

## Different Rates of LINE-1 (L1) Retrotransposon Amplification and Evolution in New World Monkeys

Stéphane Boissinot,<sup>1,\*</sup> Christian Roos,<sup>2</sup> Anthony V. Furano<sup>1</sup>

<sup>1</sup> Section on Genomic Structure and Function, Laboratory of Molecular and Cellular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0830, USA

<sup>2</sup> German Primate Center, Primate Genetics, Kellnerweg 4, Goettingen 37077, Germany

Received: 12 March 2003 / Accepted: 8 August 2003

**Abstract.** LINE-1 (L1) elements constitute the major family of retrotransposons in mammalian genomes. Here we report the first investigation of L1 evolution in New World monkeys (NWM). Two regions of the second open-reading frame were analyzed by two methods in three NWM species, the squirrel monkey (*Saimiri sciureus*), the tamarin (*Saguinus oedipus*), and the spider monkey (*Ateles paniscus*). Since these three species diverged, L1 has amplified in the *Saimiri* and *Saguinus* lineages but L1 activity seems to have been strongly reduced in the *Ateles* lineage. In addition, the active L1 lineage has evolved rapidly in *Saimiri* and *Saguinus*, generating species-specific subfamilies. In contrast, we found no evidence for a species-specific subfamily in *Ateles*, a result consistent with the low L1 activity in this species for the last ~25 My.

**Key words:** LINE-1 — Non-LTR — Retrotransposon — Primate — New World monkeys

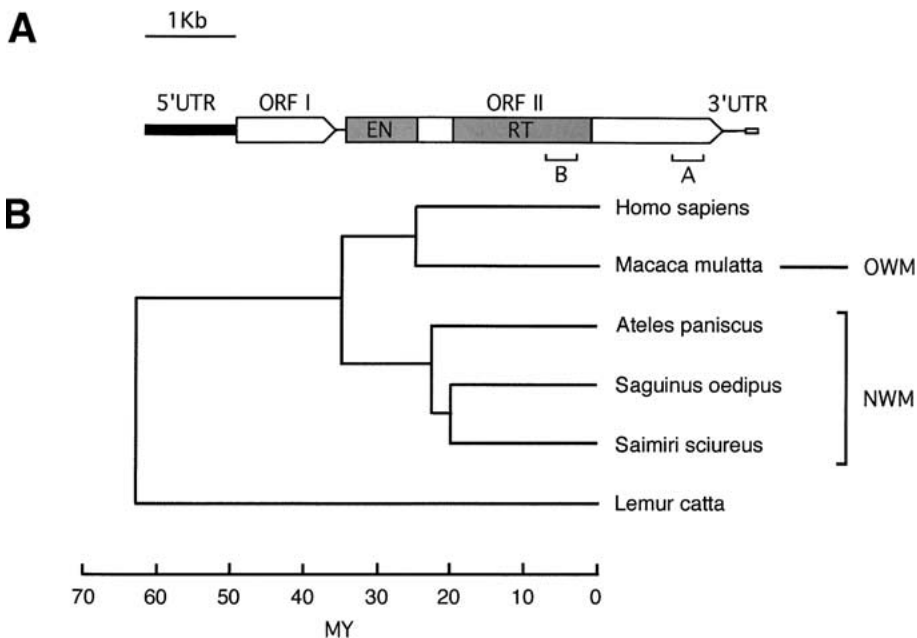
### Introduction

L1 (LINE-1) elements (Fig. 1A) are mammalian long interspersed repeats that replicate autonomously by retrotransposition (reviewed by Furano 2000). They have been evolving and replicating since before the mammalian radiation (~100 My ago) and their accumulation in mammalian genomes accounts for up to 25% of the DNA mass of these genomes. L1 retrotransposition generates mostly defective copies, which remain in the genome and diverge from each other at the pseudogene rate (Hardies et al. 1986; Pascale et al. 1993; Voliva et al. 1983). With time, novel replication-competent L1 variants arise and some can generate families of hundreds to thousands of copies. Although more than one family of closely related variants (referred to as subfamilies) can be concurrently active (Boissinot et al. 2000; Furano et al. 1994; Pascale et al. 1993; Smit et al. 1995), ultimately only one prevails (Cabot et al. 1997). This process is exemplified by the successive emergence of a single lineage of replicatively dominant L1 families during the last 60 My of human evolution (Boissinot et al. 2001; Smit et al. 1995).

The persistence of L1 activity in all mammalian orders studied so far (Burton et al. 1986) is puzzling because L1 insertions can cause genetic defects (Kazazian 1998) and L1 activity in general can pose a

\*Current address: Department of Biology, Queens College, CUNY, Flushing, NY 11367, USA

Correspondence to: Anthony V. Furano, NIH, Building 8, Room 203, 8 Center Dr. MSC 0830, Bethesda, MD 20892-0830, USA; email: avf@helix.nih.gov



**Fig. 1.** **A** Structure of a typical full-length human L1 element. The 5' untranslated region (5'UTR) has a regulatory function; open reading frame (ORF) I encodes an RNA-binding protein; ORFII encodes the L1 cDNA replicase containing highly conserved endonuclease (EN) and reverse transcriptase (RT) domains; the

3'UTR contains a conserved G-rich polypurine motif. Genomic copies of L1 end in an A-rich stretch (open rectangle) (Furano 2000). The A and B regions correspond to the two regions studied in this paper. **B** Evolutionary relationships between the primate species used in these studies (Goodman et al. 1998).

serious genetic load to its host (Boissinot et al. 2001). How serious depends, in part, on the rate at which L1 amplifies. In murine rodents the L1 amplification rate changes over evolutionary time; periods of high and low amplification rates alternate (Pascale et al. 1993; Verneau et al. 1998). Apparent L1 replication rates also differ between species; the rate of genetic defects due to L1 insertions that cause disease in human is 30 times lower than in the house mouse (Ostertag and Kazazian 2001), and it has been suggested that L1 amplification has recently decreased in humans and their immediate primate antecedents (Boissinot et al. 2000; Consortium 2001).

As L1 elements have been characterized in only a few nonhuman primate species (DeBerardinis and Kazazian 1998; Mathews et al. 2003; Stanhope et al. 1993), most of our knowledge of primate L1 is based on human studies. Here we report the first analysis of L1 evolution in an additional group of nonhuman primates, New world monkeys (NWMs; infraorder Platyrrhini, following the classification of Goodman et al. [1998]). NWMs are restricted to Central and South America, where they have evolved and diversified since their split from Catarrhini primates (Old World monkeys, apes, and human), ~35 My ago (Fig. 1B). We collected sequences from two regions of L1 in three NWM species that diverged from each other between 20 and 25 My ago. L1 amplified and evolved in the tamarin (g. *Saguinus*) and squirrel monkey (g. *Saimiri*) lineages. In contrast, both processes were drastically reduced in the spider

monkey lineage (g. *Ateles*). We also found that two L1 lineages coexisted in the common ancestor of these three species but only one of them has persisted until the present time.

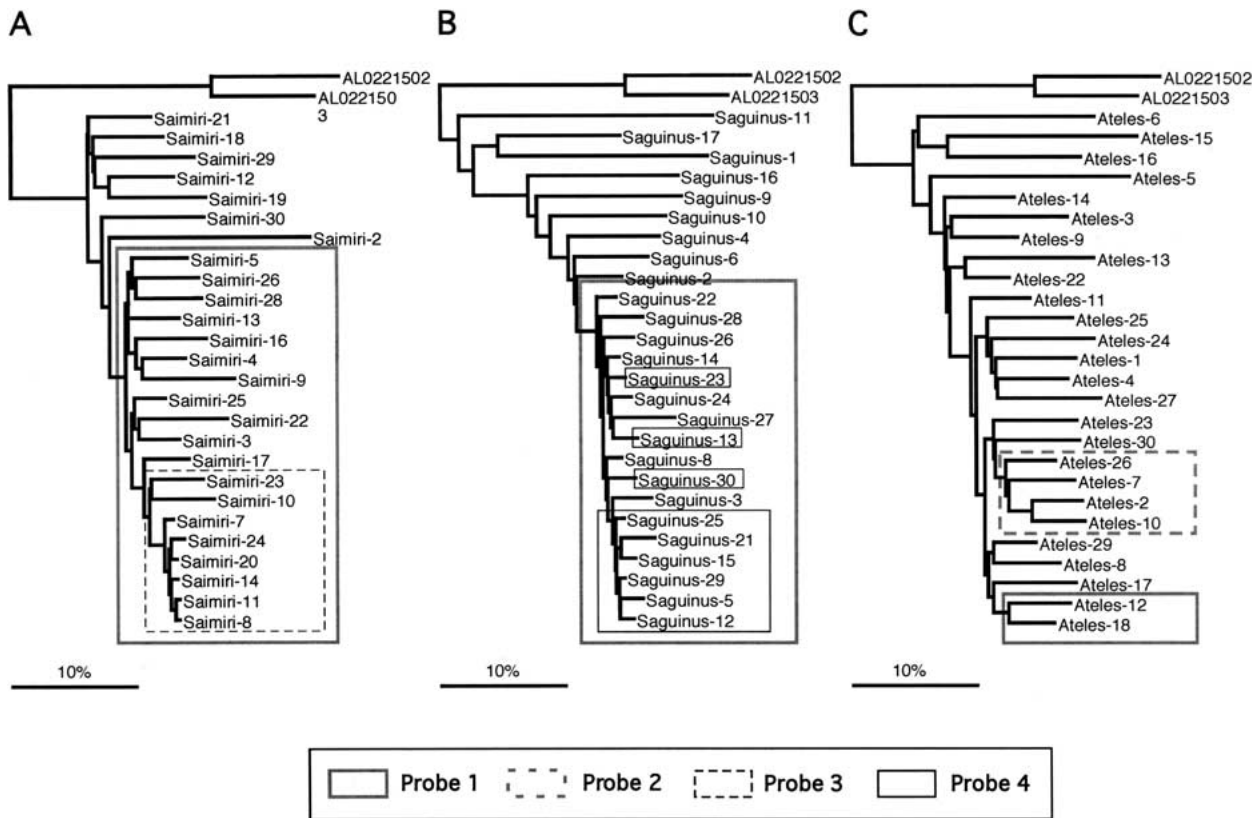
## Materials and Methods

### Sources of Samples

DNA samples of squirrel monkey (*Saimiri sciureus*), spider monkey (*Ateles paniscus*), rhesus monkey (*Macaca mulatta*), and lemur (*Lemur catta*) were obtained from individuals kept in captivity, respectively, at the University of München, the Wuppertal Zoological Garden, the Leipzig Zoological Garden, and the Münster Zoological Garden. The tamarin (*Saguinus oedipus*) and human (*Homo sapiens*) DNAs were purchased from the Coriell Institute for Medical Research. The phylogenetic relationship between these species is shown in Fig. 1B.

### PCR Amplification and Cloning

PCR primers were cognate to a highly conserved region of the second open reading frame (ORFII; Region A in Fig. 1A). Primers GAGAAATGCAAATCAAACCA (forward) and GCTGCA-TAGTATCCATTGT (reverse) amplify a 396-bp fragment located near the 3' extremity of ORFII (from position 5426 to position 5822 on alignment ALIGN\_000165 from the EMBL-ALIGN database [Boissinot and Furano 2001]). This region encompasses a cysteine-rich motif (Moran et al. 1996) that is conserved in all L1 and L1-like elements (Clements and Singer 1998). The conservation of this part of ORFII was confirmed by obtaining amplification with the same primers in a wide range of mammals including mouse, rabbit, dog, cow, pig, and elephant shrew (data not shown). PCRs were performed in capillary tubes in an Idaho Technology Air-Thermo Cycler using the reagents provided by the company. Primers were



**Fig. 2.** Phylogenetic trees of L1 elements based on region A of ORFII. Trees were built using the neighbor-joining method based on Kimura's two-parameter distances. Sequences AL022150-2 and AL022150-3 were used as outgroups because they have 75 of the 84 characters in region A that distinguish the modern human L1 family from the L1P5 family (Smit et al. 1995), an ancestral L1

family that amplified before the split between New World monkeys and Old World monkeys and apes. Boxes indicate the sequences that have the characters diagnostic of the four hybridization probes. Trees built using (A) *Saimiri sciureus* sequences, (B) *Saguinus oedipus* sequences, and (C) *Ateles paniscus* sequences.

used at a final concentration of 0.5  $\mu$ M. Fifty nanograms of genomic DNA was amplified in a total volume of 25  $\mu$ l using the following conditions: 94°C for 0 s, 50°C for 0 s, and 72°C for 10 s, for 30 cycles. PCR products were purified with the QIAquick PCR purification kit (QIAGEN) and cloned into the pGEM vector (Promega). Positive clones were sequenced in both directions using the Sequenase version 2.0 sequencing kit (Amersham).

When this first method failed to yield young L1 elements from *Ateles*, we used a cloning protocol designed to select for young L1 elements (Cantrell et al. 2000; Casavant et al. 2000). This method depends on the higher likelihood of intact ORFs in younger than in older L1 elements. Degenerate primers were used to amplify a portion of the conserved reverse transcriptase domain of ORFII (Region B in Fig. 1A). The PCR product was then cloned in a modified bacterial cloning vector (pBS II KS+ vector; Stratagene) that does not contain a functional LacZ reading frame but can produce a functional LacZ fusion peptide if an L1 ORF amplified with the degenerate primers is inserted appropriately. When electroporated into *E. coli* strain TG1, plasmids containing an L1 fragment with an intact ORF yielded blue colonies, while L1 elements with an interrupted ORF yielded white colonies. Clones from blue colonies were then sequenced. The modified vector was kindly provided by Dr. Holly Wichman (University of Idaho, Moscow).

#### DNA Blotting and Hybridization Conditions

DNAs were digested with *Nla*III. About 50 ng of each DNA digest was electrophoresed on a 6% acrylamide gel and electroblotted to

Zeta-probe membranes. Alternatively, DNA dot-blot were applied manually on Zeta-probe membranes. Membranes were hybridized to family-specific [ $^{32}$ P]GTP-labeled oligonucleotide in the presence of competitor oligonucleotides (described by Verneau et al. 1997).

#### Sequence Analysis

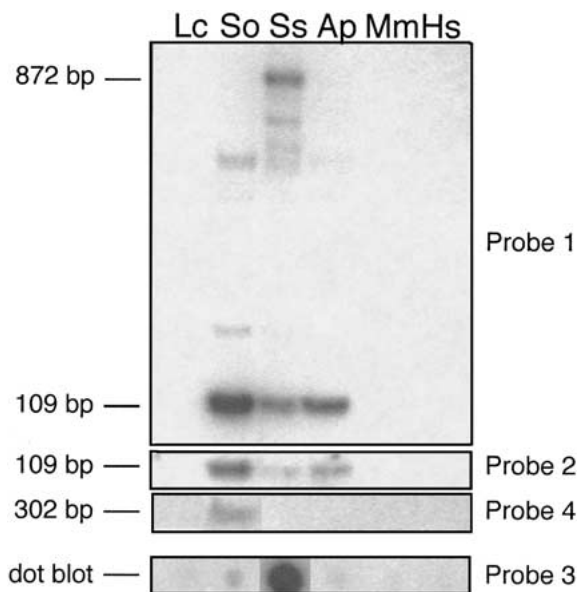
Sequences were aligned manually. Phylogenetic analyses were performed using the PHYLIP 3.5c computer package (Felsenstein 1993). The percentage nucleotide substitution between sequences was calculated using Kimura's (1980) two-parameter method. Trees were built with the neighbor-joining method (Saitou and Nei 1987) and their robustness was assessed using a bootstrap procedure. The aligned sequences (in fasta format) are available by anonymous FTP from [helix.nih.gov/usr/ftp/pub/avf/Align\\_NWM\\_Regions\\_A\\_all\\_seq](http://helix.nih.gov/usr/ftp/pub/avf/Align_NWM_Regions_A_all_seq).

## Results

### Analysis of Region A

Twenty-six PCR-derived clones from each of *Saimiri sciureus*, *Saguinus oedipus*, and *Ateles paniscus* and six clones from *Macaca mulatta* were sequenced. The sequences were aligned together with the consensus





**Fig. 4.** Hybridization of various L1 probes to blot of primates DNA. Each panel shows the result of overnight hybridization with a different probe as described under Materials and Methods. The sequences of the probes are shown in Fig. 3. The top three panels are Southern blots of *Nla*III-digested DNAs and the bottom panel is a dot blot. Lc—*Lemur catta*; So—*Saguinus oedipus*; Ss—*Saimiri sciureus*; Ap—*Ateles paniscus*; Mm—*Macaca mulatta*; Hs—*Homo sapiens*. Probe 1, hybridization with competitor at 45°C; probe 2, no competitor, at 40°C; probe 3, with competitor, at 50°C; probe 4, with competitor, at 45°C.

since these species diverged  $\sim 25$  Mya. To confirm the lack of young L1 elements in *Ateles*, more clones were collected, but this time we screened specifically for younger elements. *E. coli* colonies were transferred to Hybond-N nylon membranes (Amersham) using the procedure described in (Buluwela et al. 1989). The membranes were hybridized to the NWMs specific probe (probe 1 in Fig. 3). Twelve positive clones were picked and sequenced. Despite this additional cloning, we failed to find a group of young L1 sequences in *Ateles* as we found in *Saguinus* and *Saimiri*. The divergence of these additional sequences ranged from 3.4 to 14.1% and the five most similar sequences are 5.14% (SE = 0.79%) divergent. This apparent lack of L1 sequences younger than 8 My in *Ateles* suggests that the amplification of L1 elements was strongly reduced in the *Ateles* lineage.

Figure 5A shows a phylogenetic tree of the NWM L1 lineages. For simplicity, we selected the most similar sequences from each species; trees built with other groups of sequences (not shown) were similar to the one shown here. We added to this data set two human and two macaque sequences and three sequences representative of a group of divergent sequences identified in Fig. 2C by a dashed gray box (called L1nwm-old in Fig. 5A). This L1 tree mimics the species tree shown in Fig. 1B, suggesting that L1 has amplified in each species leading to species-spe-

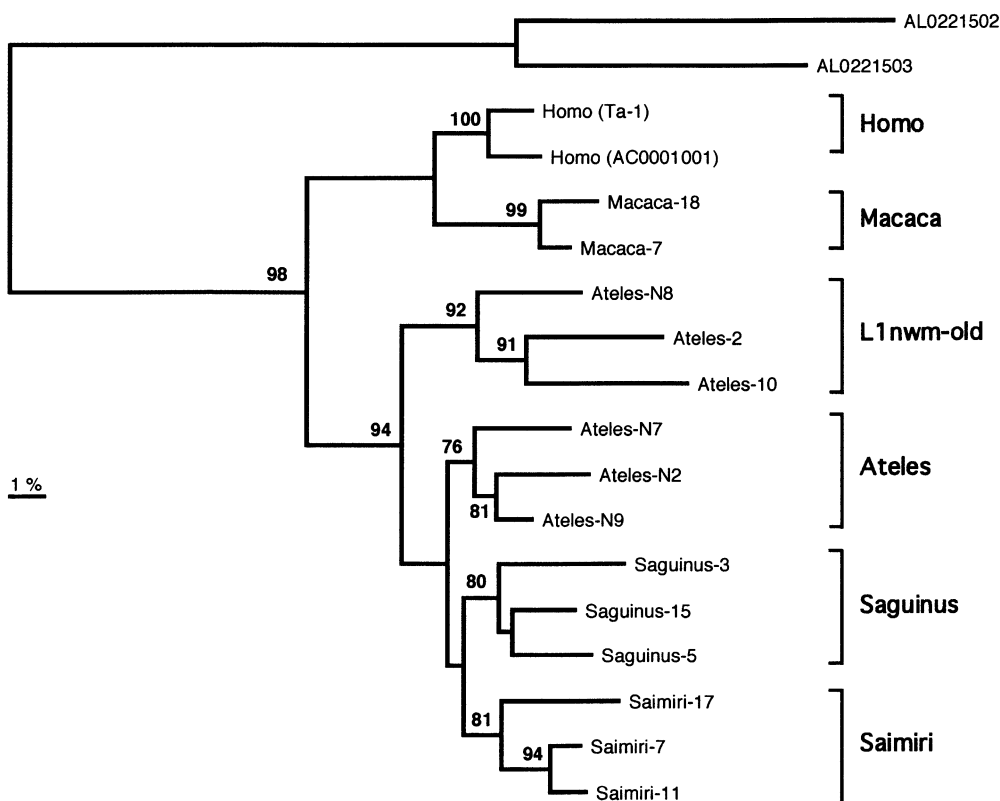
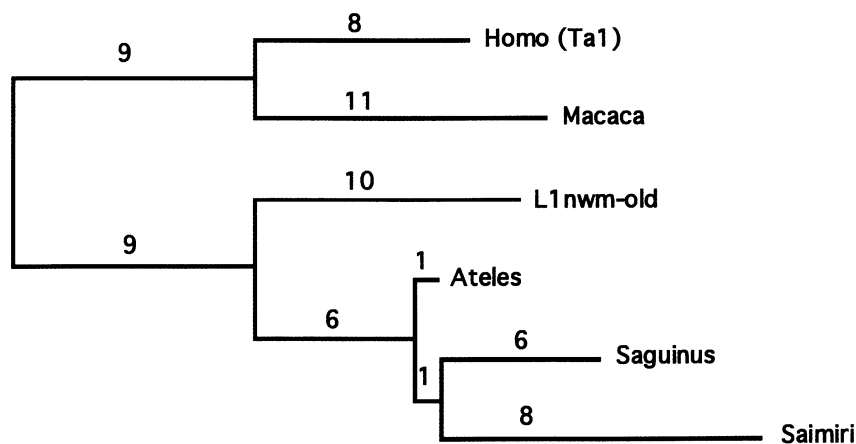
cific clusters of sequences. The *Ateles*, *Saguinus*, and *Saimiri* specific L1 clusters are supported by relatively high bootstrap values but the relationship among these three clusters is not fully resolved.

The divergent sequences cloned from *Ateles* cluster together and this group (L1nwm-old) is supported by a high bootstrap value. The L1nwm-old clade branches outside the (*Ateles* (*Saguinus*, *Saimiri*)) clade and therefore probably split from the main L1 lineage before the three species diverged from each other but after the split between NWMs and OWMs and apes. This was confirmed by hybridization with probe 2 (Fig. 4), which hybridized to the three NWM DNAs but not to other primate DNAs. The difference among the intensities of hybridization to the three species indicates that L1nwm-old amplified differentially in the three species. Thus, L1nwm-old apparently remained active (at least in *Saguinus* and *Ateles*) after the three species diverged from each other. This is supported by the fact that the least divergent L1nwm-old sequences differ by only 8%, which corresponds to 19 My. Although this is a very rough estimate, it is consistent with the hypothesis that L1nwm-old remained active in *Ateles* after *Ateles* diverged from the *Saguinus*/*Saimiri* clade, about 25 My ago. The fact that L1nwm-old sequences were recovered only from the DNA of *Ateles* is not really surprising because the abundance of young elements in *Saimiri* and *Saguinus* would dominate the population of PCR products, making the recovery of old L1 lineages problematic.

We then examined the evolution of the active L1 lineage in NWMs. We first determined the consensus sequences (Fig. 3) of the most recently active subfamily in each species and then the evolutionary relationships between these consensus sequences (Fig. 5B). The parsimony tree in Fig. 5B reveals that the active lineage of L1 has evolved at very different rates in NWMs. In *Saimiri* and *Saguinus*, L1 has evolved into species-specific subfamilies that are characterized, respectively, by eight and six diagnostic characters. The species specificity of these families was verified by hybridization with probes 3 (*Saimiri* specific) and 4 (*Saguinus* specific; Figs. 2 and 4). In contrast, the consensus built with *Ateles* sequences shows only one (non-CpG) character that would be specific to *Ateles*. However, this character is also found sporadically in older subfamilies and therefore might be a hypervariable site. Therefore, the most recently active L1 lineage has accumulated more changes in *Saimiri* and *Saguinus* than in *Ateles*.

#### Analysis of Region B

To confirm the difference between L1 amplification and evolution in the NWMs studied here, we used another method to distinguish old and new L1 fami-

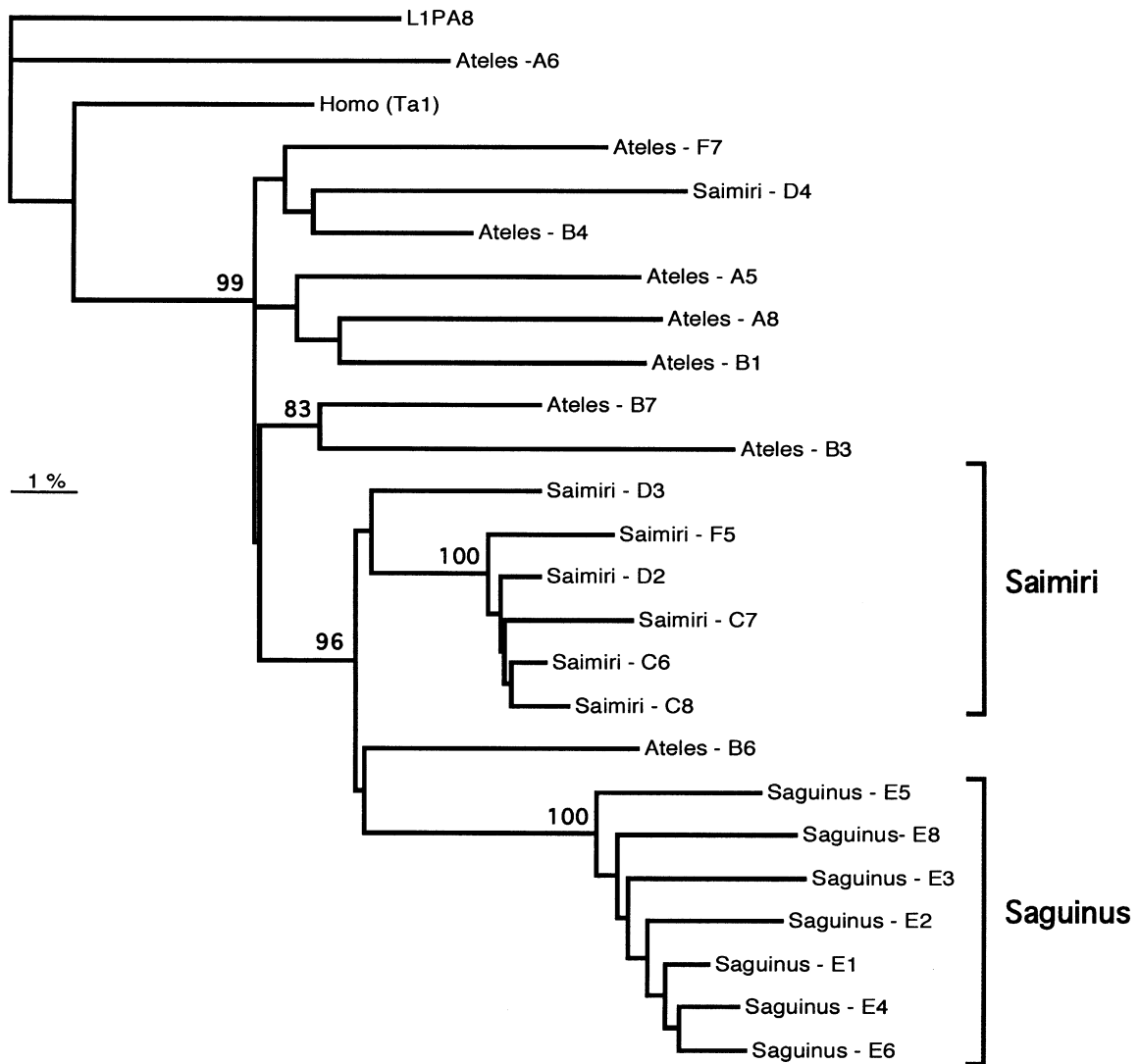
**A****B**

**Fig. 5.** Phylogenetic relationship between NWM L1 lineages. **A** Neighbor-joining tree based on Kimura's two-parameter distances. The *Ateles* sequences that begin with N are derived from clones selected with probe 1 as described under Results. The number at a particular node indicates its percentage of appearance in 500 bootstrap replicates. Only values >75% are indicated. **B** Parsimony

tree of consensus sequences. The tree is rooted using the ancestral sequence shown in Fig. 2. The branch lengths are proportional to the number of characters supporting each branch. CpG dinucleotides were excluded from this analysis because in most cases it is not possible to determine the ancestral state at these hypermutable sites.

lies. As described by Cantrell et al. (2000), PCR products of region B were cloned in frame into lacZ. Clones with intact reading frames should yield blue colonies, while clones with interrupted reading frames should yield white ones. The fraction of blue colonies was significantly higher ( $p = 0.000$ , Fisher's exact test) in *Saimiri* (63.4%; 298 colonies examined) and *Saguinus* (62.2%; 399 colonies) than in *Ateles* (35.4%; 223

colonies). This result confirms the above finding that *Saimiri* and *Saguinus* contain a larger proportion of young (i.e., with an intact reading frame) L1 elements than *Ateles*. This observation is consistent with the lack of recent L1 amplification in the *Ateles* lineage deduced from the analysis of region A. Twenty-three blue colonies were picked, sequenced (9 from *Ateles* and 7 each from *Saimiri* and *Saguinus*), and used in a



**Fig. 6.** Phylogenetic tree of L1 elements based on region B of ORFII. The tree was built using the neighbor-joining method based on Kimura's two-parameter distances. The number at a particular node indicates its percentage of appearance in 500 bootstrap replicates. Only values > 75% are indicated. The tree is rooted with the consensus of ancestral subfamily L1PA8.

phylogenetic analysis (Fig. 6). This NJ tree shows that most *Saguinus* and *Saimiri* sequences are closely related and that they cluster into species-specific clades. On the other hand, *Ateles* sequences are very divergent from each others and do not constitute a monophyletic lineage. This tree topology substantiates our conclusions from examining region A of ORFII (Fig. 5B), namely, that species-specific lineages have evolved in *Saimiri* and *Saguinus* but not in *Ateles*.

## Discussion

Here we investigated the evolutionary history of L1 retrotransposons in NWM in which L1 evolution had not yet been examined. We found that episodes of L1 amplification occurred at different times in three NWM species. Whereas L1 amplified recently

in *Saimiri* and in *Saguinus*, L1 activity seems to have been strongly reduced in the *Ateles* lineage for the last ~10 My. In both *Saimiri* and *Saguinus* the active L1 lineage has evolved and has generated new species-specific subfamilies. In contrast, we could not find convincing evidence for new L1 subfamilies in the *Ateles* lineage. In fact, our results suggest that the last amplification of a discernable L1 family in this species occurred more than 19 Mya. These results were obtained by analyzing two different regions of ORFII with different methods. As both methods were PCR-based, any bias would favor yielding younger elements (more likely to retain nonmutated PCR primer sites). Therefore, we are confident that our observations are not experimental artifacts and correspond to real differences in the rate of evolution and amplification of L1 in *Ateles*, *Saguinus*, and *Saimiri*.

Either L1 activity is so low in *Ateles* that any active family will have too few copies to be discernible above the background of ancestral L1 elements or L1 is now extinct in this species. Demonstrating the extinction of L1 is not easy. Casavant et al. (2000) suggested that such a conclusion should satisfy three criteria: (1) no taxon-specific band observed by Southern blot; (2) no recently inserted elements isolated by methods designed to enrich for young elements; and (3) no genomewide pattern of L1 distribution observed by in situ hybridization. Using these criteria, these authors showed that L1 is probably extinct in a group of South American rodents (genus *Oryzomys*) (Casavant et al. 2000).

In our case, the second criterion is clearly met but not the first and the third. The reason could be that these criteria are more likely to be met by rapidly evolving rodents than by the far more slowly evolving primates (Li 1997). For instance, Fig. 4 reveals little, if any, hybridization by probe 1 to taxon-specific bands in *Saguinus* genomic DNA, although L1 has evolved and amplified in this species. Similarly, bands corresponding to old subfamilies are not going to vanish as rapidly as in rodents because their restriction sites will not degenerate as fast as in rodents (as shown for the L1nwm-old clade, probe 2 in Fig. 4) As L1 evolution requires L1 replication, it is not surprising that species-specific L1 clades arose in *Saguinus* and *Saimiri* but not in *Ateles*. In the former two species, L1 amplification (and thus replication) was robust compared to *Ateles*, where L1 amplification was very low. Indeed, the most recently active NWM L1 family is found in *Saimiri*, where the active L1 lineage has experienced the largest number of changes.

Rather than going extinct in *Ateles*, L1 may just be in a low-activity phase. Alternating episodes of high and low activity typify L1 evolution in murine rodents (Pascale et al. 1993; Verneau et al. 1998). Among other possibilities, cyclic repression of L1 activity and subsequent escape by a novel L1 family could explain the episodic nature of L1 amplification. The host could adapt to deleterious L1 activity by repressing it directly or indirectly (e.g., altering a host factor required for L1 replication). As L1 replication is error prone (Furano 2000), a low level of L1 replication could generate and maintain a repository of variants but not produce a discernible family, especially if numerous variants are contemporaneously active. Any variant that can bypass host repression (or regain effective interaction with putative essential host factors) could produce a full-blown L1 amplification.

Primate L1 evolution may be consistent with this model. First, at times, L1 activity has been seriously deleterious as judged by strong purifying selection against only full-length L1 elements (those capable of replication [Boissinot et al. 2001]). Presumably, the

deleterious effect of L1 was due to high L1 activity. Second, L1 concurrently underwent adaptive evolution. This suggests that the host responded to the deleterious L1 activity (Boissinot and Furano 2001). Both the purifying selection against L1 elements and their adaptive evolution greatly decreased in the African apes (Boissinot and Furano 2001). Interestingly, the accumulation of both L1 and SINE (thought to be retroposed by L1) elements also dramatically decreased about the time that African apes emerged 14–16 Mya (Consortium MGS 2002). As for the currently active human L1Pa1 family, it may be too early to judge whether this family represents a departure from the current phase of low L1 activity.

In NWMs a single major L1 lineage has evolved and is responsible for most of the recent L1 amplification. However, the common ancestor of the three NWM species contained another L1 lineage (L1nwm-old) that was also active and probably generated inserts after the three species diverged from each other, as indicated by the hybridization experiment (Fig. 4). L1nwm-old seems to have been active until at least 19 Mya. As the three NWM species diverged ~25 Mya, L1nwm-old and the major active lineage could have coexisted for at least 6 My and probably longer because the major lineage was clearly differentiated before the split among the three species. Thus, two active L1 lineages coexisted for several million years during the evolution of NWMs.

The persistence of more than one active L1 lineage for any length of time is unusual in mammals, although this conclusion is based on an admittedly small number of species. In humans only a single active lineage comprising five major families has persisted for at least the last 25 My (Boissinot et al. 2000; Smit et al. 1995). Although several differentiated subsets (subfamilies) within these families have been concurrently active, one ultimately attained replicative dominance (A.V. Furano, unpublished observations; Boissinot et al. 2001). The same situation occurred in old world rats and mice (murine rodents) (Furano 2000). However, as L1 families have not been nearly as well catalogued in murine rodents as in humans, this conclusion may need to be modified.

Nonetheless, until the present work, the only unequivocal exception to the single L1 lineage model was found in the new world rodent, *Peromyscus* (the deer mouse). Here two active lineages persisted and evolved for at least 10 My (Casavant et al. 1996). One possible explanation for the existence of a single L1 lineage is competition between active L1 elements for a limiting host factor(s) essential for L1 replication. As the competition would presumably be reduced during periods of low L1 activity, the latter condition could favor the coexistence of multiple active L1 lineages. In fact, multiple L1 lineages seem to typify nonmammalian species which do not support the



high level of L1 activity possible at times in mammals (Eickbush and Furano 2002).

*Acknowledgments.* The authors thank the Wuppertal Zoological Garden, the Leipzig Zoological Garden, and the Münster Zoological Garden for providing DNA samples.

## References

- Boissinot S, Furano AV (2001) Adaptive evolution in LINE-1 retrotransposons. *Mol Biol Evol* 18:2186–2194
- Boissinot S, Chevret P, Furano AV (2000) L1 (LINE-1) retrotransposon evolution and amplification in recent human history. *Mol Biol Evol* 17:915–928
- Boissinot S, Entezam A, Furano AV (2001) Selection against deleterious LINE-1-containing loci in the human lineage. *Mol Biol Evol* 18:926–935
- Buluwela L, Forster A, Boehm T, Rabbitts TH (1989) A rapid procedure for colony screening using nylon filters. *Nucleic Acids Res* 17:452
- Burton FH, Loeb DD, Voliva CF, Martin SL, Edgell MH, Hutchison III CA (1986) Conservation throughout mammalia and extensive protein-encoding capacity of the highly repeated DNA long interspersed sequence one. *J Mol Biol* 187:291–304
- Cabot EL, Angeletti B, Usdin K, Furano AV (1997) Rapid evolution of a young L1 (LINE-1) clade in recently speciated *Rattus* taxa. *J Mol Evol* 45:412–423
- Cantrell MA, Grahn RA, Scott L, Wichman HA (2000) Isolation of markers from recently transposed LINE-1 retrotransposons. *Biotechniques* 29:1310–1316
- Casavant NC, Sherman AN, Wichman HA (1996) Two persistent LINE-1 lineages in *Peromyscus* have unequal rates of evolution. *Genetics* 142:1289–1298
- Casavant NC, Scott L, Cantrell MA, Wiggins LE, Baker RJ, Wichman HA (2000) The end of the LINE?: Lack of recent L1 activity in a group of South American rodents. *Genetics* 154:1809–1817
- Clements AP, Singer MF (1998) The human LINE-1 reverse transcriptase: effect of deletions outside the common reverse transcriptase domain. *Nucleic Acids Res* 26:3528–3535
- Clough JE, Foster JA, Barnett M, Wichman HA (1996) Computer simulation of transposable element evolution: random template and strict master models. *J Mol Evol* 42:52–58
- Consortium IHGS (2001) Initial sequencing and analysis of the human genome. *Nature* 409:860–921
- Consortium MGS (2002) Initial sequencing and comparative analysis of the mouse genome. *Nature* 420:520–562
- DeBerardinis RJ, Kazazian HH Jr (1998) Full-length L1 elements have arisen recently in the same 1-kb region of the gorilla and human genomes. *J Mol Evol* 47:292–301
- Eickbush TH, Furano AV (2002) Fruit flies and humans respond differently to retrotransposons. *Curr Opin Genet Dev* 12:669–674
- Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author, Department of Genetics, University of Washington, Seattle
- Furano AV (2000) The biological properties and evolutionary dynamics of mammalian LINE-1 retrotransposons. *Prog Nucleic Acids Res Mol Biol* 64:255–294
- Furano AV, Hayward BE, Chevret P, Catzeffis F, Usdin K (1994) Amplification of the ancient murine Lx family of long interspersed repeated DNA occurred during the murine radiation. *J Mol Evol* 38:18–27
- Goodman M, Porter CA, Czelusniak J, Page SL, Schneider H, Shoshani J, Gunnell G, Groves CP (1998) Toward a phylogenetic classification primates based on DNA evidence complemented by fossil evidence. *Mol Phylogenet Evol* 9:585–598
- Hardies SC, Martin SL, Voliva CF, Hutchison III CA, Edgell MH (1986) An analysis of replacement and synonymous changes in the rodent L1 repeat family. *Mol Biol Evol* 3:109–125
- Kazazian HH Jr (1998) Mobile elements and disease. *Curr Opin Genet Dev* 8:343–350
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120
- Li W-H (1997) Molecular evolution. Sinauer Associates, Sunderland MA
- Mathews LM, Chi SY, Greenberg N, Ovchinnikov I, Swergold GD (2003) Large differences between LINE-1 amplification rates in the human and chimpanzee lineages. *Am J Hum Genet* 72:739–748
- Moran JV, Holmes SE, Naas TP, DeBerardinis RJ, Boeke JD, Kazazian HH Jr (1996) High frequency retrotransposition in cultured mammalian cells. *Cell* 87:917–927
- Ostertag EM, Kazazian HH Jr (2001) Biology of mammalian L1 retrotransposons. *Annu Rev Genet* 35:501–538
- Pascale E, Liu C, Valle E, Usdin K, Furano AV (1993) The evolution of long interspersed repeated DNA (L1, LINE 1) as revealed by the analysis of an ancient rodent L1 DNA family. *J Mol Evol* 36:9–20
- Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Smit AFA, Tóth G, Riggs AD, Jurka J (1995) Ancestral, mammalian-wide subfamilies of LINE-1 repetitive sequences. *J Mol Biol* 246:401–417
- Stanhope MJ, Tagle DA, Shivji MS, Hattori M, Sakaki Y, Slightom JL, Goodman M (1993) Multiple L1 progenitors in prosimian primates: Phylogenetic evidence from ORF1 sequences. *J Mol Evol* 37:179–189
- Verneau O, Catzeffis F, Furano AV (1997) Determination of the evolutionary relationships in *Rattus sensu lato* (Rodentia: Muridae) using L1 (LINE-1) amplification events. *J Mol Evol* 45:424–436
- Verneau O, Catzeffis F, Furano AV (1998) Determining and dating recent rodent speciation events by using L1 (LINE-1) retrotransposons. *Proc Natl Acad Sci USA* 95:11284–11289
- Voliva CF, Jahn CL, Comer MB, Hutchison III CA, Edgell MH (1983) The LIMd long interspersed repeat family in the mouse: Almost all examples are truncated at one end. *Nucleic Acids Res* 11:8847–8859