in *pol* which together comprise about 12% of the FIV genome, for four Australian FIV isolates and compare these with other known FIV sequences, comprising three US isolates and one Japanese. Despite the considerable geographic distance between Australia and North America,

from the present study it was clear that a remarkable genetic similarity is present between Australian and Californian isolates, thereby indicating a common origin. In marked contrast, both the Japanese isolate TM 2 and the Maryland isolate revealed considerable genetic divergence from both the Californian and Australian isolates with a nucleotide sequence difference in the order of 13–16% (versus 2–5% within and between Australian and Californian isolates). This suggests that these isolates have diverged at a much earlier time. Moreover, the greater divergence between the three US isolates than that found between the Australian isolates, tends to suggest a longer period of evolution of the virus in North America.

To determine the position of the Australian isolates in the phylogeny of feline lentiviruses, we constructed a minimum length evolutionary tree based on the nucleotide sequence variation found within the *pol* region (sequence position 2425–3013). This region was chosen because it is generally the most conserved gene among this family of viruses [5, 9]. The Australian isolates, T 91, N 91, DC 91 and S 90, were found to cluster very closely with the US west coast (Californian) isolates, Petaluma, and particularly PPR, whereas TM 2 and MD tended to be genetically equidistant from both the Australian and Californian isolates. The genetic similarity of the Australian and Californian isolates strongly suggests a common ancestry, presumably of European origin. This would therefore tend to rule out the transfer of virus from wild to domestic felidae in North America, a conclusion also reached by Olmsted et al. [19] due to the high sequence divergence found between wild and domestic feline lentiviruses. The Japanese and Maryland isolates formed a separate cluster from other domestic isolates which indicates a divergence from other known domestic isolates at a much earlier time. This, along with the reported worldwide spread of FIV [10, 11, 23, 26], suggests that the emergence of FIV is not recent. Much more sequence data from a variety of wild, feral and domestic isolates will be required to detail more fully the evolutionary history of feline lentiviruses.

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