Sequencing Primate Genomes: What Have We Learned?

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genome, sequencing, variation, gene comparison, speciation, diversity

Abstract

We summarize the progress in whole-genome sequencing and analyses of primate genomes. These emerging genome datasets have broadened our understanding of primate genome evolution revealing unexpected and complex patterns of evolutionary change. This includes the characterization of genome structural variation, episodic changes in the repeat landscape, differences in gene expression, new models regarding speciation, and the ephemeral nature of the recombination landscape. The functional characterization of genomic differences important in primate speciation and adaptation remains a significant challenge. Limited access to biological materials, the lack of detailed phenotypic data and the endangered status of many critical primate species have significantly attenuated research into the genetic basis of primate evolution. Next-generation sequencing technologies promise to greatly expand the number of available primate genome sequences; however, such draft genome sequences will likely miss critical genetic differences within complex genomic regions unless dedicated efforts are put forward to understand the full spectrum of genetic variation.

INTRODUCTION

As new primate genomes become sequenced and are compared within the context of the primate phylogeny (**Figure 1**), scientists are provided with an unprecedented opportunity to reconstruct the evolutionary history of every basepair of the human genome [see (44, 50, 60) for reviews]. This review focuses on how multiple primate genomes have provided a framework to understand the mode and tempo of primate genome evolution, including an understanding of our gene repertoire and chromosomal organization. We discuss how this information has provided a new understanding of the extent of primate molecular adaptations and stimulated new hypotheses regarding selection in primate genomes. We focus on mounting evidence that gene regulation and expression as opposed to amino acid changes have driven primate adaptations to new environments. Comparisons among primate genomes have begun to reveal more basepairs affected by genome structural variation (IN/DELS, duplications, deletions, insertions, and bursts of retrotransposition events) than single nucleotide changes (16, 28, 165). Such changes occur preferentially within complex regions of the primate genome wherein, paradoxically, newly minted genes and gene families of unknown function have been discovered (77, 79, 124). Comparison of primate genome sequences has provided new insights into the molecular mechanisms that have contributed to chromosomal evolution within our species (51, 86), their influences on recombination (128, 162), and their relationship to natural selection within primates (18). Finally, we discuss the future of primate genomics and the need to strike the right balance between the number of genomes versus the quality of the sequence assemblies. We conclude with a discussion of the challenges of performing functional studies and obtaining material from other primate species that are endangered.

PRIMATE GENOME SEQUENCING: RATIONALE AND PROGRESS

With the initial draft sequence assembly of the human genome in 2001 followed by the mouse genome in 2002 (94, 160), other

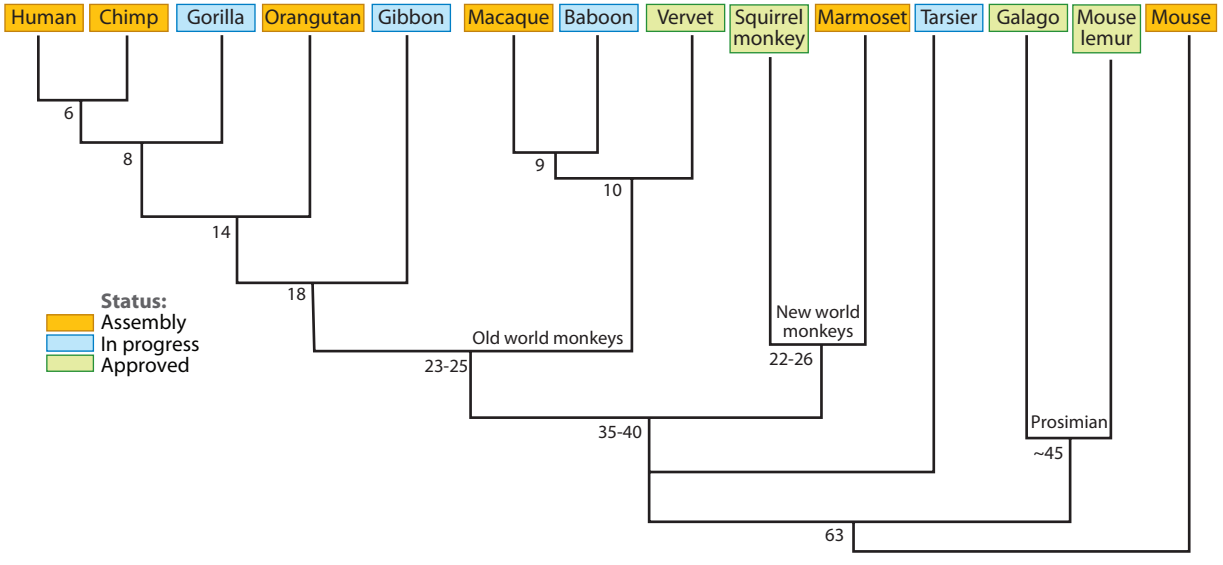

Figure 1

Primate genome sequencing status. Primate whole-genome sequencing projects (10/25/08) with respect to the generally accepted primate phylogeny and an outgroup (mouse). Divergence times are estimated based on millions of years according to Goodman et al. 1998.

primate species became high priorities for whole-genome sequencing. A series of proposals and white papers (41) identified less than a dozen key primate species as sequencing targets (**Figure 1**). From the beginning, species choice was based on two different (sometimes antagonistic and sometimes complementary) rationales: biomedical relevance and the species position within the primate phylogeny with respect to human. The first considered primate species as models of disease and biomedical research. The baboon and macaque were justified because of their use in studying the genetic basis of numerous diseases (e.g., diabetes, cardiovascular disease, hypertension) and as models of transplantation, reproduction, infection, immunity, and pharmacology. Similarly, the squirrel monkey is used as a model for understanding primate neurobiology and infectious disease (in particular, malarial research). Since many of the genes and gene families underlying biological processes such as immunity, reproduction, and drug detoxification change rapidly over short periods of evolutionary time, complete genome sequences would reveal these idiosyncratic aspects and thereby enhance the utility of these models for understanding disease. More importantly, high-quality genomes provide an immediate framework upon which to map genetic traits associated with diseases and to develop transgenic models of disease (164).

The second major rationale was to improve annotation of the human genome. Based on our current understanding of the primate phylogeny (57, 142), there are seven key points of evolutionary transition (**Figure 1**) with respect to human. Sequencing index species from each of these phylogenetic nodes (i.e., chimpanzee, orangutan, gibbon, etc.) has two advantages. Comparative sequencing will determine the ancestral and derived states of every single basepair within the human genome sequence, thereby assigning mutational events to different time points during human evolution. Second, multiple primate genome comparisons will highlight differences of functional import (bursts of regulatory changes or amino-acid changes within specific lineages) (15). We should emphasize that these fundamental motivations contrast with that of other genome projects. Most mammalian genome projects, for example, aim to identify conserved regulatory elements, exonic sequence and other regions of potential functional significance (102, 152). This focus on the differences demands a unique quality of product to eliminate potential artifacts. High-quality comparative sequencing of primate genomes is necessary in order to provide a balanced view of genome variation (including regions of structural variation, segmental duplications, lineage-specific events and chromosomal variation) and to identify true genetic differences (as opposed to sequencing or assembly artifacts) between the species.

In addition to continued refinement and sequencing of additional human genomes, 12 primate genomes are currently in progress for whole-genome sequencing (**Table 1**). In most cases, the representative individuals are females to provide adequate coverage of the X chromosome, albeit with the concomitant loss of Y chromosome genetic information. Two working draft assemblies (chimpanzee and macaque) have been published (32, 51) and three additional genome assemblies (orangutan, gibbon, and common marmoset) have been generated and are being analyzed. Although none of the genomes will currently be sequenced to the same standard as the human genome, the need for higher-quality draft assemblies has been recognized owing in part to the frustration of distinguishing artifacts from true sequence differences in the low-coverage three-fold chimpanzee draft assembly (32).

These initial analyses stressed the importance of independent assemblies of nonhuman primate genomes instead of simply mapping reads against the human reference sequence. As a result, typical working draft assemblies now target six- to eight-fold coverage in capillary whole-genome shotgun sequence reads. In five cases, these whole-genome shotgun sequence assemblies will be further supplemented by the sequencing of large-insert BAC clones mapping to structurally complex or biomedically

Table 1 Primate genome sequencing projects[1]

Species	Scientific name	Individual	Sequencing center(s)	Target	Assembly status	WGS	Query
Human	*Homo sapiens*	RPCI-11 (M)	Consortium	Finishing genome	Build37	78,410,449	TRACE_TYPE_CODE = "WGS" and SPECIES_CODE = "HOMO SAPIENS"
Chimpanzee	*Pan troglodytes*	Clint #C0471 (M)	WUGSC and BI/MIT	Draft2	PanTro2	31,339,291	TRACE_TYPE_CODE = "WGS" and SPECIES_CODE = "PAN TROGLODYTES"
Gorilla	*Gorilla gorilla*	Kamillah (F)	Sanger	Hybrid*	Not released*	8,302,852	TRACE_TYPE_CODE = "WGS" and (SPECIES_CODE = "GORILLA GORILLA" or SPECIES_CODE = "GORILLA GORILLA GORILLA")
Orangutan	*Pongo pygmaeus*	Susie ISIS#71 (F)	BCM and WUGSC	Draft2	Ponabe2	14,726,737	TRACE_TYPE_CODE = "WGS" and (SPECIES_CODE = "PONGO ABELII" or SPECIES_CODE = "PONGO PYGMAEUS" or SPECIES_CODE = "PONGO PYGMAEUS ABELII")
Gibbon	*Nomascus leucogenys*	Asia #0098 (F)	BCM and WUGSC	Draft2	In progress	28,885,424	TRACE_TYPE_CODE = "WGS" and SPECIES_CODE = "NOMASCUS LEUCOGENYS" or SPECIES_CODE = "NOMASCUS LEUCOGENYS LEUCOGENYS"
Rhesus macaque	*Macaca mulatta*	#25311 (F)	BCM, JCVI, WUGSC	Draft2	Rhemac2	23,017,908	TRACE_TYPE_CODE = "WGS" and SPECIES_CODE = "MACACA MULATTA"
Cynomolgus macaque	*Macaca fascicularis*	ND	WUGSC	Draft1	Not started		

Baboon	*Papio hamadryas*	ND	BCM	Draft1	In progress	12,717,107	TRACE_TYPE_CODE = "WGS" and SPECIES_CODE = "PAPIO HAMADRYAS"
Vervet	*Chlorocebus aethiops*	ND	WUGSC	Draft1	Not started		
Marmoset	*Callithrix jacchus*	SFPRC#17066	BCM and WUGSC	Draft2	Caljach1	25,615,089	TRACE_TYPE_CODE = "WGS" and SPECIES_CODE = "CALLITHRIX JACCHUS"
Squirrel monkey	*Saimiri boliviensis***	ND	BI/MIT	Draft1	Not started	690,374	TRACE_TYPE_CODE = "WGS" and SPECIES_CODE = "SAIMIRI BOLIVIENSIS" or SPECIES_CODE = "SAIMIRI BOLIVIENSIS BOLIVIENSIS"
Galago/ bushbaby	*Otolemur garnetti*	ND	BI/MIT	Draft1	Low coverage 2X	13,953,011	TRACE_TYPE_CODE = "WGS" and SPECIES_CODE = "OTOLEMUR GARNETTII"
Mouse lemur	*Microcebus murinus*	ND	BCM and BI/MIT	Draft1	Low coverage 2X	7,749,934	TRACE_TYPE_CODE = "WGS" and SPECIES_CODE = "MICROCEBUS MURINUS"

[1]For each genome project, we identify the specific individual whose genome is being sequenced, the responsible genome sequencing center, and number of available capillary whole-genome shotgun sequences (WGS) in the trace repository. As of 10/1/2008, Draft1 = genomes with 6–8 coverage WGS only; Draft2 = genomes with 6–8X coverage with targeted sequencing of BACs for difficult regions.

*Gorilla genome was approved by Wellcome Trust 12/2005, 2X capillary (3730) WGS data and 12 X WGS Solexa/Illumina WGS data were generated as of 12/2007; only 2X 3730 has been released as of 10/2008; Goal has shifted from WGSA of capillary reads to a hybrid assembly using next-generation technology, NIH approved targeted sequencing of complex regions within BACs.

**Specific squirrel monkey species is not yet definite.

relevant regions of the genome (a procedure known as genome refinement). This is especially crucial with respect to the phylogenetic index species such as chimpanzee, orangutan, gibbon, macaque, and squirrel monkey (indicated as Draft 2 in **Table 1**) where duplications and structural variation are now thought to account for more genetic variation than single basepair differences. Unfortunately, the gorilla genome assembly is not slated for the same standard. The Sanger Center has opted to generate an experimental hybrid assembly consisting of 12X Solexa sequencing data combined with 2X coverage of whole-genome assembly data. BAC refinement has been proposed but will be carried out as part of an NIH initiative.

FEATURES OF PRIMATE GENOME VARIATION

Although relatively few genome-wide comparisons have been completed to date (32, 51), analyses of the human, macaque, and chimpanzee genomes, as well as targeted resequencing of specific genomic segments, have revealed some important features and general principles of primate genome evolution. The alignment of the majority of genomic sequence from closely related primates is relatively trivial (38, 152) and shows a neutral pattern of single-nucleotide variation consistent with the primate phylogeny (**Figure 2**), although the rate of single-nucleotide variation has varied by a factor of three-fold within different lineages (42, 96, 143). Notably, the pattern of single-nucleotide variation also varies as a function of chromosome structure and organization (32, 51). Metacentric and acrocentric chromosomes show significant differences in variation, and regions within 10 Mb of telomeres evolve more rapidly perhaps as a consequence of biased gene conversion (**Figure 2**). On average, 10% of the genomic sequence has proven more elusive in terms of orthologous alignment. This includes segmental duplications, subtelomeric regions, pericentromeric regions, and lineage-specific repeats. Such regions typically cannot be resolved strictly by whole-genome sequence assembly (WGSA), thus larger insert clones (i.e., BAC clones) provide better substrates for resolving these areas.

Comparative sequence data highlight the value of genomic sequence from nonhuman primates to determine the ancestral and derived status of human alleles (27, 81). There have been some surprises. Phylogenetic analysis of resequenced regions among humans and the great apes reveal that as many as 18% of genomic regions are inconsistent with the Homo-Pan clade, and, rather, support a Homo-Gorilla clade (27). This has been taken as evidence of lineage-sorting and/or an ancestral hominid population size greater than five times that of the effective human population size (n = 10,000). Another surprise has been the identification of ancestral allelic variants that now occur as disease alleles within the human population, i.e., pyrin and familial Mediterranean fever (51, 136). Such findings suggest that the functional and selective effects of mutations change over time, perhaps as a result of environmental changes or compensatory genetic mutations.

COMPARATIVE GENE ANALYSES

The unique attributes of the primate order, and more specifically that of human, may be explained either as a consequence of key amino-acid changes within the coding sequences of a subset of critical genes or as a result of dramatic changes in how genes are regulated both temporally and spatially. Genome-wide analyses have provided traction for both of these views, although not with equal levels of support. A critical component of these analyses has been the construction of rigorous multiple-sequence alignments among primate genes. Despite the ease at which genomic sequences can be aligned among primate genomes, the number of genes that can be assigned to 1:1:1 orthologous group has changed only slightly with the first two nonhuman primate genomes sequenced. A three-way comparison involving chimp-human-mouse identified 7645 orthologues [by the estimate of (31)] as compared to 10,376 by human-chimp-macaque (51) over

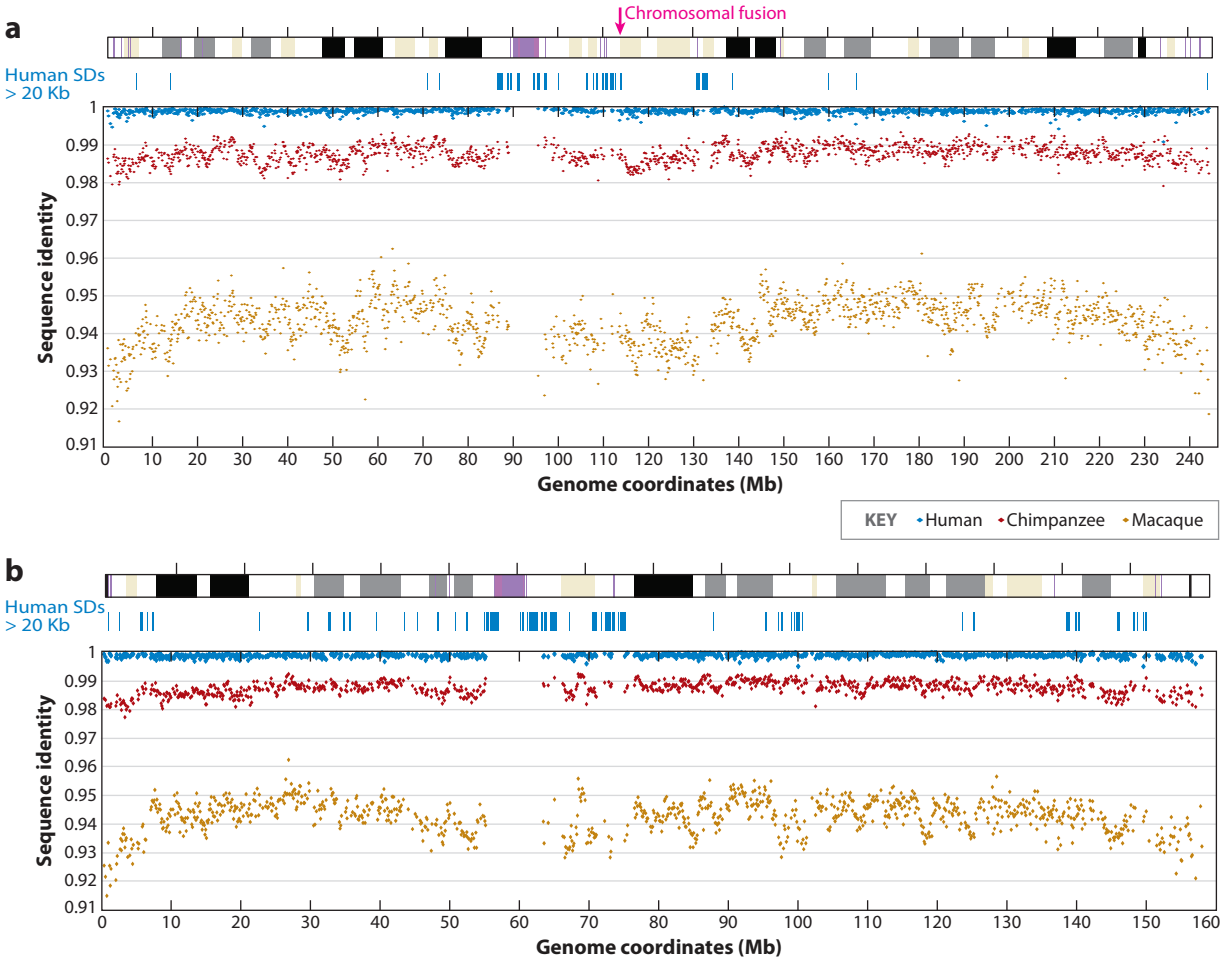

Figure 2

Human and primate sequence similarity. Whole-genome shotgun sequences from a human (*blue*), chimpanzee (*red*), and macaque (*brown*) were aligned against the human reference genome and the nucleotide divergence was computed in 100-kbp windows along (*a*) chromosome 2 and (*b*) chromosome 7. Note the increase in sequence divergence within 10 Mb of the telomere.

the total estimated 20,000 genes in the human genome. It follows then that a large fraction of human genes have not been subjected to three-way orthologous comparisons, and the pattern of selection (and its directionality) operating on ~50% of genes has not yet been adequately interrogated. With this caveat, we consider the lessons learned.

Evolution of Coding Sequences

The anthropocentric view of primate evolution, in which humans have acquired multiple idiosyncratic features needed for our success as a species, led to the tacit assumption that differences in a subset of key genes would explain the evolution of our species within the context of the primate order. Most of the first genome-wide studies of natural selection have thus been focused on coding sequence and estimates of omega (dN/dS)—the ratio of nonsynonymous versus synonymous substitutions as a metric of the signal of the intensity of such selection. The first three-way primate genome comparisons suggested that human and chimpanzee

genes had similar average omega values but significantly larger values than macaque or rodents (human = 0.169, chimpanzee = 0.175, macaque = 0.124, nonprimate mammals ~0.11) (51). This was explained by a relaxation of purifying selection during hominoid evolution as a consequence of smaller effective population sizes (see below, and see **Figure 7**).

Earlier studies that used mouse coding sequences as an outgroup (31) reported the first global estimates of genes under positive selection in human and chimpanzee, identifying more than 500 genes under positive selection (especially glycophorin C, protamines, and a gene family related to nociception). The posterior refinement with the macaque assembly, however, reduced that number to only ~200 genes (32, 51). Looking at pervasive adaptive evolution in genes within the same category led groups to identify gene ontologies enriched for positive selection (51, 115). The common conclusion of most of these studies is an overrepresentation of rapidly evolving genes in biological processes associated with immunity, olfaction, host defense, and reproduction. These enrichments, however, are not particular to human but represent adaptive changes more generally important in evolution of the primate and broadly other mammalian lineages.

A recent report using macaque as an outgroup has suggested that it is the chimpanzee lineage with an excess of positive selection compared to humans (7). Bakewell and colleagues found that even after multiple test correction, the chimpanzee has an excess of genes under positive selection (~40% more than human), although these conclusions should be tempered by the incomplete nature of the chimpanzee genome assembly and the potential for sequence errors to confound such estimates asymmetrically. Nevertheless, this view was also supported by the most comprehensive genomic comparison to date including six genomes (human, chimpanzee, macaque, mouse, rat, and dog). Given the increased power of this six-way comparison, it was possible to identify genes involved in biological processes rapidly diverging within the primate lineage in contrast to rodents. Once again, genes related to sensory perception (e.g., pain receptor NPFF2 and color vision genes OPN1SW), immunity (CCR1), and defense were overrepresented among positively selected genes (93). The previous study also found mRNA transcription, stress response, and protein metabolism as the main biological processes with excess of amino-acid replacements in chimpanzee but not in human. Despite some discrepancies, these and other studies, in general, did not find an enrichment of positively selected genes related to brain development or size.

Gene Regulation and Expression

After considerable scrutiny of coding sequences searching for traces of adaptive evolution, it has become increasingly apparent that regulatory differences must be playing a key role in specifying primate adaptations (25). This is not a new concept. Thirty years ago Wilson & King suggested that the phenotypic changes between humans and great apes were too dramatic to be explained by the rather limited degree of protein variation (91). There are many layers of complexity ranging from changes in gene expression, chromatin regulation, and patterns of alternative splicing. For example, a whole-genome comparison of alternative splicing between human and chimpanzee found that as much as 6%–8% of the analyzed exons showed evidence of differential alternative splicing (23).

Numerous microarray studies have focused on gene-expression differences between human and chimpanzee (21, 43, 84, 154) or even between male and female individuals of different primates (129). There has been a strong emphasis on the investigation of genes acting in the brain especially in light of human cognitive specializations. In this regard, however, it should be noted that chimps may, in fact, have some mental skills that are enhanced, such as faster hand-eye signaling of character recognition (69) (see **http://www.pri.kyoto-u.ac.jp/ai/video/video_library/project/project.html** for a library of impressive videos on the project). Significant gene-expression differences

between human and chimpanzee brains have been reported, but, surprisingly, other tissues (such as heart or liver) showed a larger number of differences (see (126) for a review on the use of microarrays on primate gene expression). Since the divergence of human and chimpanzee, ~100 genes have altered their gene expression patterns. Some studies suggest that gene expression differences in the brain have been biased toward the human lineage as a result of upregulation in humans (21, 43) (**Table 2**), although other studies contradict these results (52). In contrast, sex differences in brain gene expression have been a conserved feature over the course of primate evolution (129).

One interesting example of a differential gene expression between human and chimpanzee is the two- to nearly sixfold increase in gene expression of THBS2 and THBS4 (thrombospondin 2 and 4) within specific parts of the human brain when compared to chimpanzee or macaque (22). In this study, the authors showed that the levels of THBS2 and THBS4 mRNA are highly differentiated in the forebrain (cortex and caudate) but not in the cerebellum or other analyzed tissues. As these genes are involved in synaptogenesis, there was speculation that these changes in gene expression may be underlying some of the human cognitive specializations.

Recent reports suggest that the hypothetical rapid relative rate of change in gene expression in the human brain has occurred in the context of a decreased rate of coding sequence evolution (compared to the genomic average) (159). Genes whose expression is restricted to the brain show greater conservation than genes expressed in the brain along with other tissues, perhaps as a consequence of stronger selective constraint operating within brain biochemical networks. Despite this constraint, there are some interesting examples of adaptive evolution for brain-expressed genes. The glutamate dehydrogenase (GDH) gene (20) shows both evidence of increased copy as a result of duplication and traces of positive selection within the human lineage. It encodes a protein that plays an important role in neurotransmitter recycling.

There has been renewed interest on the role of posttranscriptional regulation via microRNA (miRNA) and transcription factors. The study of miRNA, for example, has become increasingly relevant because of the central role miRNA plays in regulating the transcriptome of plants and animals (8). A whole-genome comparison of brain miRNA of human and chimpanzee found that more than 10% of the human brain miRNA are primate specific whereas only 1% is human specific. Although such differences might affect a large number of potential gene targets, no bias appears to exist within the human lineage (14). For example, the authors reported 14 human- and 15 chimpanzee-specific miRNA expansions. In contrast, the evolution of protein transcription factors may have not have evolved so uniformly (17). Gilad and colleagues (52) analyzed the expression profile of ~1000 orthologous genes in human, chimpanzee, orangutan, and macaque by cDNA array hybridization and found transcription factors enriched among genes upregulated in humans. Similarly, as a class, transcription factors, in particular C2H2 zinc finger genes, show the greatest excess of amino-acid replacements within the human lineage (32), providing further support for the notion that gene expression changes have been pivotal during human evolution. These data suggest a concerted effect of positive selection and gene expression on transcription factors in altering gene expression.

Concomitant with positive selection and changes in expression of transcription factors, it follows that *cis*-regulatory regions (promoters, enhancers, etc.) might similarly show evidence of accelerated evolution. Using the background substitution rate of intronic regions, Haygood and colleagues developed a method to detect an excess of single-basepair changes within human promoters (compared to chimpanzee and macaque). Several genes were detected by this method, including an apparent excess of genes related to neural development and nutrition (63). Another approach has been to study highly conserved noncoding DNA that shows a burst

Table 2 List of genes upregulated in humans[1]

#ID Gene symbol	RefSeq ID	Gene description	Chr (Build 35)	Start	End	Direction	Tissue	Source	dN/dS Chimp (ENSEMBL)
BAT8	NM_025256	LA-B associated transcript 8 BAT8 isoform b	chr6	31,955,518	31,973,443	Up	Liver	Gilad et al. 2006	0.0862
BTAF1	NM_003972	BTAF1 RNA polymerase II, B-TFIID transcription	chr10	93,673,715	93,780,062	Up	Liver	Gilad et al. 2006	0.0000
C21orf33	NM_004649	es1 protein isoform Ia precursor	chr21	44,382,191	44,384,261	Up	Brain	Enard et al. and Caceres et al.	0.1464
C4ORF1	#N/A	#N/A				Up	Liver	Gilad et al. 2006	0.1059
CA2	NM_000067	Carbonic anhydrase II	chr8	86,578,696	86,580,973	Up	Brain	Enard et al. and Caceres et al.	2.1364
COL6A1	NM_001848	Collagen, type VI, alpha 1 precursor	chr21	46,226,090	46,249,391	Up	Brain	Enard et al. and Caceres et al.	0.1162
CROC4	NM_006365	Transcriptional activator of the c-fos promoter	chr1	153,187,128	153,212,257	Up	Brain	Enard et al. and Caceres et al.	0.9643
CTCF	NM_006565	CCCTC-binding factor	chr16	66,153,964	66,230,589	Up	Liver	Gilad et al. 2006	0.0000
DUSP6	NM_022652	Dual specificity phosphatase 6 isoform b	chr12	88,265,972	88,270,394	Up	Liver	Gilad et al. 2006	0.0683
ENTPD6	NM_001247	Ectonucleoside triphosphate diphosphohydrolase 6	chr20	25,157,785	25,160,569	Up	Brain	Enard et al. and Caceres et al.	0.4350
FCN3	NM_173452	Ficolin 3 isoform 2 precursor	chr1	27,568,218	27,573,883	Up	Liver	Gilad et al. 2006	#N/A
FLJ10326	#N/A	#N/A	chr1	216,656,117	216,709,777	Up	Liver	Gilad et al. 2006	0.0826
FOXO1A	NM_002015	Forkhead box O1	chr13	40,027,817	40,138,734	Up	Liver	Gilad et al. 2006	0.0000
GM2A	NM_000405	GM2 ganglioside activator precursor	chr5	150,628,953	150,631,513	Up	Brain	Enard et al. and Caceres et al.	0.4397
GOSR1	NM_001007024.1	Golgi SNAP receptor complex member 1 isoform 3	chr17	25,828,552	25,877,957	Up	Liver	Gilad et al. 2006	0.2787
GOSR1	NM_001007024.1	Golgi SNAP receptor complex member 1 isoform 3	chr17	25,828,552	25,877,957	Up	Brain	Enard et al. and Caceres et al.	0.2787
GTF2I	NM_032999	General transcription factor II, i isoform 1	chr7	73,516,681	73,619,673	Up	Brain	Enard et al. and Caceres et al.	0.0410

HSPA2	NM_021979	Heat shock 70-kDa protein 2	chr14	64,077,212	64,079,708	Up	Brain	Enard et al. and Caceres et al.	0.1539
LGALS4	NM_006149	Galectin-4	chr19	43,984,158	43,995,409	Up	Liver	Gilad et al. 2006	0.2471
NAGPA	NM_016256	N-acetylglucosamine-1-phosphodiester	chr16	5,014,872	5,023,920	Up	Brain	Enard et al. and Caceres et al.	0.0677
NPD009	#N/A	#N/A	chr16	8,784,670	8,785,932	Up	Liver	Gilad et al. 2006	0.4530
OSBPL8	NM_001003712.1	Oxysterol-binding protein-like protein 8 isoform	chr12	75,269,707	75,477,720	Up	Brain	Enard et al. and Caceres et al.	0.0000
PDE4DIP	NM_014644	Phosphodiesterase 4D interacting protein isoform	chr1	143,562,782	143,706,379	Up	Brain	Enard et al. and Caceres et al.	0.4444
PMS2L5	NM_174930	Postmeiotic segregation increased 2-like 5	chr7	73,751,552	73,766,505	Up	Brain	Enard et al. and Caceres et al.	#N/A
PRDX6	NM_004905	Peroxiredoxin 6	chr1	171,713,108	171,724,569	Up	Brain	Enard et al. and Caceres et al.	0.0000
RB1	NM_000321	Retinoblastoma 1	chr13	47,775,911	47,954,123	Up	Liver	Gilad et al. 2006	0.1163
RGL1	NM_015149	Ral guanine nucleotide dissociation	chr1	181,871,830	182,164,289	Up	Brain	Enard et al. and Caceres et al.	0.1225
SF3A3	NM_006802	Splicing factor 3a, subunit 3	chr1	38,195,785	38,229,180	Up	Brain	Enard et al. and Caceres et al.	0.0000
SMAD1	NM_001003688	Sma- and Mad-related protein 1	chr4	146,760,556	146,837,928	Up	Brain	Enard et al. and Caceres et al.	0.1754
SPTLC1	NM_006415	Serine palmitoyltransferase subunit 1 isoform a	chr9	93,852,933	93,917,459	Up	Brain	Enard et al. and Caceres et al.	0.1333
STAT6	NM_003153	Signal transducer and activator of transcription	chr12	55,775,457	55,791,428	Up	Liver	Gilad et al. 2006	0.4141
THBS4	NM_003248	Thrombospondin 4 precursor	chr5	79,366,746	79,414,866	Up	Brain	Enard et al. and Caceres et al.	0.0719
TIMP3	NM_000362	Tissue inhibitor of metalloproteinase 3	chr22	31,521,362	31,583,581	Up	Liver	Gilad et al. 2006	0.0000
WIRE	NM_133264	WIRE protein	chr17	35,629,100	35,691,965	Up	Brain	Enard et al. and Caceres et al.	#N/A
ZFP36L2	NM_006887	Zinc finger protein 36, C3H type-like 2	chr2	43,361,192	43,365,396	Up	Brain	Enard et al. and Caceres et al.	#N/A

[1]Examples of genes showing increased gene expression in human compared to chimpanzee. Data were taken from References 52, 126 and references therein. Only one gene is found consistently upregulated in both studies (GOSR1). The Gene Symbol, RefSeq ID, chromosomal location (Human Build35), and the tissue studied in every study are also reported. dN/dS (a measure of the selection pressure acting upon the sequence) was retrieved from ENSEMBL. Only one gene could be a candidate to have been under positive selection (CA2, $\omega = 2.13$).

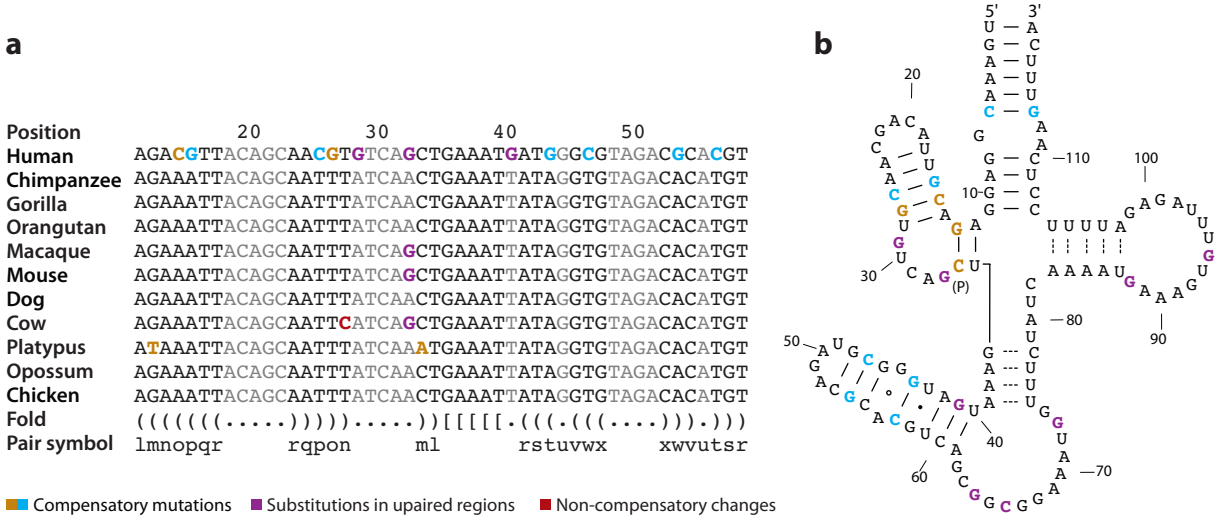

Figure 3

A human-accelerated region of single-basepair substitution, HAR1F. (*a*) A highly conserved noncoding DNA segment, HAR1F, shows an excess of substitutions within the human lineage based on a multiple sequence alignment (123). (*b*) HAR1F is transcribed into a noncoding RNA. Many of the single-basepair substitutions correspond to compensatory changes within the context of RNA secondary structure (*yellow–blue* denotes compensatory mutations, *purple* denotes substitutions in unpaired regions, and *red* denotes noncompensatory changes).

of evolutionary changes within the human lineage (123). Among such regions, HAR1 (human accelerated region 1) has the distinction of being one of the most accelerated unique regions of the genome and is itself part of a novel RNA gene (*HAR1F*) that is highly expressed within the cortex of the human brain (see **Figure 3**). Similarly, Prabhakar and colleagues identified a 546-basepair element (HACNS1) conserved among terrestrial vertebrate genomes that had acquired 16 specific changes within the human lineage. In vivo transgenic experiments showed that a subset of these human-specific changes confers patterns of expression strongly associated with limb development relative to chimpanzees or macaques. Their results implicate changes within the conserved noncoding sequences in creating a *de novo* enhancer associated with anterior wrist and proximal thumb development. Although the role of this enhancer with respect to nearby genes *CENTG2* and *GBX2* is unknown, the authors speculate that this burst of nucleotide changes may have contributed to the dexterity of the human hand or opposability of the thumb (125) (**Figure 4**).

In total, several lines of evidence strongly suggest that gene-expression differences have been a catalyst of primate evolution and adaptive specializations. These findings raise the possibility that there has been an overemphasis on coding sequence differences and, perhaps, too much attention paid to the brain (as opposed to other morphological traits). Proving that these gene regulatory differences played a major role in primate adaptation remains a significant challenge, especially in the absence of "relevant" model organisms where these mutational effects can be directly tested. However, the discovery of previously unrecognized evolutionary targets potentially important in neurodevelopment and hand dexterity predicts that mutations in these may underlie neurocognitive disease in humans. The discovery of *de novo* mutations in association with human disease for these transcripts or *cis*-acting elements would provide strong evidence of their functional import.

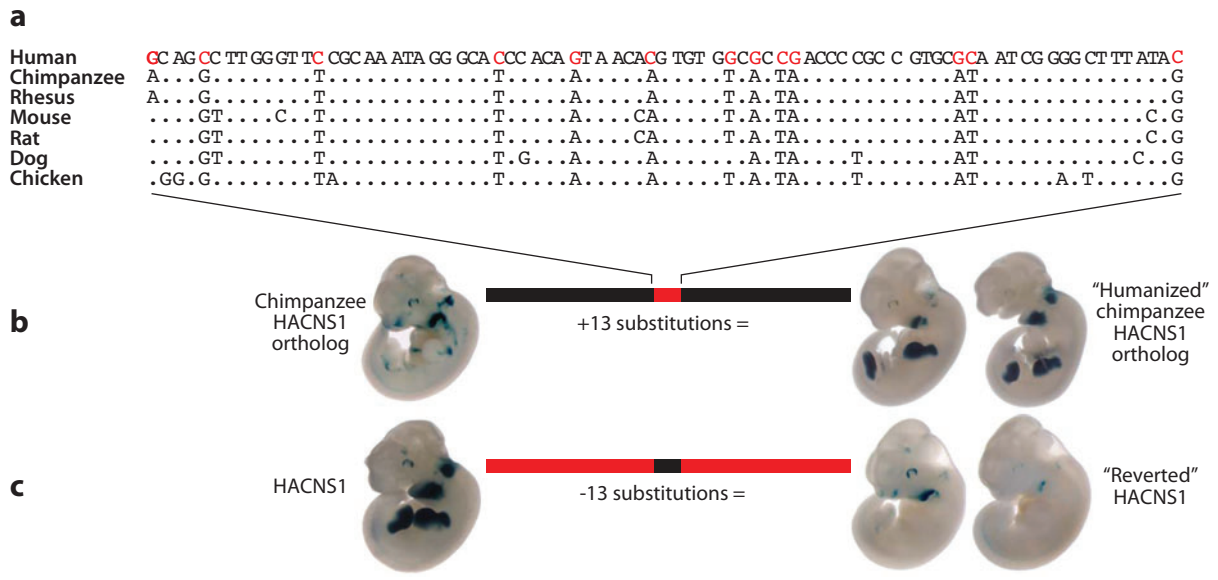

Figure 4

HACNS1 human-accelerated mutations create a limb enhancer. (*a*) Multiple sequence alignment of the putative enhancer HACNS1 with other vertebrate genomes shows an excess of 13 human-specific substitutions (*red*). (*b*) Transgenic mouse lacZ expression pattern using an enhancer in which the 13 human-specific substitutions were introduced against the orthologous chimpanzee sequence background. (*c*) Expression pattern using an enhancer reverting the substitutions in the human sequence to the nucleotide states in chimpanzee and rhesus. These results show that those substitutions drive strong gene expression within the limbs.

PRIMATE GENOME EVOLUTION

Genomes are highly dynamic entities that evolve rapidly and nonuniformly through time; the genomes of primates are no exception. As genome-wide data and new technologies have become available, structural variation and copy-number differences have emerged as another important aspect of primate genetic variation (16, 28). With limited exceptions such as the gibbon (108), there are relatively few cytological differences among primate chromosomes. Most of the cytogenetic differences between humans and apes (167) have now been well characterized and most resolved at the molecular level (24, 55, 59, 87, 88, 89, 98, 133, 149–151). However, the majority of structural changes smaller than a few megabasepairs preclude detection by standard cytogenetic approaches. With the sequencing of the non-human primate genomes, the extent of this submicroscopic variation became more evident. A comparison of human and chimpanzee genomes estimated more than ~90 Mb of DNA affected by insertion, deletion, duplication, and inversion (being ~40–45 Mb in each lineage) (28, 107). Although single-basepair changes are far more numerous, structural variants have been estimated to affect 3–4 times the number of basepairs between human and great ape (i.e., 90 million basepairs of structural variation versus 30 million basepairs of single nucleotide difference between human and chimpanzee).

Due to the limitations of the early draft assemblies, these estimates likely represent a lower bound. Newman and colleagues (114), for example, mapped chimpanzee fosmid end-pairs against the human genome assembly and detected more than 500 insertion, deletion, and inversion events, ranging in length from 12 to 40 kbp. Most of these were previously unknown. In another study comparing the human and chimpanzee genome, a total of 1576 putative regions of inversion were detected (47). Using the gorilla genome as an outgroup,

many of the validated sites were found to be relatively young events and some were polymorphic within the human lineage. Similarly, Szamalek and colleagues (148) performed gene-order comparisons for more than 10,000 orthologous genes between human and chimpanzees and identified 71 putative microrearrangements. The importance of structural variation and copy-number polymorphism in human disease and disease susceptibility and its abundance suggest that such variation may have had profound repercussions in the evolution of human and great ape primates. The role of these events in altering the pattern of gene expression and chromatin organization has not yet been addressed.

Two features of primate genomes may account for the abundance of large-scale structural variation observed in primate genomes, namely segmental duplications and retrotransposons. Segmental duplications (SDs) are segments of DNA greater than 1 kbp in length with high sequence identity (>90%) that typically map to two or more locations in the genome.

Recent comparative work among humans and non-human great-apes has shown that the human and great-ape lineage are particularly enriched for interspersed duplications with a suggested burst occurring in the common ancestor of the human and African great-apes (105). Breakpoints of large-scale structural variation between and within species preferentially map to regions of segmental duplications (1, 4, 121). When compared to other sequenced mammalian genomes, primate (especially human and great ape) segmental duplications tend to be larger, more complex, and more interspersed [(5, 29, 77, 137, 138; for a detailed description of the organization and distribution of primate SDs see (6)]. These peculiar features promote further genomic instability leading to extensive copy-number variation both within and between species enhanced by long-distance nonallelic homologous recombination and other less well understood mechanisms of genetic exchange (78). As a result of this constant genomic turnover, evolutionarily shared duplications often show as much copy-number variation as lineage-specific duplication events (120, 121).

New insights regarding the evolution of human SDs have recently come to light (77, 109). Using a computational graph-theory and phylogenetic approach, Jiang et al. revealed that human SDs are frequently organized around "core" elements that show greater EST and exon density when compared to flanking segmental duplications (77). Detailed comparative sequencing of one of these core elements on chromosome 16 in human and ape showed that the complexity of these regions has emerged as a result of the serial accretion of duplicated segments centered around the core duplication segment (**Figure 5**). In different primate lineages, the core segments have moved or been copied to new locations or entirely different chromosomes leading to a completely different suite of segmental duplications all centered around the same core duplication (78). This feature helps to explain why unique genes mapping in close proximity to these duplication blocks have a ten-fold increased probability of being duplicated when compared to a random model of genome duplication (28, 40, 140)—a phenomenon known as duplication shadowing (28).

In addition to being hotspots of genomic structural variation, segmental duplications are substrates for the emergence of new genes and gene families (39, 79). Primate segmental duplications are enriched for exons when compared to mouse segmental duplications (137). Most of the primate gene expansions correspond to regions of segmental duplication (28, 36). This includes the olfactory receptor gene family (153), which has been subjected to sudden decline in catarrhine primates (53, 54), although it may still play a critical role in kin recognition (71).

In general, gene expansions seem to have been common in primate evolution (36, 61), and many notable examples have been found [such as *PRAME* (expressed antigen of melanoma) within the human lineage and *PFKP* (sugar metabolism) and *DIP2C* (segmentation patterning) within the macaque lineage]. Examples of reported human-specific expansions

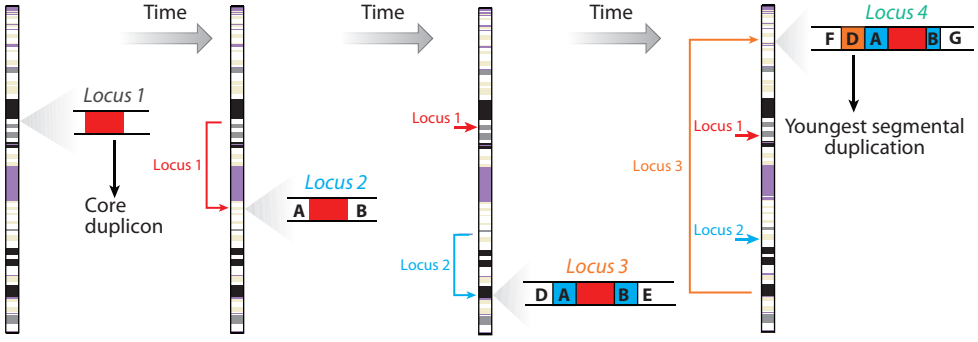

Figure 5

Core duplication hypothesis of human segmental duplications. Duplicative transposition or biased gene conversion duplicates copies to new locations with different unique sequences flanking the duplicated copy of the core; a secondary duplication moves into a third location but also carries flanking duplicons from the second locus to the third; the process repeats itself during the course of evolution, leading to the formation of large complex blocks of intrachromosomal segmental duplication along the chromosome that can now promote nonallelic homologous recombination. These events occur before and after speciation, leading to both lineage-specific and shared duplication blocks between closely related species. Segmental duplications located at the periphery are more likely to be evolutionary young or lineage specific (e.g., duplication D), whereas the core is common to all the duplication blocks. Paralogous sequence exchanges can occur between loci changing the sequence and erasing evolutionary history. Data based on primate comparative sequencing (78) and evolutionary reconstruction of human segmental duplications (77).

include *NEK2* or *ANAPC1* encoding centrosome-related proteins and aquaporin 7 (*AQP7*), which has been suggested as a candidate for a human endurance running (36). Perhaps some of the most conspicuous gene family expansions map to the core duplicons described above (**Figure 5**) and include gene families such as *NPIP*, *DUF1220*, *RANBP2*, and *TRE2* (**Table 3**) (30, 79, 119, 124, 156). These hominoid-specific gene families frequently show evidence of remarkable signatures of positive selection, are associated with bursts of segmental duplication, and demonstrate dramatic changes in their expression profile. The function(s) of these novel genes are largely unknown.

In addition to segmental duplications, mobile elements, particularly retrotransposons, have played a significant role in altering the landscape of primate genomes. Primates are distinguished from all other mammalians by the presence of Alu retroposons (169). Similar to segmental duplications, there is strong evidence of a burst of Alu activity. For example, over one third of existing human elements are thought to have retrotransposed over a 10-million-year window of evolution (30–40 Mya) (11, 35, 97).

Notably, Alu repeats are preferentially found at the boundaries of segmental duplications (4, 83) and map to gene-rich and GC-rich chromosomal regions. It has been postulated that the abundance of interspersed segmental duplications in primates may have been precipitated by the potential for 100,000s of Alu repeats to incur double-strand breaks and to provide microhomology. In this model of cascading repeat instability, the burst of Alu activity would have promoted segmental duplications and, in turn, segmental duplications promoted copy-number variation—a domino effect of increasing structural variation over time.

Although most retrotranspon activity has waned in recent human evolution, sequencing of other primate genomes has uncovered lineage-specific expansions of other elements. For example, a retroviral expansion of 100–200 copies of the retroviral PTERV1 element was

Table 3 Great ape and human gene family expansions[1]

Gene family name	Representative gene	Gene info	Evidence of positive selection	Gene expression	Estimated human copy number	Chromosome	Comment	Reference
Morpheus	NPIP	Nuclear pore complex interacting protein	++	Widely expressed	18	16	50X dN/dS	(79)
DUF1220 (NBPF)	NBPF11	Neuroblastoma breakpoint family, member 11	+ (but see 155)	Neurons (among other tissues)	11	1	Expressed in neurons	(124, 155)
RGP	RANBP2	RAN binding protein 2	+	Testis/cortex	8	2	Positive selection	(30)
LRRC37	LRRC37	Leucine-rich repeat containing 37	Unknown	Ubiquitous	3	17		(77)
TBC1D3	TRE2/USP6	Biquitin-specific protease 6	Unknown	Testis	4	17	Result of a recent evolutionary fusion of two other genes—TBC1D3 and USP32	(119)

[1]Examples of gene families embedded in "core" duplicon (77) that have been greatly duplicated specifically in great apes (NPIP, RANBP2, TRE2, NBPF11) or in primates (LRRC37). Evidence of positive selection as well as information on the gene function and expression or the estimated copy number in human (according to the number of WGS reads mapping to the region) is also provided.

discovered after sequencing of the chimpanzee genome (**Figure 6**) (82, 122, 165).

PTERV1 is found in both the gorilla and chimpanzee genome but the map locations are largely nonorthologous. Moreover, no copies of the sequence have been identified in orangutan or human, although both macaque and baboon genomes carry multiple copies—albeit at locations different from each other and the gorilla and chimpanzee. These data have been used to argue that PTERV1 arose from an external viral source that integrated into the germline. Mutations in the TRIM5α (a protein active in restriction of retroviral insertion) have been posited to explain differences in the distribution of this retrovirus. For example, the human variant of TRIM5α has been shown to be capable of restricting the ancestrally reconstructed version of the PTERV1 retrovirus (82).

TESTS OF MODELS OF SPECIATION ON GENOMIC DATA

The complex process, tempo, and mode of primate speciation has been the subject of considerable debate. However, if a limited understanding has been produced regarding divergence from a common ancestor of humans and chimpanzees, even less is known concerning the speciation within other great apes. Taking advantage of genome-wide datasets, at least two different approaches have been put forward to answer this question. One (a test of a chromosomal speciation model) focuses on the hypothetical effect of chromosomal rearrangements and suppressed recombination within primates [see review of (3)], whereas another approach focuses on identifying traces of speciation by using scans of divergence across different regions of the genome (see for instance Reference 118). Both are highly controversial but have engendered considerable discussion regarding the events underlying separation of our species from nonhuman primates.

Chromosomal Rearrangements and Speciation

Polymorphic genome structural variants (such as inversions, fusions, and fissions) may

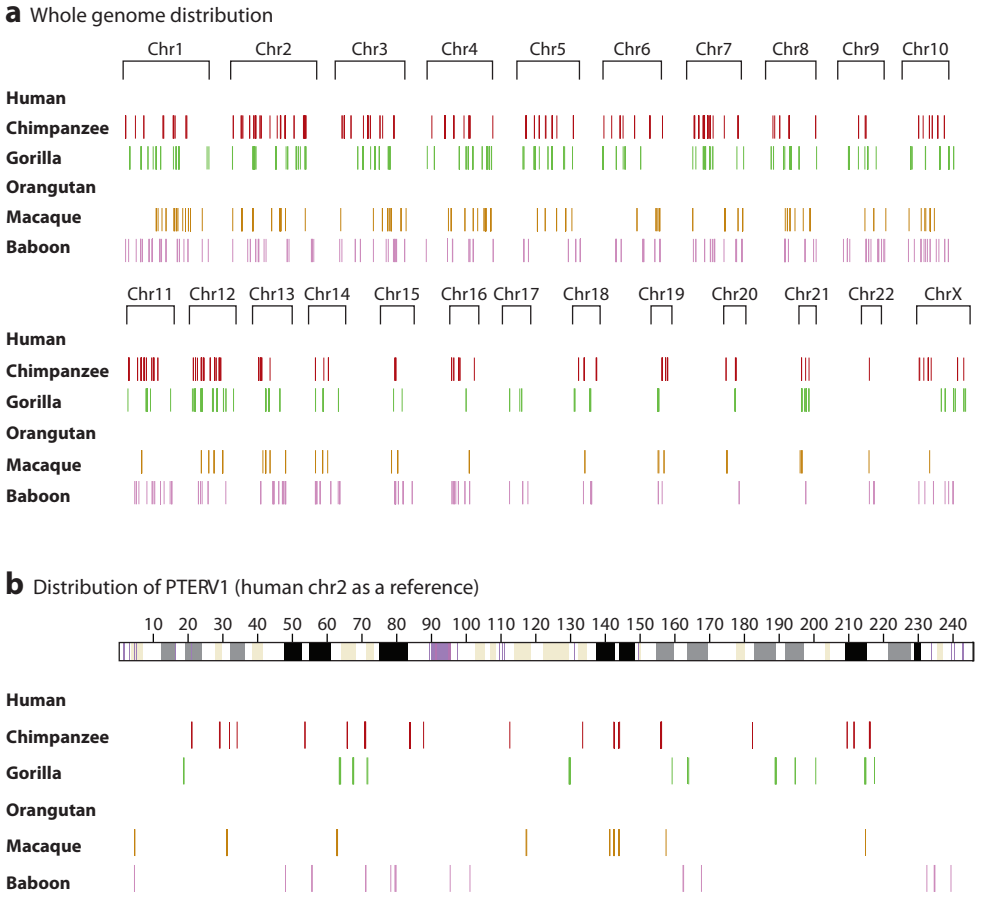

Figure 6

A burst of PTERV1 retroviral insertions in African great apes and Old World monkey species but not orangutan or human. (*a*) Whole-genome landscape of PTERV1 integration sites is shown using the human chromosomes as a reference (build35). (*b*) A detailed view on one chromosome (chromosome 2) is shown. While some species has been massively plagued by the retrovirus that integrated into the germline (chimpanzee, gorilla, macaque, and baboon), other species (human and orangutan) are unusually devoid. Furthermore, ~95% of these sites were found to be in nonorthologous locations when compared between species, suggesting episodic events early in the history of each of these species potentially from an exogenous source.

promote speciation events between contiguous (parapatric) or partly overlapping (sympatric) populations. Among the several models of chromosomal speciation (see 161), the so-called suppressed-recombination model of speciation suggests that chromosomal rearrangements serve as a genetic barrier between populations and, hence, substitutions linked with the rearranged chromosomes cannot be freely exchanged among karyotypically distinct subpopulations, eventually leading to incompatibilities and thus to speciation (112). Studies within a variety of different lineages such as *Drosophila*, *Anopheles*, murids, shrews, or sunflowers (3, 10, 116, 131, 132) have provided some support for this model of speciation.

In the first study to test predictions of suppressed-recombination chromosomal speciation models in primates, Navarro & Barton reported an association between chromosomal

rearrangements and higher evolutionary rates based on an analysis of a limited set of 115 autosomal orthologous genes from humans and chimpanzees (113). This and other observations (such as a lower level of polymorphism in rearranged chromosomes) were consistent with the predictions of the model. Several interpretations for the results were given, the most controversial being the suggestion that under the hypothesis tested, the results would hold only if the chromosomal rearrangements had been barriers in parapatry for no less than half of the time of divergence between humans and chimpanzees (130). This conclusion was striking because it ran counter to both anthropological data and the molecular dating of rearrangement events (151).

Subsequent analyses have questioned the results and inferences from the initial paper. For example, Lu and colleagues found that rapidly evolving genes may not have a homogeneous distribution among chromosomes and that rearranged chromosomes may have been linked to rapidly evolving genes due to factors unrelated to speciation. An alternative explanation was that the GenBank sequences used by the initial study and by Lu et al. (99) were biased and not representative of the rest of the genome. This latter explanation seemed to be the main reason for the initial result since subsequent genome-wide studies (32, 51, 64) found evolutionary rates threefold smaller than the original dataset. Similarly, studies (mainly from noncoding DNA) found that the average nucleotide divergence was in fact lower in rearranged chromosomes when compared to colinear—a result opposite to the predictions of Navarro & Barton (168). Other studies (32, 155) found no genome-wide differences.

Within the past year, additional studies have revisited the topic with more complete datasets. All these studies found slightly less divergence within the pericentromeric inversions that distinguish human and chimpanzee (106, 147) even when considering the effect of duplicated regions (104). Although genome-wide analyses of positive selection have provided little support for this model, analogous comparisons of gene expression differences between humans and chimpanzees for colinear and rearranged chromosomes have, however, yielded contradictory results. Based on earlier gene-expression studies of the cerebral cortex (21, 43, 90), the average gene expression difference between human and chimpanzee was statistically higher in rearranged chromosomes when compared to colinear chromosomes (103). This observation was replicated by Khaitovich and colleagues (90) but not by Zhang and colleagues (168), although the latter considered only a subset of genes (genes with statistical different expression pattern in human and chimpanzee).

Overall, it appears that there is little evidence in support of human-chimpanzee speciation via suppressed-recombination. Chromosomal speciation in primates, however, cannot be definitively ruled because (*a*) chromosomal speciation does not have to involve all rearranged chromosomes, and (*b*) speciation might have involved other noncoding functional elements, such as genes that do not encode proteins (microRNAs, for example) or other regulatory elements (such as transcription factor binding sites). The discrepancy between gene expression and positive selection data may provide some support for this view.

Ancient Hybrid Models

Coalescent models have recently been applied to the question of human and chimpanzee speciation. Based on the hypothesis that allopatric models predict similar divergence times among different regions of the genome, Osada & Wu found that coding regions shared deeper genealogies than intergenic regions as suggested by parapatric models, because coding regions are more likely to have been involved in hybrid incompatibilities or adaptive evolution (117). The analysis supported a complex parapatric view of speciation between human and chimps. Patterson and colleagues (118) addressed the question by analyzing the heterogeneity of human chimpanzee divergencies in DNA sequence based on analysis of genomic sequence from human, chimpanzee, gorilla, and

other related species (orangutan or macaque). Human-chimpanzee genetic divergences varied from ~84% of the average to up to 147%, a range of more than 4 million years. Notably, they observed a lower divergence between X-chromosomal sequences than for autosomal sequences, a circumstance they suggest could be explained if human and chimpanzee initially diverged, and then later exchanged genes before separating permanently.

They proposed that humans and chimpanzees would be more closely related through the X chromosome, by the following process: If human and chimpanzee ancestors initially speciated and then interbred, male hybrids might be partly sterile, as Haldane's rule on heterogametic sex suggested (62). A viable population could then only have arisen if the fertile females mated back to one of the ancestral populations, producing fertile male hybrids that then transmitted X chromosomes derived almost entirely from the initial population. Several concerns, however, have risen with respect to the interpretation of these results. Barton (9) noted that the scenario proposed by Patterson and colleagues is not the only compatible explanation. In fact, the heterogeneity in divergence observed between human and chimpanzee genomes is consistent with quick speciation (allopatric) from a large ancestral population (Ne ~ 45,000), a view supported by others (66). Moreover, a similar level of X-chromosome divergence could also be explained by the amount of time that copies of the X chromosomes spent in the male/female lineages, which of course depends on the male/female ratio (α) (100, 157). Additionally, Burgess & Yang (19) analyzed 7.4 Mb aligned for human, chimp, gorilla, orangutan, and macaque showing that the peculiar reduction of X-chromosome diversity may have arisen as a result of specific selective sweeps on the X chromosome prior to the human/chimp speciation (19).

In summary, applications of genome-wide datasets to develop models of primate speciation have provided no clear consensus on the underlying mechanisms. The controversy and confusion are largely a reflection of the fact that sequence divergence data are much less uniform than anticipated. As population genetic models evolve to more adequately incorporate these data from additional primate genomes, greater insight into speciation should emerge.

PRIMATE DIVERSITY AND RECOMBINATION

Primate Diversity

Analysis of genetic variation within and between primate populations is a powerful tool to understand the evolutionary history, demography, and effective ancestral population size of primate populations. High-throughput analyses of SNPs, haplotypes, and CNVs, for example, have significantly resolved the population ancestry and geographic origin of humans (72, 95, 127). Although studies of nonhuman primates are less developed, the availability of reference genomes and the sampling of genetic diversity (largely SNPs) from multiple individuals of different species have begun to provide other contrasting models of primate demography. From the first restriction fragment length polymorphism (RFLP) studies of ape mitochondrial DNA (46) suggesting that chimpanzees and gorillas possessed two to three times more genetic diversity than humans, it has been evident that humans have less genetic diversity than that of our closest great-ape relatives. Later analysis of nuclear loci confirmed the existence of fundamentally different levels of genetic diversity, although at a lower level—approximately 50% more than humans (76, 80).

Wild primate populations are notoriously difficult to census, suggesting that genetic approaches to estimating effective population size can help understand the evolution of current populations and their demographic history. Previous studies of chimpanzee genetic diversity suggested that chimpanzees can be differentiated in three main subpopulations: Eastern (*Pan troglodytes schweinfurthii*), Central (*Pan troglodytes troglodytes*), and Western (*Pan troglodytes verus*). From all three

subpopulations, Central chimpanzees show the greatest genetic diversity and the largest effective population size (12, 13, 26, 48, 49, 80, 144, 163, 166). The effective population size of Central chimpanzees has been estimated to be much higher than human and Western chimpanzees perhaps as a result of three- to four-fold population expansion after separation from the Western chimpanzees (**Figure 7**). Other studies suggest an even larger effective population size (~100,000) (26). Given that the estimated effective population size for that common ancestor of bonobo and chimp is approximately 25,000 (13,000 to 30,000), which in turn is smaller than

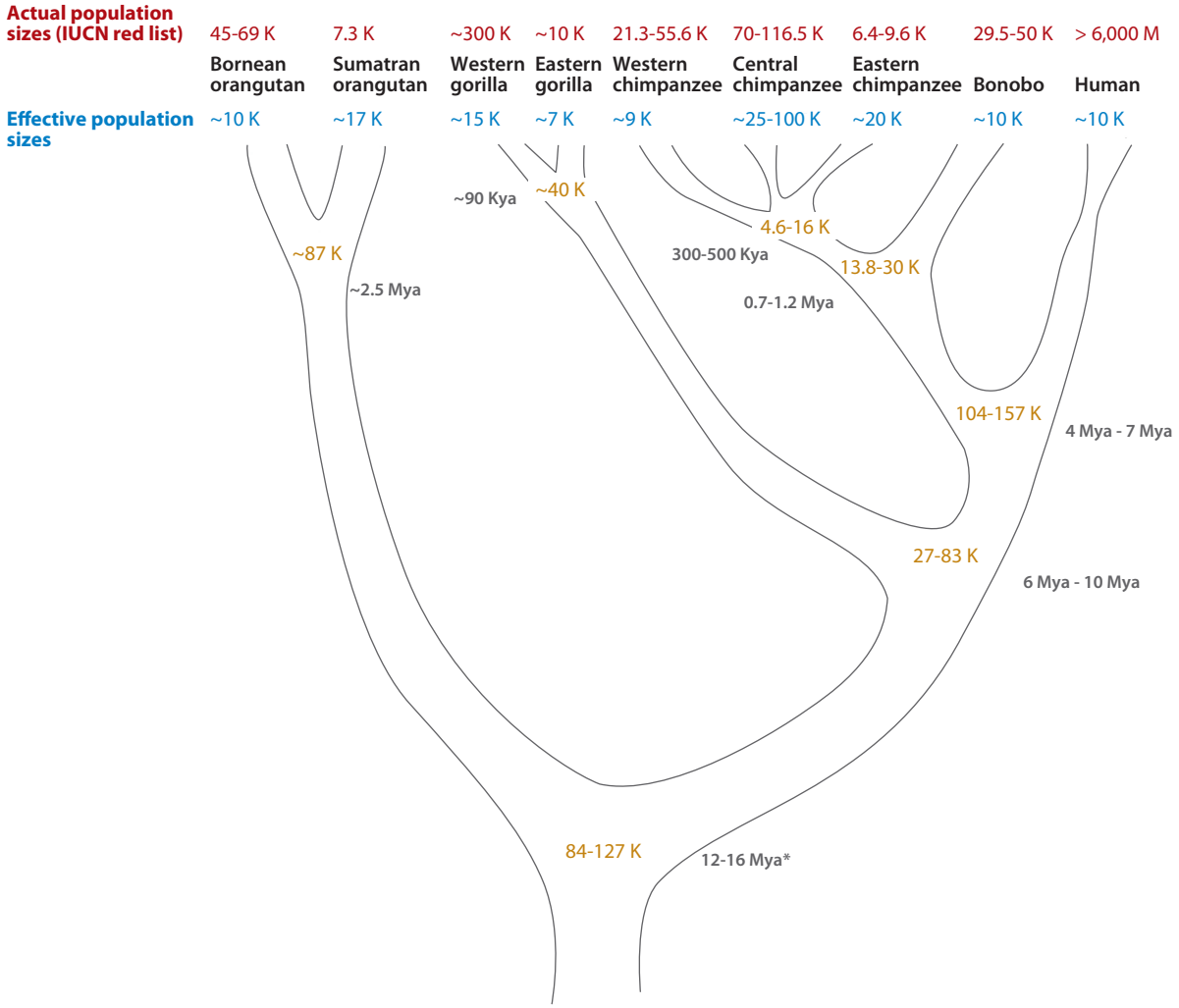

Figure 7

Demographic parameters and speciation times of humans and great apes. Current population sizes (*red*; IUCSN list of threatened species at **http://www.iucnredlist.org/**), effective population sizes, and estimated ancestral population sizes (*blue*) are shown in thousands of individuals for each hominid species and subspecies. Divergence times in either millions of years (Mya) or thousands of years (Kya) are indicated. Population genetic parameters are based on diversity data presented in the following papers: Wall (158), Won & Hey (163), Becquet & Przeworski (13), Hobolth et al. (66), Burgess & Yang (19), Steiper (141), and Caswell et al. (26). * correspond to most of the studies but Burgess & Yang (19) suggested a lower bond of 14 Mya to 22 Mya.

that estimated from human and chimpanzee (~100,000) (13, 19, 26, 66, 158, 163), the data suggest that human and chimpanzees have experienced a drastic reduction in their effective population sizes.

Similarly, the genetic diversity of Western gorillas and Eastern gorillas and orangutan subspecies also suggests lower effective population sizes than their ancestral population (~40,000 and ~87,000, respectively) (13, 19, 66) and lower than the common ancestral human, chimp, gorilla, and orangutan population sizes (70,000 and up to 127,000) (19). On a much smaller scale, and using an approach that allows the ratio of past and present population effective size to be estimated (145), Goossens and colleagues (58) analyzed microsatellite allelic variation in endangered Kinabantangan (Bornean) orangutans and inferred a drastic reduction in population size over the past several decades. In this instance, the reduction in population size has been attributed to direct effects of recent human activities, especially habitat reduction through logging and agricultural activities. Taken together, a consensus emerges that the ancestral effective population size in hominoid lineage was five- to tenfold higher than almost all primate effective population sizes, suggesting a continuous reduction of effective individuals during great-ape evolution.

The recent sequence of the macaque genome has been accompanied by a detailed diversity analysis of a few Kb (~150 Kb) in 47 macaque individuals from two populations (9 from Chinese and 38 from Indian populations) (51, 65). This survey showed that Chinese macaques have an excess of rare variants when compared to the Indian population where intermediate frequency variants predominate. These findings contrasted with earlier studies based on mtDNA and a much smaller set of SNPs that showed much more modest levels of differentiation (45, 139). Demographic inferences of these findings indicate that the Chinese population was first expanded and that later there was a drastic reduction of population size in the Indian population (population size in the Chinese population is now estimated to be one order of magnitude higher than the Indian population). These findings have immediate practical application in mapping of genetic traits. The patterns of LD (linkage disequilibrium) decay suggest that Indian macaques are especially useful for disease association studies since their LD patterns extend even farther than humans, hence fewer markers would be required to discover significant associations. On the other hand, the Chinese rhesus would be more useful in winnowing the interval associated with any phenotypic trait (65) as a consequence of greater decay in the patterns of LD disequilibrium.

Recombination

Among the forces shaping primate genomes are adaptations of cellular mechanisms that facilitate reproduction, including the structural components of recombination pathways and impacts of genome organization. Recombination hotspots are defined as narrow regions of the genome (1–2 Kb) in which recombination occurs to a higher degree than the genome average. Recombination has been assessed from direct genotyping of recombination events in sperm (2, 74) or inferred by population genetic analysis of genome sequence data (33). The former method, although time-consuming, identified the existence of recombination hotspots in human males (2, 74). An alternative approach is to make use of the patterns of LD generated to estimate the recombination rate required to produce the population distribution of observed haplotypes. Importantly, the majority of hotspots detected by sperm typing have been also detected by LD-based approaches (75; but see 85). Myers et al. (110) proposed that the human genome contains ~25,000 hotspots (approximately one hotspot every 50 Kb) and that there are sequence motifs associated with those hotspots that could be experimentally demonstrated to modulate the intensity of recombination (110). These results suggested that recombination rates could be regulated (at least in part) by

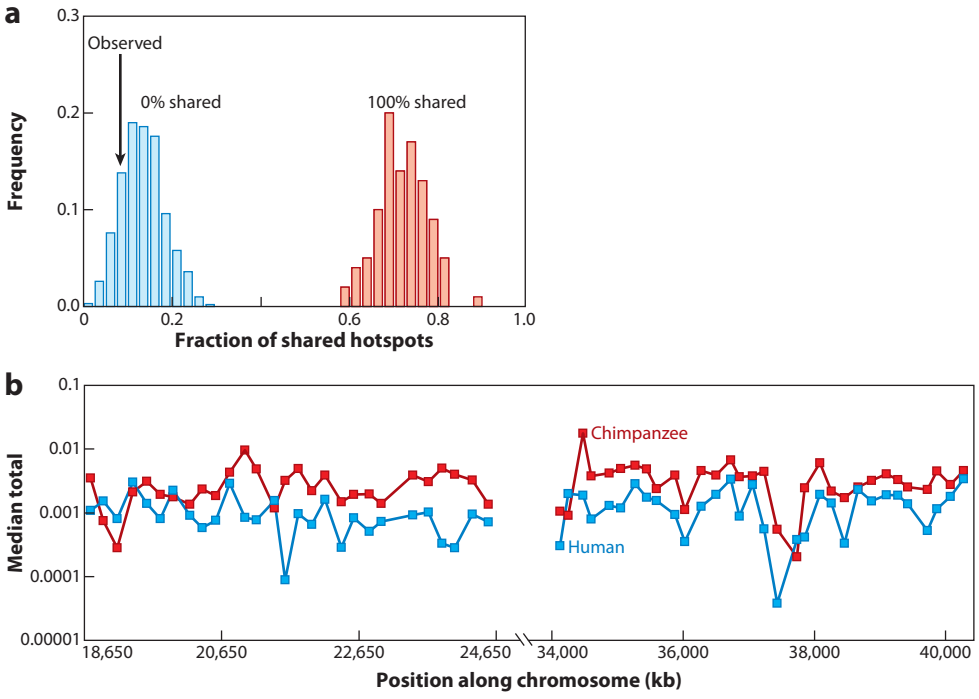

Figure 8

Recombination hotspots vary between chimpanzee and human. (*a*) Distribution of shared hotspots under two statistical models. In the first model (*blue*) all the hotspots are independent and in the second (*red*) all hotspots are shared. The observed proportion of shared hotspots (8%) suggests that allelic hotspots of recombination evolve rapidly and change over short periods of evolutionary time. (*b*) On a broader scale, the recombination rates (measured in 50-Kb windows) correlate, suggesting that the landscape of recombination remains relatively constant.

cis-acting sequences, and motifs have been identified associated with both allelic and non-allelic recombination (111). Genetic linkage maps in baboon, green monkey, and macaque have shown that the recombination maps are significantly shorter than the human (34, 73, 134). The available results suggest that humans and, perhaps, hominoids more generally have evolved higher rates of recombination with implications of greater genetic diversity in a background of a reduced rates of single nucleotide substitution (42, 56, 143).

One of the most interesting findings that has emerged from comparative maps of fine-scale recombination has been that recombination hotspots are not necessarily conserved between human and chimpanzee (128, 162) (**Figure 8**). These findings establish that positional recombination rates may change rapidly over time, a concept reinforced by the finding that recombination rates are highly variable among different humans (92). However, when analyzed on a broader scale (~50 Kb), a weak correlation in rates of recombination exists between humans and chimpanzees (128, 162) and among other mammals (37). This has opened the possibility that the regional background rate of recombination remains relatively constant but that the actual hotspots are transient, perhaps as a result of competition. Yet this difference in recombination must occur in a background where the sequence (and thus the sequence motifs) are nearly identical, suggesting that other structural changes in *trans*-regulation, epigenetic factors, or selection against alternative alleles in the motifs

affect the specificity of recombination and ultimately rates of recombination in different primate lineages.

THE FUTURE

A complete framework for understanding of the human genome requires knowledge of its evolution, necessitating comparative studies with other species, including closely related primates. With the emergence of next-generation sequencing technology (101) and the concomitant reduction in sequencing costs, it is no longer outside the realm of possibilities that "complete" genome sequencing of all species of the primate order will be achieved within the next ten years. The prospects of a "500 Primate Genomes Sequencing" project should be tempered with the need to sequence the genomes to a standard of high quality. In light of the complex organization of the human genome and the finding that so much of the genetic difference maps to these complex regions, simply aligning sequencing reads to a reference genome and cataloguing the single-nucleotide differences between and within species should not be the end goal. As evidenced by the sequencing of the human and chimpanzee genomes, sequencing primate genomes well is nontrivial. Even whole-genome sequence assembly of primate genomes using "Sanger" capillary sequences leads to the significant loss of information, including the absence of entire genes and gene families (**Figure 9**). The excitement of sequencing more primate genomes thus should be balanced by insisting that the most dynamic regions are not excluded as part of the process. This requires a greater investment of resources and careful attention to details, which in this era of "slash-and-burn" genomics is becoming a dying art.

The opportunity, however, to sequence the true extent of primate diversity is time-limited. It is a commentary on the success of human beings that the rapid population expansion that has taken place in our

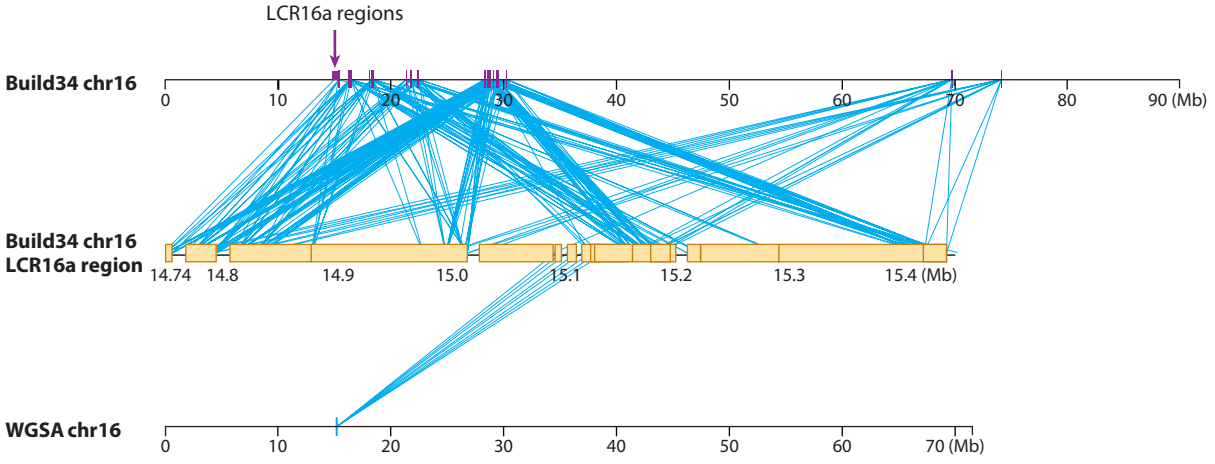

Figure 9

Limitations of whole-genome shotgun sequencing of primate genomes. A comparison of two genome assembly methods for human chromosome 16 is shown (138). Top line represents the chromosome 16 assembly based on hierarchical sequencing of large insert clones (IHGSC, 2005) (94) while bottom line represents the genome based strictly on whole-genome shotgun sequence assembly of sequence reads from capillary sequencers (70). The WGSA assembly is ~20 Mbp shorter than the clone-based assembly and is missing primarily duplicated sequences (*middle line*). The missing material is highly duplicated (28 distinct regions), carries a rapidly evolving human-great ape gene family (78), and is a breakpoint for microdeletions associated with mental retardation and autism. The WGSA assembly is missing most of this material and fails to map most of the loci. This effect is expected to be exacerbated with next-generation sequence technology where the average read length is currently significantly shorter than capillary sequencing methods.

species over the past approximately 10,000 years has led to dramatic reductions in other primate species. Fully 48% of all primate species are threatened; 70% of Asian primates face extinction and two dozen are critically endangered (**http://www.primate-sg.org/redlist08.htm;** 68). All of the great apes, to which humans are most closely related (**Figure 1**)—comprising chimpanzees, bonobos, gorillas, and orangutans—are endangered or critically endangered. Declining populations of primates are the object of conservation efforts to secure habitat and reduce threats to population viability. Knowledge of the genetic structure of primate populations, their demographic history, and significant life-history attributes that can be inferred from assessments of genetic diversity can contribute to efforts to conserve self-sustaining wild primate populations. Thus, there is clear opportunity of comparative genomics studies to contribute to primate conservation actions (135).

Additional whole-genome sequencing projects for primates serve societal interests in identifying the functional elements of the human genome and provide a basis for evaluating genetic diversity and nucleotide sequence evolution not only for species whose genomes have been sequenced, but for closely related species as well. The practical consideration of obtaining appropriate samples for preliminary studies, partial or complete genome sequencing, and postassembly resequencing has received relatively little attention, yet represents a significant community need. The U.S. National Science Foundation recently established the Integrated Non-human Primate Biomaterials and Information Resource (**www.ipbir.org**) to assemble, characterize and distribute high-quality DNA samples of known provenance with accompanying demographic, geographic, and behavioral information in order to stimulate and facilitate research in primate genetic diversity and evolution, comparative genomics, and population genetics. Many samples in the IPBIR were derived from primates in zoos or U.S. National Primate Centers, and a large proportion of the cell cultures serving as sources of DNA for distribution came from the Frozen Zoo® of the Zoological Society of San Diego.

In addition to access to DNA from index individuals, nucleic acid preparations from additional unrelated individuals are essential for evaluating genetic diversity, SNP discovery, copy-number variation, recombination parameters, effective population size, and parameters of selection, including selective sweeps. SNP identification and analysis is of direct interest as SNP assays are a basic currency of genetic variation, relevant to population genetics, disease association, and phylogeographic studies alike. The potential utility of high-throughput SNP assays is manifested in the aforementioned studies of geographic population of rhesus macaques and the effort to identify factors associated with variability in responses to SIV infection that distinguish rhesus macaques of Indian and Chinese origin (45, 65). Recently, comparison of SNPs in cynomolgus (*Macaca fascicularis*) and rhesus macaque (*M. mullata*) identified shared polymorphisms, raising the possibility that these two well-recognized species may be more closely related than suggested by mitochondrial DNA or morphological comparisons (146). This unexpected finding serves as an additional example of the significance of genomic studies for the understanding of evolutionary processes in primate speciation and phenotypic diversification, relevant to medical primatology and conservation of wild primate populations alike.

Among the primate order of mammals, comparative genomic studies have advanced more rapidly for taxa closely related to humans, chimpanzees, macaques, and baboons. As complete genome-sequencing projects advance for other primate families, including the New World monkeys (Cebidae) and strepsirhine primates (lemurs, lorises, aye-aye, pottos, and galagos), new insights are anticipated as, particularly for a lemur genome project, new information about primate adaptations and evolution can be anticipated (67).

The availability of complete genome sequences from additional primate species and

the elucidation of intraspecific and population diversity at the nucleotide sequence and cytogenetic levels can be applied to conservation efforts for wild populations of threatened primates. Constraints in sample collection may require exploration of new technological approaches as, for example, current studies of intraspecific variation in wild populations of great apes depend on noninvasive samples such as feces and hair. Genome sequencing projects for primates should typically include components evaluating genetic diversity of geographically distinct populations. Ideally, new partnerships between field researchers, governments, conservation organizations, and genome biologists can advance both the understanding of genomic mechanisms of primate adaptations and evolution, while enriching the understanding of primatology and contributing to primate conservation.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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