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# Gorilla genome structural variation reveals evolutionary parallelisms with chimpanzee

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**ABSTRACT**

Structural variation has played an important role in the evolutionary restructuring of human and great ape genomes. Recent analyses have suggested that the genomes of chimpanzee and human have been particularly enriched for this form of genetic variation. Here, we set out to assess the extent of structural variation in the gorilla lineage by generating 10-fold genomic sequence coverage from a western lowland gorilla and integrating these data into a physical and cytogenetic framework of structural variation. We discovered and validated over 7,665 structural changes within the gorilla lineage, including sequence resolution of inversions, deletions, duplications and mobile element insertions. A comparison with human and other ape genomes shows that the gorilla genome has been subjected to the highest rate of segmental duplication. We show that both the gorilla and chimpanzee genomes have experienced independent yet convergent patterns of structural mutation that have not occurred in humans, including the formation of subtelomeric heterochromatic caps, the hyperexpansion of segmental duplications, and bursts of retroviral integrations. Our analysis suggests that the chimpanzee and gorilla genomes are structurally more derived than either orangutan or human genomes.

## INTRODUCTION

The nature of the genetic differences between humans and other great apes has fascinated scientists since the discovery of DNA in the 1950s(Sarich and Wilson 1973; Yunis and Prakash 1982; Goodman et al. 1989). The genetic relationship and phylogeny of humans and great apes is well established based primarily on studies of single nucleotide variation(Koop et al. 1986; Enard and Paabo 2004). A surprising finding has been the extent of larger forms of structural variation among hominid genomes well below the limit of cytogenetic resolution(Locke et al. 2003; Fortna et al. 2004; Cheng et al. 2005; Bailey and Eichler 2006; Gibbs et al. 2007; Marques-Bonet et al. 2009a). Interestingly, the hominid genomes appear to be enriched with respect to structural variation but the extent to which this has impacted each of the major lineages is not yet completely known. To date, three hominid genomes have been sequenced and assembled to the working draft stage using capillary-based approaches (human(Consortium. 2001; Consortium. 2004), chimpanzee(Consortium. 2005) and orangutan(Locke et al. 2011)). Projects are underway to sequence additional apes including the bonobo, gorilla and gibbon. Many of these remaining ape genomes will be sequenced and assembled using a combination of next-generation sequencing and capillary whole-genome shotgun sequence data sets(Marques-Bonet et al. 2009b). Studies of structural variation, however, are complicated by difficulties in detecting and accurately resolving the sequence structure of these regions. In this study we set out to systematically investigate the pattern of structural variation in the gorilla genome combining capillary-based clone sequencing and next-generation genome sequencing in conjunction with detailed cytogenetic characterization and experimental validation. We present a comprehensive overview of inversions, deletions, segmental duplications and retrotranspositions within the gorilla genome. Comparisons with humans and other apes reveal that parallel and independent mutational processes have more dramatically restructured chimpanzee and gorilla genomes when compared to other hominid genomes.

## RESULTS

In order to investigate the gorilla's pattern of genome structural variation, we undertook a three-pronged approach. First, we tested 788 human BAC clones by Fluorescence *in situ* hybridization (FISH) comparing the probe order on human and gorilla chromosomal metaphases, thus, providing a refined cytogenetic framework of large-scale and intermediate-sized rearrangement events (**Supplementary Note**). Next, we completely end sequenced 176,880 BAC clones (http://www.genome.gov/Pages/Research/Sequencing/BACLibrary/primateProposal.pdf) from a gorilla BAC library (CH277) and mapped them to the human reference genome [NCBI build 35 (NCBI35)] to generate a clone-based framework of the gorilla genome(Eichler and DeJong 2002). This approach defined potential rearrangements based on discordant end-sequence placements. Last, we obtained blood DNA from Kwan, a middle-aged silverback gorilla, and generated 9.6-fold sequence coverage using massively parallel Illumina sequencing. While this sequence coverage means that each base is represented on average 9–10 times, the paired-end sequences flanking a portion of the insert that is not sequenced means a larger fraction of the genome is spanned by anchored matepairs (34-fold). These data were used to identify regions of copy number variation based on sequence read-depth and paired-end mapping revealing smaller forms of structural variation including mobile element insertions (>300 bp) using end-sequence profiling approaches(Tuzun et al. 2005; Hormozdiari et al. 2009; Hormozdiari et al. 2010a). The experimental and molecular data were integrated (**Table 1 and Supplementary Note**) allowing us to correctly reclassify events that particularly distinguished translocations from duplicative transposition events and inversions from segmental duplications (SDs). For example, translocations could be distinguished from duplicative transpositions because read-depth and array comparative genomic hybridization (arrayCGH) predicted copy number changes of a segment of DNA but with no evidence of chromosomal rearrangement using cytogenetic markers. In those cases where we were able to completely sequence the corresponding BAC clone, the breakpoints could be resolved at the single basepair level. We summarize the pattern of gorilla genome structural variation from the perspective of size and class and then compare our findings to human and other great ape genomes.

**Large-scale Rearrangements and Duplicative Transpositions.** Yunis and Prakash originally reported 11 large-scale cytogenetic differences between human and gorilla (8 pericentric inversions, 1 paracentric inversion, 1 translocation and 1 fusion)(Yunis and Prakash 1982). Using FISH and data from other studies (Dutrillaux 1980; Yunis and Prakash 1982; Montefalcone et al. 1999; Muller et al. 2000; Carbone et al. 2002; Eder et al. 2003; Locke et al. 2003; Misceo et al. 2003; Muller et al. 2003; Ventura et al. 2003; Ventura et al. 2004; Misceo et al. 2005; Cardone et al. 2006; Cardone et al. 2007; Stanyon et al. 2008), we refined all classical evolutionary breakpoints that distinguish the human and gorilla karyotypes (**Table S1**). It should be noted that cytogenetics typically only resolves Mbp level variation while FISH analyses utilizing overlapping probes can be used to resolve events ~50 kbp in size depending on the complexity of the region. Using gorilla BAC end sequence (BES) data mapped against the human genome (NCBI35), we identified 424 putative chromosomal rearrangements (**Table S2 and Supplementary Note**) including six of the eleven original classical rearrangements. The remaining five were confirmed by FISH but mapped to large and nearly identical SDs that could not be traversed by BES. We selected 14 representative BAC

clones corresponding to the classical gorilla-human breakpoints and completely sequenced them using capillary-based sequencing methods (**Table 2**). Detailed breakpoint analyses (**Figure 1, Figures S1–S5**) showed SDs (translocation 5;17 and inversion 7) or common repeat elements (*Alus* and LINEs) at or in close proximity to all breakpoints. Due to the abundance of these elements in the hominid genome and the small number of sequenced sites, this enrichment is not significant. In most cases, the repetitive sequences were not homologous, suggesting that mechanisms other than non-allelic homologous recombination were responsible for these evolutionary rearrangements. None of these rearrangements disrupted unique genes.

The BAC read-pair analysis predicted an unusually large number of putative inversions and translocations, which is inconsistent with previous chromosomal analyses and our own cytogenetic framework. We selected a subset of these events (6 putative translocations and 14 inversions) (**Table S3**) for further investigation. For each rearrangement, if the predicted translocations and inversions were *bona fide*, we would expect a change in the order of flanking probes (inversions) or a change in chromosomal location (translocation) when comparing human and gorilla. For each of these breakpoints, we selected gorilla BAC clones spanning the putative rearrangement breakpoints as well as gorilla BAC clones located distally and proximally to each breakpoint and tested their order between human and gorilla. In all cases, no change in the order of flanking unique sequences was observed. These FISH results suggested the presence of duplicated sequences at new locations in the gorilla genome.

We completely sequenced 20 of the corresponding BAC clones (3.2 Mbp of finished capillary-based sequence) to resolve the structure of these selected loci (**Table S3**). In each case, we confirmed duplicative transpositions and gorilla-specific juxtapositions of SDs as opposed to inversions and translocations. For example, BLAST sequence similarity searches of the sequenced gorilla BACs from chromosome 5 (Figure 1b, AC23944) indicate that this portion of the gorilla genome consists of a mosaic of four diverse SDs originating from different locations on human chromosome 5. The *Miropeats* analysis shows the extent of each duplicated segment (ranging in length from 9–86 kbp). Sequence read-depth analysis among the various species (whole-genome shotgun sequence detection (WSSD) tracks, see **Supplementary Note**) suggests that different segments have been duplicated at different time points during evolution. We conclude that this particular architecture is unique to gorilla originating from a series of duplicative transpositions to gorilla chromosome 5p13.2. We note that none of the 20 sequenced regions from BACs were collinear with the human genome. These regions carry, on average, three reconfigured or newly interspersed duplications with an average length of 29 kbp. We estimate 79% map interstitially within gorilla chromosomes with another 21% mapping to subtelomeric or pericentromeric regions. These data reveal extensive duplicative transposition in the gorilla genome creating a complex pattern of SDs unique to this lineage (see below).

**Deletions.** We initially detected 79 large deletions (>50 kbp) compared to human using a combination of interspecies arrayCGH, BES mapping, and depletion in sequence read-depth. Of these events, 89% (70/79) were confirmed experimentally by arrayCGH and/or FISH (**Table S4**). Based on human genome annotation, these regions contained 61 genes that were either completely (38) or partially (23) deleted in Kwan (**Table S4**). We examined 52 of these regions by FISH and found that 62% (32/52) of these apparent deletions correspond to regions of duplication in the human where the gorilla simply showed reduced copy number. Only 16 of the regions contained unique hominid genomic regions that had been completely deleted in the gorilla lineage (**Figure S6, Table S4**). In order to detect smaller deletions in the gorilla genome, we searched for clusters of discordant read pairs based on mapping gorilla sequence (**Supplementary Note**) against the human reference genome(Tuzun et al. 2005; Hormozdiari et al. 2009). We experimentally validated 1,820 deletion intervals (6.7 Mbp) using a customized microarray (**Supplementary Note**). This included 580 partial and 13 complete gene deletions (**Table S5, Supplementary Note**). Many of the completely deleted genes belong to well-known gene families including olfactory receptors (*OR10K1, OR5L2, OR5D16, OR1M1,* and *OR7G2*), keratin-associated proteins *(KRTAP13-3* and *KRTAP13-4*) and HLA genes (*HCP5* (HLA complex P5) and *HCG26* (HLA complex group 26)). One particularly intriguing deletion included SELV (selenoprotein V), thought to be important in the metabolism of dietary selenium implicated in cancer prevention, immune function, aging, male reproduction and other physiological processes(Kryukov et al. 2003). Among the partial gene deletions, 36 span more than a single exon—the largest being *DNAH14* with 45 deleted exons. In total, we discovered and validated 1,863 deletion intervals in the gorilla genome corresponding to 12.69 Mbp.

**Mobile Elements.** We initially excluded regions with more than 80% repeat content from our deletion analysis due to difficulties in validating these calls by arrayCGH. However, such deletions likely correspond to mobile element insertions in the human genome that occurred since the two lineages diverged. We specifically searched for the aforementioned events by identifying deletions where the corresponding locus in human was composed largely of a particular common repeat. We predicted 2,481 *Alu* (887 kbp), 1,861 L1 (5.59 Mbp), 663 SVA (1.17 Mbp), 524 LTR (1.27 Mbp), and 110 HERV (409 kbp) insertions in human when compared to gorilla(Hormozdiari et al. 2010b). A subset of these were full length, including 2,372 *Alu* (≥275 bp) and 564 L1 (≥5.8 kbp) elements. Of these mobile elements, 37% (2,066/5,639) mapped within 1,650 genes (**Table S6**). We performed the reciprocal analysis by searching for retrotranspositions specifically within the gorilla lineage. We detected the insertion breakpoints of various classes of active mobile elements (*Alu*, L1, SVA, and LTR) as well as nonhuman primate-specific endogenous retroviruses (PTERV1 and PTERV2)(Yohn et al. 2005) and predicted a total of 263 PTERV1, 4,272 *Alu*, 325 SVA, 113 LTR, and 299 full-length L1 insertions (**Table S7**). In order to estimate the rate of false positives, we tested 30 full-length gorilla *Alu* retrotransposons by PCR analysis of gorilla and human DNA (**Supplementary Note**). Of the events, 90% (27/30) validated as fixed insertions (**Figure S7**) with the remaining three being polymorphic. Consistent with recent analyses of human and other ape

genomes, these results predict an acceleration of SVA retrotransposition in the chimpanzee-human ancestral lineage with a more recent surge of *Alu* retrotransposons in the human branch (**Table 3**). While we found no evidence of PTERV2 integrations in gorilla, we did identify 263 full-length integrations of PTERV1—an endogenous retrovirus initially discovered in chimpanzee(Yohn et al. 2005). A comparison to a previously developed integration map of chimpanzee revealed that 99.6% of these integrations are non-orthologous, mapping to different locations in the gorilla and chimpanzee genomes (**Figure 2**). Both experimental and sequence analyses confirm that this mobile element is completely absent from human and orangutan genomes. This provides strong support that PTERV1 arose from an exogenous source that retrotransposed independently in both gorilla and chimpanzee lineages less than six million years ago.

**Segmental Duplications.** We developed an SD map of the gorilla genome based on detecting regions with excess sequence read-depth as described previously(Bailey et al. 2002; Alkan et al. 2009; Marques-Bonet et al. 2009a). We detected 99 Mbp of SDs (>20 kbp in length and >95% identity)(Cheng et al. 2005; Marques-Bonet et al. 2009a). We validated the duplications by interspecies arrayCGH, discovering 68 complete or partial gene duplications in the gorilla (**Supplementary Note**). Although most of the duplications are shared with other hominids (**Figure 3 and Supplementary Note**), we note an apparent excess of gorilla-specific duplications when compared to human, chimpanzee or orangutan. Comparing the SD maps of five primate genomes, we assigned shared and lineage-specific SDs (**Figure 3b**) and computed a genomic duplication rate along each branch under a maximum likelihood model, which assumes 20% homoplasy. The addition of gorilla duplication data into a maximum likelihood framework predicts a more significant ($p<5.6 \times 10^{-8}$) excess of SD in the human-African great ape ancestor(Marques-Bonet et al. 2009a) with an estimated rate 4- to 5-fold higher when compared to the human or chimpanzee branches. Surprisingly, the gorilla-specific branch is also significantly accelerated compared to the human (~2 to 4X), but less so when compared to the common ancestral branch of humans and chimpanzees.

This difference becomes more dramatic in the gorilla when adjusting for copy number (**Figure 3a**) indicating that several sequences have expanded more prominently. We tested and validated by FISH (**Table S8**) 11 gorilla-specific duplications showing the highest copy number increase (11–45 copies). Nine of these 11 regions contain genes completely and or partially expanded specifically in the gorilla lineage (e.g. *NDUFA8*—unknown function, *LRPAP1*—low density lipoprotein receptor-related protein, *DOK7*—downstream of tyrosine kinase 7, *HGF*—activator preproprotein, *LETM1*—leucine zipper-EF-hand containing transmembrane, and *FGFR3*—fibroblast growth factor receptor 3 isoform 2). Most of these expansions map to the termini of gorilla chromosomes indicating that the subtelomeric regions of gorilla have become increasingly complex as a result of duplicative transpositions (see above). In this regard, the most expanded gorilla-specific duplication (n = 45 copies) maps within 10 kbp of the evolutionary fusion point that led to the formation of human chromosome 2 (chr2: 114145970-114215607). We also compared

the copy number of shared duplications among humans, chimpanzees and gorillas searching for regions of hyperexpansion (>500 copies) in one lineage when compared to the other two (**Table S9**). Only in the gorilla and chimpanzee genomes were such hyperexpanded SDs identified with expansions of 1000–1500 copies mapping primarily to acrocentric, pericentromeric, subtelomeric and subterminal cap regions of African ape chromosomes (**Table S9**).

**Subterminal Caps.** One of the most striking karyotypic differences between humans and African apes is the presence of subterminal heterochromatic caps at the ends of ape chromosomes (**Figure 4a**). Evident by G banding(Yunis and Prakash 1982) and postdenaturation DAPI staining, these regions have been classified as subterminal heterochromatin found exclusively among gorillas (80/96 chromosomes), the common chimpanzee (*Pan troglodytes*) (42/96 chromosomes), and the pygmy chimpanzee (*Pan paniscus*) (42/96 chromosomes). They are thought to be composed primarily of a 32 bp satellite repeat sequence (pCht7/13 sequence) arrayed in tandem(Royle et al. 1994). In addition, it is known that the formation of the subterminal cap in chimpanzee was accompanied by the hyperexpansion of SDs, which map near the human chromosome 2 fusion point (113997859-114024033, NCBI35)(Fan et al. 2002; Cheng et al. 2005). In gorilla, we find no evidence of an association of chromosome 2 sequences with the heterochromatic caps, but rather our copy number and FISH analysis suggests that a segment of chromosome 10 (19557646-19564636, NCBI35) is one of the primary components of the gorilla cap (**Figure 4a**). To confirm these results, we selected three large-insert BAC clones corresponding to the cap regions of both gorilla and chimpanzee and subjected these to capillary-based sequence and assembly. The sequence analysis shows dramatic differences in the organization of heterochromatic caps between the two species. While both possess tracts of pCht satellite sequence ranging in size from 10–50 kbp, chromosome 2 SDs are predominate in the chimpanzee cap whereas chromosome 10 duplications define the cap organization in gorilla (**Figure 4b**). These duplications appear to have expanded in concert with the satellite sequence creating a higher-order tandem array structure of several hundred copies in each species. Since subterminal heterochromatic block have also been reported among the lesser apes (Wijayanto et al., 2005), we tested pCht satellite probes on gibbon metaphase chromosomes and observed no hybridization signal above background (data not shown). Combined these data strongly suggest that the heterochromatic caps have evolved independently in both chimpanzee and gorilla and possibly all ape species.

## DISCUSSION

Structural variation has been extensive and episodic during human-great ape evolution. In this study, we identified and validated over 7,665 (**Table 4**) structural variant events in the gorilla when compared to human. It is important to note that our analysis is based primarily on a single gorilla genome (Kwan). Consistent with other studies, we expect (10–30%) of these variants to be polymorphic (i.e. not fixed within the gorilla lineage) (Chen and Li 2001; Ebersberger et al. 2002; Marques-Bonet et al. 2009a). Nevertheless, we find that the gorilla branch shows a significant increase in the rate of SD when compared to human or

chimpanzee ($p<5.6 \times 10^{-7}$) being more similar to the rate predicted in the human-African ape ancestor. Notably, our estimated *Homo-Pan* ancestral rate of duplication appears higher than rates estimated for the chimpanzee and human terminal branches. In general, our data support a model where SD activity slowed after hominid speciation events in all lineages, with this deceleration being the least evident for gorilla. This slowdown is supported by the observation that the sequence identity spectrum of SDs in humans peaks at 99.2%(Bailey and Eichler 2006) and the finding of few large-scale SD differences between human and Neandertal, which separated less than 1 million years ago (Green et al., 2010). The basis for deceleration is unknown but it is possible that extensive differences in the SD architecture facilitated genetic isolation of emerging species during evolution(White 1978). We caution, however, that we cannot accurately estimate the time of such SDs with respect to hominid speciation events so such correlations remain speculative. Thus, a reasonable line of inquiry going forward will be to compare the extent of genetic diversity in an unbiased fashion within each great ape lineage and compare these to divergence estimates between species.

In this study, we document hundreds of duplicative transposition differences between human and gorilla that alter the structure of duplication blocks between the two lineages. Most of these structural differences are opaque to standard whole-genome shotgun sequence assembly methods or would be incorrectly classified without integration into a higher-level cytogenetic framework. Our structural variation analysis also suggests that the genomes of chimpanzee and gorilla have experienced several independent genomic rearrangements that did not occur during the evolution of the orangutan and human. The African ape genomes have been bombarded by retroviral integrations that entered the germline after the two lineages diverged. Neither orangutan nor human genomes carry these retroelements(Yohn et al. 2005) and the fact that fine mapping of the integration sites are largely non-orthologous argues for ancient parallel infections(Kaiser et al. 2007). Gorilla and chimpanzee have independently acquired subtelomeric heterochromatin caps, and this chromosome feature has been associated with the hyperexpansion of different SDs in the two lineages. Our molecular analyses suggest that these events occurred independently and in parallel early during the evolution of the *Pan* and *Gorilla* lineages adding many new Mbp of DNA that altered the chromosome and chromatin architecture of these two species compared to all other primates. We propose that the orangutan and human genomes represent the hominid archetype, while the African ape genomes are more structurally derived with respect to these properties.

## METHODS

**FISH.** BAC and human fosmids clones (n = 1022) were used as probes to develop a comparative cytogenetic framework and to test rearrangements specific to the gorilla lineage. Ancestral state determined based on comparison to other primate species. Metaphases from nonhuman primates were obtained from lymphoblastoid or fibroblast cell lines of the following species: common chimpanzee (*Pan troglodytes*, PTR); gorilla (*Gorilla gorilla*, GGO) and Borneo orangutan (*Pongo pygmaeus pygmaeus*, PPY) as representative of great apes; and rhesus monkey (*Macaca mulatta*, MMU, Cercopithecinae) as representative of Old World Monkeys. FISH experiments were essentially performed as previously described(Ventura et al. 2003).

**Gorilla Genome Sequencing.** Peripheral blood DNA was isolated from a male silverback gorilla, Kwan (Studbook #1107, b. 02/03/1989), housed at the Lincoln Park Zoo. Paired-end whole-genome sequence data were generated on an Illumina Genome Analyzer II using a modified protocol (see **Supplementary Note**). Sequence data has been deposited into the SRA under accession SRP002878.

**BAC Sequencing.** End sequences from a gorilla BAC library (CH277) were retrieved from the NCBI Trace Archive and mapped to the human reference genome (NCBI35) to identify and clone rearrangement breakpoints as described previously(Newman et al. 2005). A subset of clones were selected for complete insert sequencing using capillary sequencing methods(McPherson et al. 2001) in order to obtain high quality finished sequence within duplicated regions. Rearrangements were visualized using Miropeats(Parsons 1995) and previously described in-house visualization tools(Kidd et al. 2009).

**Structural Variation Discovery.** Gorilla sequence reads were aligned to the human reference genome using the mrFAST and mrsFAST mapping algorithms(Alkan et al. 2009; Hach et al. 2010). Deletions and mobile element insertions were detected using VariationHunter(Hormozdiari et al. 2009; Hormozdiari et al. 2010c) while SDs (>20 kbp) were detected and copy number quantified using measures of read-depth(Alkan et al. 2009) (see **Supplementary Note**).

**ArrayCGH.** We designed two oligonucleotide microarrays (n = 385,000) targeted to regions of gorilla deletions and duplications and performed cross-species arrayCGH as previously described (GEO accession number: GSE27072; samples: GSM665036, GSM665334, GSM665336, GSM665992, GSM665993, GSM667894, and GSM668114; and platforms GGO 2.1 custom: GPL11674 and Human 2.1 standard: GPL9684).

**PCR.** 30 PCR assays were designed to test the specificity and polymorphism of predicted *Alu* insertions in the gorilla genome. We only tested loci not embedded within other repetitive elements or SDs to facilitate reliable primer design.

## DATA ACCESS

Gorilla Sequence data (western lowland) has been deposited into the SRA under accession SRP002878.
We designed two oligonucleotide microarrays GEO accession number: GSE27072; samples: GSM665036, GSM665334, GSM665336, GSM665992, GSM665993, GSM667894, and GSM668114; and platforms GGO 2.1 custom: GPL11674 and Human 2.1 standard: GPL9684.
A subset of clones were selected for complete insert sequencing: AC243004.1, AC242656.3, AC242655.3, AC242627.3, AC242595.2, AC243002.1, AC241241.3, AC241522.2, AC240968.2, AC240954.2, AC239638.4, AC243003.2, AC243178.1, AC240953.2, AC239379.3, AC239356.2, AC239357.3, AC239280.2, AC239360.3, AC239282.3, AC239362.3, AC239380.3, AC239639.1, AC239363.3, AC239796.3, AC239381.3, AC239444.4, AC239382.3, AC239359.3, AC239358.3, AC239361.3 AC239281.3, AC239393.3, AC239640.2.

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**FIGURE LEGENDS**

**Figure 1. Sequencing of large-scale gorilla chromosome rearrangements.** (A) Sequence characterization of a gorilla inversion on chromosome 12(Egozcue and Chiarelli 1967; Miller et al. 1974). A schematic of the 42.5 Mbp inversion (*top panel* ideogram) is refined by paired-end BES and confirmed by FISH (*bottom right panel*). A gorilla BAC clone (CH277-205P14) corresponding to the long-arm breakpoint is completely sequenced (AC240968). Miropeats analysis(Parsons 1995) compares two regions on human chromosome 12 (*middle panel*; blue and orange) to the gorilla sequence. (Repeat elements, LINE, SINEs, LTRs, SDs, and genes are annotated based on the human genome.) ClustalW alignment (*bottom left panel*) pinpoints an *AluSx* element (purple) at the precise breakpoint. (B) Sequence characterization of a duplicative transposition region in gorilla. A region on chromosome 5p13 near the retinoic acid-induced 14 gene (*RAI14*) has acquired at least four SDs in the gorilla lineage (*top panel*). Sequencing of a gorilla BAC clone CH277-50D8, (AC239444.4) and miropeats analysis (*middle panel*) show SDs ranging in size from 10–28 kbp along chromosome 5. Read-depth analyses (WSSD tracks) predict that these gorilla duplicative transpositions are focused on a more ancient duplication carrying the spinal muscular atrophy type 4 gene (*PSMA4*) with flanking duplications becoming increasingly lineage-specific. FISH analysis with the clone as a probe (*lower panel*) shows multiple signals. Unique probes proximal and distal to the region indicate no change in the order between human and gorilla. Duplicative transpositions have occurred without a large-scale chromosomal rearrangement. (C) Duplicative transposition at translocation fusion point. Gorilla chromosome XVII arose as a lineage-specific fusion between chromosome 5 and 17(Stankiewicz et al. 2001) (*top panel*). Cloning and sequencing of the breakpoint (CH277-159N16, AC240953) show the presence of a >85 kbp complex gorilla-specific duplication block at the breakpoint. The duplication block is a mosaic composed of at least five distinct SDs originating primarily from chromosome 5 (see **Supplementary Note** for more detail).

**Figure 2. Endogenous retroviral integration map.** A comparison of chimpanzee (n = 275, blue) and gorilla (n = 265, red) PTERV1 sites of integration based on mapping to the human genome. None of the map positions in the two genomes are orthologous except one (indicated in green and corresponding to gorilla chr2: 143467521-143467682; chimpanzee chr2: 143467889-143468851). The endogenous retroviral element is absent in human and orangutan genomes and appears to have expanded largely independently in the two lineages after they diverged.

**Figure 3. Segmental duplication distributions.** (A) SDs (>20 kbp) were classified as lineage-specific or shared based on a three-way comparison of human, chimpanzee and gorilla genomes. The inclusion of gorilla suggests that most SDs are shared among humans and African apes but not with Asian apes(Marques-Bonet et al. 2009a) (**Supplementary Note**). Numbers are in Mbp; all SDs were validated by interspecies arrayCGH; *Mbp adjusted for copy number. (B) Using parsimony, we assigned the number of Mbp to different terminal and ancestral branches in the human-ape phylogeny. The copy-number-corrected

Mbp are shown (emboldened) and a calculated rate of Mbp/million years (in brackets) is estimated. A simple maximum-likelihood ratio test showed a dramatic SD burst in the African ape ancestor and in the common ancestor of humans and chimpanzees. The gorilla lineage-specific rate is greater than any other hominid.

**Figure 4. Subterminal heterochromatic cap architecture in chimpanzee and gorilla**. (A) Different FISH hybridization patterns using human fosmid probes (ABC8_40868200_C16 and ABC8_40925900_F12) corresponding to one hyperexpanded SD in gorilla (chr10: 19530349-19564732) and one in chimpanzee (chr2: 113978394-114020431). Extracted metaphase chromosomes (*top panel*) and cohybridization experiments (*lower panel*) in chimpanzee and gorilla reveal differences in the composition of the heterochromatic cap in each species. (B) Complete sequence analysis of three large-insert BAC clones sampled from gorilla and chimpanzee genomes confirm large-scale differences in the sequence organization. pCht satellite sequence (purple) interdigitates between different SDs (color bars) depending on the species. These SDs have expanded in copy from 500–1000 copies (Y-axis represents copy number count based on read-depth) in chimpanzee (blue) and gorilla (red). This architecture has emerged in a species-specific fashion in conjunction with the evolution of the subterminal heterochromatic satellite.

**Figure S1. Sequencing of large-scale gorilla chromosome rearrangements.** (A) Sequence characterization of a gorilla inversion on chromosome 7(Egozcue and Chiarelli 1967; Miller et al. 1974). A schematic of the inversion (*top panel* ideogram) is refined by paired-end BES and confirmed by FISH (*bottom right panel*). A gorilla BAC clone (CH277-325N15) corresponding to the long-arm breakpoint is completely sequenced (AC242656.3). Miropeats analysis(Parsons 1995) compares two regions on human chromosome 7 (*middle panel*; blue and orange) to the gorilla sequence. (Repeat elements, LINE, SINEs, LTRs, SDs, and genes are annotated based on the human genome.) ClustalW alignment (*bottom left panel*) defines the precise breakpoint in an SD-rich territory.

**Figure S2. Sequencing of large-scale gorilla chromosome rearrangements.** (A) Sequence characterization of a gorilla inversion on chromosome 8(Egozcue and Chiarelli 1967; Miller et al. 1974). A schematic of the inversion (*top panel* ideogram) is refined by paired-end BES and confirmed by FISH (*bottom right panel*). A gorilla BAC clone (CH277-481C13) corresponding to the long-arm breakpoint is completely sequenced (AC242627.3). Miropeats analysis(Parsons 1995) compares two regions on human chromosome 8 (*middle panel*; blue and orange) to the gorilla sequence. (Repeat elements, LINE, SINEs, LTRs, SDs, and genes are annotated based on the human genome.) ClustalW alignment (*bottom left panel*) pinpoints a L1P3 (LINE) element (red) at the precise breakpoint.

**Figure S3. Sequencing of large-scale gorilla chromosome rearrangements.** (A) Sequence characterization of a gorilla inversion on chromosome 10(Egozcue and Chiarelli 1967; Miller et al. 1974).

A schematic of the inversion (*top panel* ideogram) is refined by paired-end BES and confirmed by FISH (*bottom right panel*). A gorilla BAC clone (CH277-103D21) corresponding to the long-arm breakpoint is completely sequenced (AC241522.2). Miropeats analysis(Parsons 1995) compares two regions on human chromosome 10 (*middle panel*; blue and orange) to the gorilla sequence. (Repeat elements, LINE, SINEs, LTRs, SDs, and genes are annotated based on the human genome.) ClustalW alignment (*bottom left panel*) pinpoints L1m4 (LINE) (red) *AluSc* elements (blue) at the precise breakpoint.

**Figure S4. Sequencing of large-scale gorilla chromosome rearrangements.** (A) Sequence characterization of a gorilla pericentric inversion on chromosome 18(Egozcue and Chiarelli 1967; Miller et al. 1974). A schematic of the inversion (*top panel* ideogram) is refined by paired-end BES and confirmed by FISH (*bottom right panel*). A gorilla BAC clone (CH277-492f03) corresponding to the long-arm breakpoint is completely sequenced (AC243003.2). Miropeats analysis(Parsons 1995) compares two regions on human chromosome 18 (*middle panel*; blue and orange) to the gorilla sequence. (Repeat elements, LINE, SINEs, LTRs, SDs, and genes are annotated based on the human genome.) ClustalW alignment (*bottom left panel*) pinpoints an *AluSq* element (blue) at the precise breakpoint.

**Figure S5. Schematic representation of breakpoint inversions in gorilla genome.** Each inverted chromosome has been reported in the human (*left panel*) and gorilla (*right panel*) configuration, and repetitive elements flanking the breakpoints have been drawn by arrows defining the orientation of the found elements. Genomic locations of the breakpoints have been reported by vertical blue bars.

**Figure S6. Example of deletion in gorilla genome.** Deletion detected by read depth of coverage and array (A) and BAC end mapping (BEM) (B) in gorilla genome (green bar presents the supposed deleted region). FISH experiments show the heterozygous state of this deletion (C).

**Figure S7. *Alu* new insertion in gorilla genome.** Ten PCR validation sites for Kwan gorilla genome of the novel *Alu* integration sites (G, gorilla, ~450 bp) compared to human (H, human, ~150 bp). Marker 100 bp (by Biolabs) is displayed in the left lane.

# TABLES

**Table 1. Gorilla Sequence and FISH Resources**

| Resource | Technology | # Reads/Clones | Average read length | Average insert size (bp) | Data Release |
|---|---|---|---|---|---|
| WGS libraries | Illumina | 1,619,928,596 | 36 | 244 | SRA: SRP002878 |
| BAC clones | Sanger finished | 34 | 750 | 161,627 | GenBank Accessions (see Tables 2 and S3) |
| BAC end sequence (BES) | Sanger paired end | 353,761 | 776 | 160,447 | Trace Archive |
| BAC clones | FISH | 788 | NA | 170,000 | http://www.biologia.uniba.it/primates/ |

**Table 2. BAC Sequenced Chromosomal Breakpoints**

| Rearrangement | Breakpoint | Clone | Accession | Size (bp) | Location by BES | Human Mapping | Gorilla Mapping | Repeat elements at/near BP | Class |
|---|---|---|---|---|---|---|---|---|---|
| Inv chr7 | 7q arm | CH277-505P01 | AC243004.1 | 36298 | chr7:76489360-102048010 | 7q15; 7q22 | VIIq | Seg Dup | Inversion |
| | | CH277-325N15* | AC242656.3 | 95809 | chr7:76558315-102048039 | 7q15; 7q22 | VIIq | | |
| Inv chr8 | 8q arm | CH277-11L17 | AC242655.3 | 180892 | chr8:31006213-86005534 | 8p12; 8q21 | VIIIq | L1P3 (LINE) | Inversion |
| | | CH277-481C13 | AC242627.3 | 158706 | chr8:31148163-85832627 | 8p12; 8q21 | VIIIq | | |
| | | CH277-402K23 | AC242595.2 | 102724 | chr8:31148078-85914766 | 8p12; 8q21 | VIIIq | | |
| | | CH277-401D02 | AC243002.1 | 55127 | chr8:31287889-86049917 | 8p12; 8q21 | VIIIq | | |
| Inv chr10 | 10p arm | CH277-125A6* | AC241241.3 | 198809 | chr10:27573884-80780850 | 10p12 | Xp, Xq | L1m4 (LINE)/*AluSc* | Inversion |
| | | CH277-103D21* | AC241522.2 | 188246 | chr10:27675315-80631965 | 10p12 | Xp, Xq | | |
| Inv chr12 | 12q arm | CH277-205p14 | AC240968.2 | 146531 | chr12:21041953-63546183 | 12p12; 12q14 | XIIp | *AluSx*/L1M5 (LINE) | Inversion |
| | | CH277-242i19 | AC240954.2 | 116473 | chr12:21163982-63679105 | 12p12; 12q14 | XIIp | | |
| Inv chr18 | 18q arm | CH277-230I8 | AC239638.4 | 59640 | chr18:211564-16874356 | 18pter; 18q11 | XVIIIq | *AluSq2* | Inversion |
| | | CH277-492F03 | AC243003.2 | 36580 | chr18:177407-16836303 | 18pter; 18q11 | XVIIIq | | |
| | | CH277-545D07 | AC243178.1 | 187650 | chr18:287777-16800762 | 18pter; 18q11 | XVIIIq | | |
| t5:17 | 5q arm | CH277-159N16* | AC240953.2 | 169000 | chr5:79817957-80044726 | 5q14 | XVIIp | Seg Dup | Translocation |
| | | | | | chr17:16517994-16524015 | 17p11 | | | |

**Note:** All positions are relative to build35. *Clone duplicated in human, chimpanzee, gorilla and orangutan. Bp, breakpoint

**Table 3. Mobile Element Comparison among Hominid Genomes**

| | Human (1) | Human (2) | Chimpanzee | Gorilla | Orangutan |
|---|---|---|---|---|---|
| ***Alu*** | 584 | 7082 | 2340 | 4272 | 250 |
| **L1** | 52 | 1814 | 1979 | 299 | 5000 |
| **SVA** | 14 | 970 | 400 | 325 | 1800 |
| **PTERV** | ND | ND | 275 | 263 | ND |

**Note:** Human mobile elements (Venter vs. reference genome) (1) (Xing et al., 2009); human-specific mobile elements compared to chimpanzee (2) (The Chimpanzee Sequencing and Analysis Consortium 2005); gorilla-specific mobile elements detected by *VariationHunter* (see text for details); orangutan-specific elements (in press). Mobile element prediction is based on comparison to human genome (NCBI35). ND, not detected

**Table 4. Summary of Gorilla Genome Structural Variation**

| SV Type | | # intervals | # basepairs |
|---|---|---|---|
| **Duplications** | | | |
| | All | 1,258 | 87,103,094 |
| | GGO specific | 88 | 6,813,344 |
| **Deletions** | | | |
| | >=50 kbp | 44 | 6,298,067 |
| | <50 kbp | 1,820 | 6,290,005 |
| **Mobile elements*** | | | |
| | *Alu* | 4,274 | 1,325,597 |
| | L1 | 299 | 872,642 |
| | LTR | 123 | 125,879 |
| | SVA | 325 | 450,450 |
| | PTERV1 | 263 | 2,033,779 |
| **Large chromosomal rearrangements** | | | |
| | fusion | 1 | *NA* |
| | inversions | **9 | *NA* |
| | translocation | 1 | *NA* |
| | duplicative transpositions | ***418 | *NA* |

**Note:** Alu calls include FRAM element (2), LTR includes THE1 (11). # of basepairs are estimated with the length of the consensus sequence of the predicted retrotransposon subclass. *only full-length sequence considered. **5/9 detected by BEM. ***47/424 validated by FISH with 20/47 validated by sequence (Table S3).

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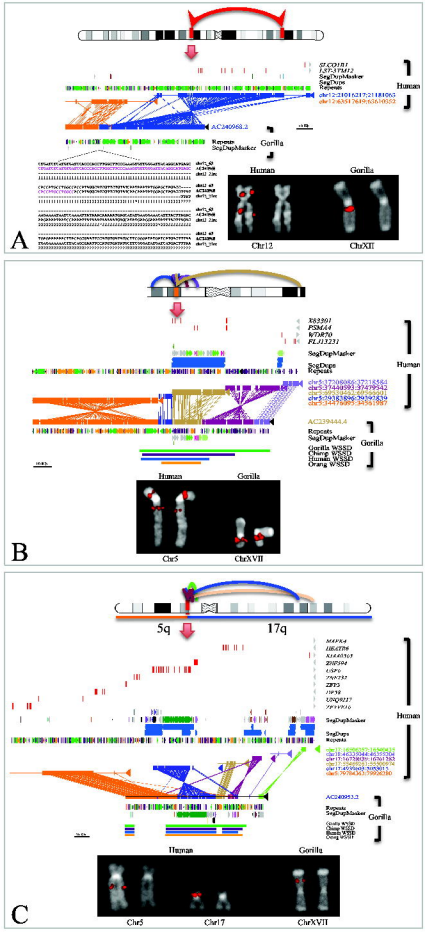
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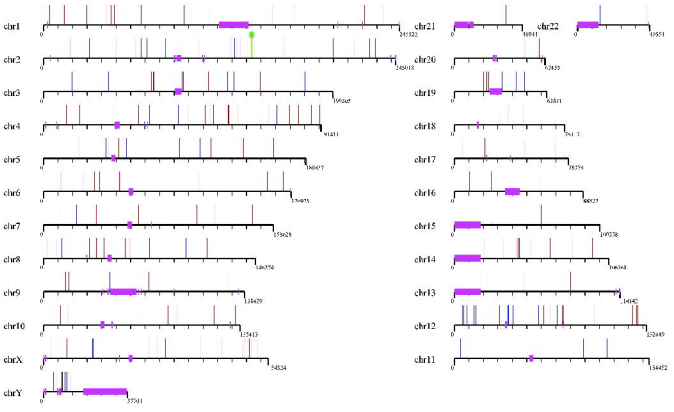
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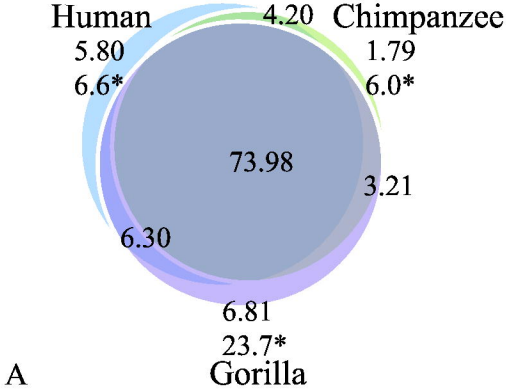
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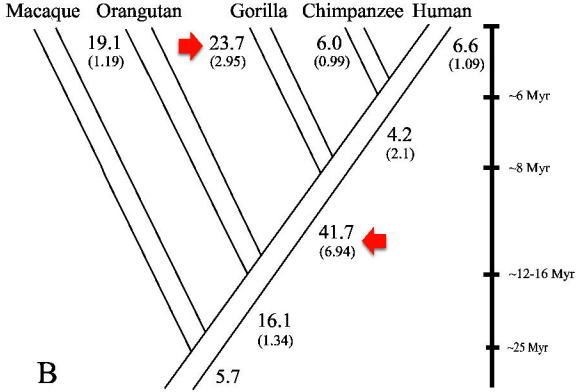
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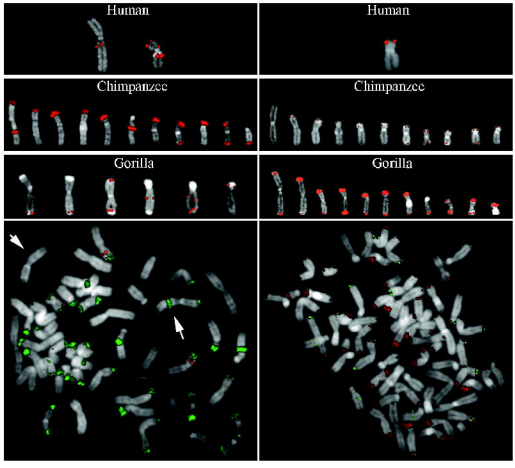
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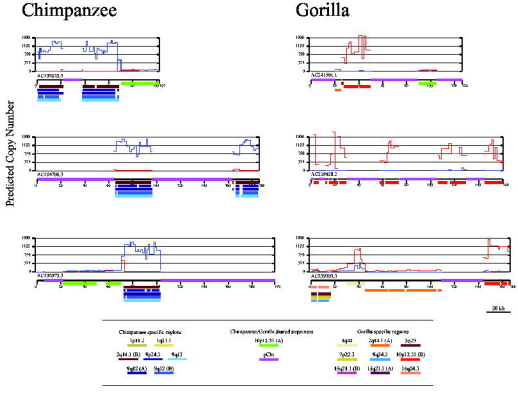
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