January 2008

Rapid Rates of Lineage-Specific Gene Duplication and Deletion in the α-Globin Gene Family

Federico G. Hoffmann
University of Nebraska - Lincoln, fhoffmann2@unl.edu

Juan C. Opazo
University of Nebraska - Lincoln, jopazo2@unl.edu

Jay F. Storz
University of Nebraska - Lincoln, jstorz2@unl.edu

Published in *Molecular Biology and Evolution* **25**:3 (2008), pp. 591–602; doi 10.1093/molbev/msn004

Accepted for publication January 1, 2008; published online on January 4, 2008. Associate editor for *MBE*: David Irwin.

Rapid Rates of Lineage-Specific Gene Duplication and Deletion in the α-Globin Gene Family

Federico G. Hoffmann, Juan C. Opazo,* and Jay F. Storz

School of Biological Sciences, University of Nebraska–Lincoln

* *Present address:* Instituto de Ecologia y Evolucion, Universidad Austral de Chile, Valdivia, Chile

Correspondence email: jstorz2@unl.edu

Abstract

Phylogeny reconstructions of the globin gene families have revealed that paralogous genes within species are often more similar to one another than they are to their orthologous counterparts in closely related species. This pattern has been previously attributed to mechanisms of concerted evolution such as interparalog gene conversion that homogenize sequence variation between tandemly duplicated genes and therefore create the appearance of recent common ancestry. Here we report a comparative genomic analysis of the α-globin gene family in mammals that reveal a surprisingly high rate of lineage-specific gene duplication and deletion via unequal crossing-over. Results of our analysis reveal that patterns of sequence similarity between paralogous α-like globin genes from the same species are only partly explained by concerted evolution between preexisting gene duplicates. In a number of cases, sequence similarity between paralogous sequences from the same species is attributable to recent ancestry between the products of de novo gene duplications. As a result of this surprisingly rapid rate of gene gain and loss, many mammals possess α-like globin genes that have no orthologous counterparts in closely related species. The resultant variation in gene copy number among species may represent an important source of regulatory variation that affects physiologically important aspects of blood oxygen transport and aerobic energy metabolism.

Keywords: birth-and-death evolution, concerted evolution, gene duplication, gene family, α-globin, hemoglobin

Supplemental Materials: Supplemental figures S1–S3 and tables S1 and S2 follow the "References"; they are also available at *Molecular Biology Evolution* online: http://mbe.oxfordjournals.org/

Introduction

Phylogeny reconstructions of gene family evolution often reveal that paralogous genes within species are more similar to one another than they are to their orthologous counterparts in closely related species. This pattern is a hallmark of concerted evolution and is typically attributed to the homogenizing effects of interparalog gene conversion or unequal crossing-over (Zimmer *et al.* 1980; Ohta 1984, 1990, 2000). Gene conversion involves a nonreciprocal recombination event between paralogous sequences and is thought to be the most important mechanism of concerted evolution in small multigene families (Dover 1982; Nagylaki and Petes 1982; Nagylaki 1984a, 1984b; Ohta 1990). Unequal crossing-over is a reciprocal recombination event that produces a sequence duplication on one chromatid or chromosome and a corresponding deletion in the other. Repeated rounds of unequal crossing-over can result in concerted evolution in cases where one paralogous sequence is propagated at the expense of other tandemly duplicated loci, thereby progressively homogenizing sequence variation among members of the gene family (Ohta 1980, 1984; Gojobori and Nei 1984; Li *et al.* 1985). The role of both gene conversion and unequal crossing-over in homogenizing sequence variation among tandemly duplicated genes has been especially well documented in the globin gene families (Jeffreys 1979; Slightom *et al.* 1980; Liebhaber *et al.* 1981; Scott *et al.* 1984; Storz, Baze, *et al.* 2007; Storz, Sabatino, *et al.* 2007).

The α- and β-like globin genes encode individual subunit polypeptides of the tetrameric hemoglobin protein. The progenitors of the α- and β-globin gene families arose via tandem duplication of an ancestral globin gene approximately 450–500 MYA (Goodman *et al.* 1975, 1987; Czelusniak *et al.* 1982), and in amniote vertebrates the two gene families are located on different chromosomes. Most marsupial and placental mammals possess four different α-like globin genes: ζ-globin (HBZ), α^D-globin (HBK), α^A-globin (HBA), and θ-globin (HBQ). The HBZ and HBA genes both encode α-chain subunits of hemoglobin, but they are expressed at different stages of development. HBZ is expressed in primitive erythroid cells in the yolk sac during the earliest stages of embryogenesis, and HBA is expressed in definitive erythrocytes during fetal development and postnatal life (Higgs *et al.* 1989; Hardison 2001; Nagel and Steinberg 2001). In contrast to the HBZ and HBA genes, the HBK and HBQ genes do not appear to encode subunit polypeptides of hemoglobin in mammals, and their functions have yet to be illuminated. The duplication events that produced the HBZ, HBK, and HBA genes predated the origin of tetrapod vertebrates (Goodman *et al.* 1975, 1987; Hoffmann and Storz 2007), whereas the HBQ gene appears to be the product of a mammal-specific duplication of the HBA gene (Cooper *et al.* 2005).

The majority of mammals studied to date possess either one or two functional copies of HBZ and either two or three functional copies of HBA. It has often been assumed that the same tandemly duplicated HBZ and HBA genes were inherited from the common ancestor of all mammals (Zimmer *et al.* 1980; Flint *et al.* 1988; Hardison 2001). According to this scenario, the 5′ HBA gene in one species is assumed to be orthologous to the 5′ HBA gene of all other species and likewise for the other HBA and HBZ paralogs (Flint *et al.* 1988; Hardison 2001). The fact that paralogous α-like globin genes within the genome of the same species are often identical or nearly identical in sequence has typically been attributed to concerted evolution (Zimmer *et al.* 1980; Liebhaber *et al.* 1981; Proudfoot *et al.* 1982; Michelson and Orkin 1983). According to this explanation, the homogenization of sequence variation between paralogous α-globin genes erases phylogenetic history and creates the appearance of recent common ances-

try (Zimmer *et al.* 1980; Liebhaber *et al.* 1981; Proudfoot *et al.* 1982; Michelson and Orkin 1983; Higgs *et al.* 1989; Hardison 2001). As stated by Graur and Li (2000, p. 314) in explaining the observed sequence similarity between paralogous HBA genes in humans and other mammals: "... one had to assume either that multiple gene duplication events occurred independently in many evolutionary lineages or that the two genes are quite ancient, having been duplicated once in the common ancestor of these organisms, but their antiquity was subsequently obscured by concerted evolution. Ultimately, the most parsimonious solution was to choose the latter alternative." Although this interpretation of the observed phylogenetic patterns may be the most parsimonious, results of our comparative genomic analysis reveal that it is not completely correct. Here we report a detailed analysis of sequence variation in coding regions and flanking regions of mammalian α-like globin genes that reveals a surprisingly high rate of lineage-specific gene duplication and deletion via unequal crossing-over. Results of our analyses reveal that observed patterns of sequence similarity between paralogous HBZ and HBA genes are only partly explained by concerted evolution. In many cases, the appearance of recent common ancestry between paralogous sequences is real as new α-like globin genes have originated multiple times independently in different lineages of placental mammals.

The objective of this study was to assess the relative importance of concerted evolution and birth-and-death evolution in shaping the genomic structure of the α-globin gene family in mammals. Specifically, we used genomic sequence data 1) to characterize the genomic structure of the mammalian α-globin gene family, 2) to assign orthologous and paralogous relationships among duplicate copies of α-like globin genes, and 3) to assess whether sequence similarity between paralogs within the same species' genome is typically attributable to concerted evolution between preexisting gene duplicates or recent ancestry between duplicated genes that originated independently in different lineages.

Materials and Methods

DNA Sequence Data and Bioinformatic analyses

Genomic sequences that spanned all or most of the α-globin gene cluster were identified in either GenBank or Ensembl databases by BlastN alignment to known α-like globin sequences. When possible, we focused on sequences from a single genomic contig, genomic scaffold, or full chromosome, depending on the nature of the available data. The basic annotation was derived from the database records in most cases, but we also identified globin genes in unannotated sequences using GENSCAN (Burge and Karlin 1997) and by comparing known exon sequences with genomic contigs using the program Blast2 sequences version 2.2 (Tatusova and Madden 1999) from the National Center for Biotechnology Information Blast suite: http://www.ncbi.nlm.nih.gov/blast. Annotated genes were considered to be functional when they met the following criteria: there were no premature stop codons, there were no frameshift mutations, and a stop codon was present at codon position 42 of the third exon. Because of incomplete sequence coverage of the gene cluster, there were some genomic sequences in our data set for which we could not ascertain the full extent of conserved synteny. These include genomic sequences from the cat (*Felis domesticus*) and the stripe-face dunnart (*Sminthopsis macroura*). Genomic sequences were masked using RepeatMasker (http://www.repeatmasker.org), and genomic sequence alignments were conducted using Pipmaker (Schwartz *et al.* 2000), Multipipmaker (Schwartz *et al.* 2003), and Mulan (Ovcharenko *et al.* 2005). In order to identify tandemly duplicated genes or sets of genes, we used percent identity plots to identify short chromosomal regions that were locally alignable to one or more additional regions within the same genomic contig. For the intragenomic dot plot analyses, we focused on contigs that included 50 kb of flanking sequence upstream and downstream of the α-globin gene cluster.

Phylogeny Reconstruction

We explored phylogenetic relationships of α-globin genes at several levels. In all cases, sequences were aligned using ClustalX (Thompson *et al.* 1997). We inferred phylogenetic relationships in a maximum likelihood framework using Treefinder version June 2007 (Jobb *et al.* 2004) and assessed support for the nodes with 1,000 bootstrap pseudoreplicates. In analyses restricted to protein-coding sequences, an independent model of nucleotide substitution was used for each codon position. Phylogenetic results were robust to variation in the model of nucleotide substitution selected; here, we report results obtained under the general time-reversible model (Rodriguez *et al.* 1990) in which rate variation followed a discrete gamma distribution (GTR + Γ). Due to the fact that intronic sequences from distantly related species were often unalignable, we restricted the analysis to coding sequence. We followed a similar strategy to reconstruct phylogenetic trees for all putatively functional HBZ and HBA genes. Phylogeny reconstructions that deviated from the expected species phylogeny were investigated using the approximately unbiased test (Shimodaira 2002), as implemented in Treefinder.

To reconstruct the history of gene duplications and deletions in the α-globin gene cluster of primates, we compared the coding sequences of the genes and the corresponding upstream and downstream flanking regions. Because gene conversion tracts are often restricted to coding regions (Chen *et al.* 2007), in many cases orthologous relationships between duplicated genes can still be reliably inferred by examining flanking sequence that lies outside of gene conversion tracts (Hardison and Gelinas 1986; Hardison and Miller 1993; Storz, Baze, *et al.* 2007). In order to identify interparalog conversion tracts in primates that possess three or more copies of HBA, we used the program GENECONV (Sawyer 1989) with the G-scale parameter (mismatch penalty) set to 2.0. For the HBZ and HBA genes, we conducted phylogeny reconstructions on three different partitions of the alignment: the coding sequence, upstream flanking sequence, and downstream flanking sequence. For the HBZ genes, phylogeny reconstructions were based on a fragment that started 500-bp upstream of the start codon and ended 500-bp downstream of the stop codon. In the case of the HBA genes, the first set of analyses included all primates and was based on an alignment that started 1-kb upstream of the start codon and ended 1-kb downstream of the stop codon. A second analysis focused on resolving relationships among the

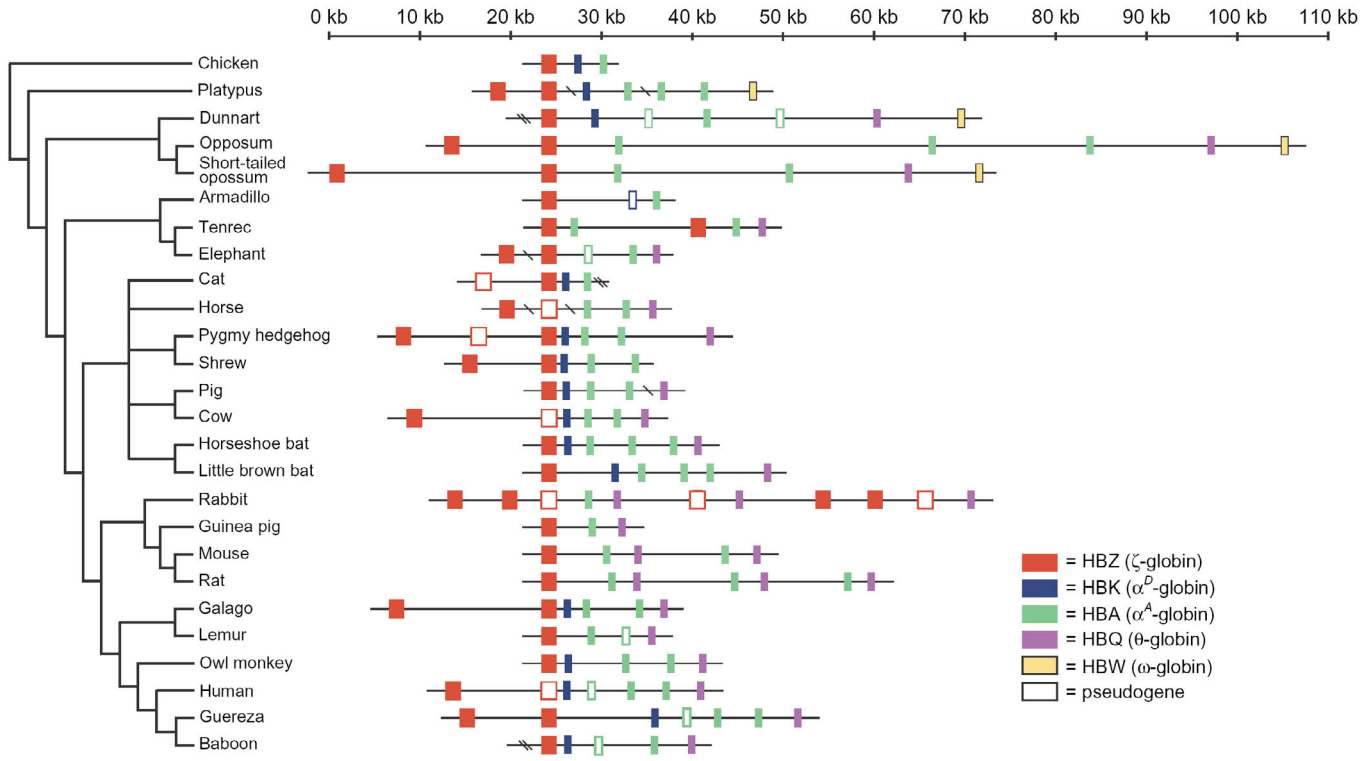

Figure 1.— Genomic structure of the α-globin cluster in mammals. Phylogenetic relationships among mammalian species are based on a loose consensus of recent studies (Murphy *et al.* 2001, 2007; Hallstrom *et al.* 2007; Wildman *et al.* 2007). Diagonal slashes indicate gaps in genomic coverage. Segments containing such gaps were not drawn to scale. Pseudogene fragments containing less than two complete exons were not included. The orientation of the clusters is from 5′ (on the left) to 3′ (on the right).

HBA genes of anthropoid primates, the group that includes New World monkeys, Old World monkeys, and apes. For the anthropoids, we aligned a fragment that started 2-kb upstream of the start codon and ended 1-kb downstream of the stop codon.

Results and Discussion

Genomic Sequence Data

We obtained genomic sequences that spanned all or most of the α-globin gene cluster of 40 mammalian species (supplementary table S1, Supplementary Material). These genomic contigs ranged in size from 10 to 100 kb. This sample of genomic sequences included representatives of the three subclasses of mammals: Prototheria (monotremes), Metatheria (marsupials), and Eutheria (placental mammals). The sample of placental mammals included representatives of each of the four superorders: Afrotheria, Xenarthra, Laurasitheria, and Euarchontoglires.

Following the nomenclature of Aguileta *et al.* (2006), we refer to the ζ-globin gene, the α^D-globin gene, the α^A-globin gene, and the θ-globin gene, as HBZ, HBK, HBA, and HBQ, respectively. Because mammalian α-globin genes have undergone multiple rounds of duplication that have resulted in tandemly repeated sets of paralogous gene copies (Zimmer *et al.* 1980; Czelusniak *et al.* 1982; Proudfoot *et al.* 1982; Hardison and Gelinas 1986; Cheng *et al.* 1987; Goodman *et al.* 1987; Flint *et al.* 1988, 2001), we index each duplicated gene with the symbol –T followed by a number that corresponds to the linkage order in the 5′ to 3′ orientation (Aguileta *et al.* 2006).

Genomic Structure of the Mammalian α-Globin Gene Cluster

Results of intragenomic dot plot analyses revealed that tandemly duplicated gene regions were exclusively restricted to the α-globin gene cluster (supplementary Figure S1, Supplementary Material). Genomic sequence comparisons among monotremes, marsupials, and placental mammals revealed conserved synteny across the entire α-globin gene cluster. In representatives of all three subclasses of mammals, the 5′ end of the α-globin gene cluster is located downstream of the ortholog of the human *C16orf35* gene and the 3′ end of the gene cluster is located upstream of the ortholog of the human *Luc7L* gene. The one notable exception to this pattern is the house mouse (*Mus musculus*). In this species, the 5′ end of the α-globin gene cluster is located on Chromosome 11 but the 3′ end of the cluster, including pseudogene copies of HBA and HBQ, has been translocated to Chromosome 17 (Flint *et al.* 2001; Tufarelli *et al.* 2001).

In nearly all the genomic sequences in our data set that had complete coverage of the α-globin gene cluster, the HBZ and HBQ genes were located at the 5′ and 3′ ends of the cluster, respectively (Figure 1). As is generally the case in the globin gene clusters of vertebrates, the embryonic HBZ genes were located upstream of the adult HBA genes. The only exceptions involved

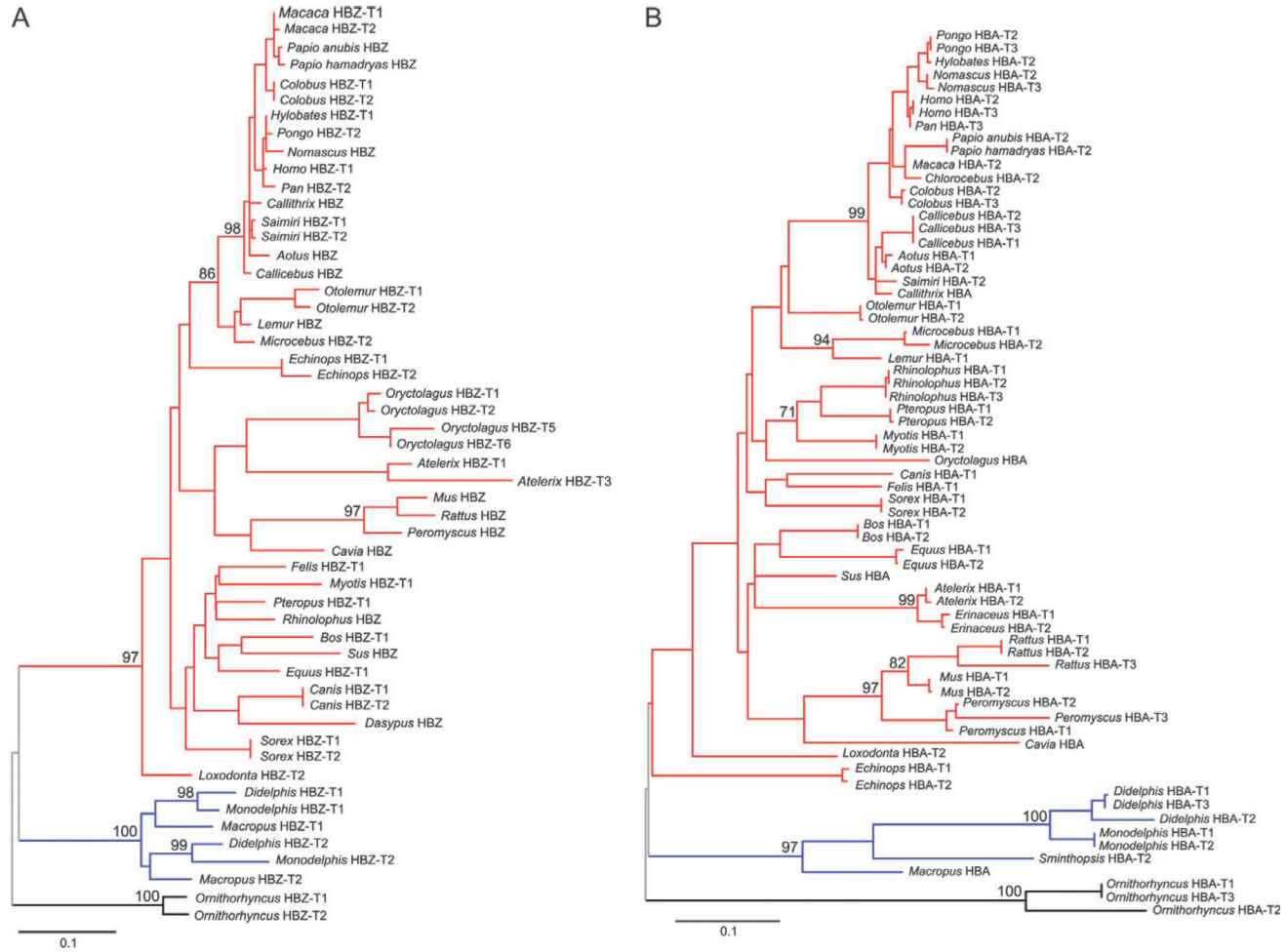

Figure 2.— Maximum likelihood phylogram depicting relationships among copies of HBZ (*A*) and HBA (*B*) in mammals. The placental mammal clade is shown in red, the marsupial clade is shown in blue, and the monotreme clade is shown in black. Bootstrap support for the relevant nodes was evaluated using 1,000 pseudoreplicates in unconstrained searches.

en bloc duplications in the tenrec (*Echinops telfairi*) and the rabbit (*Oryctolagus cuniculus*), where HBZ genes in the 3′ duplication block were located downstream of HBA genes in the 5′ duplication block (Figure 1).

We found that all species possess at least one functional copy of HBZ and HBA (Figure 1). By contrast, HBK is missing from the genomes of the glires (Rodentia + Lamogomorpha) and Afrotherians, and HBQ is missing from the genomes of the shrew (*Sorex araneus*), the armadillo (*Dasypus novemcinctus*), and the platypus (*Ornithorhyncus anatinus*).

In contrast to the HBZ, HBA, and HBQ genes, the HBK gene was never present in more than one copy. In our data set, the number of putatively functional genes ranged from 2 in the armadillo (HBZ and HBA) to 8 in the rabbit (4 copies of HBZ, 1 copy of HBA, and 3 copies of HBQ). The observed variation in gene copy number is primarily attributable to tandem duplications of single genes, although there is also evidence for *en bloc* duplications involving sets of 2–3 closely linked genes. For example, triplication of an ancestral HBA–HBQ gene pair is evident in the α-globin gene cluster of the rat (*Rattus norvegicus*) (Storz, Hoffmann, *et al.* 2008), and the rabbit has several variant copies of an HBZ–HBZ–HBA–HBQ repeat motif, as first reported by Cheng *et al.* (1987).

Ancestral State of the Mammalian α-Globin Gene Cluster

Based on a comparative analysis of the α-globin gene cluster of marsupials and placental mammals, Cooper *et al.* (2006) proposed that the α-globin gene cluster in the common ancestor of therian mammals (marsupials + placentals) contained 7 α-like globin genes, in addition to a single copy of ω-globin (HBW) at the 3′ end of the cluster: 5′-HBZ-T1, HBZ-T2, HBK, HBA-T1, HBA-T2, HBA-T3, HBQ, HBW-3′. The HBW gene is a β-like globin gene that has previously been described only in marsupials (Wheeler *et al.* 2001, 2004; De Leo *et al.* 2005). The location of the HBW gene at the 3′ end of the α-globin gene cluster reflects the ancestral linkage arrangement of α- and β-like globin genes in the common ancestor of amniote vertebrates (Wheeler *et al.* 2001, 2004). The availability of genomic sequence from a monotreme taxon, the platypus, provides an opportunity to evaluate the hypothesized structure of the ancestral α-globin gene cluster at the stem of the mammalian radiation. We identified 6 α-like globin genes in the platypus: 5′-HBZ-T1, HBZ-T2, HBK, HBA-T1, HBA-T2, HBA-T3-3′. We also confirmed the presence of HBW at the 3′ end of the cluster (Figure 1). However, we found no evidence of an HBQ gene in the α-globin gene cluster of the platypus. Thus, aside from the absence of HBQ, the platypus α-globin

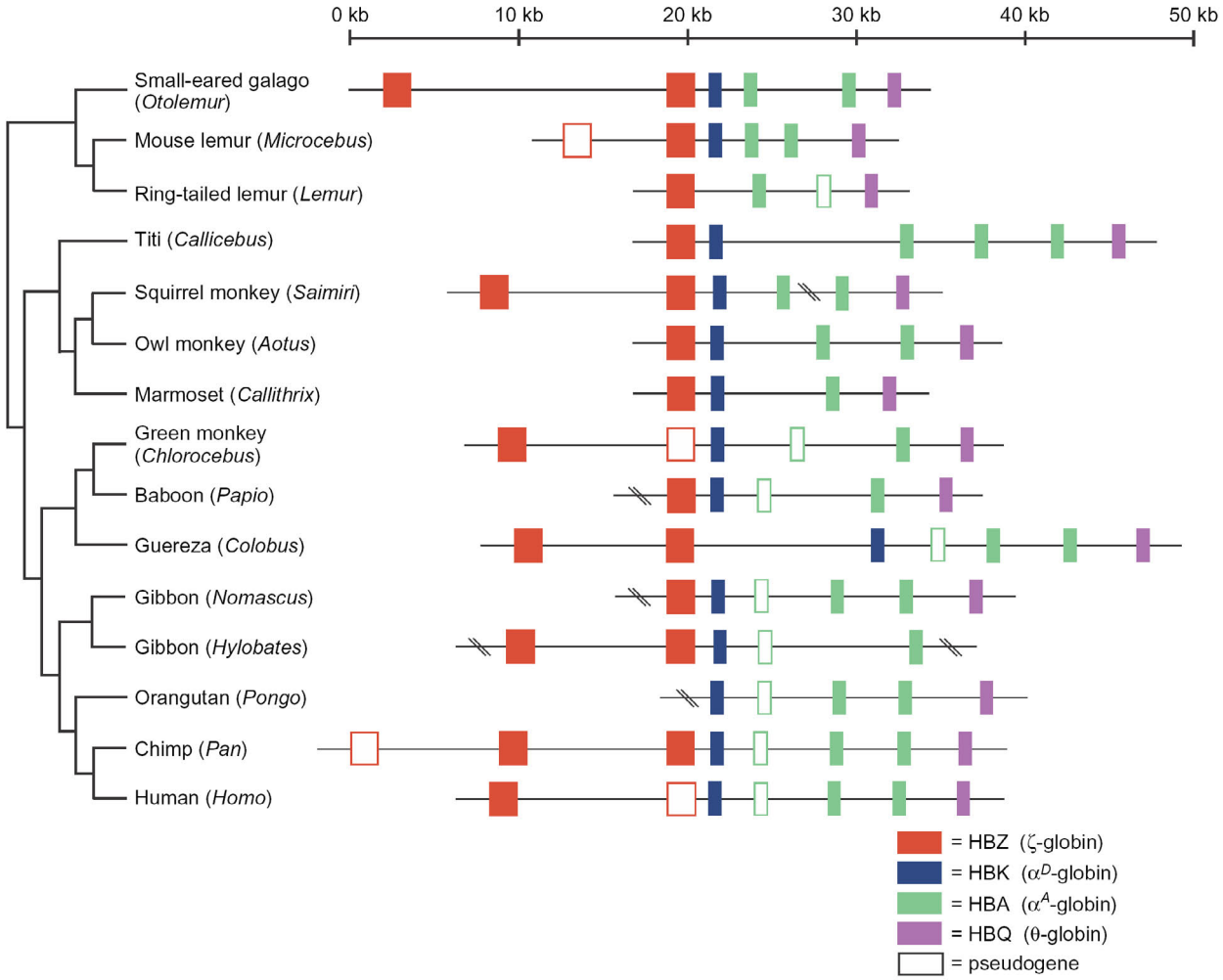

Figure 3.— Genomic structure of the α-globin cluster in primates. Phylogenetic relationships among species follow Goodman *et al.* (2005) and Opazo *et al.* (2006). Diagonal slashes indicate gaps in genomic coverage. Segments containing such gaps were not drawn to scale. Pseudogene fragments containing less than two complete exons were not included. The orientation of the clusters is from 5′ (on the left) to 3′ (on the right).

cluster is similar to the ancestral therian α-globin gene cluster proposed by Cooper *et al.* (2006). The existence of duplicated copies of HBZ and HBA in the α-globin gene cluster of monotremes, marsupials, and placental mammals suggests that duplicate copies of both genes may have been present in the common ancestor of all extant mammals. The absence of an HBQ gene in the α-globin gene cluster of the platypus suggests that the duplication that gave rise to HBQ occurred after the divergence between monotremes and therian mammals. Conversely, the fact that we found no trace of HBW in any of the eutherian mammals examined indicates that the loss of HBW from the 3′ end of the α-globin gene cluster predates the radiation of extant placental mammals. Accordingly, the principle of parsimony suggests the following gene arrangement in the last common ancestor of monotremes, marsupials, and placental mammals: 5′-HBZ-T1, HBZ-T2, HBK, HBA-T1, HBA-T2, HBA-T3, HBW-3′. This inferred ancestral gene arrangement is identical to the consensus gene arrangement in extant mammals, except that in placental mammals HBQ has been added and HBW has been lost.

Evolution of Duplicate Copies of HBZ and HBA

In several cases, phylogeny reconstructions of mammalian α-like globin genes did not recover the expected set of species relationships but deviations from the expected species relationships were not statistically significant. This discordance between the inferred gene trees and the expected species trees is not surprising given the number of informative sites in the alignment relative to the number of taxa. The trees in Figure 2 correspond to results of phylogeny reconstructions for the mammalian HBA and HBZ genes where sequences were constrained to match the systematic relationships among the major mammalian subclasses: (Monotremes (Marsupials, Placentals)).

The phylogenies obtained show the hallmark of concerted evolution: paralogous copies of HBA and HBZ form monophyletic clades within species, to the exclusion of sequences from other species (Figure 2). The HBZ genes of marsupials represent the one notable exception to this general pattern (Figure 2*A*). Within marsupials, the HBZ-T1 and HBZ-T2 paralogs from the tammar wallaby (*Macropus eugenii*), opossum

(*Didelphis virginiana*), and short-tailed opossum (*Monodelphis domestica*) are reciprocally monophyletic to one another. In each case, the HBZ-T1 and HBZ-T2 clades both recover the expected species phylogeny: (*Macropus* (*Didelphis*, *Monodelphis*)). Dot plot comparisons between HBZ paralogs in monotremes, marsupials, and placental mammals are consistent with the inferences drawn from phylogenetic analyses. In monotremes and placental mammals, there are good sequence matches between the paralogous HBZ genes within the same species. However, dot plot comparisons between the HBZ-T1 and HBZ-T2 paralogs of marsupials revealed the presence of a ~1-kb block of nonhomology in the second intron (supplementary Figure S2, Supplementary Material).

Evolution of the α-Globin Gene Cluster in Primates

To investigate mechanisms of gene family evolution in more detail, we focused our analysis of sequence variation on the α-globin gene cluster of primates, the taxon for which we have the most genomic sequence data. In addition to the previously characterized human α-globin gene cluster on Chromosome 16 (GenBank accession number NG_000006 [Flint *et al.* 2001]), we characterized the genomic structure of the α-globin gene cluster in an additional 14 primate species (3 prosimians, 4 New World monkeys, 3 Old World monkeys, and 4 apes). The primate species represented in our data set possess either 1 or 2 copies of HBZ, 1 copy of HBK, 1 to 3 copies of HBA, and 1 copy of HBQ (Figure 3).

Because systematic relationships among the species in our study have been the subject of intensive study (Goodman *et al.* 2005; Opazo *et al.* 2006), we have a solid phylogenetic framework for reconstructing gains and losses of α-like globin genes over the course of primate evolution (Figure 3). As described below, we found that the vast majority of de novo duplications and deletions of α-like globin genes in primates can be attributed to unequal crossing-over events. An unequal crossing-over event between two chromosomes that both carry a tandemly duplicated pair of genes will produce two daughter chromosomes that carry either one or three copies of the gene, the historical record of this event is written in the pattern of sequence variation in upstream and downstream flanking regions (Figure 4).

Evolution of the Primate α-Globin Gene Cluster

The objective of our initial analyses was to assign orthologous relationships among the multiple HBZ and HBA genes found in primates. Although phylogeny reconstructions based on coding sequence indicate that orthologous relationships among HBZ and HBA genes have been obscured by a history of concerted evolution, the true history of gene duplication and species divergence is revealed by sequence variation in flanking regions (Figures 5 and 6). In the case of the 5′ HBZ genes of primates, gene conversion tracts appear to be restricted to the exons and introns of the genes as phylogenetic analyses based on upstream and downstream flanking sequence recover the expected species relationships with strong bootstrap support. In all primate species that possess multiple HBZ copies, our phylogeny reconstructions reveal evidence for 1:1 orthology among the full set of 5′ HBZ genes and among all the 3′ HBZ genes with the exception of the guereza (*Colobus guereza*; Figures 5 and 7*A*).

Assigning orthology among the HBA genes was complicated because gene conversion tracts often extend upstream and downstream of the coding region (supplementary table S2, Supplementary Material). Analyses based on 1 kb of flanking sequence upstream of the start codon strongly suggest that the 5′ HBA gene of prosimians is orthologous to the 5′ HBA pseudogene of most Old World monkeys and apes. Likewise, analyses based on 1 kb of flanking sequence downstream of the stop codon indicate that the 3′ HBA genes of most primates are 1:1 orthologs (Figures 6 and 7*B*). Interestingly, none of the New World monkeys appear to possess an ortholog of the 5′ HBA gene of prosimians, Old World monkeys, and apes. Based on these data, there are two equally parsimonious reconstructions of the ancestral α-globin gene cluster of primates. One possibility is that the ancestral α-globin gene cluster contained duplicate copies of both HBZ and HBA, and the other possibility if that it contained duplicate copies of HBZ and triplicate copies of HBA. The following sections assume that the former scenario is correct, based on the fact that prosimians do not possess an ortholog of the HBA gene that would have been the middle gene of the 3-gene set in the common ancestor of New World monkeys, Old World monkeys, and apes (=the HBA-T1 gene of *Callicebus*; Figure 7*B*). It should be noted that our inferences about the numbers of gene gains and losses are identical under both scenarios.

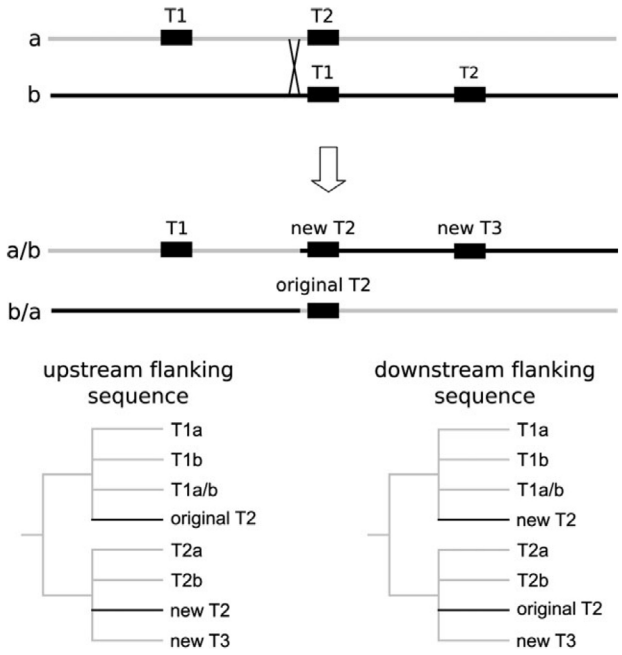

Figure 4.— Unequal crossing-over occurs as a result of mispairing between paralogous gene duplicates on sister chromatids during mitosis in germ line cells or between homologous chromosomes during meiosis (Lam and Jeffreys 2006, 2007). In the case where two sister chromatids carry a tandemly duplicated pair of genes (T1 and T2), unequal crossing-over results in a gene duplication on one daughter chromosome (yielding an additional "T3" copy) and a corresponding gene deletion on the other daughter chromosome (yielding a solitary "T1" copy). The trees depict the resultant pattern of sequence matches. Notice that the "original T2" gene on chromosome b/a, has upstream flanking sequence that matches the T1 genes on chromosomes a and b, but it has downstream flanking sequence that matches the T2 genes on chromosomes a and b.

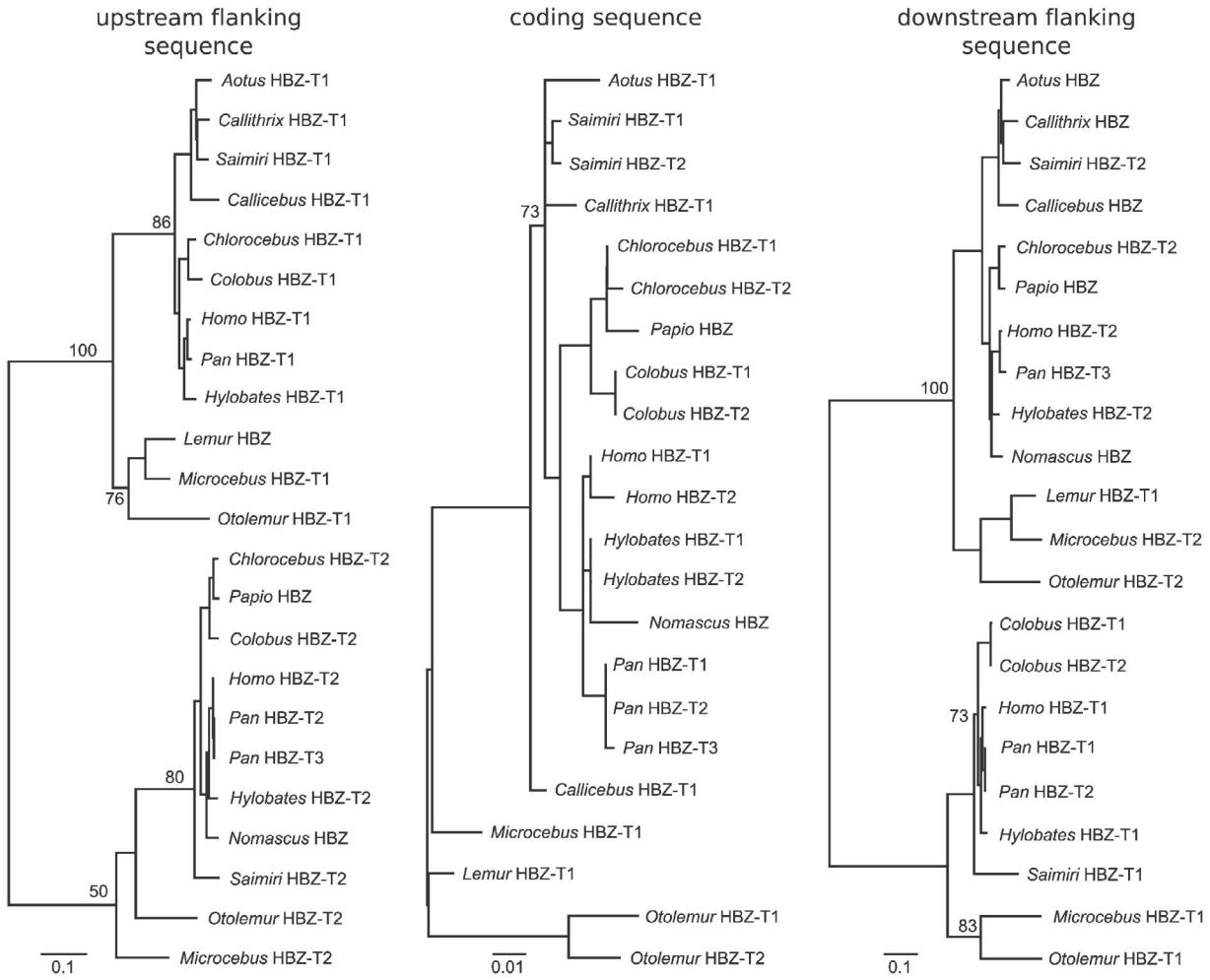

Figure 5.— Maximum likelihood phylograms depicting relationships among HBZ genes in primates based on 500 bp of 5′ flanking sequence (left column), the coding sequence (center column), and 500 bp of 3′ flanking sequence (right column). Pseudogenes are identified by the "ps" suffix. Bootstrap support for the relevant nodes was evaluated using 1,000 pseudoreplicates.

Prosimians (Strepsirrhini)

We analyzed genomic sequence data from three species of prosimian primates: the small-eared galago (*Otolemur garnettii*), the mouse lemur (*Microcebus murinus*), and the ring-tailed lemur (*Lemur catta*). All three species possess duplicated copies of HBA, and the mouse lemur and the galago also possess duplicated copies of HBZ (Figure 3). This suggests that the common ancestor of these three prosimian species had an α-globin gene cluster with the following structure: 5′-HBZ-T1, HBZ-T2, HBK, HBA-T1, HBA-T2, HBQ-3′. If this ancestral gene arrangement is correct, then single copies of HBZ and HBK have been secondarily lost in the ring-tailed lemur. Phylogeny reconstructions of flanking sequence indicate that the HBZ-T1 pseudogene of the mouse lemur is orthologous to the HBZ-T1 gene of the galago and that the HBZ-T2 genes in the galago and the mouse lemur are 1:1 orthologs as well (Figure 5). In contrast, the ring-tailed lemur has a single copy of HBZ. Whereas the 5′ flanking sequence of this gene matches the 5′ flanking sequence of the HBZ-T1 gene in the galago and mouse lemur, the 3′ flanking sequence of the ring-tailed lemur HBZ gene matches the 3′ flanking sequence of HBZ-T2 in these other two species (Figure 5). This suggests that one HBZ gene was deleted from the α-globin gene cluster of the ring-tailed lemur by an unequal crossing-over event similar to that shown in Figure 4.

In the case of the HBA paralogs, all prosimians have two copies, one of which has become a pseudogene in the ring-tailed lemur (Figure 3). Despite the fact that coding regions of the HBA paralogs have been homogenized by gene conversion, analyses of the flanking regions reveal that the HBA-T1 genes of all prosimians are 1:1 orthologs and likewise for the HBA-T2 copies (Figures 6 and 7*B*).

Anthropoid Primates (Platyrrhini and Catarrhini)

The α-globin gene cluster of anthropoid primates appears to have undergone an especially high rate of turnover due to lineage-specific gains and losses of HBZ and HBA genes (Figure 7). Genomic sequence comparisons indicate that the α-globin gene cluster in the ancestor of New World monkeys (platyrrhines) contained two copies of HBZ and two copies of HBA and that the α-globin gene cluster in the common ancestor of Old World monkeys and apes (catarrhines) contained

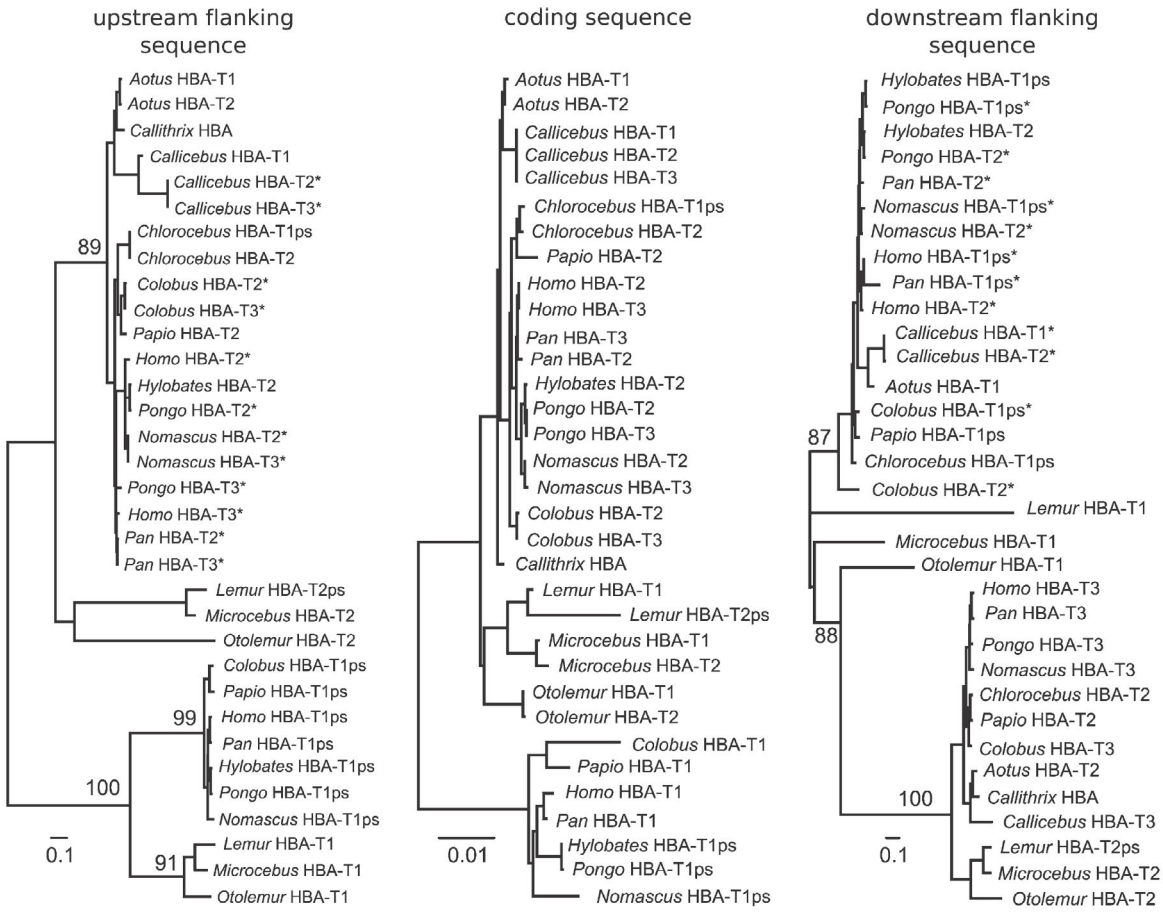

Figure 6.— Maximum likelihood phylograms describing relationships among HBA paralogs in primates based on 1 kb of upstream flanking sequence (left column), the coding sequence (center column), and 1 kb of 3′ flanking sequence (right column). Pseudogenes are identified by the "ps" suffix. Bootstrap support for the relevant nodes was evaluated using 1,000 pseudoreplicates. In the left and right columns, asterisks denote cases where gene conversion tracts extend part way into flanking sequence (the 3′ end of upstream flanking sequence or the 5′ end of downstream flanking sequence).

two copies of HBZ and three copies of HBA. As in prosimians, phylogeny reconstructions based on coding sequence indicate that orthologous relationships among HBZ and HBA genes have been obscured by a history of concerted evolution, but analyses of flanking sequence enable us to resolve orthologous relationships in the majority of cases (Figures 5 and 6). Three of the platyrrhines in our data set, the titi monkey (*Callicebus moloch*), the owl monkey (*Aotus nancymaae*), and the marmoset (*Callithrix jacchus*), possess a single copy of HBZ, and the fourth species, the squirrel monkey (*Saimiri boliviensis*), has duplicate copies of HBZ. Comparisons of flanking sequence indicate that the *Saimiri* HBZ-T1 gene is orthologous to the HBZ-T1 gene of Old World monkeys and apes (catarrhines), whereas HBZ-T2 is orthologous to the 3′ HBZ gene of catarrhines (Figures 5 and 7*A*). This indicates that an HBZ paralog was lost independently in each of the platyrrhine species that have a single HBZ gene, and analysis of upstream and downstream flanking sequence indicates that these losses were due to unequal crossing-over, as in the ring-tailed lemur.

Phylogenetic analyses suggest that the presence of three copies of HBA in the ancestor of catarrhines was due to unequal crossing-over between chromosomes that originally possessed two copies of HBA. This conclusion is consistent with the observed distribution of homology blocks found in the α-globin gene cluster of apes (Shaw *et al.* 1991; Bailey *et al.* 1997). The upstream flanking sequence of HBA-T2 of most catarrhines is more closely related to the upstream sequence of the 3′ HBA gene of prosimians and platyrrhines. Conversely, the downstream sequence HBA-T2 of most catarrhines is more closely related to the downstream sequence of the 5′ HBA gene of prosimians and platyrrhines (Figure 6). These results suggest that the 5′ HBA gene of most platyrrhines is orthologous to the HBA-T2 gene of most catarrhines (Figures 6 and 7*B*). Additional phylogenetic analysis of a 2-kb alignment of flanking sequence upstream the start codon of the HBA genes of anthropoids (supplementary Figure S3, Supplementary Material) provided additional insights into the true set of orthologous relationships, although the presence of gene conversion tracts is also evident in the analyses of flanking sequences. Taken together, our analyses of upstream and downstream flanking sequence also suggest that, with the exception of the olive baboon (*Papio anubis*), the white-handed gibbon (*Hylobates klossi*), and the green monkey (*Chlorocebus aethiops*), all HBA-T2 genes of catarrhines are 1:1 orthologs (Figure 7*B*). The analysis of flanking

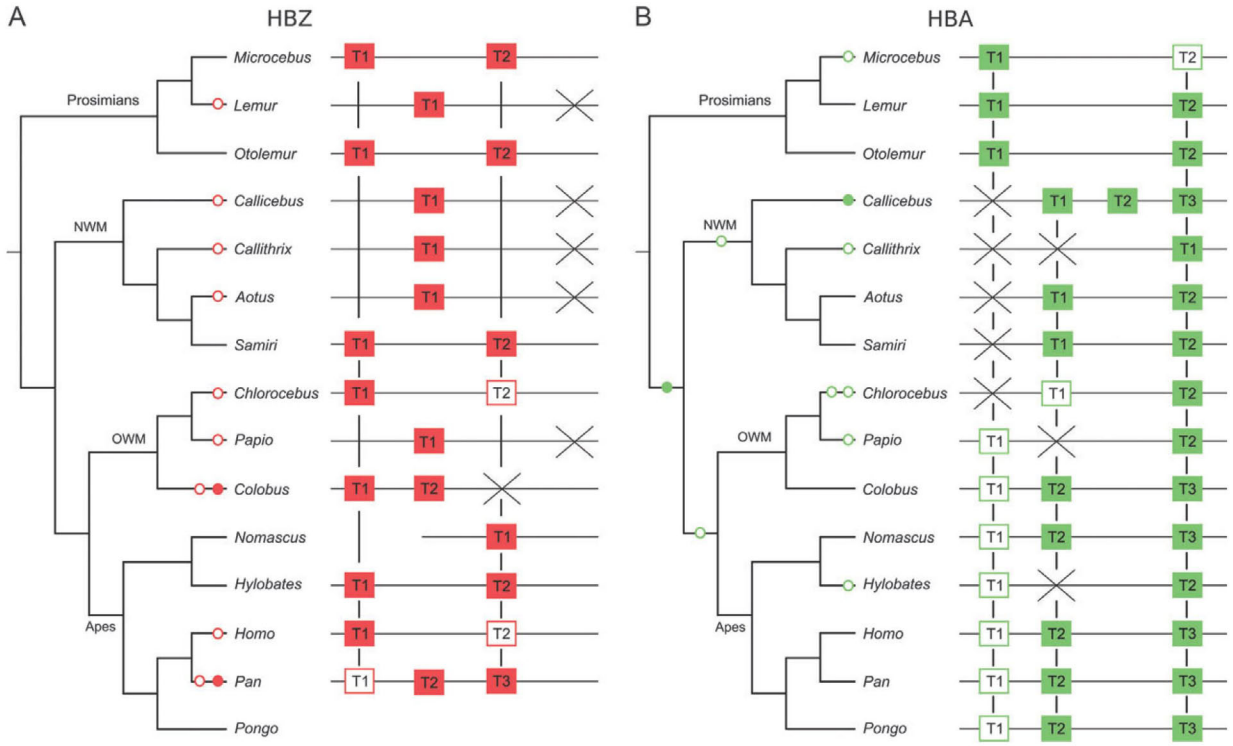

Figure 7.— Orthologous relationships among the HBZ (*A*) and HBA (*B*) genes of anthropoid primates, as inferred from an analysis of 5′ and 3′ flanking sequence. Solid boxes denote putatively functional genes, open boxes denote pseudogenes, and crosses denote gene deletions. Vertical lines are used to indicate orthologous relationships. Gene gains (solid circles) and gene losses (open circles) were mapped onto the primate phylogeny. Gene losses include inactivations (creation of a pseudogene) as well as wholesale gene deletions. The crosses on the fourth column of panel A identify cases where an HBZ copy was deleted, but we were not able to ascertain the orthology of the deleted copy. Due to incomplete coverage, we did not include HBZ sequences from the orangutan (*Pongo*) in panel A. The orientation of the clusters is from 5′ (on the left) to 3′ (on the right).

sequences also revealed that the HBA-T2 gene of the dusky titi (*C. moloch*) originated via an independent, unequal crossing-over event. Finally, orthologs of the HBA-T1 gene of prosimians have been inactivated in all catarrhines other than *Chlorocebus*, and they have been deleted independently in *Chlorocebus* and in all platyrrhines. Accordingly, we infer that the ancestral HBA-T1 gene was deleted in the stem lineage of the platyrrhine clade and that the α-globin gene cluster in the common ancestor of anthropoid primates had the following gene arrangement: 5′-HBZ-T1, HBZ-T2, HBK, HBA-T1, HBA-T2, HBA-T3, HBQ-3′.

The lineage-specific gains and losses of HBZ and HBA genes in primates mirrors patterns that have been described in rodents, where unequal crossing-over events gave rise to functionally distinct copies of HBA in the deer mouse (*Peromyscus maniculatus*) and the Norway rat (*R. norvegicus*) (Storz, Hoffmann, *et al.* 2008). Although phylogeny reconstructions reveal that interparalog gene conversion is pervasive in coding regions, our analysis of flanking sequences revealed several instances where monophyly of paralogous sequences from the same species is attributable to lineage-specific gene duplications. In primates, for example, we identified four α-like globin genes that were the products of de novo duplication events: HBZ-T2 in *Pan*, HBZ-T2 in *Colobus*, HBA-T2 in *Callicebus*, and HBA-T2 in the common ancestor of platyrrhines and catarrhines. The latter gene has been secondarily lost several times independently, having been deleted in *Callithrix*, *Hylobates*, and *P. anubis* and inactivated in *Chlorocebus*.

As an explanation for patterns of sequence similarity among paralogous genes within the same species, results of our comparative genomic analysis of the α-globin gene family in mammals suggest that concerted evolution may not be as important as many previous workers had assumed. This conclusion is consistent with the results of several other comparative genomic studies of gene family evolution (Nei *et al.* 2000; Piontkivska *et al.* 2002; Eirin-Lopez *et al.* 2004; Nei and Rooney 2005; Rooney and Ward 2005).

Evolutionary Implications of Variation in Copy Number among Species

Results of our study reveal a high rate of differential gene gain and loss among the α-globin gene clusters of different mammalian species. Over the course of mammalian evolution, we have documented the "birth" of new genes via duplication as well as "death" via inactivation or deletion. This "genomic revolving door" (Demuth *et al.* 2006) of gene gain and loss has resulted in continual turnover in the membership of the α-globin gene family. Consequently, many mammals possess α-like globin genes that have no orthologous counterparts in closely related species. In other cases, the ortholog of an apparently functional gene in one species is a pseudogene in another spe-

cies. For example, the ortholog of the HBZ-T3 gene in chimpanzee is a pseudogene in human, and the ortholog of the HBZ-T1 gene in humans is a pseudogene in chimpanzee (Figure 7*A*). Results of our detailed study of the α-globin gene family mirror the results of a genome-wide survey of size variation among mammalian gene families (Demuth *et al.* 2006). The analysis of Demuth *et al.* (2006) revealed that at least 6% of genes between human and chimpanzee are not orthologous. As these authors point out, this striking difference in gene content between the human and chimpanzee genomes stands in stark contrast to the well-documented 1.5% difference between orthologous nucleotide sequences.

The addition or subtraction of genes is expected to produce dosage imbalances that may often have deleterious effects. The adverse effects of such dosage imbalances have been well documented in the case of the adult α- and β-globin genes, as whole or partial gene deletions produce the thalassemia pathologies (Forget 2001; Higgs 2001; Lam and Jeffreys 2007). Although changes in gene dosage are generally expected to have deleterious effects, variation in gene copy number may also represent a source of potentially adaptive regulatory variation. It has been suggested that phenotypic differences among species are more commonly attributable to changes in gene regulation than changes in protein structure (King and Wilson 1975; Carroll 2005). The variation in globin gene copy number that we have documented among different mammalian lineages may constitute an important source of regulatory variation that affects physiologically important aspects of blood oxygen transport and aerobic energy metabolism.

Acknowledgements

We thank K. Campbell, D. Irwin, A. Runck, and four anonymous reviewers for helpful comments and suggestions. This work was funded by a Postdoctoral Fellowship in Population Biology to F.G.H. from the University of Nebraska–Lincoln, an National Science Foundation grant to J.F.S. (DEB-0614342), a Layman Award to J.F.S., and an Interdisciplinary Research Grant to J.F.S. from the Nebraska Research Council.

References

Aguileta G, Bielawski JP, Yang Z. Proposed standard nomenclature for the alpha- and beta-globin gene families. Genes Genet Syst (2006) 81:367–371.

Bailey AD, Shen CC, Shen CK. Molecular origin of the mosaic sequence arrangements of higher primate alpha-globin duplication units. Proc Natl Acad Sci USA (1997) 94:5177–5182.

Burge C, Karlin S. Prediction of complete gene structures in human genomic DNA. J Mol Biol (1997) 268:78–94.

Carroll SB. Evolution at two levels: on genes and form. PLoS Biol (2005) 3:e245.

Chen JM, Cooper DN, Chuzhanova N, Ferec C, Patrinos GP. Gene conversion: mechanisms, evolution and human disease. Nat Rev Genet (2007) 8:762–775.

Cheng JF, Raid L, Hardison RC. Block duplications of a zeta-zeta-alpha-theta gene set in the rabbit alpha-like globin gene cluster. J Biol Chem (1987) 262:5414–5421.

Cooper SJ, Wheeler D, De Leo A, Cheng JF, Holland RA, Marshall Graves JA, Hope RM. The mammalian alphaD-globin gene lineage and a new model for the molecular evolution of alpha-globin gene clusters at the stem of the mammalian radiation. Mol Phylogenet Evol (2006) 38:439–448.

Cooper SJ, Wheeler D, Hope RM, Dolman G, Saint KM, Gooley AA, Holland RA. The alpha-globin gene family of an Australian marsupial, *Macropus eugenii*: the long evolutionary history of the theta-globin gene and its functional status in mammals. J Mol Evol (2005) 60:653–664.

Czelusniak J, Goodman M, Hewett-Emmett D, Weiss ML, Venta PJ, Tashian REJ. Phylogenetic origins and adaptive evolution of avian and mammalian haemoglobin genes. Nature (1982) 298:297–300.

De Leo AA, Wheeler D, Lefevre C, Cheng JF, Hope R, Kuliwaba J, Nicholas KR, Westerman M, Graves JA. Sequencing and mapping hemoglobin gene clusters in the Australian model dasyurid marsupial *Sminthopsis macroura*. Cytogenet Genome Res (2005) 108:333–341.

Demuth JP, De Bie T, Stajich JE, Cristianini N, Hahn MW. The evolution of mammalian gene families. PLoS ONE (2006) 1:e85.

Dover G. Molecular drive: a cohesive mode of species evolution. Nature (1982) 299:111–117.

Eirin-Lopez JM, Gonzalez-Tizon AM, Martinez A, Mendez J. Birth-and-death evolution with strong purifying selection in the histone H1 multigene family and the origin of orphon H1 genes. Mol Biol Evol (2004) 21:1992–2003.

Flint J, Taylor AM, Clegg JB. Structure and evolution of the horse zeta globin locus. J Mol Biol (1988) 199:427–437.

Flint J, Tufarelli C, Peden J. (14 co-authors). Comparative genome analysis delimits a chromosomal domain and identifies key regulatory elements in the alpha globin cluster. Hum Mol Genet (2001) 10:371–382.

Forget BG. Molecular mechanisms of β-thalassemia. In: Disorders Of Hemoglobin: Genetics, Pathophysiology and Clinical management—Steinberg MH, Forget BG, Higgs DR, Nagel RL, eds. (2001) Cambridge (UK): Cambridge University Press. 252–276.

Gojobori T, Nei M. Concerted evolution of the immunoglobulin VH gene family. Mol Biol Evol (1984) 1:195–212.

Goodman M, Grossman LI, Wildman DE. Moving primate genomics beyond the chimpanzee genome. Trends Genet (2005) 21:511–517.

Goodman M, Miyamoto MM, Czelusniak J. Pattern and process in vertebrate phylogeny revealed by coevolution of molecules and phylogenies. In: Molecules and morphology in evolution. Conflict or Compromise?—Patterson C, ed. (1987) Cambridge (UK): Cambridge University Press. 140–176.

Goodman M, Moore GW, Matsuda G. Darwinian evolution in the genealogy of haemoglobin. Nature (1975) 253:603–608.

Graur D, Li W-H. Fundamentals of molecular evolution (2000) Sunderland (MA): Sinauer Associates.

Hallstrom BM, Kullberg M, Nilsson MA, Janke A. Phylogenomic data analyses provide evidence that Xenarthra and Afrotheria are sister groups. Mol Biol Evol (2007) 24:2059–2068.

Hardison R. Organization evolution and regulation of the globin genes. In: Disorders Of Hemoglobin: Genetics, Pathophysiology and Clinical management—Steinberg MH, Forget BG, Higgs DR, Nagel RL, eds. (2001) Cambridge (UK): Cambridge University Press. 95–115.

Hardison R, Miller W. Use of long sequence alignments to study the evolution and regulation of mammalian globin gene clusters. Mol Biol Evol (1993) 10:73–102.

Hardison RC, Gelinas RE. Assignment of orthologous relationships among mammalian alpha-globin genes by examining flanking regions reveals a rapid rate of evolution. Mol Biol Evol (1986) 3:243–261.

Higgs DR. Molecular mechanisms of α-thalassemia. In: Disorders Of Hemoglobin: Genetics, Pathophysiology and Clinical management—Steinberg MH, Forget BG, Higgs DR, Nagel RL, eds. (2001) Cambridge (UK): Cambridge University Press. 405–430.

Higgs DR, Vickers MA, Wilkie AO, Pretorius IM, Jarman AP, Weatherall DJ. A review of the molecular genetics of the human alpha-globin gene cluster. Blood (1989) 73:1081–1104.

Hoffmann FG, Storz JF. The αD-globin gene originated via duplication of an embryonic α-like globin gene in the ancestor of tetrapod vertebrates. Mol Biol Evol (2007) 24:1982–1990.

Jeffreys AJ. DNA sequence variants in the G gamma-, A gamma-, delta- and beta-globin genes of man. Cell (1979) 18:1–10.

Jobb G, von Haeseler A, Strimmer K. TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. BMC Evol Biol (2004) 4:18.

King MC, Wilson AC. Evolution at two levels in humans and chimpanzees. Science (1975) 188:107–116.

Lam KW, Jeffreys AJ. Processes of copy-number change in human DNA: the dynamics of α-globin gene deletion. Proc Natl Acad Sci USA (2006) 103:8921–8927.

Lam KW, Jeffreys AJ. Processes of de novo duplication of human α-globin genes. Proc Natl Acad Sci USA (2007) 104:10950–10955.

Li W-H, Luo C-C, Wu C-I. Evolution of DNA sequences. In: Molecular evolutionary genetics—MacIntyre R, ed. (1985) New York: Plenum. 1–94.

Liebhaber SA, Goossens M, Kan YW. Homology and concerted evolution at the alpha 1 and alpha 2 loci of human alpha-globin. Nature (1981) 290:26–29.

Michelson AM, Orkin SH. Boundaries of gene conversion within the duplicated human alpha-globin genes. Concerted evolution by segmental recombination. J Biol Chem (1983) 258:15245–15254.

Murphy WJ, Eizirik E, Johnson WE, Zhang YP, Ryder OA, O'Brien SJ. Molecular phylogenetics and the origins of placental mammals. Nature (2001) 409:614–618.

Murphy WJ, Pringle TH, Crider TA, Springer MS, Miller W. Using genomic data to unravel the root of the placental mammal phylogeny. Genome Res (2007) 17:413–421.

Nagel RL, Steinberg MH. Role of epistatic (modifier) genes in the modulation of the phenotypic diversity of sickle cell anemia. Pediatr Pathol Mol Med (2001) 20:123–136.

Nagylaki T. Evolution of multigene families under interchromosomal gene conversion. Proc Natl Acad Sci USA (1984a) 81:3796–3800.

Nagylaki T. The evolution of multigene families under intrachromosomal gene conversion. Genetics (1984b) 106:529–548.

Nagylaki T, Petes TD. Intrachromosomal gene conversion and the maintenance of sequence homogeneity among repeated genes. Genetics (1982) 100:315–337.

Nei M, Rogozin IB, Piontkivska H. Purifying selection and birth-and-death evolution in the ubiquitin gene family. Proc Natl Acad Sci USA (2000) 97:10866–10871.

Nei M, Rooney AP. Concerted and birth-and-death evolution of multigene families. Annu Rev Genet (2005) 39:121–152.

Ohta T. Evolution and variation of multigene families. Lecture notes in biomathematics. Vol. 37 (1980) New York: Springer.

Ohta T. Some models of gene conversion for treating the evolution of multigene families. Genetics (1984) 106:517–528.

Ohta T. How gene families evolve. Theor Popul Biol (1990) 37:213–219.

Ohta T. Evolution of gene families. Gene (2000) 259:45–52.

Opazo JC, Wildman DE, Prychitko T, Johnson RM, Goodman M. Phylogenetic relationships and divergence times among New World monkeys (Platyrrhini, Primates). Mol Phylogenet Evol (2006) 40:274–280.

Ovcharenko I, Loots GG, Giardine BM, Hou M, Ma J, Hardison RC, Stubbs L, Miller W. Mulan: multiple-sequence local alignment and visualization for studying function and evolution. Genome Res (2005) 15:184–194.

Piontkivska H, Rooney AP, Nei M. Purifying selection and birth-and-death evolution in the histone H4 gene family. Mol Biol Evol (2002) 19:689–697.

Proudfoot NJ, Gil A, Maniatis T. The structure of the human zeta-globin gene and a closely linked, nearly identical pseudogene. Cell (1982) 31:553–563.

Rodriguez F, Oliver JL, Marin A, Medina JR. The general stochastic model of nucleotide substitution. J Theor Biol (1990) 142:485–501.

Rooney AP, Ward TJ. Evolution of a large ribosomal RNA multigene family in filamentous fungi: birth and death of a concerted evolution paradigm. Proc Natl Acad Sci USA (2005) 102:5084–5089.

Sawyer S. Statistical tests for detecting gene conversion. Mol Biol Evol (1989) 6:526–538.

Schwartz S, Elnitski L, Li M, Weirauch M, Riemer C, Smit A, Green ED, Hardison RC, Miller W. MultiPipMaker

and supporting tools: alignments and analysis of multiple genomic DNA sequences. Nucleic Acids Res (2003) 31:3518–3524.

Schwartz S, Zhang Z, Frazer KA, Smit A, Riemer C, Bouck J, Gibbs R, Hardison R, Miller W. PipMaker–a web server for aligning two genomic DNA sequences. Genome Res (2000) 10:577–586.

Scott AF, Heath P, Trusko S, Boyer SH, Prass W, Goodman M, Czelusniak J, Chang LY, Slightom JL. The sequence of the gorilla fetal globin genes: evidence for multiple gene conversions in human evolution. Mol Biol Evol (1984) 1:371–389.

Shaw JP, Marks J, Shen CK. The adult alpha-globin locus of Old World monkeys: an abrupt breakdown of sequence similarity to human is defined by an Alu family repeat insertion site. J Mol Evol (1991) 33:506–513.

Shimodaira H. An approximately unbiased test of phylogenetic tree selection. Syst Biol (2002) 51:492–508.

Slightom JL, Blechl AE, Smithies O. Human fetal G gamma- and A gamma-globin genes: complete nucleotide sequences suggest that DNA can be exchanged between these duplicated genes. Cell (1980) 21:627–638.

Storz JF, Baze M, Waite JL, Hoffmann FG, Opazo JC, Hayes JP. Complex signatures of selection and gene conversion in the duplicated globin genes of house mice. Genetics (2007) 177:481–500.

Storz JF, Hoffmann FG, Opazo JC, Moriyama H. Forthcoming. Adaptive functional divergence among triplicated α-globin genes in rodents. Genetics (2008).

Storz JF, Sabatino SJ, Hoffmann FG, Gering EJ, Moriyama H, Ferrand N, Monteiro B, Nachman MW. The molecular basis of high-altitude adaptation in deer mice. PLoS Genet (2007) 3. e45, 448–459.

Tatusova TA, Madden TL. BLAST 2 Sequences, a new tool for comparing protein and nucleotide sequences. FEMS Microbiol Lett (1999) 174:247–250.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res (1997) 25:4876–4882.

Tufarelli C, Frischauf AM, Hardison R, Flint J, Higgs DR. Characterization of a widely expressed gene (LUC7-LIKE; LUC7L) defining the centromeric boundary of the human alpha-globin domain. Genomics (2001) 71:307–314.

Wheeler D, Hope R, Cooper SB, Dolman G, Webb GC, Bottema CD, Gooley AA, Goodman M, Holland RA. An orphaned mammalian beta-globin gene of ancient evolutionary origin. Proc Natl Acad Sci USA (2001) 98:1101–1106.

Wheeler D, Hope RM, Cooper SJ, Gooley AA, Holland RA. Linkage of the beta-like omega-globin gene to alpha-like globin genes in an Australian marsupial supports the chromosome duplication model for separation of globin gene clusters. J Mol Evol (2004) 58:642–652.

Wildman DE, Uddin M, Opazo JC, Liu G, Lefort V, Guindon S, Gascuel O, Grossman LI, Romero R, Goodman M. Genomics, biogeography, and the diversification of placental mammals. Proc Natl Acad Sci USA (2007) 104:14395–14400.

Zimmer EA, Martin SL, Beverley SM, Kan YW, Wilson AC. Rapid duplication and loss of genes coding for the alpha chains of hemoglobin. Proc Natl Acad Sci USA (1980) 77:2158–2162.

Supplemental Materials follow.

Figure S1. Intragenomic comparisons of pairwise sequence identity in the α-globin gene clusters of the short-tailed opossum (left), human (center), and rat (right). The plots correspond to a genomic contig that extends 50 kb upstream of the gene at the 5' end of the cluster (HBZ) and 50 kb downstream of the gene at the 3’ end of the cluster (HBQ). The central diagonal represents perfect sequence identity, and off-diagonal lines represent chromosomal regions that are locally alignable to one or more additional regions within the same genomic contig.

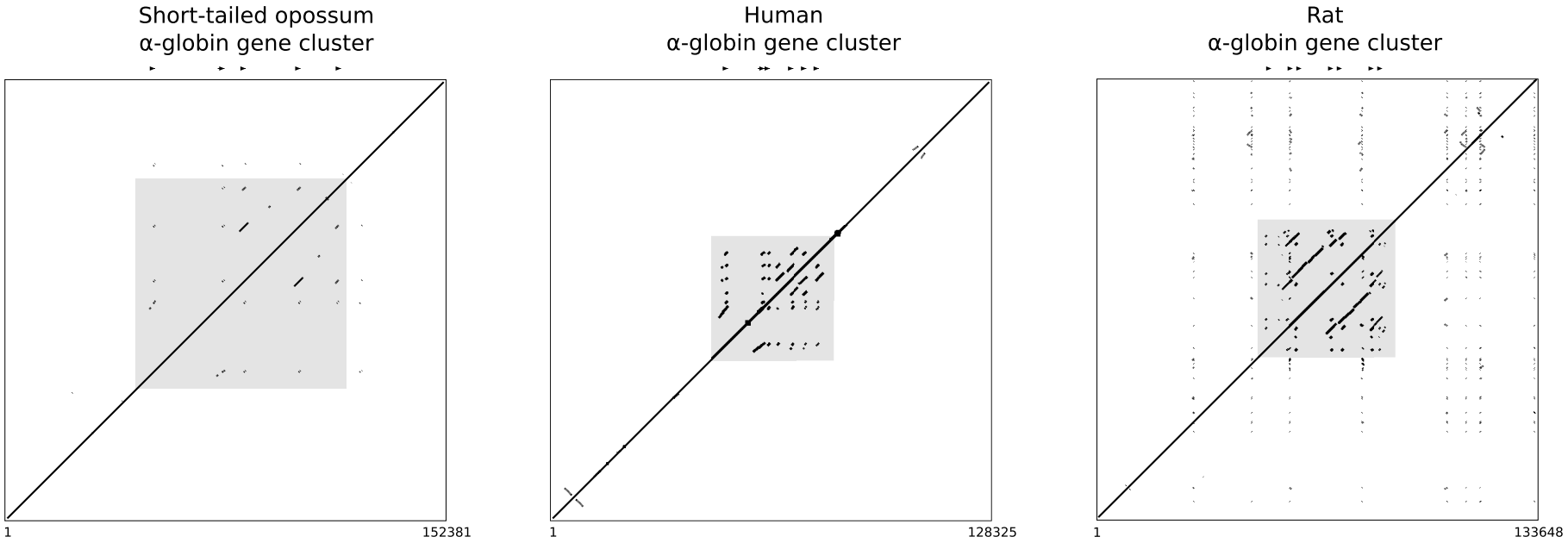

Figure S2. Intragenomic dot-plot comparisons between duplicated copies of HBZ for a placental mammal (pygmy hedgehog), a marsupial (short-tailed opossum), and a monotreme (platypus).

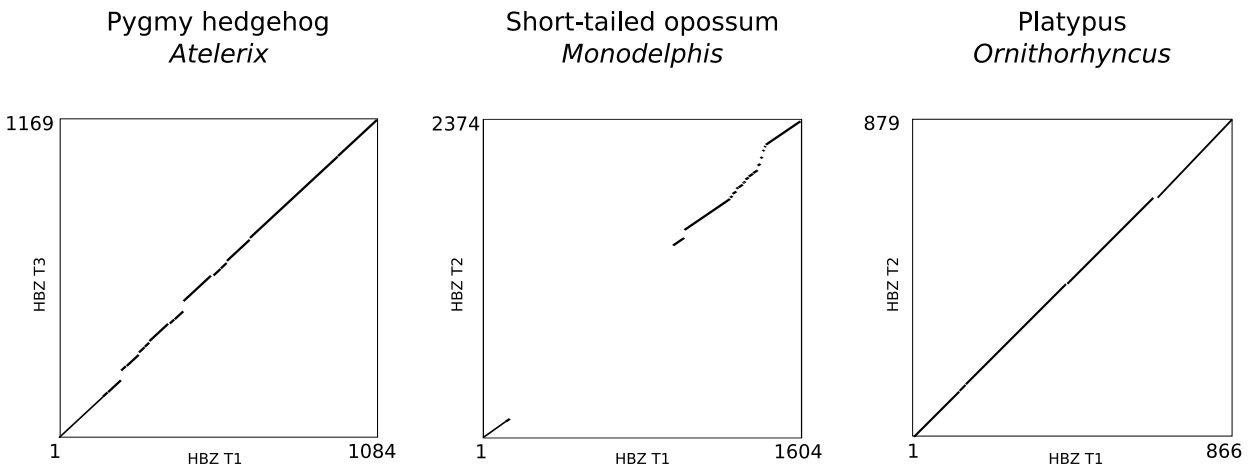

Figure S3. Maximum likelihood phylogram describing relationships among HBA paralogs in anthropoind primates based on 2 kb of upstream flanking sequence. Pseudogenes are identified by the "ps" suffix. Bootstrap support for the relevant nodes was evaluated using 1000 pseudoreplicates. Asterisks denote cases where gene conversion tracts extend part way into flanking sequence.

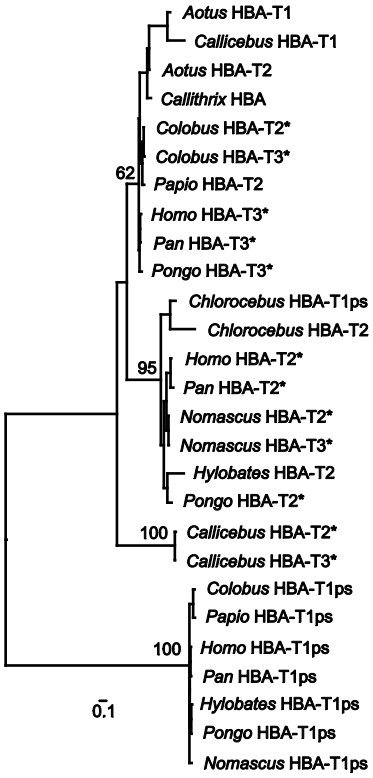

Table S1. Summary of DNA sequences used in the evolutionary analysis of α-like globin genes.

Taxon ID	Common Name	Accession Number (GenBank or ENSEMBL)
Eutherian Mammals (Placental)		
Afrotheria		
Echinops telfairi	Tenrec	AC174835
Loxodonta africana	African Elephant	AC158446, AC160597
Xenarthra		
Dasypus novemcintus	Nine-banded Armadillo	AC151647
Laurasiatheria		
Atelerix albiventris	African Pygmy Hedgehog	AC150435, AC171754
Bos taurus	Cow	AC150547
Canis familiaris	Dog	AC183635
Erinaceus europaeus	European Hedgehog	Ensemble, sc235869, sc_219931
Equus caballus	Horse	AC203695
Felis catus	Domestic Cat	AC130194
Myotis lucifugus	Little Brown Bat	AC182000, AC186242
Pteropus vampyrus	Flying Fox	AC205226
Rhinolophus ferrumequinum	Greater Horseshoe Bat	AC164632
Sorex araneus	Common Shrew	AC166625, AC192919
Sus scrofa	Pig	AC145444, AC130971
Euarchontoglires		
Aotus nancymaae	Owl Monkey	AC174393
Callicebus moloch	Titi Monkey	AC145465
Callithrix jacchus	Marmoset	AC158422
Cavia porcellus	Guinea pig	AC181986
Chlorocebus aethiops	Green Monkey	AC193704
Colobus guereza	Guereza Monkey	AC148220, AC174625
Homo sapiens	Human	NG_000006
Hylobates lar	White-handed Gibbon	AC146900
Lemur catta	Ring-tailed Lemur	AC145163
Macaca mulatta	Macaque	AC191460
Microcebus murinus	Mouse Lemur	AC174387
Mus musculus	House Mouse	AY016021
Nomascus leucogenys	White-cheeked Gibbon	AC190359
Oryctolagus cuniculus	European Rabbit	AC164931
Otolemur garnetti	Galago	AC146622
Pan troglodytes	Chimpanzee	AC145460
Papio anubis	Olive Baboon	AC115532
Papio hamadryas	Hamadryas Baboon	AC145461
Peromyscus maniculatus	Deer Mouse	EU053203
Rattus norvegicus	Norway Rat	NW_047334
Saimiri boliviensis	Squirrel Monkey	AC146643
Metatheria (Marsupials)		
Didelphis virginiana	Opossum	AC148752, AC139599
Macropus eugenii	Tammar Wallaby	AY459989, AY459590, AY789121, AY789122
Monodelphis domestica	Short-tailed Opossum	MonDom 5.0 at ENSEMBL
Sminthopsis macroura	Dunnart	AC146781
Prototheria (Monotremes)		
Ornithorhyncus anatinus	Platypus	AC203513
FISH		
Danio rerio	Zebra fish	NM_131257

Table S2. Genomic positions of interparalog gene conversion tracts in primate species with triplicated copies of HBA. Sequences included in the analyses extend 2 kb upstream of the start codon and 1 kb downstream of the stop codon.

Species	Genes	p-value*	Alignment position	Length (bp)	Affected gene region
Callicebus	HBA -T1, HBA-T2	$<10^{-4}$	2210-3741	1532	Exon 1 → 3 flanking sequence
	HBA-T2, HBA-T3	$<10^{-4}$	5-1664	1660	5 flanking sequence
Colobus	HBA -T1ps, HBA-T2	$<10^{-4}$	2880-3384	505	Intron 2 → 3 flanking sequence
	HBA-T2, HBA-T3	$<10^{-4}$	12-2876	2865	5 flanking sequence→ intron 2
Nomascus	HBA -T1ps, HBA-T2	$<10^{-4}$	2900-3822	923	Intron 2 → 3 flanking sequence
	HBA-T2, HBA-T3	$<10^{-4}$	24-2852	2829	5 flanking sequence→ intron 2
Pongo	HBA -T1ps, HBA-T2	$<10^{-4}$	2921-3809	889	Intron 2 → 3 flanking sequence
	HBA-T2, HBA-T3	$<10^{-4}$	1159-2793	1635	5 flanking sequence→ intron 2
Pan	HBA -T1ps, HBA-T2	$<10^{-4}$	2932-3531	600	Intron 2 → 3 flanking sequence
	HBA-T2, HBA-T3	$<10^{-4}$	1097-2753	1657	5 flanking sequence→ intron 2
Homo	HBA -T1ps, HBA-T2	$<10^{-4}$	2890-3803	915	Intron 2 → 3 flanking sequence
	HBA-T2, HBA-T3	$<10^{-4}$	1141-2799	1659	5 flanking sequence→ intron 2

*Calculated from the permutation test of Sawyer (1989).