4-2016

Analysis of western lowland gorilla (Gorilla gorilla gorilla) specific Alu repeats

Thomas Beckstrom

Analysis of western lowland gorilla (*Gorilla gorilla gorilla*) specific Alu repeats

By

Thomas Beckstrom

Undergraduate honors thesis under the direction of

Dr. Mark A. Batzer

Department of Biological Sciences

Submitted to the LSU Roger Hadfield Ogden Honors College in partial fulfillment

of

the Upper Division Honors Program.

April 2016

Louisiana State University

& Agricultural and Mechanical College

Baton Rouge, Louisiana

Introduction

I. Alu Elements

Alu elements are a family of primate-specific SINEs (**S**hort **IN**terspersed **E**lements) of ~300 base pairs (bp) long and present in the genomes of all living primates [1-3]. *Alu* elements were derived from 7SL RNA, the RNA component of the signal recognition particle, in the common ancestor of all living primates [4]. In the past ~65 million years *Alu* elements have become widely distributed in primate genomes [1, 5]. *Alu* elements are now present at copy numbers of >1,000,000 in all surveyed great ape genomes [1] (Table S.1). Despite their high copy number the majority of *Alu* elements are genomic fossils, non-propagating relics passed down over millions of years after earlier periods of replicative activity [1, 6]. It is hypothesized that a relatively small number of "master" elements are responsible for the continued spread of all active subfamilies [7, 8].

As non-autonomous retrotransposons, *Alu* elements do not encode the enzymatic machinery necessary for self-propagation [1, 2]. This is accomplished by appropriating the replication machinery [2, 9] of a much larger, autonomous retrotransposon called LINE1 (L1) via a process termed target-primed reverse transcription (TPRT) [10-13].

II. Retrotransposons as Phylogenetic Markers

The effective use of SINEs as phylogenetic markers was first demonstrated in 1993 in a study seeking to resolve relationships between Pacific salmonid species [14]. Subsequent to this study, SINE-based phylogenetic methods have been applied across a wide range of species to determine evolutionary relationships [15, 16]. In particular, *Alu* elements have proven to be extremely useful tools for elucidating evolutionary relationships between primate species [1, 17]. The essentially homoplasy free presence of

an *Alu* element of the same subfamily at a given locus between two or more primate species is almost always definitive evidence of shared ancestry [18]. The possibility of confounding events is very small, and easily resolved by the sequencing and examining of the element in question [1, 18]. In the past 15 years *Alu*-based phylogenetic methods have been used with great success to resolve evolutionary relationships among the Tarsiers [19, 20], New World [21] and Old World monkeys [22-24], gibbons [25], lemurs [26, 27], and great apes [28].

In addition to phylogenetic applications *Alu* elements also function as effective markers for the study of population genetics via examination of polymorphic elements between members of the same species [2, 29, 30]. *Alu* elements are also linked to numerous genetic diseases, and the insertion of an element at an importune genomic location can have grave consequences for the individual involved [3, 31, 32]. Additionally, *Alu* elements are thought to be a causal factor in genomic instability [33-36].

III. Alu Subfamilies

Alu elements are classified in multiple major subfamilies and numerous smaller, derivative subfamilies based on specific sequence mutations [37-40]. All extant primates share older elements, while all primate lineages examined also have younger, lineage-specific subfamilies [41]. *Alu* subfamily evolution is parallel, not linear, and various subfamilies have been found to be actively retrotransposing at the same time in all primate genomes surveyed; each primate lineage thus possesses its own *Alu* subfamilies [1, 42, 43].

The *Alu*J subfamily is the most ancient *Alu* lineage, and was largely active from ~65 million years ago (mya) to ~55 mya, at which point *Alu*S evolved and supplanted *Alu*J as the predominant active subfamily [37, 41]. Due to the antiquity of the lineage, *Alu*J subfamilies are present in all extant primates, including Strepsirrhines [27, 44]. *Alu*S, on the other hand, evolved from *Alu*J after the Strepsirrhine-Haplorrhine divergence, and so is only found in New World and Old World primates [2, 37, 45]. The *Alu*Y subfamily subsequently evolved from *Alu*S in the Old World primate lineage, and remains the predominant active subfamily in catarrhines [1, 41, 45].

A number of *Alu*Y-derived subfamilies continue to be active in great apes [1], and polymorphic lineage-specific *Alu* elements have been well documented between existing human populations [2], indicating a continued activity level for these mobile elements. A rate of 1 new element in every ~20 live births has been proposed as the current rate of *Alu* element activity in the extant human population, but the large size of this population coupled with human generation time would make it very difficult for new elements to come to fixation outside of small population groups [46, 47]. Research into *Alu* element activity in Sumatran and Bornean orangutans has indicated a comparatively low-level of continued retrotransposition activity in these apes [48], suggesting some alteration of the propagation of *Alu* within this lineage [49].

IV. Western Lowland Gorilla

The western lowland gorilla (*Gorilla gorilla gorilla*), a subspecies of the western gorilla (*Gorilla gorilla*), is a critically endangered great ape endemic to the forests and lowland swamps of central Africa [50, 51]. Western lowland gorillas are gregarious, living in family groups comprised of a dominant male, multiple females, subadult males,

and juvenile offspring [52]. Western lowland gorillas are in danger of extinction due to human activity. Their wild population size is shrinking in the face of anthropogenic pressure and diseases such as Ebola [50]. Gorillas are a close evolutionary relative of humans and the *Pan* lineage of chimpanzees and bonobos, with the most widely accepted date for a common ancestor 6-9 mya [28, 53-55], though a date as early as 10 mya has been recently proposed [56].

The genome of "Kamilah", a female western lowland gorilla living at the San Diego Zoo, was initially assembled from 5.4 Gbp of capillary sequence and 166.8 Gbp of Illumina read pairs, and further refined using bacterial artificial chromosome (BAC) and fosmid end pair capillary technology [57]. This sequence is available from the Wellcome Trust-Sanger Institute.

Previous analyses of *Alu* elements in gorillas have been limited to analysis in the context of wider research projects [28, 58-61] and have not focused specifically on subfamily analysis. Here we examine the western lowland gorilla genome (build gorGor3.1) [57] to identify gorilla-specific *Alu*Y subfamilies and assess the activity levels, copy number, and age of these subfamilies. Our final analysis resulted in the identification of 1,075 *Gorilla* specific *Alu* element insertions.

Results and discussion

I. Computational examination of the western lowland gorilla genome

A total of 1,085,174 *Alu* elements were identified in the genome of the western lowland gorilla (Additional file 1). Of these, 286,801 were identified as belonging to the ancient *Alu*J subfamily, and 599,237 were identified as members of the *Alu*S subfamily.

57,427 elements were too degraded or incompletely sequenced to be assigned a subfamily designation by RepeatMasker, and were simply identified as "*Alu*". We identified 141,709 members of the *Alu*Y subfamily. This subfamily is of particular interest due to its relatively young age and known continued mobility in other great ape genomes [1, 62]. Approximately one-third (57,458) of these putative *Alu*Y elements were >250bp in length. Gorilla-specific elements were subsequently identified by comparison of orthologous loci in the genomes of human, common chimpanzee, and orangutan [63]. Putative unique, gorilla-specific *Alu*Y insertions were estimated at 4,127 copies. This number is similar (96.5%) to the 4,274 potentially gorilla-specific *Alu* elements identified using other approaches [58]. Individual examination demonstrated that the majority of our 4,127 loci were in fact shared insertions. These loci were manually examined for gorilla specificity using BLAT [64]. This manual examination excluded 2,858 loci from further analysis due to the presence of shared insertions missed by Lift Over (2,626 insertions) or the lack of orthologous flanking regions in the genomes of other species that preclude polymerase chain reaction (PCR) verification (232 insertions). This resulted in a total of 1,269 likely gorilla-specific *Alu* insertion loci for inclusion in subfamily structure analysis.

These 1,269 loci were analyzed for subfamily structure using the COSEG program. COSEG removed 194 probable gorilla-specific *Alu* insertions from the dataset due to the presence of truncations or deletions in diagnostic regions of the element, leaving 1,075 probable gorilla-specific *Alu* insertion loci for further analysis. COSEG then divided the loci into 10 subfamilies based on diagnostic mutations in the sequence of the individual *Alu* elements and provided subfamily consensus sequences (Figure 1). The

consensus sequences were then aligned with known human *Alu*Y subfamilies from the RepBase database of repetitive elements [65] (Figure 2). A gorilla-specific nomenclature system was created to designate subfamilies using the suffix "*Gorilla*" preceded by the subfamily affiliation based on a comparison to identified human subfamilies (e.g. "*Alu*Yc5a1_*Gorilla*"). Subfamilies were named in accordance with established practice for *Alu* subfamily nomenclature [41]. The first identified *Alu*Yc5-derived subfamily was, for example, designated *Alu*Yc5a3_*Gorilla*. The "a" denotes the fact that this is the first Yc5-derived subfamily identified. The "3" denotes the number of diagnostic mutations by which this gorilla-specific subfamily differs from the human *Alu*Yc5 consensus sequence [41]. Subfamily age estimates were calculated using the BEAST (**B**ayesian **E**volutionary **A**nalysis by **S**ampling **T**rees) program [66].

II. AluY subfamily activity in the western lowland gorilla genome

Computational and PCR analysis of the western lowland gorilla genome has identified 1,075 independent, gorilla-specific *Alu*Y insertion loci. Computational analysis of this dataset indicates the presence of 10 distinct subfamilies identifiable by the presence of diagnostic mutations specific to each lineage. The 1,075 elements identified in our study almost certainly do not represent the total number of *Alu*Y specific to western lowland gorilla genome. Any loci under our arbitrary length of >250 were excluded from our dataset. It is also likely that a number of *Alu*Y loci are located in portions of the genome where sequence data is incomplete; within repeat regions, for example. Additionally, some *Alu*Y loci were excluded when no orthologous genomic region was present in the species being used for comparison.

The largest newly identified gorilla-specific *Alu* subfamily was designated as *Alu*Y_*Gorilla*. This designation was established via computational evaluation and manual alignment of the 759 elements assigned to this subfamily. The consensus sequence for these elements was found to be 100% identical to the canonical *Alu*Y human consensus sequence (Figure 2). This subset of classic *Alu*Y elements continued to propagate in the *Gorilla* lineage after the divergence from the shared common ancestor with the *Homo-Pan* lineage. We assayed and verified a total of 135 loci from this subfamily via PCR (18%). The 43 elements belonging to the *Alu*Ya1_*Gorilla* subfamily differ from the *Alu*Y consensus sequence by one diagnostic mutation at nucleotide position 133. We assayed and verified via PCR 21 elements in this subfamily (49%). This sequence should not be confused with the *Homo-Pan Alu*Ya subfamily.

The *Alu*Ya1b4 subfamily is derived from *Alu*Ya1_*Gorilla* and is a small and very likely young subfamily of 13 elements that shared the diagnostic mutation at position 133 of Ya1 but has also accrued four additional diagnostic mutations. We assayed and verified via PCR seven elements in this subfamily (54%). A second identified *Alu*Y lineage in gorilla is the *Alu*Yc3_*Gorilla* subfamily. We assayed and verified via PCR 20 of the 69 elements in this subfamily (29%). The consensus sequence for the 69 members identified in this subfamily is a 100% match to the human *Alu*Yc3 subfamily consensus sequence (Figure 2).

Two additional gorilla-specific *Alu*Yc-derived subfamilies share the characteristic 12bp deletion at position 87-98 that is a hallmark of human *Alu*Yc5. These two subfamilies possess independent diagnostic mutations that make them distinct from the *Alu*Yc5 consensus sequence. These two subfamilies are designated as *Alu*Yc5a3_*Gorilla*

(55 elements identified) and *Alu*Yc5b2_*Gorilla* (46 elements identified). *Alu*Yc5a3_*Gorilla* has three additional diagnostic mutations differentiating it from the *Alu*Yc5 consensus as a mark of identification. In keeping with *Alu* subfamily naming convention this subfamily has thus been deemed "Yc5a3", "a" as the first Yc5-like subfamily identified in the gorilla genome and "3" for the three diagnostic mutations differentiating it from the canonical Yc5 consensus. We assayed and verified 27 members of this subfamily via PCR (49%). *Alu*Yc5b2 also shares the characteristic 12bp deletion of the human *Alu*Yc5, but has two independent diagnostic mutations (Figure 2). We assayed and verified via PCR 19 members of this subfamily (41%). It is probable that *Alu*Yc5a3_*Gorilla* and AluYc5b2_*Gorilla* derived from *Alu*Yc5 around the time of the *Homo*/*Pan*-*Gorilla* speciation event.

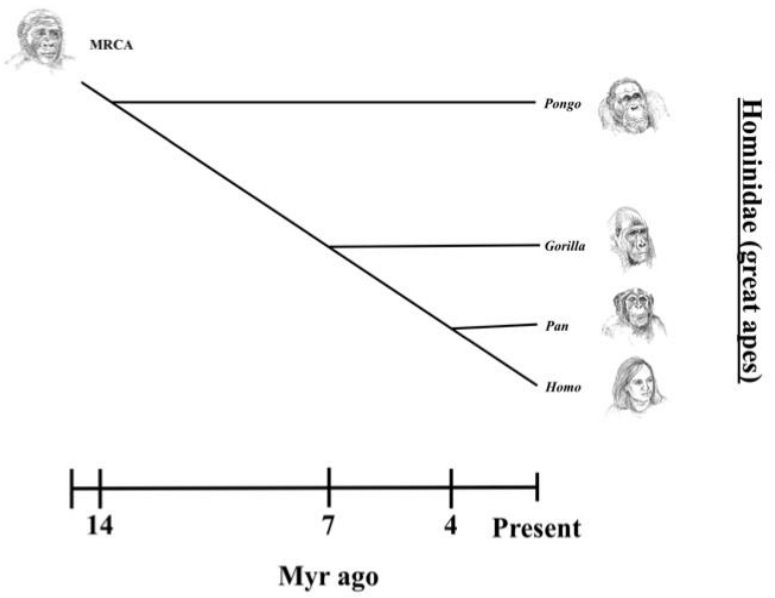
A third lineage nearly identical to human *Alu*Yb3a2 was identified as *Alu*Yb3a2b2_*Gorilla* (25 elements identified). This *Alu* subfamily contains two additional diagnostic mutations. Termed *Alu*Yb3a2b2_*Gorilla*, this lineage is an independent evolution in the *Gorilla gorilla gorilla* genome and not a derivative of the human-specific *Alu*Yb3a2. The *Alu*Yb lineage is human specific, meaning any identical or apparently derived *Alu* lineages in other primate genomes must be examples of independent evolution [67]. This is confirmed by the lack of orthologs at the same location in the human genome. We assayed and verified 14 members of this subfamily via PCR (56%). An additional subfamily present at only 17 copies and derived from *Alu*Yb3a2b2_*Gorilla* was identified and termed *Alu*Yb3a2b2a2_*Gorilla*, due to two diagnostic mutations separating these otherwise identical subfamilies. We assayed and verified via PCR nine elements in this subfamily (53%). The low copy-number of these

subfamilies coupled with their lack of impairing point mutations, even with the caveat that some subfamily members may have been overlooked, leads us to posit that they are among the youngest and potentially still active subfamilies in the western lowland gorilla genome.

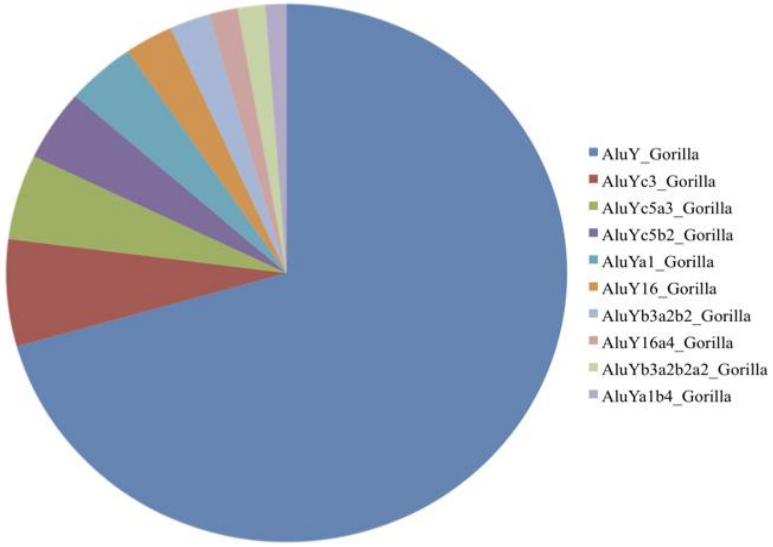
Two additional subfamilies were identified that, while clearly *Alu*Y derived, do not follow the consensus sequences of established subfamilies available via RepBase. The first, termed *Alu*Y16_*Gorilla* is identified clearly by the presence of an A-rich insert at position 219 followed by a 16bp deletion, and is present in 30 copies. We assayed and verified via PCR 10 members of this subfamily (33%). The second subfamily, apparently derived from the first and designated *Alu*Y16a4_*Gorilla*, is present in 18 copies and can be distinguished from *Alu*Y16_*Gorilla* by a 20bp deletion occurring after the A-rich region at position 219. 17 elements from this subfamily were assayed via PCR (94%), with 100% of these 17 being verified as gorilla-specific. One locus (gorGor3.1 chrX:74544052-74544324) lacked sufficient orthologous 5' sequence in non-gorilla outgroups to successfully design a working primer, but was included in the total count based on computational verification. The accumulation of non-diagnostic mutations in these two subfamilies may indicate that they are more ancient.

Approximately 25% of the 1,075 gorilla-specific *Alu*Y elements computationally identified in this study were verified by PCR, with the remaining ~75% verified by manual examination of computational data. It is important to note that we had no false positives in this study, and 100% of the elements computationally identified as gorilla-specific that were subsequently assayed via PCR were confirmed to be, in fact, gorilla-specific.

A.


MRCA
Pongo
Gorilla
Pan
Homo
Hominidae (great apes)
14
7
4
Present
Myr ago

B.


AluY_Gorilla
AluYc3_Gorilla
AluYc5a3_Gorilla
AluYc5b2_Gorilla
AluYa1_Gorilla
AluY16_Gorilla
AluYb3a2b2_Gorilla
AluY16a4_Gorilla
AluYb3a2b2a2_Gorilla
AluYa1b4_Gorilla

C.

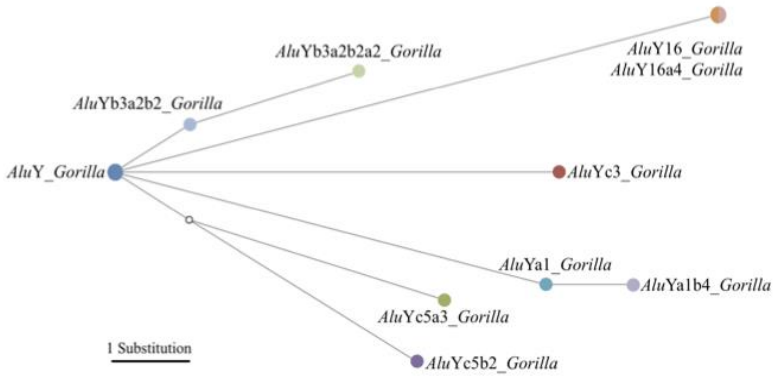

AluYb3a2b2a2_Gorilla
AluY16_Gorilla
AluY16a4_Gorilla
AluYb3a2b2_Gorilla
AluY_Gorilla
AluYc3_Gorilla
AluYa1_Gorilla
AluYa1b4_Gorilla
AluYc5a3_Gorilla
1 Substitution
AluYc5b2_Gorilla

Figure 1: Analysis of gorilla-specific *Alu* subfamilies.
A) A schematic diagram of a tree of evolutionary relationships of the four genera in Family Hominidae (great apes) based on divergence dates of 6-9mya for the *Gorilla-Homo/Pan* speciation event [28, 53-55] **B)** A pie chart showing a color-coded distribution of Gorilla-specific *Alu*Y subfamilies. *Alu*Y_*Gorilla* is the largest subfamily, representing slightly less than 3/4 of the total copy number identified **C)** A stepwise analysis of the relationships between Gorilla-specific *Alu*Y subfamilies generated from a Network analysis of the consensus sequences for each subfamily. The color of the dots representing each subfamily are correlated with the colors in the pie chart in Figure 1.B.

One means of identifying potential master elements [7] is to look for subfamily members with mutation-free polyA-tails [68]. A possible source element for the *Alu*Y_*Gorilla* subfamily, for instance, was identified on chrX:5135584-5135921, with a mutation-free 30bp polyA-tail and intact promoter region. A posited source element for the *Alu*Yc5b2 subfamily was identified on chr9:17925753-17926051, also with a mutation-free 30bp polyA-tail and intact promoter region.

*Alu*Y retrotransposition rates appear to be lower in the western lowland gorilla genome than in the human or chimpanzee genomes [69], while higher than that seen in the orangutan genome [48, 49]. Factors influencing rates of retrotransposition are myriad [1, 46]. Active retrotransposons are frequently polymorphic within a population, and are easily lost during events like speciation or population bottlenecks [70, 71]. The number of active elements, and the amplification rate of elements surviving such an event, can vary greatly and impact overall retrotransposition activity in the host genome.

A possible explanation for this lower activity level include inhibition of retrotransposition in the *Gorilla* lineage by the interaction of host factors such as members of the APOBEC family of proteins with the enzymatic machinery of L1 [1, 72]. Interference with L1 and *Alu* retrotransposition by APOBEC has been documented [72-74]. Analysis of the activity level of *Gorilla*-specific L1 elements could elucidate this,

but has not yet been done. Additionally, environmental stress factors may impact retrotransposition rates [75]. It is possible that one or a combination of these retrotransposition-inhibiting factors could be responsible for the lower level of *Alu*Y activity in the western lowland gorilla genome.

A median joining tree of relationships between gorilla-specific *Alu*Y subfamilies was generated from a stepwise alignment [76] using the Network program (Figure 1) [42, 77]. The tree generated supports the divergence of all gorilla-specific subfamilies from the *Alu*Y*_Gorilla* subfamily, and analysis of subfamily ages using BEAST places the date for this subfamily divergence at the stem of the *Gorilla* lineage. Initial divergence of gorilla-specific subfamilies occurred shortly after the speciation event separating the *Gorilla* lineage from the *Homo-Pan* lineage 6-9 mya [28, 53-55], and master elements have continued to produce copies of each subfamily at varying rates since [7].

III. Divergence dates of gorilla-specific AluY subfamilies

BEAST analysis of individual subfamily ages suggests no delay or change in transposon activity in western lowland gorilla following the divergence of the *Gorilla* and *Homo-Pan* lineages. The age of the gorilla-specific lineages ranges from 6.5-6.71 mya based on a baseline divergence of 7 mya for the most recent common ancestor of *Gorilla* and *Homo-Pan*. This indicates that all of the identified subfamilies originated around the time of the speciation event that separated these two lineages. This result is consistent with the ongoing propagation of these subfamilies before, during, and after the speciation event at a relatively constant rate. This indicates that the "master genes" [7] from which these subfamilies are derived already existed and were retrotranspositionally active prior to the aforementioned speciation event, and have remained active subsequently.

Examination of *Alu* elements indicates retrotranspositionally active elements are relatively rare, and that most *Alu* activity is the result of a small number of "master" copies engaging in retrotranspositional activity over time [7]. Our results suggest that the ten gorilla-specific *Alu*Y subfamilies identified in this study diverged and are still diverging from master elements already present in the genome of the common ancestor of the *Gorilla* and *Homo-Pan* lineages. A table listing each subfamily, the "master gene" or ancestral *Alu* subfamily from which it was likely derived, the % divergence from the consensus sequence of the master element, copy number, and suggested age of the most recent common ancestral element are available in the Additional files section of this paper as Additional file 3.

Materials and methods

I. Computational methodology

The genome of the Western lowland gorilla (*Gorilla gorilla gorilla*) was downloaded and analyzed for the presence of *Alu* elements using an in-house installation of the RepeatMasker program [62]. The *Gorilla gorilla gorilla* genome is available for download and analysis via the website of the Wellcome Trust-Sanger Institute [78]. The resulting dataset was parsed into separate files based on the *Alu* subfamily designations assigned by RepeatMasker. The file containing elements designated as members of the *Alu*Y subfamily was then further parsed to remove 84,251 elements under the length of 250bp using the estimation that shorter elements were likely to be older elements present in multiple species and therefore not useful for our analysis. The "Fetch Sequences" function from the online version of the Galaxy suite of programs [63, 79-81] was then used to retrieve the individual DNA sequence present at each of these loci using the

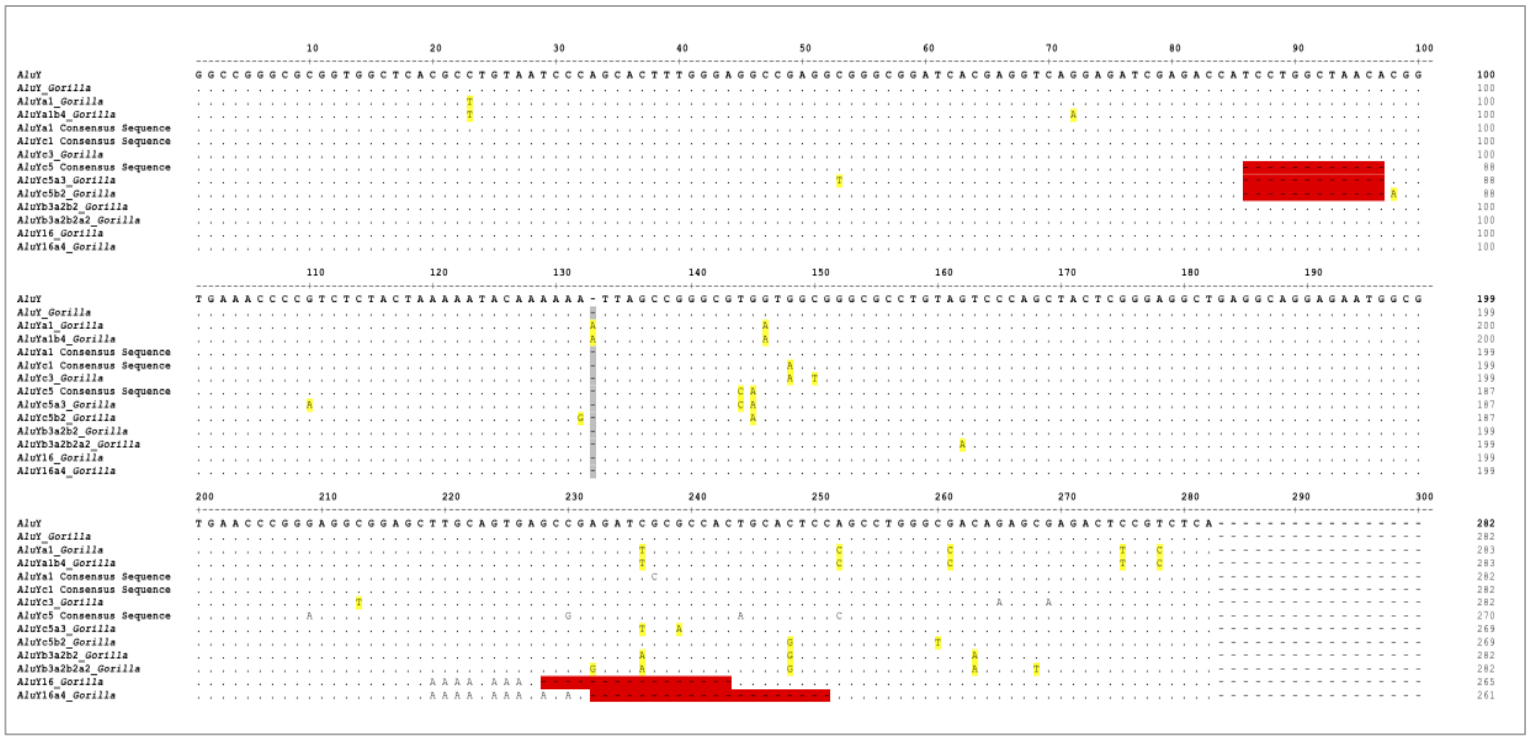


Figure 2: *Alu* sequence alignment. The consensus sequence for the *Alu*Y subfamily is shown at the top, with western lowland gorilla-specific *Alu* subfamilies listed below. The dots below the consensus denote the same base with insertions and deletions noted by dashes and mutations with the appropriate bases. The consensus sequences for the *Alu*Ya1, *Alu*Yc1, and *Alu*Yc5 subfamilies included for comparative purposes. Subfamily-specific diagnostic mutations are highlighted in yellow. Lineage-specific deletions are highlighted in red. *Alu*Y_*Gorilla* is 100% identical to the *Alu*Y consensus sequence. The shared 12-bp deletion identifying the *Alu*Yc5-derived *Gorilla* subfamilies is located at position 86. The 16-bp and 20-bp deletions identifying the *Alu*Y16_*Gorilla* and *Alu*Y16a4_*Gorilla* subfamilies are visible at position 228 and 232.

gorilla genome build gorGor3.1, and the Lift Over function was used to examine these loci for gorilla lineage specificity by comparison to the closely related genomes of human (*Homo sapiens*; hg19), chimpanzee (*Pan troglodytes*; panTro2), and Sumatran orangutan (*Pongo pygmaeus abelii*; ponAbe2). An additional 200 bp of flanking sequence on each side of the loci assayed was included in this analysis for validation of orthologous loci between the nine primate species considered in this study (Table 1).

Loci selected for verification were examined for further evidence of gorilla-specificity using the BLAST-Like Alignment Tool (BLAT) available at the University of

California at Santa Cruz (UCSC) Genome Browser website [82]. Putative gorilla-specific loci were compared to the available genomes of 3 other primate species, human (hg19), chimpanzee (panTro2), and orangutan (ponAbe2) [64, 83]. Elements found to be absent in these species and with sufficient orthologous flanking across species were marked for PCR primer design and experimental validation. Loci determined to be shared insertions, as well as those lacking sufficient orthologous flanking for effective primer design, were removed from further consideration [64].

The COSEG program [84], designed to identify repeat subfamilies using significant co-segregating mutations, was then run on the remaining putative gorilla-specific insertions to identify and group specific subfamilies together. COSEG ignores non-diagnostic mutations during analysis, providing an accurate representation of relationships between subfamilies of elements by ignoring potentially misleading mutational events [43]. COSEG uses a minimum subfamily size of 50 elements as the default setting. We arbitrarily defined subfamilies as groups of >10 elements to increase the detail of subfamily structure resolved. A subset of a minimum of 10% of each identified subfamily was then chosen for verification using locus-specific PCR, with a total of 279 loci assayed and verified (Figure 1).

A multi-species alignment comprised of the species listed above was created for each locus using BioEdit [85]. Oligonucleotide primers for the PCR assays were designed in shared regions flanking each putative gorilla-specific element chosen for experimental verification using the Primer3Plus program [86]. These primers were then tested computationally against available primate genomes using the *in-silico* PCR tool on the UCSC Genome Bioinformatics website [83].

Subfamily age estimates were calculated using the BEAST program [66, 87]. BEAST has previously been used to estimate dates of divergence using transposon data [88]. For each subclade, the consensus sequence for each subfamily was determined from the COSEG output [43]. The progenitor element was determined by RepeatMasker analysis of each consensus sequence. Elements were aligned using the SeaView software program and MUSCLE algorithm [76, 89]. The progenitor element was then used as an out-group to root the tree of each subclade. BEAST was calibrated with a baseline divergence date of 7 mya for the split between the *Gorilla* and *Homo-Pan* lineages. A divergence date of 7 mya is within the generally accepted 6-9 mya range for this divergence [28, 53-55]. BEAST was run with the following parameters: Site Heterogeneity= "gamma"; Clock= "strict clock"; Species Tree Prior= "birth death process"; Prior for Time of Most Recent Common Ancestor (tmrca)= "Normal distribution" with mean of 7.0 million years and 1.0 standard deviation"; ucld.mean= "uniform model" with initial rate set at 0.033; Length of Chain= "10,000,000"; All other parameters were left at default settings.

The Network program [90] was run on gorilla-specific *Alu*Y subfamily consensus sequences to generate a stepwise tree of relationships between identified subfamilies [42, 77]. The consensus sequences for the gorilla-specific *Alu*Y subfamilies were aligned using the MUSCLE algorithm [76] and converted to the .rdf file format using the DNAsp program [91]. The .rdf file was then imported to Network, and a median-joining analysis was run. The resulting output file demonstrating evolutionary relationships between subfamilies is presented in Figure 1.C.

The computational research performed in this study inspired the creation of a programmatic pipeline written in the Python programming language to analyze a large number of potential *Alu* insertions for species specificity and subsequently design locus specific PCR primers to verify species-specific insertions. The pipeline accepts *Alu* sequences in fasta format. To determine species specificity, sequences are first aligned to the genome of a closely related outgroup using a locally installed copy of the BLAST-like alignment tool (BLAT) [82]. The best scoring alignment above a cutoff score (~700) for each locus is used for specificity analysis. Gap sizes in the target and query sequence of each alignment are calculated. Loci displaying a gap pattern indicative of a species-specific insertion are then collected and aligned to the genomes of any additional outgroups to find the corresponding orthologous loci.

Specific insertions with intact orthologous loci in all outgroups are chosen for primer design. Sequences from all orthologous loci for each insertion are collected and aligned using MUSCLE [76] and masked with RepeatMasker. A base-to-base comparison of each alignment was performed to mark non-repeat regions of 100% similarity between all sequences for Primer3 input. Primer3 was used to generate primers in the acceptable regions using default parameters with the exception of "max-poly x" and "GC-clamp" which were set to 4 and 2, respectively. Primers were then verified by confirming the expected length and uniqueness of each amplicon using inSilico PCR tool of the Kent Utilities software package [86].

II. PCR and DNA sequencing

To verify gorilla-specificity, locus specific PCR was performed with a 9-species primate panel comprised of DNA samples from the following species: Western lowland

gorilla (*Gorilla gorilla gorilla*); Human HeLa (*Homo sapiens*); Common chimpanzee (*Pan troglodytes*); Bonobo (*Pan paniscus*); Bornean orangutan (*Pongo pygmaeus*); Sumatran orangutan (*Pongo abelii*); Northern white-cheeked gibbon (*Nomascus leucogenys*); Rhesus macaque (*Macaca mulatta*); African green monkey (*Chlorocebus aethiops*). Information on all DNA samples used in PCR analysis is listed in Table 1.

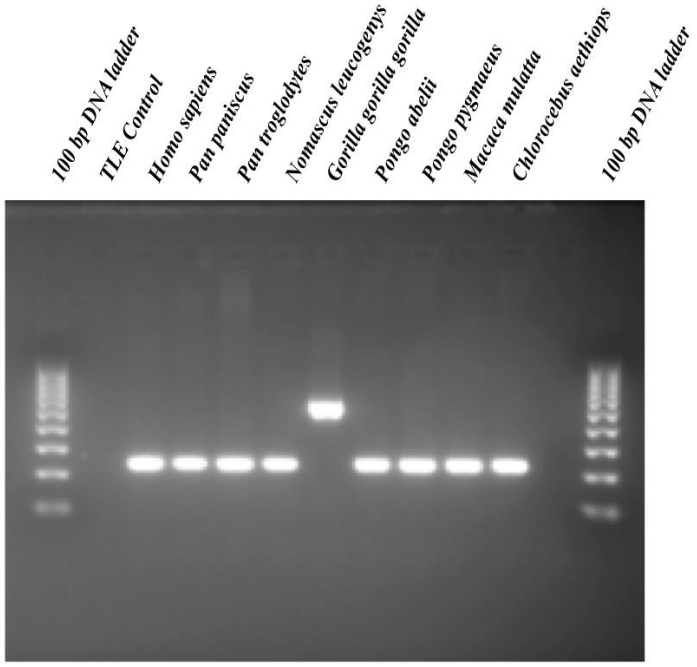


Figure 3: Phylogenetic assay of a western lowland gorilla-specific *Alu* insertion (Primer Pair Gor112). An agarose gel chromatograph of the gorilla specific *Alu* insertion Gor112. The filled site is approximately 550 bp (lane 7) and the empty site is 250 bp (lanes 3-6 & 8-11). Lanes (1) 100 bp DNA ladder; (2) negative control; (3) human; (4) bonobo; (5) common chimpanzee; (6) northern white-cheeked gibbon; (7) western lowland gorilla; (8) Sumatran orangutan; (9) Bornean orangutan; (10) Rhesus macaque; 11) green monkey; (12) empty; (13) 100 bp DNA ladder.

PCR amplification of each locus was performed in 25µl reactions using 15ng of template DNA, 200nM of each primer, 200µM dNTPs in 50mM KCl, 1.5mM $MgCl_2$, 10mM Tris-HCl (pH 8.4), and 2 units of *Taq* DNA polymerase. PCR reaction conditions were as follows: an initial denaturation at 95°C for 1 minute, followed by 32 cycles of denaturation at 95°C, annealing at the previously determined optimal annealing

temperature (60°C with some exceptions), and extension at 72°C for 30 seconds each, followed by a final extension of 72°C for 1 minute. PCR products were analyzed to confirm gorilla specificity of all loci on 2% agarose gels stained with 0.25ug ethidium bromide and visualized with UV fluorescence (Figure 3). A list of all 279 assayed loci, corresponding primer pairs, and optimal annealing temperatures for each are available as Additional File 2 in the Additional files for this study. Additionally, all PCR tested loci containing unidentified bases in the original sequence data were subjected to chain-termination sequencing to verify base pair composition [92]. Sequence data generated from this project for gorilla-specific *Alu*Y subfamilies has been deposited in GenBank under the accession numbers (KF668269-KF668278).

Conclusions and Future Work

*Alu*Y subfamily activity appears to be greatly reduced in the western lowland gorilla genome when compared to the human and chimpanzee genomes. The level of activity seen, while not as low as that observed in the genome of the orangutan, is consistent with a change in host surveillance or intrinsic retrotransposition capacity. *Alu* subfamily age estimates provide further support for the master gene model of *Alu* retrotransposition with a relatively low number of ancient lineages responsible for ongoing retrotranspositional activity. The 1,075 lineage specific *Alu*Y insertion loci and the ten subfamilies identified should provide future researchers with a rich source of genetic systems for conservation biology and evolutionary genetics.

Although it was not used extensively in this study, the computational pipeline for *Alu* analysis and DNA primer design could be used to reanalyze the entirety of the gorilla genome assembly to produce a more complete representation of *Gorilla*-specific *Alu* insertions. With the additional species-specific *Alu* insertions represented, a more

exhaustive subfamily analysis could be performed. Beyond the gorilla genome, the pipeline could be applied to additional primate genome assemblies as they become available, drastically reducing the time required to characterize *Alu* activity within a species and generate informative phylogenetic data.

Acknowledgements

I would like to thank Dr. Mark Batzer for guiding me in this project as my thesis director. His continuing support and mentorship over my four years at LSU have been invaluable to my undergraduate experience. I would also like to thank Dr. Adam McLain for introducing me to this project and teaching me the original computational methods used to analyze *Alu* insertions. Additionally, I would like to thank Miriam Konkel for her advice and support during my efforts in computational analysis for this study. I am grateful to Brygg Ulmer for his help in using the Melete computational cluster for testing my programmatic analyses. I thank Jerilyn Walker for her constant help and encouragement in learning the lab techniques used in this study. I extend my thanks to my fellow researchers, past and present, in the Batzer Laboratory, especially Glenn Carman, Will Gensler, Emily Bennett, Sreeja Sanampudi, and Hunter Strohmeyer. Additional thanks is given to the following people for their contributions to this research: Mitchell L. Fullerton, Thomas J. Meyer, Christopher Faulk, Scott Herke, and George Cook. I would like to thank the members of my thesis defense committee: Dr. Mark Batzer, Dr. Miriam Konkel, and Dr. Sophie Warny.

I thank the following organizations and people for contributing the DNA samples vital to this study: the American Type Culture Collection, The Coriell Institute for Medical Research, the Integrated Primate Biomaterials and Information Resource, and Dr. Lucia Carbone (http://carbonelab.com). This research was supported by National Institutes of Health Grant RO1 GM59290 (M.A.B.). Additionally, this research was supported in part by a grant to Louisiana State University from the Howard Hughes

Medical Institute through the Precollege and Undergraduate Science Education Program (T.O.B.).

References

1. Konkel MK, Walker JA, Batzer MA: **LINEs and SINEs of primate evolution.** *Evolutionary Anthropology* 2010, **19:**236-249.
2. Batzer MA, Deininger PL: **Alu repeats and human genomic diversity.** *Nat Rev Genet* 2002, **3:**370-379.
3. Cordaux R, Batzer MA: **The impact of retrotransposons on human genome evolution.** *Nat Rev Genet* 2009, **10:**691-703.
4. Ullu E, Tschudi C: **Alu sequences are processed 7SL RNA genes.** *Nature* 1984, **312:**171-172.
5. Roy-Engel AM, M.A. Batzer, P.L. Deininger: **Evolution of human retrosequences: Alu.** In *Encyclopedia of Life Sciences.* Chichester, United Kingdom: John Wiley & Sons, Ltd.; 2008
6. Cordaux R, Lee J, Dinoso L, Batzer MA: **Recently integrated Alu retrotransposons are essentially neutral residents of the human genome.** *Gene* 2006, **373:**138-144.
7. Deininger PL, Batzer MA, Hutchison CA, 3rd, Edgell MH: **Master genes in mammalian repetitive DNA amplification.** *Trends Genet* 1992, **8:**307-311.
8. Han K, Xing J, Wang H, Hedges DJ, Garber RK, Cordaux R, Batzer MA: **Under the genomic radar: the stealth model of Alu amplification.** *Genome Res* 2005, **15:**655-664.
9. Dewannieux M, Esnault C, Heidmann T: **LINE-mediated retrotransposition of marked Alu sequences.** *Nat Genet* 2003, **35:**41-48.
10. Luan DD, Korman MH, Jakubczak JL, Eickbush TH: **Reverse transcription of R2Bm RNA is primed by a nick at the chromosomal target site: a mechanism for non-LTR retrotransposition.** *Cell* 1993, **72:**595-605.
11. Luan DD, Eickbush TH: **RNA template requirements for target DNA-primed reverse transcription by the R2 retrotransposable element.** *Mol Cell Biol* 1995, **15:**3882-3891.
12. Cost GJ, Feng Q, Jacquier A, Boeke JD: **Human L1 element target-primed reverse transcription in vitro.** *EMBO J* 2002, **21:**5899-5910.
13. Feng Q, Moran JV, Kazazian HH, Jr., Boeke JD: **Human L1 retrotransposon encodes a conserved endonuclease required for retrotransposition.** *Cell* 1996, **87:**905-916.
14. Murata S, Takasaki N, Saitoh M, Okada N: **Determination of the phylogenetic relationships among Pacific salmonids by using short interspersed elements (SINEs) as temporal landmarks of evolution.** *Proc Natl Acad Sci U S A* 1993, **90:**6995-6999.
15. Shedlock AM, Okada N: **SINE insertions: powerful tools for molecular systematics.** *Bioessays* 2000, **22:**148-160.
16. Shedlock AM, Takahashi K, Okada N: **SINEs of speciation: tracking lineages with retroposons.** *Trends Ecol Evol* 2004, **19:**545-553.
17. Minghetti PP, Dugaiczyk A: **The emergence of new DNA repeats and the divergence of primates.** *Proc Natl Acad Sci U S A* 1993, **90:**1872-1876.
18. Ray DA, Xing J, Salem AH, Batzer MA: **SINEs of a nearly perfect character.** *Syst Biol* 2006, **55:**928-935.

19. Zietkiewicz E, Richer C, Labuda D: **Phylogenetic affinities of tarsier in the context of primate Alu repeats.** *Mol Phylogenet Evol* 1999, **11:**77-83.
20. Schmitz J, Ohme M, Zischler H: **SINE insertions in cladistic analyses and the phylogenetic affiliations of Tarsius bancanus to other primates.** *Genetics* 2001, **157:**777-784.
21. Ray DA, Xing J, Hedges DJ, Hall MA, Laborde ME, Anders BA, White BR, Stoilova N, Fowlkes JD, Landry KE, et al: **Alu insertion loci and platyrrhine primate phylogeny.** *Mol Phylogenet Evol* 2005, **35:**117-126.
22. Xing J, Wang H, Han K, Ray DA, Huang CH, Chemnick LG, Stewart CB, Disotell TR, Ryder OA, Batzer MA: **A mobile element based phylogeny of Old World monkeys.** *Mol Phylogenet Evol* 2005, **37:**872-880.
23. Xing J, Wang H, Zhang Y, Ray DA, Tosi AJ, Disotell TR, Batzer MA: **A mobile element-based evolutionary history of guenons (tribe Cercopithecini).** *BMC Biol* 2007, **5:**5.
24. Li J, Han K, Xing J, Kim HS, Rogers J, Ryder OA, Disotell T, Yue B, Batzer MA: **Phylogeny of the macaques (Cercopithecidae: Macaca) based on Alu elements.** *Gene* 2009, **448:**242-249.
25. Meyer TJ, McLain AT, Oldenburg JM, Faulk C, Bourgeois MG, Conlin EM, Mootnick AR, de Jong PJ, Roos C, Carbone L, Batzer MA: **An Alu-based phylogeny of gibbons (hylobatidae).** *Mol Biol Evol* 2012, **29:**3441-3450.
26. McLain AT, Meyer TJ, Faulk C, Herke SW, Oldenburg JM, Bourgeois MG, Abshire CF, Roos C, Batzer MA: **An alu-based phylogeny of lemurs (infraorder: Lemuriformes).** *PLoS One* 2012, **7:**e44035.
27. Roos C, Schmitz J, Zischler H: **Primate jumping genes elucidate strepsirrhine phylogeny.** *Proc Natl Acad Sci U S A* 2004, **101:**10650-10654.
28. Salem AH, Ray DA, Xing J, Callinan PA, Myers JS, Hedges DJ, Garber RK, Witherspoon DJ, Jorde LB, Batzer MA: **Alu elements and hominid phylogenetics.** *Proc Natl Acad Sci U S A* 2003, **100:**12787-12791.
29. Batzer MA, Stoneking M, Alegria-Hartman M, Bazan H, Kass DH, Shaikh TH, Novick GE, Ioannou PA, Scheer WD, Herrera RJ, et al.: **African origin of human-specific polymorphic Alu insertions.** *Proc Natl Acad Sci U S A* 1994, **91:**12288-12292.
30. Perna NT, Batzer MA, Deininger PL, Stoneking M: **Alu insertion polymorphism: a new type of marker for human population studies.** *Human biology* 1992, **64:**641-648.
31. Deininger PL, Batzer MA: **Alu repeats and human disease.** *Molecular genetics and metabolism* 1999, **67:**183-193.
32. Hancks DC, Kazazian HH, Jr.: **Active human retrotransposons: variation and disease.** *Curr Opin Genet Dev* 2012, **22:**191-203.
33. Cook GW, Konkel MK, Major JD, 3rd, Walker JA, Han K, Batzer MA: **Alu pair exclusions in the human genome.** *Mob DNA* 2011, **2:**10.
34. Hedges DJ, Deininger PL: **Inviting instability: Transposable elements, double-strand breaks, and the maintenance of genome integrity.** *Mutat Res* 2007, **616:**46-59.

35. Konkel MK, Batzer MA: **A mobile threat to genome stability: The impact of non-LTR retrotransposons upon the human genome.** *Semin Cancer Biol* 2010, **20:**211-221.
36. Cook GW, Konkel MK, Walker JA, Bourgeois MG, Fullerton ML, Fussell JT, Herbold HD, Batzer MA: **A comparison of 100 human genes using an alu element-based instability model.** *PLoS One* 2013, **8:**e65188.
37. Jurka J, Smith T: **A fundamental division in the Alu family of repeated sequences.** *Proc Natl Acad Sci U S A* 1988, **85:**4775-4778.
38. Slagel V, Flemington E, Traina-Dorge V, Bradshaw H, Deininger P: **Clustering and subfamily relationships of the Alu family in the human genome.** *Mol Biol Evol* 1987, **4:**19-29.
39. Willard C, Nguyen HT, Schmid CW: **Existence of at least three distinct Alu subfamilies.** *J Mol Evol* 1987, **26:**180-186.
40. Britten RJ, Baron WF, Stout DB, Davidson EH: **Sources and evolution of human Alu repeated sequences.** *Proc Natl Acad Sci U S A* 1988, **85:**4770-4774.
41. Batzer MA, Deininger PL, Hellmann-Blumberg U, Jurka J, Labuda D, Rubin CM, Schmid CW, Zietkiewicz E, Zuckerkandl E: **Standardized nomenclature for Alu repeats.** *J Mol Evol* 1996, **42:**3-6.
42. Cordaux R, Hedges DJ, Batzer MA: **Retrotransposition of Alu elements: how many sources?** *Trends Genet* 2004, **20:**464-467.
43. Price AL, Eskin E, Pevzner PA: **Whole-genome analysis of Alu repeat elements reveals complex evolutionary history.** *Genome Res* 2004, **14:**2245-2252.
44. Liu GE, Alkan C, Jiang L, Zhao S, Eichler EE: **Comparative analysis of Alu repeats in primate genomes.** *Genome Res* 2009, **19:**876-885.
45. Kapitonov V, Jurka J: **The age of Alu subfamilies.** *J Mol Evol* 1996, **42:**59-65.
46. Cordaux R, Hedges DJ, Herke SW, Batzer MA: **Estimating the retrotransposition rate of human Alu elements.** *Gene* 2006, **373:**134-137.
47. Xing J, Zhang Y, Han K, Salem AH, Sen SK, Huff CD, Zhou Q, Kirkness EF, Levy S, Batzer MA, Jorde LB: **Mobile elements create structural variation: analysis of a complete human genome.** *Genome Res* 2009, **19:**1516-1526.
48. Locke DP, Hillier LW, Warren WC, Worley KC, Nazareth LV, Muzny DM, Yang SP, Wang Z, Chinwalla AT, Minx P, et al: **Comparative and demographic analysis of orang-utan genomes.** *Nature* 2011, **469:**529-533.
49. Walker JA, Konkel MK, Ullmer B, Monceaux CP, Ryder OA, Hubley R, Smit AF, Batzer MA: **Orangutan Alu quiescence reveals possible source element: support for ancient backseat drivers.** *Mob DNA* 2012, **3:**8.
50. Strier KB: *Primate behavioral ecology.* 3rd ed. edn. Boston: Allyn and Bacon; 2007.
51. Fleagle JG: *Primate adaptation and evolution.* 2nd ed. edn. San Diego ; London: Academic Press; 1999.
52. Fleagle JG, Janson CH, Reed KE: *Primate communities.* Cambridge: Cambridge University Press; 1999.
53. Steiper ME, Young NM: **Primate molecular divergence dates.** *Mol Phylogenet Evol* 2006, **41:**384-394.
54. Glazko GV, Nei M: **Estimation of divergence times for major lineages of primate species.** *Mol Biol Evol* 2003, **20:**424-434.

55. Chen FC, Li WH: **Genomic divergences between humans and other hominoids and the effective population size of the common ancestor of humans and chimpanzees.** *Am J Hum Genet* 2001, **68:**444-456.
56. Langergraber KE, Prufer K, Rowney C, Boesch C, Crockford C, Fawcett K, Inoue E, Inoue-Muruyama M, Mitani JC, Muller MN, et al: **Generation times in wild chimpanzees and gorillas suggest earlier divergence times in great ape and human evolution.** *Proc Natl Acad Sci U S A* 2012, **109:**15716-15721.
57. Scally A, Dutheil JY, Hillier LW, Jordan GE, Goodhead I, Herrero J, Hobolth A, Lappalainen T, Mailund T, Marques-Bonet T, et al: **Insights into hominid evolution from the gorilla genome sequence.** *Nature* 2012, **483:**169-175.
58. Ventura M, Catacchio CR, Alkan C, Marques-Bonet T, Sajjadian S, Graves TA, Hormozdiari F, Navarro A, Malig M, Baker C, et al: **Gorilla genome structural variation reveals evolutionary parallelisms with chimpanzee.** *Genome Res* 2011, **21:**1640-1649.
59. Lee J, Han K, Meyer TJ, Kim HS, Batzer MA: **Chromosomal inversions between human and chimpanzee lineages caused by retrotransposons.** *PLoS One* 2008, **3:**e4047.
60. Sen SK, Han K, Wang J, Lee J, Wang H, Callinan PA, Dyer M, Cordaux R, Liang P, Batzer MA: **Human genomic deletions mediated by recombination between Alu elements.** *Am J Hum Genet* 2006, **79:**41-53.
61. Hormozdiari F, Konkel MK, Prado-Martinez J, Chiatante G, Herraez IH, Walker JA, Nelson B, Alkan C, Sudmant PH, Huddleston J, et al: **Rates and patterns of great ape retrotransposition.** *Proc Natl Acad Sci U S A* 2013, **110:**13457-13462.
62. **RepeatMasker Open-3.0** [http://www.repeatmasker.org]
63. Giardine B, Riemer C, Hardison RC, Burhans R, Elnitski L, Shah P, Zhang Y, Blankenberg D, Albert I, Taylor J, et al: **Galaxy: a platform for interactive large-scale genome analysis.** *Genome Res* 2005, **15:**1451-1455.
64. Kent WJ: **BLAT--the BLAST-like alignment tool.** *Genome Res* 2002, **12:**656-664.
65. Jurka J, Kapitonov VV, Pavlicek A, Klonowski P, Kohany O, Walichiewicz J: **Repbase Update, a database of eukaryotic repetitive elements.** *Cytogenet Genome Res* 2005, **110:**462-467.
66. Drummond AJ, Rambaut A: **BEAST: Bayesian evolutionary analysis by sampling trees.** *BMC Evol Biol* 2007, **7:**214.
67. Carter AB, Salem AH, Hedges DJ, Keegan CN, Kimball B, Walker JA, Watkins WS, Jorde LB, Batzer MA: **Genome-wide analysis of the human Alu Yb-lineage.** *Human genomics* 2004, **1:**167-178.
68. Roy-Engel AM, Salem AH, Oyeniran OO, Deininger L, Hedges DJ, Kilroy GE, Batzer MA, Deininger PL: **Active Alu element "A-tails": size does matter.** *Genome Res* 2002, **12:**1333-1344.
69. Hedges DJ, Callinan PA, Cordaux R, Xing J, Barnes E, Batzer MA: **Differential alu mobilization and polymorphism among the human and chimpanzee lineages.** *Genome Res* 2004, **14:**1068-1075.
70. Hedges DJ, Batzer MA: **From the margins of the genome: mobile elements shape primate evolution.** *Bioessays* 2005, **27:**785-794.

71. Belancio VP, Hedges DJ, Deininger P: **Mammalian non-LTR retrotransposons: for better or worse, in sickness and in health.** *Genome Res* 2008, **18:**343-358.
72. Schumann GG: **APOBEC3 proteins: major players in intracellular defence against LINE-1-mediated retrotransposition.** *Biochemical Society transactions* 2007, **35:**637-642.
73. Hulme AE, Bogerd HP, Cullen BR, Moran JV: **Selective inhibition of Alu retrotransposition by APOBEC3G.** *Gene* 2007, **390:**199-205.
74. Bogerd HP, Wiegand HL, Hulme AE, Garcia-Perez JL, O'Shea KS, Moran JV, Cullen BR: **Cellular inhibitors of long interspersed element 1 and Alu retrotransposition.** *Proc Natl Acad Sci U S A* 2006, **103:**8780-8785.
75. Farkash EA, Kao GD, Horman SR, Prak ET: **Gamma radiation increases endonuclease-dependent L1 retrotransposition in a cultured cell assay.** *Nucleic Acids Res* 2006, **34:**1196-1204.
76. Edgar RC: **MUSCLE: multiple sequence alignment with high accuracy and high throughput.** *Nucleic Acids Res* 2004, **32:**1792-1797.
77. Bandelt HJ, Forster P, Rohl A: **Median-joining networks for inferring intraspecific phylogenies.** *Mol Biol Evol* 1999, **16:**37-48.
78. **Wellcome Trust-Sanger Institute Gorilla Genome Homepage** [http://www.sanger.ac.uk/resources/downloads/gorilla/]
79. Blankenberg D, Von Kuster G, Coraor N, Ananda G, Lazarus R, Mangan M, Nekrutenko A, Taylor J: **Galaxy: a web-based genome analysis tool for experimentalists.** *Current protocols in molecular biology / edited by Frederick M Ausubel [et al]* 2010, **Chapter 19:**Unit 19 10 11-21.
80. Goecks J, Nekrutenko A, Taylor J: **Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences.** *Genome biology* 2010, **11:**R86.
81. **Galaxy** [http://galaxyproject.org]
82. **UCSC Genome Browser** [http://genome.ucsc.edu]
83. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D: **The human genome browser at UCSC.** *Genome Res* 2002, **12:**996-1006.
84. **COSEG** [http/:http://www.repeatmasker.org:COSEGDownload.html]
85. Hall TA: **BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.** *Nucleic Acids Symp Ser* 1999, **41:**95-98.
86. Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R, Leunissen JA: **Primer3Plus, an enhanced web interface to Primer3.** *Nucleic Acids Res* 2007, **35:**W71-74.
87. **BEAST** [http://beast.bio.ed.ac.uk]
88. Hellen EH, Brookfield JF: **The diversity of class II transposable elements in mammalian genomes has arisen from ancestral phylogenetic splits during ancient waves of proliferation through the genome.** *Mol Biol Evol* 2013, **30:**100-108.
89. Gouy M, Guindon S, Gascuel O: **SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building.** *Mol Biol Evol* 2010, **27:**221-224.

90. **NETWORK** [http://www.fluxus-engineering.com/sharenet.htm]
91. Librado P, Rozas J: **DnaSP v5: a software for comprehensive analysis of DNA polymorphism data.** *Bioinformatics* 2009, **25:**1451-1452.
92. Sanger F, Nicklen S, Coulson AR: **DNA sequencing with chain-terminating inhibitors.** *Proc Natl Acad Sci U S A* 1977, **74:**5463-5467.

Table 1: DNA sample data of all species examined in this study.

Taxonomic Name	Common Name	Origin	ID Number
Gorilla gorilla gorilla	Western lowland gorilla	Coriell[a]	AG05251
Homo sapiens	Human, HeLa	ATCC[b]	HeLa CCL-2
Pan troglodytes	Common Chimpanzee	IPBIR[c]	NS06006
Pan paniscus	Bonobo	IPBIR[c]	PR00661
Pongo pygmaeus	Bornean orangutan	Coriell[a]	AG05252A
Pongo abelii	Sumartran orangutan	Coriell[a]	GM06213A
Nomascus leucogenys	Northern white-cheeked gibbon	Carbone Lab[d]	NLL606
Macaca mulatta	Rhesus macaque	Coriell[a]	NG07098
Chlorocebus aethiops	African green monkey	ATCC[b]	CCL70

[a] Coriell Institute for Medical Research, 403 Haddon Avenue, Camden, NJ 08103, USA.
[b] From cell lines provided by American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108, USA.
[c] Integrated Primate Biomaterials and Information Resource (IPBIR), http://ccr.coriell.org/Sections/Collections/.
[d] Laboratory of Dr. Lucia Carbone, Oregon Health & Science University, Beaverton, Oregon, http://carbonelab.com/.

Additional Files

Additional file 1: Enumeration of *Alu* elements in ape genomes. The RepeatMasker program was run on the ape genomes currently available for download via Genbank. An in-house Perl script was then used to tally *Alu* elements by total copy number, and total copy number per each of the three major subfamilies.
Link: <https://biosci-batzerlab.biology.lsu.edu/supplementary_data/McLain_2013_additional_file_1.xlsx>

Additional file 2: All primer pairs used in this study listed with chromosomal location of the locus assayed and optimal annealing temperature.
Link: < https://biosci-batzerlab.biology.lsu.edu/supplementary_data/McLain_2013_additional_file_2.xlsx>

Additional file 3: Estimated age of gorilla-specific *Alu* subfamilies based on BEAST analysis. The BEAST program was run on each gorilla-specific subfamily with a baseline divergence age of 7 mya to determine the age of the subfamilies, the most likely progenitor or ancestral element, and the % divergence from the consensus sequence of the ancestral subfamily.
Link: < https://biosci-batzerlab.biology.lsu.edu/supplementary_data/McLain_2013_additional_file_3.xlsx>

Additional file 4: A complete listing of all 1075 verified gorilla-specific *Alu*Y insertions
Link: <https://biosci-batzerlab.biology.lsu.edu/supplementary_data/McLain_2013_additional_file_4.xlsx>