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Gibbon genome and the fast karyotype evolution of small apes

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Abstract

Gibbons are small arboreal apes that display an accelerated rate of evolutionary chromosomal rearrangement and occupy a key node in the primate phylogeny between Old World monkeys and great apes. Here we present the assembly and analysis of a northern white-cheeked gibbon (*Nomascus leucogenys*) genome. We describe the propensity for a gibbon-specific retrotransposon (LAVA) to insert into chromosome segregation genes and alter transcription by providing a premature termination site, suggesting a possible molecular mechanism for the genome plasticity of the gibbon lineage. We further show that the gibbon genera (*Nomascus*, *Hylobates*, *Hoolock* and *Symphalangus*) experienced a near-instantaneous radiation ~5 million years ago, coincident with major geographical changes in Southeast Asia that caused cycles of habitat compression and expansion. Finally, we identify signatures of positive selection in genes important for forelimb development (*TBX5*) and connective tissues (*COL1A1*) that may have been involved in the adaptation of gibbons to their arboreal habitat.

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Author information. The *N. leucogenys* WGS project has been deposited in GenBank under the project accession ADFV00000000.1. All short-read data have been deposited into the Short Read Archive (http://www.ncbi.nlm.nih.gov/sra) under the accession SRP043117. E.E.E. is on the scientific advisory board (SAB) of DNAnexus, Inc. and was an SAB member of Pacific Biosciences, Inc. (2009-2013) and SynapDx Corp. (2011-2013).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Gibbons (Hylobatidae) are critically endangered[1] small apes that inhabit the tropical forests of Southeast Asia (Fig. 1) and belong to the superfamily Hominoidea along with great apes and humans. In the primate phylogeny, gibbons diverged between Old World monkeys and great apes, providing a unique perspective from which to study the origins of hominoid characteristics.

Gibbons have several distinctive traits, the most striking of which is the unusually high number of large-scale chromosomal rearrangements in comparison to the inferred ancestral ape karyotype[2]. The four gibbon genera (*Nomascus*, *Hylobates*, *Hoolock*, and *Symphalangus*) occupy different regions of Southeast Asia and bear distinctive karyotypes, with diploid chromosome numbers ranging from 38 to 52 (Fig. 1). Given the relatively recent differentiation of these genera (4-6 million years ago (mya)), this constitutes an extraordinary rate of karyotype change.

In order to investigate the mechanisms behind the plasticity of the gibbon genome, understand the evolutionary relationships among the four extant gibbon genera, and study the evolution of putatively functional sequences related to gibbon-specific adaptations, we sequenced and assembled the genome of a female northern white-cheeked gibbon (*Nomascus leucogenys*) named ‘Asia’. The reference assembly (Nleu1.0) provides on average 5.7-fold Sanger read coverage over 2.9 gigabase pairs (Gbp) (Table 1) (Table ST1.1). Our quality assessment (EDF 1) confirmed its equivalence to other Sanger sequence-based non-human primate draft assemblies (e.g., orangutan, rhesus[3,4]) (Supplementary Information S1, Supplementary Files 1-2). We also obtained ~15x whole-genome shotgun (WGS) short-read data (Illumina) for two individuals of each gibbon genus and high-coverage exome data (>60X) for two of the same individuals in order to derive error models for single nucleotide polymorphism (SNP) calls (Supplementary Information S2; Tables ST2.1-3). the gibbon genome was especially evident when human-gibbon chromosome alignments were compared with those between human and great apes, rhesus macaque (Old World monkey), and marmoset (New World monkey) (Fig. 2a). Interestingly, this higher rate of reshuffling applied only to large-scale chromosomal rearrangements (>10 Mbp), while smaller scale rearrangements (10-100 kbp) were comparable with other species (Fig. 2b) (Supplementary Information S1).

We identified 96 gibbon-human synteny breakpoints in Nleu1.0 and classified them as to whether they could be defined at the base-pair level (Class I, N=42) or only narrowed to an interval due to greater complexity (Class II, N=54). As previously reported[5], breakpoints were significantly depleted of genes (Fig. SF5.2 and Supplementary File 3) and breakpoint intervals contained a mixture of repetitive sequences that inserted exclusively into the gibbon genome[2,5,6] (Fig. 2c). To assess breakpoint segmental duplication (SD) content, we identified gibbon-specific SDs using *in silico* methods followed by experimental validation (EDF 2) (Fig. SF3.1, Supplementary Information S3 and File 4). Of note, both gibbon-specific SDs and gene family expansion analyses suggested the gibbon genome has not undergone a greater rate of duplication than other hominoids, further supporting a model in which accelerated evolution has been limited to gross chromosomal rearrangements (Supplementary Information S6; Fig. SF6.1).

SD enrichment was the best predictor of gibbon-human synteny breakpoints, as shown through permutation analyses (p-value <0.0001); however, breakpoints were also enriched for *Alu* elements (Table ST5.1; Supplementary Information S5; Fig. SF5.2). While non-allelic homologous recombination (NAHR) between highly similar sequences can mediate large-scale rearrangements[7], the majority of gibbon chromosomal breakpoints bore signatures of non-homology based mechanisms (Fig. 2c). These included the insertion of non-templated sequences (2-51 nt) and/or the absence of identity, suggesting non-homologous end joining (NHEJ). The presence of micro-homologies (2-26 nt) in a small portion of the breakpoints (13/42) pointed to additional alternative mechanisms such as microhomology-mediated end joining (MMEJ)[8] or microhomology-mediated break-induced replication (MMBIR)[9]. The origin of the complex breakpoint interval structures was less obvious and reinforced the observation that breakpoints tend to be receptacles for repeats.

To explore the possibility that chromatin conformation, rather than sequence, might predispose regions to breakage, we investigated the relationship between gibbon breakpoints and CCCTC-binding factor (CTCF), an evolutionarily conserved protein with multiple functions, including mediating intra-and interchromosomal interactions[10]. We therefore performed chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) of CTCF-bound DNA using lymphoblast cell lines established from eight gibbon individuals (Supplementary Information S5). We observed an enrichment of gibbon-human breakpoints in CTCF-binding events (p-value = 0.0028), heightened when we considered a ~20 kbp window centered around each breakpoint (p-value of <0.0001). Notably, this enrichment was maintained only for CTCF-binding events shared with other primates (human, orangutan and rhesus macaque)[11] but not those specific to gibbon (p-value=0.0019) (Fig. SF5.4).

Thus, gibbon-human breakpoints co-localized with distinct genomic features and epigenetic marks; however, since many of these features were shared with other primates, other factors unique to the gibbon lineage must have been present to trigger the increased frequency of chromosomal rearrangements.

LAVA insertions in the gibbon genome

The gibbon genome contains all previously described classes of transposable elements that are mostly shared with the other primates. One exceptional addition is the LAVA element, a novel retrotransposon that emerged exclusively in gibbons[12] and has a composite structure comprised of portions of other repeats (3’- L1-*Alu*S-VNTR-*Alu*$_{\text{-like}}$ -5’) (Fig. 3a). Searches of Nleu1.0 retrieved 1,797 LAVA insertions, 1,256 of which were 3’-intact elements, many carrying signs of target-primed reverse transcription (TPRT)[13]. The distribution of 3’-intact LAVA elements uncovered a significant overlap with genes (Pearson chi-squared, p=0.017) and Gene Ontology (GO) analyses using the Database for Annotation, Visualization, and Integrated Discovery (DAVID)[14] showed a significant functional enrichment exclusive to the ‘microtubule cytoskeleton’ category (FDR=0.031, p-value=0.001) (Supplementary Information S7 and File 6) (EDF 3). Additional analyses with meta-pathway database tools[15-16] refined this enrichment to pathways related to chromosome segregation, including ‘establishment of sister chromatid cohesion’ and ‘mitotic metaphase and anaphase’ (Table

ST7.3). Genes with LAVA insertions include proteins that function as checkpoints for cell division and for spindle integrity/architecture (e.g., MAP4, CEP164, BUB1B)[17-19], participate in kinetochore assembly and attachment to the spindle (e.g., MAD1L1, CLASP2)[20,21], and play a role in chromosome segregation during cell division (e.g., KIFAP3, KIF27)[22] (EDT 1).

Intragenic LAVA insertions were skewed toward introns (Pearson chi-squared, p=0.0001) and were less frequent than expected when within <1 kbp of the nearest exon junction (EDF 3). The majority (74%) of intronic LAVA elements were found in the antisense orientation. We hypothesized that intronic antisense LAVA insertions may cause early transcription termination (ETT) by providing a polyadenylation site in antisense orientation, as previously described for L1 elements[23,24] (EDF 3). Indeed, we found 84.1% of the 3'-intact LAVA elements encoded a perfect polyadenylation signal at their 3'-end in antisense orientation.

To obtain experimental evidence that LAVA elements disrupt transcription, we performed a reporter assay in which the 3'-end of a luciferase gene construct lacking a transcriptional termination site was fused to the 3'-terminal fragments of LAVA_E and LAVA_F elements, mimicking the arrangement observed in gibbon genes (Fig. 3b-left). Luciferase activity exceeding background level by ~50% was observed from the LAVA_F reporter construct (Fig. 3b-right), indicating faithful termination of luciferase transcription. Further, 3' Rapid Amplification of cDNA Ends (RACE) experiments confirmed that the transcription termination site had been supplied from the LAVA element (EDF 3). Thus antisense intronic LAVA insertions can cause ETT with some variability possibly due to the genomic context of the polyadenylation site, which explained the difference between the two reporter constructs.

We also investigated LAVA induced ETT *in vivo* by analyzing RNA-seq data generated for Asia (Table ST2.4). Specifically, we looked for paired-end reads only partially aligning to an antisense LAVA element due to untemplated residues and then identified cases for which presence of a poly(A) tail was preventing full-length alignment. This analysis revealed that elements from a variety of sub-families have the potential to cause ETT, including those identified for LAVA elements inserted in the microtubule cytoskeleton genes (e.g. B2R2, C4B, B1R2) (EDT 1). Of note, we observed that ETT occurred at relatively low levels as we identified a significant number of read pairs indicative of normal transcription and splicing for LAVA-terminated genes (Table ST7.5). This is to be expected, as full inactivation of many of these genes would be incompatible with life. On the other hand, as alternative splicing and RNA-pol II transcript termination/ polyadenylation are tightly coupled processes, LAVA-mediated ETT could also act by differently affecting distinct isoforms and/or influence the ratio between isoforms. Finally, LAVA insertions may also impact gene expression by functioning as exon traps, as shown for SVA elements[25]. One putative example of an exon trapping event was identified for *HORMAD2*, a gene that monitors the formation of synapsis during crossover[26] (Supplementary Information S7, Table ST7. 6, Fig. SF7.1-2).

Since genome reshuffling began in the common ancestor of all extant gibbon species, LAVA insertions must have occurred in key genes before the four genera diverged. We experimentally confirmed the mode and tempo of all 23 LAVA insertions in genes from the microtubule cytoskeleton category using both site-specific PCR and *in silico* methods (EDF 4) and found that most of the insertions (15/23) were shared by the four gibbon genera (Supplementary File 6). Eleven of the genes match the structural requirements for ETT and five of them are also shared. These genes include *MAP4*, involved in spindle architecture, and *CEP164*, a G2/M checkpoint whose inactivation results in an aberrant spindle during cell division[18,19] (EDT 1).

The complex evolutionary history of gibbons

We explored the relationship between LAVA family expansion and evolution of the gibbon lineage and, through analyses of diagnostic mutations, identified 22 LAVA subfamilies (Fig. 3c). In addition, we tested for presence/absence of 200 LAVA loci from among the evolutionarily youngest elements in each subfamily (EDF 4) across 17 unrelated gibbon individuals and found that 52% of loci were shared among all four genera, whereas 27% were *Nomascus*-specific. The remaining LAVA insertions showed a variety of confounding phylogenetic relationships consistent with incomplete lineage sorting (ILS) of ancestral polymorphisms, perhaps as a result of a rapid radiation of gibbon genera (Supplementary Information S7; Table ST7.1-2). We used a maximum likelihood method[27] to obtain age estimates for the 22 LAVA subfamilies. In the case of the two oldest subfamilies, LAVA_A1 and LAVA_A2, we obtained estimates of ~18 mya and ~17 mya, respectively (Table ST7.3). A coalescent-based methodology implemented in the software G-PhosCS[28] using Nleu1.0 estimated a gibbon-great ape population divergence time of ~16.8 mya (95% CI: 15.9-17.6 mya) assuming a split time with macaque of 29 mya (Supplementary Information S4). Hence, the LAVA element likely originated around the time of the divergence of gibbons from the ancestral great ape/human lineage.

The evolutionary history of the gibbon lineage and, in particular, the timing and order of splitting among the four genera, is still a subject of debate[29]. To address this issue we generated medium coverage (mean ~15X) WGS short read data for two individuals from each of the four genera, including two different *Hylobates* species (*H. moloch* and *H. pileatus*) (Table ST2.1-2). While phylogenetic analysis of assembled whole mitochondrial DNA genomes using BEAST[30] strongly supported monophyletic groupings for each gibbon genus, the branching order of the four genera remained unresolved (Fig. SF9.1-2; Supplementary Information S9).

Neighbor Joining trees constructed from pairwise sequence divergence, *k*, across ~11,000 genic (200 bp) and ~12,000 non-genic (1 kb) autosomal loci supported a supermatrix sequence topology of (((*Siamang* (SSY), *Hoolock* (HLE)), *Nomascus* (NLE)), (*H. pileatus* (HPL)), *H. moloch* (HMO)) (Fig. 4a), though bootstrap confidence for the node separating NLE and *Hylobates* was low (~52%). This topology was also the most frequently observed when constructing *k*-based Unweighted Pair Group Method with Arithmetic Mean (UPGMA) trees along the genome using non-overlapping 100 kbp sliding windows. However all 15 possible rooted topologies for the four genera were observed at considerable frequencies (EDF 5), consistent with the extensive ILS observed in the LAVA element analysis.

In order to infer the most likely bifurcating species topology amongst the four genera while taking into account ILS, we employed a novel coalescent-based ABC methodology using the autosomal nongenic and genic loci (*Veeramah et al. submitted*) (Supplementary Information S8). The topology described above had the highest combined posterior probability, though support was relatively low (*p*(Model)=17%) and other topologies, including one with NLE and *Hylobates* interchanged as the most external taxa, had comparable probabilities (Fig. 4a).

The estimated internal branch lengths under the best species topology using our ABC framework and G-PhoCS were very short, supporting a rapid speciation process for the four gibbon genera (Fig 4b-right). Given this observation and uncertainty in the best topology, we also estimated parameters under an instantaneous speciation model (Fig. 4b-left). Assuming an overall autosomal mutation rate of 1 x 10^{-9}/site/year, we placed the beginning of the speciation process at ~5 mya under both models, with the two *Hylobates* species diverging ~1.5 mya.

Consistent with the ABC analysis, SSY and HLE share the largest number of alleles across the whole genome (Table ST8.5). However, NLE and the two *Hylobates* samples are both significantly closer to SSY than HLE as assessed by the D-statistic[31]. This result could be explained by two independent gene flow events between SSY and both NLE and *Hylobates*. However fertile intergenic hybrids have yet to be observed either in the wild or captivity[32]; an alternative explanation would be long-term population structure in the gibbon ancestral population. Both the ABC and G-PhoCS analyses suggest that the ancestral gibbon effective population size (N_e) was large (80,000-130,000) but neither of these frameworks can distinguish this from a structured ancestral population.

The coalescent-based analysis (Fig 4a), along with estimates of genome-wide heterozygosity (Fig ST8.2), suggests a larger long-term N_e for both *N. leucogenys* and *H. moloch* compared to the other species. Analysis using the pairwise sequentially Markovian coalescent (PSMC) model[33] indicates that these two species underwent an increase in N_e during the Late Pleistocene era (500-100 thousand years ago (kya) followed by a subsequent decrease in N_e 100-50 kya (Fig. 4c) (Supplementary Information S8). It is important to point out that fluctuation in N_e could result from changes in the actual number of individuals in the population, changes in population structure, and/or variable gene flow.

Functional sequence evolution

Accelerated substitution rates are a hallmark of adaptive evolution, and genomic regions with excess lineage-specific substitutions have been found to have functional roles[34]. We identified 240 short (153 bp median length) regions with accelerated substitution rates in the gibbon lineage (gibARs). We observed that gibARs were primarily intergenic (66%) and tended to co-localize near the same genes as LAVA elements (p-value=81E-06; odds ratio of 2.74 (1.79–4.07, 95% CI)). Consistent with this finding, a GO enrichment test for genes within +/−100 kbp of each gibAR (in comparison with background genes) revealed enrichment for the ‘chromosome organization’ category (Benjamini-Hochberg FDR <5%) (EDF 6). Given evidence of functional roles gathered for human accelerated regions[35], we speculate that the gibARs may create functional elements (e.g., enhancer, protein-binding domains) to modulate the transcriptional effect of local LAVA insertions (Supplementary Information S12 and File 9).

We assessed the potential presence of positive selection in 13,638 human genes with one-to-one orthologs in gibbon using a branch-site likelihood ratio test[36] (Supplementary Information S10). One of the most striking features of gibbons is their use of brachiation (i.e., arboreal locomotion using only the arms). We uncovered evidence related to traits possibly associated with this adaptation such as the gibbon's longer arms, more powerful shoulder flexors, rotator muscles, and elbow flexors[37]. First, some genes whose functions relate to these anatomical specializations appear to have undergone positive selection in gibbons. They include *TBX5* (p-value=0.00015), required for the development of all forelimb elements[38]; *COL1A1* (pro-alpha1 chains of type I collagen) (p-value=3.39E-11), the fibril-forming collagen main protein of bones, tendons, and teeth[39]; and *CHRNA1* (acetylcholine receptor subunit alpha precursor) (p-value=0.00039), involved in skeletal muscle contraction[40]. These genes have not been identified as positively selected in other primates to date. We also observed that some genes involved in chondrogenesis (*SNX19*, *ID2*, and *EXT1*) were associated with gibARs. Finally, the chondroadherin gene (*CHAD*)[41] coding for a cartilage matrix protein is specifically duplicated in all gibbon genera (EDF 2).

DISCUSSION

Our sequencing, assembling, and analysis of the gibbon genome has provided numerous insights into the accelerated evolution of the gibbon karyotype and identified genetic signatures related to gibbon biology. First, SDs and repetitive sequences were the best predictors of gibbon-human breakpoints, although we excluded a causal role given the predominance of non-homology-based repair signatures. Furthermore, accelerated rearrangement was confined to large-scale chromosomal events, pointing to a mechanism responsible for causing gross chromosomal changes, rather than global genomic instability. This is in line with our hypothesis that the high rate of chromosomal rearrangements may have been due to LAVA-induced premature transcription termination of chromosome segregation genes. This effect may have occurred at a low enough level to be compatible with life but sufficient to increase the frequency of chromosome segregation errors. Of note, the link between erroneous chromosome segregation and increased chromosomal rearrangement has been recently demonstrated by others through *in vitro* experiments[25,26].

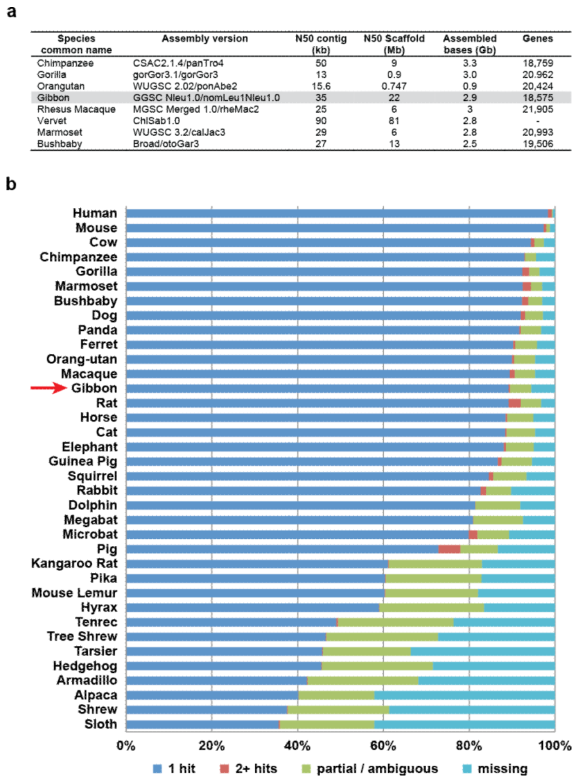
The question remains how such a high number of chromosomal rearrangements could become fixed in such a relatively short time. One possibility is that a combination of geographic isolation and post-mating reproductive barriers accelerated the radiation of the four gibbon genera. Our estimates dated the lineage-splitting event to the Miocene-Pliocene transition, when major changes in the distribution of tropical and subtropical forests were caused by the elevation of the Yunnan Plateau and rise in sea levels[42,43]. Furthermore, fluctuation in sea levels beginning in the Early Pliocene appears to have brought about cycles of forest fragmentation and amalgamation, leading to alternating range compression and expansion for many mammalian groups[44].

Together these results advance our knowledge of the unique traits of the small apes and highlight the complex evolutionary history of these species. Moreover, our analyses of the shattered gibbon genome helped gain insight into the mechanisms of chromosome evolution and uncovered a novel source of genome plasticity.

METHODS

Sanger-based whole-genome sequencing was performed as described for other species. The genome assembly was generated using the ARACHNE genome assembler assisted with alignment data from the human genome (Supplementary Information S1). The source DNA for the sequencing was derived from a single female (Asia; studbook no. 0098, ISIS no. NLL605) housed at the Virginia Zoo in Norfolk, VA. Short-read libraries were constructed at the Oregon Health & Science University (OHSU) following standard Illumina protocols and sequenced on an Illumina HiSeq 2000. Analyses were performed with custom analysis pipelines. See Supplementary Information for additional methods.

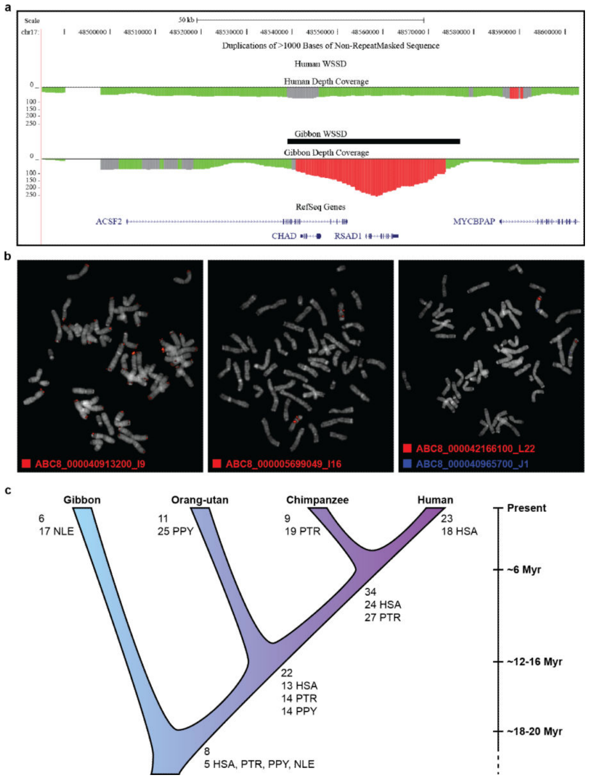
Extended Data



Species common name	Assembly version	N50 contig (kb)	N50 Scaffold (Mb)	Assembled bases (Gb)	Genes
Chimpanzee	CSAC2.1.4/panTro4	50	9	3.3	18,759
Gorilla	gorGor3.1/gorGor3	13	0.9	3.0	20.962
Orangutan	WUGSC 2.02/ponAbe2	15.6	0.747	0.9	20,424
Gibbon	GGSC Nleu1.0/nomLeu1Nleu1.0	35	22	2.9	18,575
Rhesus Macaque	MGSC Merged 1.0/rheMac2	25	6	3	21,905
Vervet	ChlSab1.0	90	81	2.8	-
Marmoset	WUGSC 3.2/calJac3	29	6	2.8	20,993
Bushbaby	Broad/otoGar3	27	13	2.5	19,506

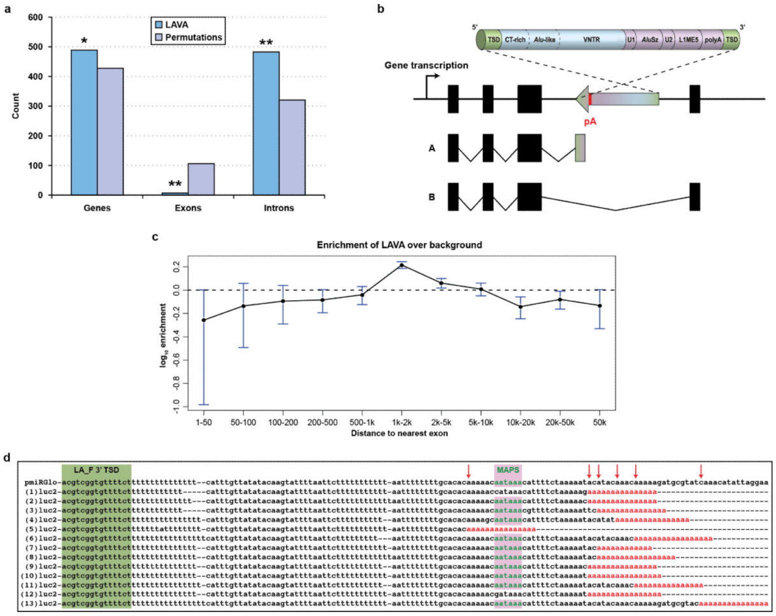
Extended Data Figure 1. The gibbon assembly: statistics and quality control

a, The table compares the gibbon assembly statistics to those of other primates sequenced with a similar strategy. **b**, The plot represents the percentage of the 10,734 single-copy gene HMMs (hidden Markov models) for which just one gene (blue) is found in the different mammalian genomes in Ensembl 70. Other HMMs match more than one gene (red). The missing HMMs (cyan) either do not match any protein or the score is within the range of what can be expected for unrelated proteins. The remaining category (green) represents HMMs for which the best matching gene scores better than unrelated proteins but not as well as expected. (See Supplementary Information section 1.4 for more details.)



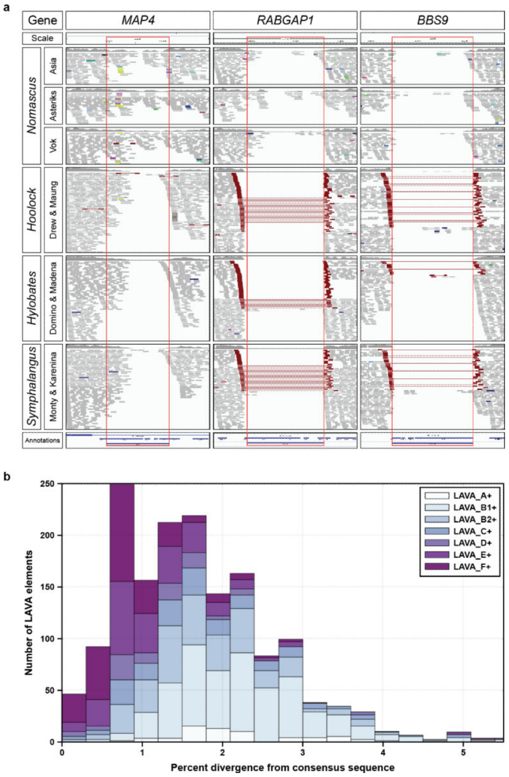
Extended Data Figure 2. Analysis of gibbon-human synteny blocks and identification and validation of gibbon segmental duplications

a, The image shows a representative gibbon-only WSSD (whole-genome shotgun sequence detection) call by Sanger read depth. The duplication identified in this case overlaps with the gene *CHAD* that codes for a cartilage matrix protein. **b**, Examples of FISH hybridizations on gibbon metaphases using duplicated human fosmid clones that were identified by the (WGS) detection strategy (red signals). A) Interchromosomal duplication. B) Interspersed intrachromosomal duplication. C) Intrachromosomal tandem duplication confirmed using cohybridization with a single control probe (blue signals). **c**, Megabases of lineage-specific and shared duplications for primates based on GRChr37 read depth analysis. Copy-number-corrected values by species are shown below.



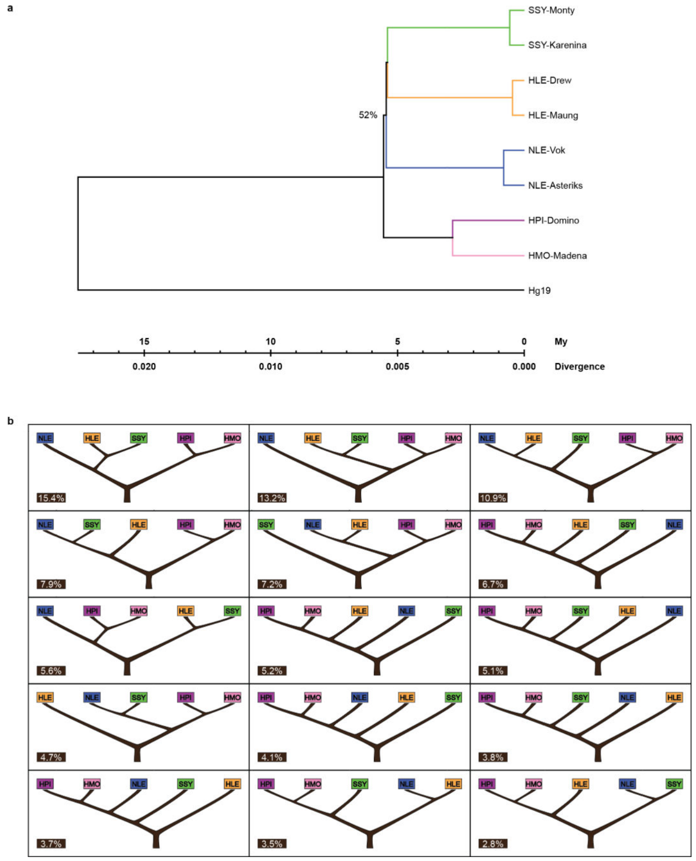
Extended Data Figure 3. Analysis of LAVA element insertion in genes and early termination of transcription

a, The histogram shows the results of permutation analyses. We find a significant association between LAVA elements and genes. Moreover, insertions are significantly enriched in introns and depleted in exons, most likely as a result of selection against insertions in exons. **b**, Schematic representation of the mechanism through which LAVA intronic insertions in anti-sense orientation might cause early termination of transcription: A) truncated transcript; B) normal transcript (pA=polyadenylation site). **c**, We calculated the distance to the nearest exon for each intronic LAVA and compared this to what would be expected for random insertions (i.e., background). We found fewer insertions than expected by chance within 1 kbp of the nearest exon. **d**, Identification of pmiRGlo_LA_F polyadenylation sites by 3'RACE. Alignment of thirteen 3'RACE PCR clone sequences and the pmiRGlo_LA_F sequence. LAVA_F 3' TSD is highlighted by dark background; the major antisense LAVA_F polyadenylation signal (MAPS) is in red. The termination sites are marked with arrows on the LAVA_F sequence. Poly(A)tails of the identified transcripts are colored in red.



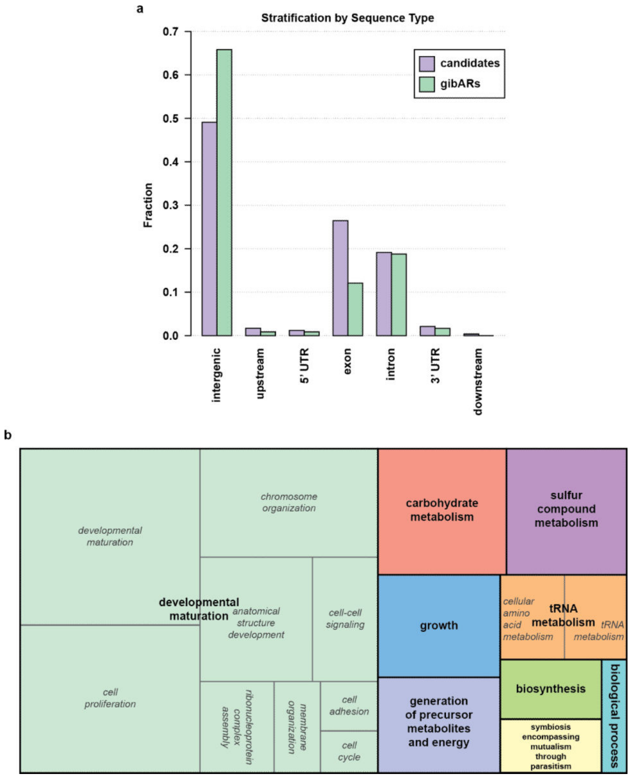
Extended Data Figure 4. Evolution of the LAVA element

a, Screenshots from the Integrative Genomics Viewer (IGV) browser for loci *MAP4*, *RABGAP1*, and *BBS9*. Each column shows portions of the IGV visualization of a LAVA insertion locus identified in Nleu1.0 and its flanking sequence. Red rectangles indicate the margins of each LAVA insertion. Read pairs are colored in red when their insert size is larger-than-expected, indicating the presence of a LAVA insertion. *MAP4* is a shared LAVA insertion, while *RABGAP1* and *BBS9* are *Nomascus*-specific. **b**, LAVA elements containing at least 300 bp of the LA section of LAVA elements were selected and reanalyzed using RepeatMasker to determine subfamily affiliation and divergence from the consensus sequence. LAVA elements are grouped based upon their subfamily affiliations (see legend top right). The x-axis shows the percent divergence from the respective consensus sequence, and the y-axis shows the number of elements with a certain percent divergence from the consensus sequence.



Extended Data Figure 5. Analysis of the phylogenetic relationships between gibbon genera

a, Neighbor-joining trees for gibbons using non-genic loci. **b**, UPGMA trees for 100 kbp nonoverlapping sliding windows moving along the gibbon genome reporting the top 15 topologies (see also Supplementary Table ST8.3). The percentage of total support for each topology is given within each subpanel.



Extended Data Figure 6. Analysis of the relationship between gibbon accelerated regions (gibARs) and genes

a, Intergenic regions are enriched in gibARs. Different sequence types are shown on the x-axis, and the y-axis displays the fraction of gibARs and candidate regions annotated to the respective class. gibARs are significantly enriched in intergenic regions (p = 4.7E-6) and significantly depleted in exons (p = 7.3E-6). p-values for each class were calculated with the Fisher's exact test. Introns are comparably prevalent in candidates and gibARs, while in UTR and flanking region counts are too low to draw meaningful conclusions (data not shown). **b,** TreeMap from REVIGO for GOslim Biological Process terms with a Benjamini-Hochberg FDR of 5%. Each rectangle is a cluster representative; larger rectangles represent ‘superclusters’ including loosely related terms. The size of the rectangles reflects the p-value.

Extended Data Table 1

Genes from the ‘microtubule cytoskeleton’ GO category with LAVA insertions

Gene	Function	LAVA strand	Polyadenylation signal	Orthology	Subfamily
CEP164	**G2/M checkpoint** and nuclear divisions	antisense	**TTTATT**	Shared	LAVA_B2R2
MAP4	**Spindle** architecture	antisense	**TTTATT**	Shared	LAVA_B1R2
STAU2	RNA-decay	antisense	**TTTATT**	Shared	LAVA_C4A
KIFAP3	**Kinesin,** motor protein moving on microtubules	antisense	**TTTATT**	*Nomascus*	LAVA_B1B
SNTB2	**Syntrophin**	antisense	**TTTATT**	*Nomascus*	LAVA_B2R2

Gene	Function	LAVA strand	Polyadenylation signal	Orthology	Subfamily
BBS9	Localizes to non-membranous **centriolar satellites**	antisense	TGTTTA	*Nomascus*	LAVA_E
DNHD1	**Dynein,** motor protein moving on microtubules during mitosis	antisense	TTTGTT	Shared	LAVA_B2R2
SHROOM3	Regulator of the **microtubule cytoskeleton**	antisense	TTTGTT	Shared	LAVA_C2
EVI5	**Centrosome stability** and dynamics/ completion of cytokinesis	antisense	TTTGTG	Shared	LAVA_B1R2
SMC3	**Cohesin**	antisense	TTTAGT	*Nomascus*	LAVA_B1F2
MAD1L1	**Kinetochore**-bound checkpoint protein	antisense	TT--TA	Shared	LAVA_D1
BUB1B	**Spindle checkpoint**	antisense	TGTTTA	Shared	LAVA_F1
HOOK3	**Centrosomal** assembly	antisense	TGTTTA	*Nomascus*	LAVA_E
TRAF5	TNF receptor-associated factor 5	antisense	TGTTTA	*Nomascus*	LAVA_F2
DYNC1LI1	Intracellular trafficking and **mitosis**	antisense	**TTTATT**	Shared	LAVA_C4B
C2CD3	Distal **centriole** formation	sense	TTTATT	Shared	LAVA_B1G
CLASP2	Regulation of **spindle** and **kinetochore** function	sense	CTTACT	Shared	LAVA_B1R2
DNAH3	**Dynein,** motor protein moving on microtubules during mitosis	sense	**TTTATT**	Shared	LAVA_B2R1
INVS	Cell rounding and **spindle positioning** during mitosis	sense	**TTTATT**	Shared	LAVA_C4B
KIF27	**Kinesin,** motor protein moving on microtubules	sense	**TTTATT**	Shared	LAVA_B1D
MFN2	Mitochondrial fusion	sense	**TTTATT**	*Nomascus*	LAVA_E
NINL	**Centrosome,** microtubule organization in interphase cells	sense	**TTTATT**	Shared	LAVA_B1F2
RABGAP1	Interaction **with** Mad2-**spindle checkpoint**	sense	**TGTTTA**	*Nomascus*	LAVA_E

Genes highlighted in gray carry LAVA insertions that are shared, antisense, and carry a perfect antisense polyadenylation site.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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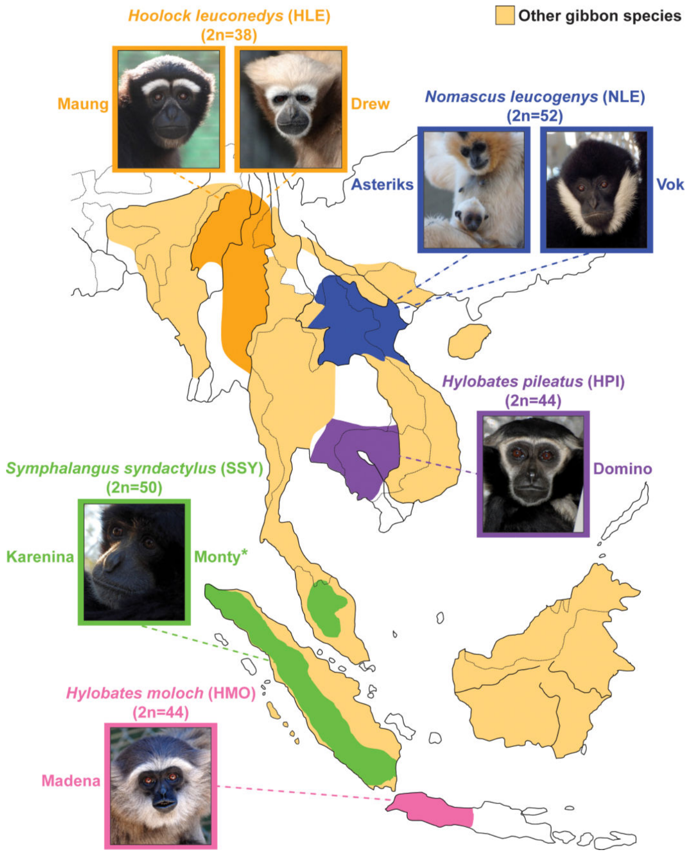

Figure 1. Geographic distribution of gibbon species used in the study

We sequenced two individuals from each gibbon genus and two different species (*H. moloch* and *H. pileatus*) for the genus *Hylobates*. The extant geographic localization for each genus is illustrated on the map. Individuals in the photos are the ones sequenced in this study. (**deceased animal*)

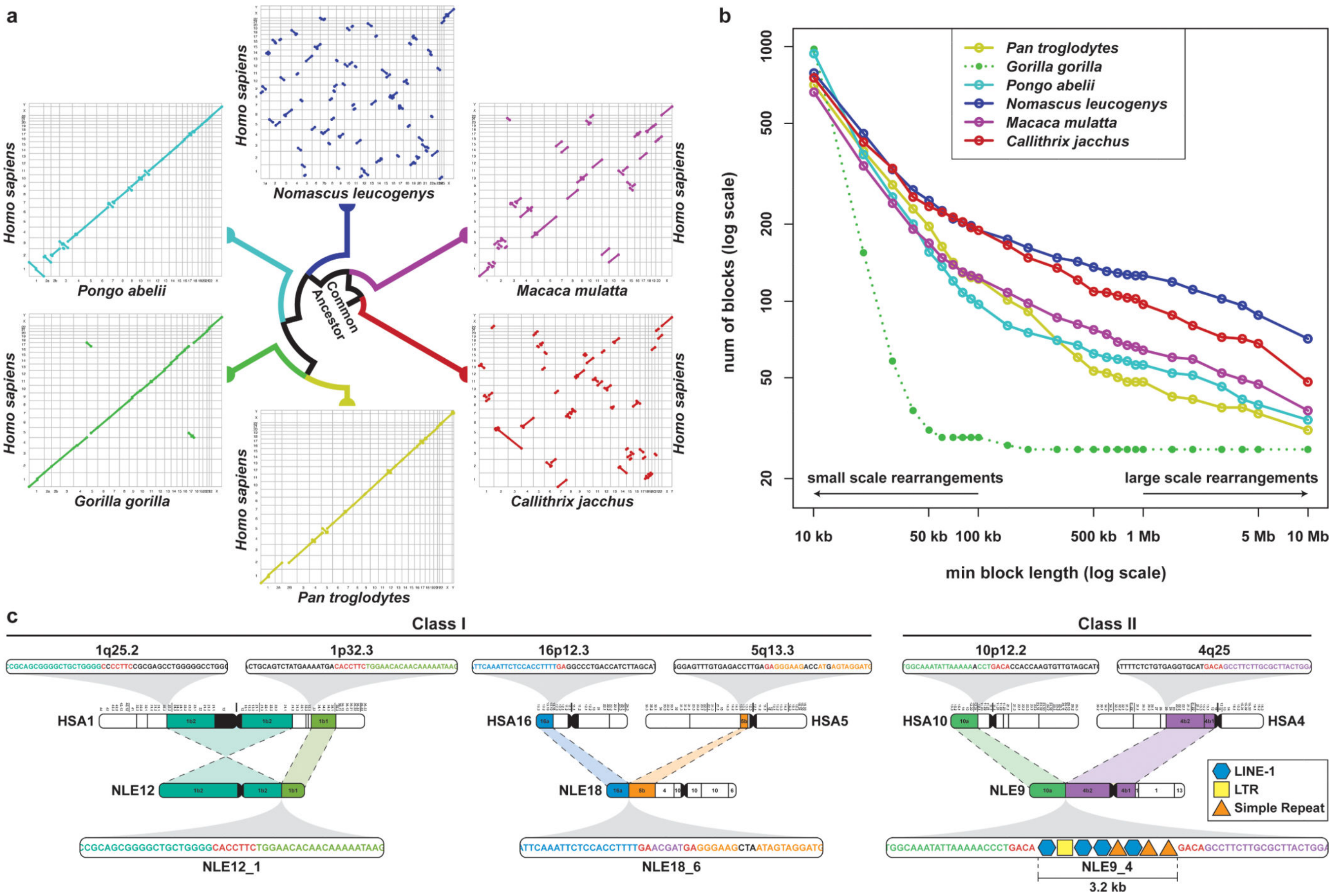

Figure 2. Analysis of gibbon-human synteny and breakpoints

a, Oxford plots for human chromosomes (on the y-axis) vs. chimpanzee, gorilla, orangutan, gibbon, rhesus macaque, and marmoset chromosomes (on the x-axis). Each line represents a collinear block larger than 10 Mbp. The gibbon genome displays a significantly larger number of large-scale rearrangements than all the other species. In the gorilla plot, chromosomes 4 and 19 stand out as the product of a reciprocal translocation between chromosomes syntenic to human chromosomes 5 and 17. **b**, The graph shows the number of collinear blocks in primate genomes with respect to the human genome. The number of collinear blocks is a proxy for the number of rearrangements and decreases as the size of the blocks becomes larger. The gibbon genome has undergone a greater number of large-scale rearrangements; however, the number of small-scale rearrangements is comparable with the other species. [Note: the extremely low number of large rearrangements in the gorilla genome (dotted green line) is a reflection of the use of the human genome as a template in the assembly process]. **c**, Examples of gibbon-human synteny breakpoints. The first two are Class I breakpoints (i.e., base-pair resolution) originated through non-homology based mechanisms. NLE12_1 is the result of an inversion in human chromosome 1and NLE18_6 is the result of a translocation between human chromosomes 16 and 5 with an untemplated insertion in the gibbon sequence shown in purple; in both cases, microhomologies in the human sequences are shown in red. The last example (NLE9_4) is a Class II breakpoint (3.2 kbp) containing a mixture of repetitive sequences.

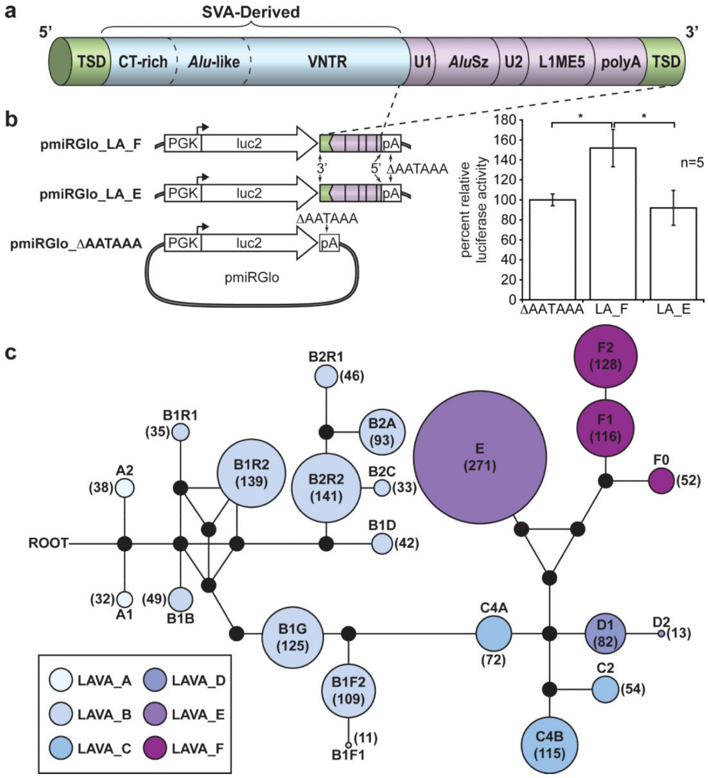

Figure 3. The LAVA element and evidence for LAVA-mediated early transcription termination

a, Schematic view of the LAVA element highlights the main components that originated from common repeats (L1, *Alu*, VNTR, and *Alu*-like). Target site duplications (TSDs) and polyA-tail are also indicated. **b**, Luciferase reporter constructs used to assay for LAVA-mediated early transcriptional termination (left panel) and results of the luciferase reporter assay (right panel) showing increased luciferase activity by ~50% relative to the background for pmiRGlo_LA_F (*P=0.0013) (see Supplementary Information S7.8). **c**, A median-joining network showing the relationships among the 22 LAVA subfamilies generated by comparing the 3'-intact LAVA elements. Colored circles represent subfamilies and their size is proportional to the number of elements in the subfamily (numbers inside each circle). Black dots represent hypothetical sequences connecting adjacent subfamilies. All possible relationships are shown. (Branch lengths are not drawn to scale.)

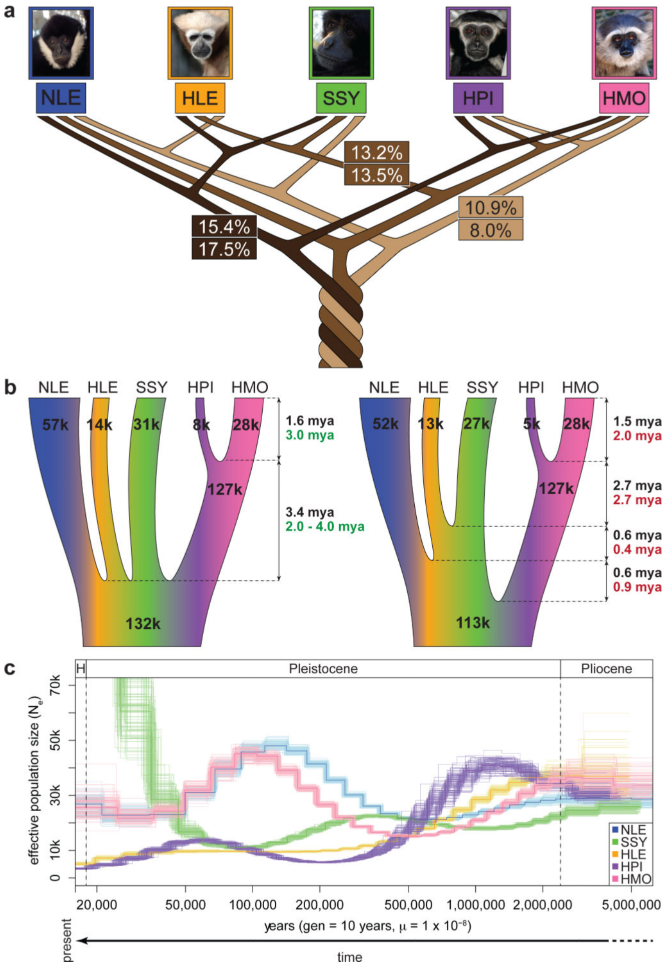

Figure 4. Gibbon phylogeny and demography

a, The three most frequently observed UPGMA gene trees (numbers at the top) constructed across the genome at 100 kbp sliding windows and posterior probabilities (numbers at the bottom) for the same species topologies from a coalescent-based ABC analysis. The relatively low numbers observed suggest presence of substantial ILS amongst the gibbon genera. **b**, Parameters estimates describing gibbon population demography assuming an instant radiation for all four genera (left) and the most probable bifurcating species topology (right). Black, green and red numbers indicate divergence times and N_e as calculated by ABC, BEAST and G-PhoCS analysis respectively (Supplementary Information S9). **c**, PSMC analysis estimating changes in historical N_e. Note: the large increase in N_e observed in our PSMC plot for SSY in recent times is likely exaggerated due to higher sequencing error and mapping biases in non-NLE samples (see details in Supplementary Section S8). A generation time of 10 years[45-46] was used to obtain a per generation mutation rate of $1x10^{-8}$ per year.

Table 1

Gibbon assembly statistics

Assembly (Nleu1.0/nomLeu1)	
Total sequence length	2,936,052,603 bp
Ungapped length	2,756,591,777 bp
Total contig length	2.77 Gbp (92.36%)
Number of contigs >1 kbp	197,908
N50 contig length	35,148 bp
Number of scaffolds >3 kbp	17,976
N50 scaffold length	22,692,035 bp
Average read depth	5.6X