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Comparative primate genomics: emerging patterns of genome content and dynamics

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Preface

Advances in genome sequencing technologies have created new opportunities for comparative primate genomics. Genome assemblies have been published for several primates, with analyses of several others underway. Whole genome assemblies for the great apes provide remarkable new information about the evolutionary origins of the human genome and the processes involved. Genomic data for macaques and other nonhuman primates provide valuable insight into genetic similarities and differences among species used as models for disease-related research. This review summarizes current knowledge regarding primate genome content and dynamics and offers a series of goals for the near future.

Introduction

Current technologies for large-scale DNA sequencing have opened new avenues for the study of nonhuman primate genomes. While the major focus of genomics research is human genetics and its relationship to disease, investigators are also pursuing comparative primate genomics. Two basic motivations exist for the detailed study of nonhuman primate genomes: the application of this information in studies using primates as models for the analysis of human disease, and comparative evolutionary analyses that reconstruct the history and mechanisms of genomic change, with a particular focus on the origin of the human genome. One unexpected outcome from new genomic data for the great apes (chimpanzees, bonobos, gorillas, orangutans) and humans is a new perspective on the process of speciation and genetic divergence among these evolutionary lineages.

The first nonhuman primate genome sequenced and published was that of the chimpanzee, *Pan troglodytes*[1], followed soon by the rhesus macaque, *Macaca mulatta*[2]. Both genomes were analysed using shotgun sequencing employing exclusively Sanger sequencing methods. As a result these projects entailed considerable cost and effort. A legacy of further primate sequencing projects that were initiated when Sanger sequencing was the only option is now reaching its end (Table 1). The genomes of all extant great ape species have been sequenced to draft quality. Analysis of a gibbon genome, the only remaining group of extant hominoids, is underway, and other nonhuman primate genome assemblies are at various stages of completion (see Supplementary information S1 (table)). Remarkably, researchers

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have also produced extensive sequence information for two extinct hominins, the Neanderthals[3] and the Denisovans[4] (Box 1). Investigators are also producing substantial information concerning primate transcriptomes and genetic variation within species.

The widespread availability of next-generation sequencing technology promises even more rapid progress. The amount of genomic information available for nonhuman primates is certain to grow at an accelerating pace. Our understanding of comparative primate genome content, diversity and evolution will necessarily change as new data appear. Conclusions based on current information may, therefore, soon be amended. Nevertheless, substantial progress has been made in the last several years, justifying an assessment of the insights gained to date.

This review begins by summarizing available information about the content of and differences among primate genomes. Next, we present some new insights regarding genomic differentiation and speciation, with particular reference to human evolution. Finally, we illustrate some of the ways genomic data are expanding and improving the use of nonhuman primates in studies concerning human health and disease.

Genomic differences among primates

Comparisons of annotated genome sequences across species allow researchers to directly identify genomic elements that are shared and that are species-specific. We have known for many years that protein-coding sequences exhibit a greater similarity between primate species than do intronic and inter-genic sequences. Past studies also indicated that a large proportion of human protein-coding genes are found in most or all primates. But detailed comparisons across all components of the genome have been impossible until recently. Investigators can now address fundamental questions concerning the content and function of genomic features across multiple species, thus providing new insight into the genetic basis of phenotypic similarity and differences across humans and other primates.

Differences in single-copy alignable sequence

With time, genomes accumulate mutations (single basepair substitutions) that may, through genetic drift or selection, become fixed differences distinguishing one species from its close relatives. Divergence in single-copy sequences occurs steadily among primate genomes but not at a uniform rate in all branches of the primate tree. Alignment of sequences across species demonstrates that pair-wise differences between species correlate fairly well with evolutionary divergence times inferred from other information. The human–chimpanzee sequence divergence is estimated at 1.1–1.4%[1, 5]. The time of separation of the human from the chimpanzee lineage remains somewhat controversial[6], but is generally dated to 5–9 million years ago (Figure 1). The uncertainty concerning the date of the human-chimpanzee divergence results from several factors, including the lack of a reliable paleontological record for that event, and ambiguity concerning the appropriate mutation rate to use to infer the time of divergence from DNA sequences alone. The difference in single copy sequence between human and rhesus macaque is approximately 6.5%[2], and the divergence of those two lineages is more confidently dated at 25–28 million years ago. Dates for the human-chimpanzee divergence calculated using estimates of mutation rate that are derived from

other between-species differences (e.g. the human-macaque or human-orangutan divergence) differ from dates based on mutation rates obtained through pedigree-based analyses of current human mutation.

Despite the uncertainties, various analyses suggest that single copy DNA accumulates individual basepair substitutions more slowly through time among gorillas, chimpanzees, bonobos and humans than in other primates such as Old or New World monkeys. This is not entirely unexpected given differences in generation time[7, 8]. One exception may be the aye-aye (*Daubentonia madagascarensis*), a Malagasy lemur with extraordinarily unique morphology. Synonymous substitutions are reported to accumulate more slowly than expected in the aye-aye, based on comparisons with other species[9]. Additional sequencing projects may identify other primate lineages that do not fit current expectations.

Small insertions and deletions

While the difference in genome sequences between humans and chimpanzees is recently estimated at 1.4%[5], this is correct only for nucleotide substitutions in regions where the two genomes can be directly aligned. As Roy Britten first noted[10], small insertions and deletions (<100 bp) account for more total nucleotide differences among closely related species than do single base changes in alignable sequences. Both the human and chimpanzee genomes each contain about 1.5% unique sequence, not found in the other, primarily due to small indels. The rhesus macaque sequence alignable to human is 93.5% identical, but when including small indels it is only 90.8% identical to human[2]. These indel differences among species are found more frequently in intronic and inter-genic regions than in protein coding exons, primarily because indels in coding sequences will generally have negative consequences on protein function. For example, only indels involving a multiple of three nucleotides do not induce frameshifts that result in substantial changes in protein amino acid sequence. Available comparisons show that small indels are most common and presumably better tolerated in non-coding regions. However, as more non-coding regions with functional significance are identified[11, 12], some indels in flanking or inter-genic segments will become more interesting, and potentially gain importance for understanding changes that affect enhancers and other regulatory sequences that influence gene expression and phenotypic differences among species.

Alu and other repetitive elements

The insertion of Alu repeats and other retroposons is an on-going process in primate genomes. Taken together, repetitive elements make up about 50% of the total genome in humans, apes and monkeys. But the number of species-specific insertions differs substantially across species, from about 5,000 in humans to 2,300 in chimpanzees, and only 250 in orangutans[13]. It is not entirely clear why the rate of accumulation differs. Nevertheless, *de novo* Alu insertions constitute a major source of genomic change, but have not affected all primates equally[14]. Retroposons also facilitate duplication or deletion events, which affect much larger DNA segments[15] and thus can have broader effects on gene and genome content.

Copy number differences and gene family changes

The majority of protein-coding genes have 1:1 homologues among humans, the great apes and Old World monkeys sequenced to date, but gene content is not identical among species. Particular gene families have expanded or contracted in individual lineages. For example, 1,358 genes were identified as new duplications in the rhesus genome compared with human[2]. The HLA gene cluster, which is critical for response to pathogens as well as other immunological processes, is expanded in macaques relative to humans[16]. Other interesting cases are changes in zinc-finger transcription factor genes, which show gains and losses that distinguish humans, chimpanzees and orangutans[17], as well as the marked expansion of genes containing DUF1220 protein domains in humans[18–20], which might be related to the expansion of brain size in our species.

However, the draft quality of current nonhuman primate genome assemblies makes it difficult to define all copy number variations accurately. One can compare gene lists from different assemblies, but gaps and other issues in assemblies create ambiguity[21, 22]. Available evidence suggests that humans and chimpanzees experienced more rapid changes in gene copy number than did orangutans or rhesus macaques[13]. Among the great apes, gorillas exhibit more copy number variants than others[5]. Complete analyses await additional data, including better genome assemblies and information concerning copy number polymorphism within nonhuman primates.

Segmental duplications

Segmental duplications (that is, chromosomal regions >1 kb that are ≥90% identical to other segments in the same genome) are a significant aspect of primate genome structure and dynamics. Duplication and deletion of these segments is active in the human genome. Some of these mutations are apparently neutral, but many lead to adverse consequences and disease[23]. Just as segmental duplications create variation among humans, they are drivers of evolutionary change across primate genomes. About 5% of the human and chimpanzee genomes, and 3.8% of the orangutan genome, consist of segmental duplications[13, 24]. The human and great ape genomes are enriched with dispersed duplications, having experienced an interval after their divergence from Old World monkeys when the production of new duplications was particularly active[25, 26]. Many expansions of specific protein-coding gene families result from segmental duplications, which sometimes involve repeated expansions of a given sequence[24, 27, 28]. Some genes within segmental duplications show evidence of positive selection acting on coding sequence as well as copy number[18, 20, 29, 30]. Among the great apes, some of these expansion events have occurred as independent parallel events in different lineages, strengthening the interpretation that these genomic changes are often the result of positive selection for both gene copy number and gene sequences[25, 31].

Genetic variation within primate species

Individual primate genome projects have assessed within-species genetic variation in different ways, with a broad range of sample sizes and population genetic parameters used to quantify variation. Earlier studies that analysed small samples or only small fractions of the genome had suggested that, for the most part, nonhuman primate species exhibit higher

levels of intra-species genetic variation than humans (e.g.[32–35] and others), and this pattern holds in the larger datasets published more recently. Great ape species have all been reduced to low total population census numbers, but studies using whole-genome data indicate that genetic diversity within great ape species is consistent with effective population sizes as large or even larger than humans[13, 24]. The Great Ape Genome Project investigated genome-wide variation within and between all six great ape species[36] and found that some subspecies and species show levels of intra-specific diversity roughly equivalent to non-African humans (Figure 2). Chimpanzees from east and central Africa, and the Nigeria-Cameroon subspecies, as well as western lowland gorillas and both orangutan species, exhibit significantly higher variation than is found among humans. Application of coalescent and incomplete lineage-sorting models allowed the researchers to re-estimate effective population size for different ape species and subspecies. Each species has a unique history of population expansion and decline, while each chimpanzee subspecies has an independent history[36]. This observation of separate unique demographic histories for regional populations or subspecies within a species is likely true for most or all primates[34].

While the great apes have high intra-species diversity despite low present day census sizes, the rhesus macaque is much more widely distributed geographically, with larger extant population numbers. As part of the rhesus genome sequencing project, DNA from Chinese- and Indian-origin rhesus macaques was sequenced for 150 kb from five genomic regions[34]. The density of SNPs was significantly higher than is found in human populations. Only about one-third of SNPs were shared between the two geographic populations, which indicates that most variation is region-specific. Similar results were obtained in a survey of 3’ UTR sequences in a small number of macaques[37]. A study of whole genome sequences for three Indian-origin rhesus macaques found >3 million variants present in at least two of the datasets examined[33]. Including singletons, about 14 million single nucleotide variants were found[33], a density substantially greater than that found in humans. For example, the 1000Genomes Project estimated the average number of SNPs (including singletons) per human individual at 3.6 million[38].

Overall levels of variation are high in nonhuman primates, including additional non-hominoid species that have very low population numbers and are in serious danger of extinction[39]. However, the amount of functionally significant variation within particular species is not yet clear. Significant numbers of non-synonymous substitutions that are predicted to be possibly damaging have been identified in even small numbers of macaques[33, 40]. But a recent comparison of protein-coding variation between humans and rhesus macaques found little difference between species[41]. It is possible that macaques and other nonhuman primates are segregating greater total within-species variation compared with humans but equivalent levels of damaging or adverse variation.

Differences in gene expression

Alan Wilson and his colleagues predicted years ago that much of the adaptively significant phenotypic change that distinguishes species results from changes in gene expression, rather than mutations in protein-coding sequences[42, 43]. Recent information concerning comparative primate gene expression is consistent with this prediction. It is likely that a

large proportion of adaptive evolution involves changes in transcription factor binding, possibly rivaling adaptation through protein evolution[44]. Overall, the description of nonhuman primate transcriptomes lags behind knowledge for human and mouse, but researchers are now developing larger information resources, such as the Allen Institute for Brain Science atlas of gene expression for the rhesus macaque brain (http://www.blueprintnhpatlas.org) and the Nonhuman Primate Reference Transcriptome Resource[45].

Nevertheless, comparisons of gene expression across primates have already proven valuable. Differences in gene expression among humans, chimpanzees and rhesus macaques are influenced by natural selection[46–48], and include substantial differences in alternative splicing[47, 49]. RNA sequencing from the livers of humans and other mammals, including 11 primates, found strong evidence for positive selection in a number of the genes expressed[39]. The results of this study show enrichment for changes affecting genes involved in peroxisome function, such as GGH, PEX7 and HACL1[39]. Patterns of DNA methylation in the prefrontal cortex differ between humans and chimpanzees and correlate with differences in gene expression[50]. Three-way comparisons find greater overall similarity in gene expression between chimpanzees and humans as compared to gorillas, which matches the overall phylogeny (Figure 1). However, and notably, genes falling in specific regions of the genome (i.e. chromosomal segments exhibiting incomplete lineage sorting, see text below and Figure 3) show a contrary pattern[5]. Studies of gene expression have recently been extended to wild populations of baboons[51], an approach with outstanding potential for future discoveries.

Primate evolutionary dynamics

One of the primary motivations for comparative primate genomics is the desire to understand the origin of the human genome. Whole genome information now available for our closest relatives is altering and refining ideas about the processes of speciation, diversification and genome evolution for this clade. This new picture, though still incomplete, reveals previously unappreciated complexity in the processes that produced the modern human genome. The theory and modeling of speciation (Box 2) is a complex topic with a long history and extensive literature, and consequently is outside the scope of this article. However, the remarkable insights concerning gene exchange among the early human and chimpanzee ancestors[52], as well as among ancient hominins[3, 4, 53, 54], are dramatic indications that this history is of greater interest than previously recognized (Box 1). In parallel, these between-species comparisons are greatly increasing our ability to identify genes or genomic regions that have undergone positive selection during recent human evolution, thereby pointing us to genetic changes and phenotypes that have been important in human and nonhuman primate adaptation. Comparisons also demonstrate that fundamental genetic processes such as recombination can undergo rapid change, as local hotspots of recombination are not conserved in humans and chimpanzees despite the overall high sequence similarity and the general conservation of large-scale patterns of recombination[55].

Signatures of selection

There is substantial evidence for positive selection on protein-coding genes in various nonhuman primate species. Two classes of genes provide consistent evidence of positive selection, those involved in the immune system and pathogen resistance and genes involved in reproductive biology and gametogenesis[2, 5, 13, 56]. These results are quite reasonable, as the constant pressure of infectious disease is a plausible driver of selection on the primate immune system, while reproductive competition within species likely accounts for the evidence of selection on those systems. Within individual species, positive selection has been detected for a wide array of phenotypes. The whole genome comparisons among human, chimpanzee and gorilla suggest that these three species have experienced approximately equal levels of positive selection[5]. This analysis also indicates shared positive selection in hominoids related to neurodevelopment and brain morphology. The orangutan genome provides evidence of selection on glycolipid metabolism and hearing acuity that is specific to that lineage[13]. Studies in marmosets and other callitrichine primates provide evidence for selection on multiple genes related to phyletic reduction in body size and the development of a unique form of dizygotic twinning in which co-twins exchange hematopoietic stem cells early in gestation, and consequently become life-long hematopoietic chimeras[57].

Genes involved in the evolution of unique human traits have received much attention, and been reviewed elsewhere[58]. Detailed description of the genetic basis of human-specific traits has obvious interest to evolutionary biologists, anthropologists and the broader public. Studies have now attributed human-specific adaptations to deletions of gene regulatory elements (enhancers)[59], rapid lineage-specific evolution of such elements[60], changes in gene copy number[19, 61], and other types of genetic changes[58].

Initial genomic divergence and incipient speciation

Whole genome sequence data from humans, chimpanzees and gorillas are not consistent with simple models of reproductive isolation, allopatric genetic divergence or the rapid development of species boundaries (Box 2). Two processes that are known to shape genome evolution in other groups of animals, incomplete lineage sorting and gene flow, are now critical elements in discussions concerning mechanisms of human and nonhuman primate genome differentiation.

Incomplete lineage sorting

Incomplete lineage sorting (ILS) occurs when a polymorphic ancestral species, with two or more alleles (haplotypes) at a given locus divides into two lineages. Both alleles can be retained in the descendant branches, and when one of those lineages divides again, the phylogenetic tree for that locus (the gene tree) may or may not match the branching order for the species-level evolutionary tree (Figure 3). The likelihood of discrepancy between the species-level phylogeny and any one particular gene tree increases as either the time between the two successive branching events decreases or effective population size increases[62]. Prior analyses of a few genes suggested that different regions within the human, chimpanzee and gorilla genomes exhibit different evolutionary relationships, i.e. different gene trees[63, 64]. Following assembly of the gorilla genome[5], researchers determined the

evolutionary relationships for arbitrary segments across the human-chimpanzee-gorilla genomes. They found, as expected, that for most of the genome, chimpanzees are more closely related to humans than to gorillas. But for ~15% of the genome, chimpanzee DNA sequences share a more recent common ancestor with the homologous sequences in the gorilla genome, not the human. For another 15%, gorillas and humans are most closely related. ILS from a polymorphic common ancestor is a likely contributing factor, but not the only possibility. Gene flow among differentiating lineages may also be a factor. This developing picture of evolutionary process complexity also applies in other cases. Bonobos and chimpanzees are undoubtedly sister taxa, more closely related to each other than to any other species. Nevertheless, for 1.6% of the genome, sequences in bonobos are more similar to homologues in humans than chimpanzees, whereas 1.7% places bonobos as the outlying group[65]. ILS is the likely explanation and may be quite common in primates[66].

Gene flow among incipient lineages

Analysis of the human, chimpanzee and gorilla genomes indicates that genetic exchange between divergent lineages is not restricted to recent periods (Box 1). The common ancestor of humans and chimpanzees began differentiating 5–12 million years ago, depending on the assumed mutation rate[1, 52]. Using coalescent models, Mailund et al. estimated that those diverging lineages experienced reciprocal gene flow for about 3 million years. In other words, the separation of the last common ancestor of humans and chimpanzees into two independent lineages was not a rapid event but included an extended period of progressive genetic divergence simultaneous with gene flow[52]. Such divergence with continuing gene flow is also true for Bornean (*Pongo pygmaeus*) and Sumatran (*P. abelli*) orangutans[13, 52].

Whole genome data are not yet available for enough species of Old or New World monkeys, or strepsirrhine primates, to support similar analyses of evolutionary divergence and exchange across partially isolated lineages. However, smaller datasets suggest that the complexity documented for humans and apes may be common across primates. The number of documented hybrid zones between morphologically and/or behaviorally distinct primate populations is increasing[67]. Active hybrid zones facilitate the study of the process of genetic differentiation, including demographic and phenotypic correlates of hybridization. Various reviews of primate hybridization are available[67, 68], but some examples will illustrate general principles.

Baboons (genus *Papio*) exhibit unusual phenotypic diversity and evolutionary complexity[69, 70]. Baboon taxonomy has been controversial, but six morphologically distinct species with parapatric geographic ranges are now widely recognized[69, 71–73]. Hybridization occurs where distinct baboon ‘types’ meet[73–75], despite morphological differences and an increased frequency of developmental abnormalities in hybrids[76]. The evidence suggests a long history of gene flow[71, 77, 78]. Among baboon species the gene trees from different non-recombining genetic elements (mtDNA, Y-chromosomes, etc.) do not necessarily match observable phenotypic similarity among populations[69, 71, 77].

Rhesus macaques and cynomolgus macaques (*Macaca fascicularis*) are closely related but universally regarded as separate species[72]. Across mainland Indochina, somewhat similar to the African baboons, these macaque species form a hybrid zone with apparently substantial

gene flow. Y-chromosomes from rhesus macaques are found in animals that are phenotypically cynomolgus macaques[79]. Autosomal gene flow occurs from rhesus into cynomolgus macaque populations, affecting mainland but not other populations of cynomolgus that are isolated on Indonesian islands and in the Philippines[80]. Thus, these two species present clear evidence for gene flow between well-differentiated species.

One recently discovered species of African monkey (*Rungwacebus kipunji*) exhibits evidence of ancient hybridization. Of the two geographically isolated populations of kipunji[81, 82], one carries mtDNA sequences associated with *Cercocebus* mangabeys while the other carries mtDNA more closely related to *Papio* baboons. Despite this apparent genetic introgression from baboons, the second population retains morphological and nuclear DNA similarities with its conspecific sister population. In another case of phylogenetic complexity, the genus *Cercopithecus*, commonly known as guenons, contains about 24 species[72, 83]. But their phylogeny has proved difficult to resolve. Novel Alu insertions generate a phylogeny[84] with multiple inconsistencies suggesting either ILS or ancient hybridization among differentiated species. Chromosome painting analysis also indicates inter-species hybridization[85], and field studies document active hybrid zones[68].

Thus, simple allopatric speciation models and associated ideas positing the rapid origin of species boundaries do not generally hold either for humans or other primates. (The bonobo-chimpanzee speciation may be one notable exception[52], possibly related to a rapid shift in the Congo River that may have created a robust barrier to gene flow). Newer models of speciation address these complexities[86–89] and provide frameworks for future studies. Unresolved questions regarding primate genome evolution include: what are the demographic circumstances associated with extended periods of progressive genetic differentiation despite continuing genetic exchange; what types of genes are able to transfer between lineages and what genes or genetic pathways are first to develop significant differences between diverging lineages; and finally, what changes correlate with the cessation of gene flow between differentiating lineages?

Biomedical relevance

The two most commonly used nonhuman primates in biomedical research are the rhesus macaque (*M. mulatta*) and the cynomolgus or long-tailed macaque (*M. fascicularis*). Their importance as models for studies of human health and disease justifies extensive analysis of these genomes (see Supplemental Information S1 (table)). These two species are members of the genus *Macaca*, a very successful radiation of Old World monkeys that contains 18 extant species[72] and is distributed across Asia from Afghanistan to Japan and the Philippines, with relict populations in Morocco. Other important primate model organisms are now also receiving attention. The genomes of the marmoset (*Callithrix jacchus*), sooty mangabey (*Cercocebus atys*), African green monkey (*Chlorocebus aethiops*) and olive baboon (*Papio anubis*) have been sequenced and assembled. Genome assemblies for mouse lemur (*Microcebus murinus*) and pig-tailed macaque (*M. nemestrina*) are in progress. These species are all used as animal models in disease-related research, and therefore whole genome assemblies, transcriptome data and other information is valuable. For example, both the sooty mangabey and African green monkey are important model species for Simian

immunodeficiency virus (SIV) research, because these animals are natural hosts that tolerate long-term infection with specific SIV viruses without developing disease[90]. Development of sequence data and related tools facilitates analyses of how these species tolerate SIV infection that is pathogenic in other primates.

Differences between species in disease-relevant variants

Comparisons of the cynomolgus, rhesus and human genomes are producing information directly relevant to specific biomedical questions. The rhesus and cynomolgus genomes are <1% different in single copy sequence, but the two species do carry specific differences in cytochrome p450 genes involved in drug metabolism[91]. Although most p450 genes are expressed at similar levels in humans, rhesus and cynomolgus, particular loci (e.g. *CYP17A1*) are not. Knowledge of genomic differences should improve the interpretation of pharmacological studies using these species.

Humans and the two macaque species also exhibit differences in other genetic pathways relevant to disease, such as melanocortin receptor activity, methyltransferase activity and the parathyroid hormone receptor 1[92], Moreover, importantly, macaques have an expanded array of MHC Class I genes that are central to their response to infectious agents and other immune system processes[16].

Several nonhuman primates carry sequences for coding genes that are associated with increased risk of specific diseases in humans. Rhesus macaques carry variants in *OTC*, *PAH* and *NAGLU* that predispose some humans to disease (i.e. OTC deficiency, a potentially severe disruption of the urea cycle, and phenylketonuria, a relatively common metabolic disorder affecting amino acid levels)[2]. Chimpanzees carry ‘disease’ alleles in genes related to cancer (*MLH1*), diabetes mellitus (*PPARG*) and Alzheimer disease (e.g. *APOE*)[1]. Gorillas exhibit alleles at *PGRN* that in humans are associated with dementia, and variants at *TCAP* that are associated with hypertrophic cardiomyopathy in humans[5].

Polymorphism within species and disease phenotypes

Macaques and other primate species generally have higher levels of within-species genetic variation than humans (see above). Thousands of non-synonymous and splice site variants have been identified in rhesus macaques[33, 41], and such variability may influence the response of individual monkeys to experimental protocols. This naturally occurring genetic variation can be exploited to identify novel relationships between specific genes and disease-related phenotypes[93, 94] or to study the phenotypic consequences of variation in genes already implicated in human disease risk[95–97]. Variation in *OPRM1*, the gene encoding mu-opioid receptor, illustrates the parallels in monkeys and humans, as naturally occurring non-synonymous variation in rhesus OPRM1 influences both the behavioral response of animals to alcohol consumption and the pharmacogenetic response to treatment, similar to non-synonymous variation in humans[96]. Large-scale DNA re-sequencing of macaques, baboons, African green monkeys, marmosets and other laboratory primates will undoubtedly identify many functionally important genetic variants useful for investigating genetic mechanisms of disease in experimentally controlled primate models[33, 41].

Transcriptomics

Analysis of gene expression in primate models of disease will be fundamental to future studies. Primates are, for example, critical for the development and testing of new drugs. Expression of drug-metabolizing p450 genes, as well as some amino acid sequences, differ between cynomolgus and rhesus macaques[98], which has implications for pharmacokinetics. Using linkage analysis and quantified differences in gene expression among pedigreed African green monkeys, researchers have mapped eQTLs[99]. Analyses of rhesus show that gene expression in the immune system is sensitive to differences in social dominance rank, a fundamental aspect of macaque behavior[100], thus indicating that common social interactions can influence gene function related to immunology and disease. Primate microRNAs will be critical to understanding disease models[101] as well as evolutionary adaptation[102, 103]. Species differences in microRNA expression may affect expression of transcription factors[104], with important consequences for multiple pathways.

Future directions

The initial draft assemblies for nonhuman primates all provide much useful information, but are not complete or reliable enough to support all current scientific goals[21, 22]. One limitation of draft genomes is the presence of gaps in chromosomal sequences, resulting in missing exons or genes. Although growing use of RNA sequencing to identify transcribed genes is improving the completeness and annotation of nonhuman genome assemblies, the available assemblies still contain gaps. For example, the recent assembly of the gorilla genome incorporates 2.8 gigabases of sequence into contigs[5]. However, when Scally et al. aligned the human, chimpanzee, gorilla and orangutan genome assemblies in order to conduct “whole genome” analyses of sequence differences, they were only able to produce a four-way “great ape plus human” alignment that included 2.0 gigabases[5], due in part to gaps and other problems among the ape assemblies. Another significant issue that affects the ability of researchers to perform comprehensive analyses is problems with identifying and properly assembling segmental duplications and gene copy number differences among species[21]. Mis-assemblies are also a recurrent problem among draft assemblies produced using next-gen short read technologies only[22].

Improved assemblies with longer contigs and more complete coverage in high-quality sequence data (i.e. comprehensive delineation of segmental duplications and fewer genes with gaps and errors) are needed. Deeper sequence coverage will improve some assemblies, but new technologies that provide longer reads will yield better assemblies by filling remaining gaps. The Pacific Biosciences RS II platform is one plausible option for upgrading primate genomes[105].

In addition, annotation of functional elements can improve with contiguity and quality of the reference genome as well as access to transcript data. Identification and validation of transcripts for both protein-coding and other transcribed sequences is a high priority. Long non-coding RNAs, microRNAs and other genome features are today poorly annotated for most nonhuman primates. Experimental study of those genomic elements in primate model systems is likely to produce significant dividends for both biomedical and evolutionary studies.

With the sequencing technologies now available, researchers are able to generate large amounts of DNA and RNA sequence data rapidly. This is creating an increasing need for software tools to process comparative data and speed interpretation. The natural emphasis among researchers in human genetics has been the development of computational tools that are specifically designed to analyze human genomes, some of which are not easily applicable to nonhuman species. However, some new tools are readily useful in analyses of nonhuman primates[106, 107], and several online databases are collecting, organizing and synthesizing comparative genomic data. [http://biologiaevolutiva.org/greatape/index.html; http://www.rhesusbase.org; http://www.genome.uscs.org; http://www.ensembl.org] However, the speed with which comparative data is being generated creates an ever-growing need for additional computational tools designed to meet the needs of comparative analysis.

Most efforts in primate sequencing to date have been directed toward the great apes, as is natural given their phylogenetic relationships to humans. The sequencing of species from other branches of the primate evolutionary tree, in particular New World monkeys and strepsirrhine primates, will provide increased power to identify conserved genomic segments unique to primates, or to subsets of primates (e.g. catarrhines). Each new species sequenced adds evolutionary perspective and generates new potential models of human genetic disorders.

Little is known about genetic variation in most primate species, although they generally display as much or more variation than do humans. Re-sequencing in commonly used laboratory primates will discover new variants of interest for biomedical research. Furthermore, there is substantial opportunity to use this naturally occurring functional variation to explore gene-gene or gene-environment interactions[108, 109].

Finally, nonhuman primates can facilitate investigation of epigenetic control of genome function. Experimental manipulation of environmental factors influencing the human epigenome will be feasible in better characterized primate genomes. Detailed analysis and manipulation of the primate microbiome may also have a substantial impact.

Conclusions

Comparative primate genomics is in a phase of rapid growth, as information about transcriptomes, intra-species polymorphism and other aspects of genomics is being generated at a rapid pace. The major impact to date has been to provide novel information concerning the history and mechanisms of human genome evolution including evidence for a complex history of genetic divergence and exchange among ancestral evolutionary lineages (Figure 1). Nonhuman primate genomics is also expanding the scope of biomedical research with innovative analyses of primate models of human disease. Despite recent progress, both evolutionary and biomedical studies would benefit significantly from additional information. There is real opportunity to examine the continuum from microevolutionary processes controlling within-species variation (e.g. positive and negative fitness effects of segregating polymorphisms within species) to macroevolutionary processes affecting between-species differences.

Either from the perspective of understanding the origin of humans or elucidating the genetic basis of human disease, nonhuman primates are indispensable resources for comparative and experimental study. Genomics is now central to all of biology and so it is both sensible and timely that comparative primate genomics is receiving increased attention. Analyses to date have provided valuable and sometimes unexpected results. There will be many further advances, including a few more surprises, and ultimately a much richer understanding of genome structure, function and dynamics as investigators with a wide range of interests continue to generate new information concerning the genomes of nonhuman primates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Biographies

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Glossary

catarrhine	any member of the primate evolutionary lineage that includes Old World monkeys (superfamily Cercopithecoidea) or hominoids (superfamily Hominoidea). The catarrhines include all extant apes, anthropoid monkeys native to Asia and Africa, and humans
coalescent models	an approach used in population genetics to investigate various aspects of population history and dynamics. These models are based on the genealogy or relationships within a gene tree among alleles of a specific DNA sequence. All alleles found in a population or set of related populations can be traced back to a common ancestral

	sequence, and the statistical properties of those allelic relationships are exploited to investigate questions of population genetics and history.
effective population size	A basic concept from population genetics that describes the number of individuals required in an ideal breeding population (equal numbers of breeding males and females, with equal reproductive success among them) of constant size to sustain a given amount of within-population genetic variation. Because genetic variation in a given population is affected by current and past demographic factors, estimation of effective population size allows researchers to infer aspects of population history.
hominins	members of the evolutionary lineage leading to humans after divergence from the ancestors of chimpanzees. Hominins include species directly ancestral to modern humans, and related species such as Neanderthals or older branches such as australopithecines. Tribe Hominini.
Hybrid zones	Geographic areas, often but not always elongated and narrow in shape, where two distinct primate species occur together, mate and produce hybrid offspring that are fertile and reproductively successful themselves.
Incomplete lineage sorting	The process by which, as a result of segregation of an ancestral polymorphism, the evolutionary relationships among a series of homologous DNA sequences present in a set of distinct populations do not match the phylogenetic relationships among those overall populations, i.e. that gene trees do not match population trees. See Figure 2.
Old World monkeys	members of the branch of primates that includes extant anthropoid primates (monkeys) native to Asia and Africa, superfamily Cercopithecoidea
New World monkeys	members of the branch of primates that includes extant anthropoid primates (monkeys) native to South and Central America, parvorder Platyrrhini
positive selection	natural selection acting on phenotypes and the relevant DNA sequences that results in directional change toward a new sequence and phenotype. Contrasted with negative selection that acts to eliminate deleterious traits, and therefore acts against any new mutations that generate them
strepsirrhine primates	members of the branch of primates that includes lemurs, lorises, galagoes and cheirogaleids, suborder Strepsirrhini
allopatric	having separate, non-overlapping geographic distributions
parapatric	having geographic distributions that are adjoining but do not overlap

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Key Points

- Whole genome assemblies are now available for all the great apes and several other nonhuman primate species. The published analyses document between-species differences in gene content, segmental duplications, retroposon insertions and other genomic features.
- Next-gen sequencing has made whole genome sequencing and draft assembly more practical, and consequently additional nonhuman primate genome assemblies, with detailed annotation and other associated analyses, are in progress.
- The available data concerning nonhuman primate population genomics indicate that these species exhibit as much or more within-species genetic variation than is found among humans, with some species showing substantially higher rates of polymorphism.
- Current information indicates that differences between species in patterns of gene expression are common, have been influenced by natural selection and are likely to contribute to phenotypic differences among species.
- Analyses suggest that the evolutionary radiation that produced the extant human, chimpanzee and gorilla lineages (i.e. the speciation events) resulted from a complex process characterized by incomplete lineage sorting and/or gene flow among partially differentiated lineages.
- Whole genome analysis as well as more targeted sequencing in nonhuman primate species used in disease-related research has identified specific variants relevant to human disease risk, and finds differences among primate model species that are directly relevant to disease mechanisms and other biomedically significant phenotypes such as drug metabolism.

Box 1. Genome analysis of ancient hominins

The fossil record for recent human evolution (i.e. the last several hundred thousand years) is substantial. A great deal is known about morphology, biogeography and the archeological evidence for behavior concerning several extinct hominin species. Remarkably, through dramatic advances in techniques for investigating ancient DNA, we now have access to extensive genome sequence data for Neanderthals, an extinct hominin population from Europe and western Asia that diverged at least 250,000 years ago from the lineage leading to modern humans[3]. This work has shown that 1–4% of DNA sequences carried by modern humans outside Africa are derived from Neanderthals, the result of interbreeding and gene flow[54]. Another extinct hominin population (the Denisovans) were only recently recognized using genome sequence produced by extracting DNA from a finger bone found in the Altai Mountains[4]. The Denisovans diverged from human ancestors 170,000–700,000 years ago. Gene flow from the Denisovans into the modern human population has so far been detected only among aboriginal Australians and populations in Melanesia and southeast Asia[4]. These findings indicate ancestral human populations interbred to some biologically significant degree with other populations that were distinct in their genetics, and at least in the case of Neanderthals, distinct also in morphology. There is also evidence that introgression from Neanderthals into modern humans introduced alleles now associated with disease among modern humans, and that negative selection after this hybridization may have been driven by adverse effects of that hybridization on male fertility[110].

Box 2. Initial genomic divergence and incipient speciation

The theory and modeling of speciation is a complex topic that has generated a large amount of discussion. Historically, the founders of the modern evolutionary synthesis (e.g. Ernst Mayr, Theodosius Dobzhansky) argued that genetic and reproductive isolation among populations precedes phenotypic and/or genetic differentiation that is significant enough to justify recognizing those populations as distinct species[111]. Ernst Mayr's 'biological species concept' and the allopatric speciation model long dominated discussion[111]. By contrast, the model of punctuated equilibrium[112] posited that most adaptively important genetic differentiation occurs during or immediately after initial divergence and isolation of incipient species (see also[113]). More recently, other models and theories have addressed the greater complexity now known to be inherent in speciation and the genetic differentiation of many lineages[86–89]. For various types of species, the process of genetic divergence and incipient speciation appears to be more complex than the traditional allopatric speciation model proposed.

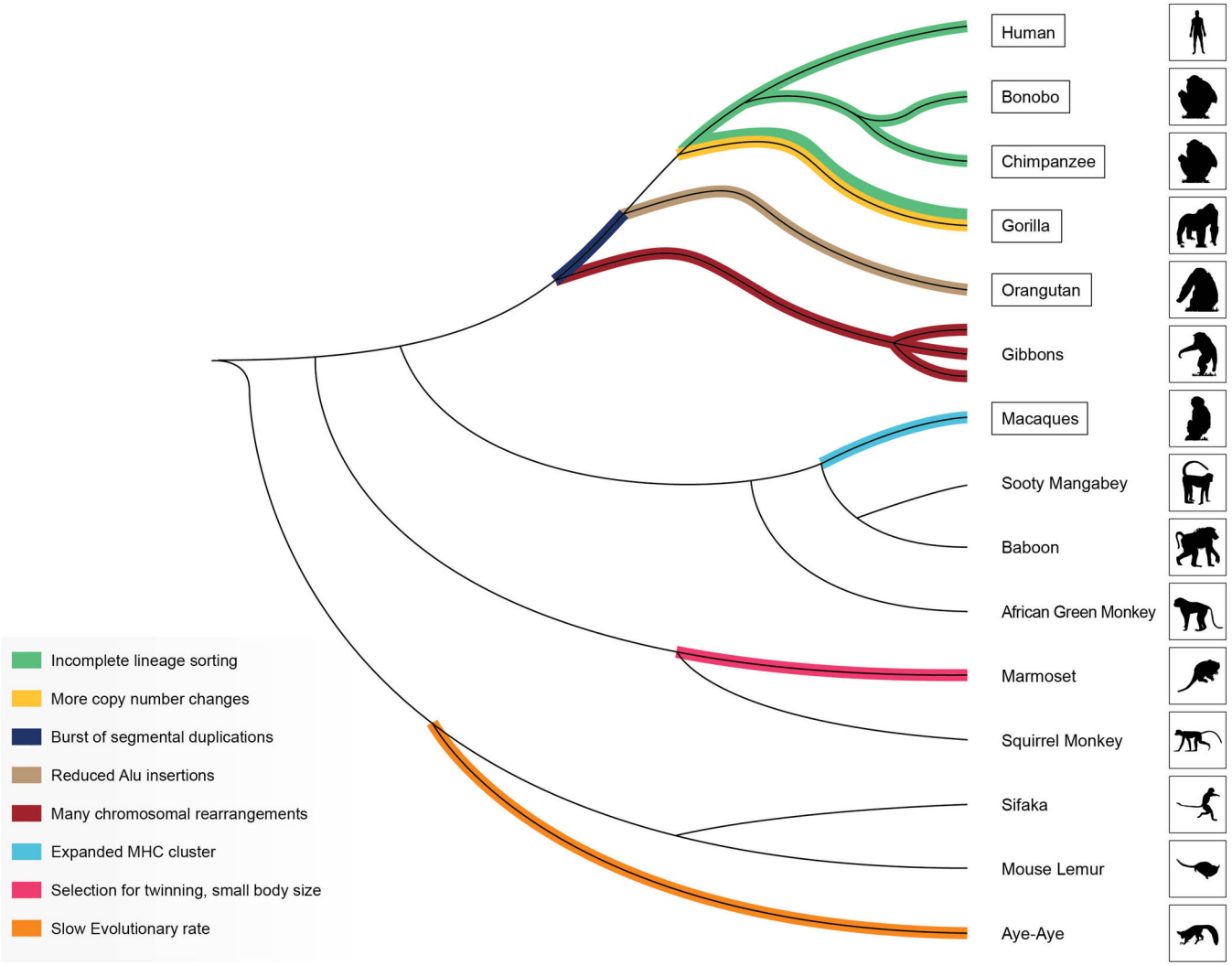
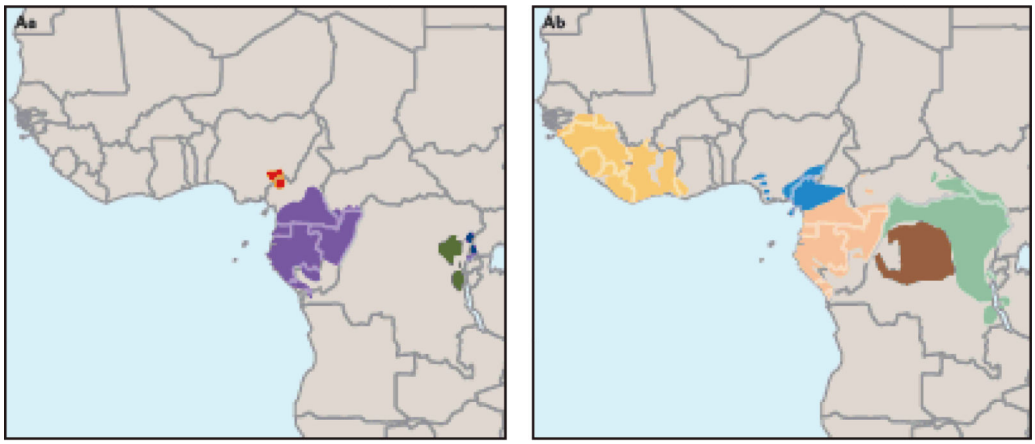


Figure 1. Primate phylogenetic tree

This diagram presents the evolutionary relationships among species for which genome sequences are published, available or in progress. The genomes for species enclosed in boxes are already published. Among the macaques, the rhesus and cynomolgus macaque genomes are published[2, 91], but sequencing of other macaque genomes is underway. Selected lineages are highlighted to indicate specific genomic features of interest, or unexpected genomic traits, such as reduced rate of Alu insertion in the orangutan genome[13] or the lower evolutionary rate in the aye-aye[9].



		Species and subspecies	Genetic variation	Conservation status
Aa	1	Cross river gorilla	0.001 < Het < 0.0015	Critically endangered
	2	Western lowland gorilla	0.0015 < Het < 0.002	Critically endangered
	3	Eastern lowland gorilla	Het < 0.001	Endangered
	4	Mountain gorilla	NA	Critically endangered
Ab	5	Western chimpanzee	Het < 0.001	Endangered
	6	Nigeria–Cameroon chimpanzee	0.001 < Het < 0.0015	Endangered
	7	Central chimpanzee	0.0015 < Het < 0.002	Endangered
	8	Eastern chimpanzee	0.0015 < Het < 0.002	Endangered
	9	Bonobo	Het < 0.001	Endangered
Ba	10	Bornean orang-utan	0.0015 < Het < 0.002	Endangered
	11	Sumatran orang-utan	Het > 0.002	Critically endangered
	12	Gibbons and siamang	0.001 < Het < 0.002	Endangered and critically endangered
Bb	13	Rhesus macaque	Het > 0.002	Least concern
	14	Cynomolgus macaque	Het > 0.002	Least concern
	15	Pigtailed macaque	NA	Vulnerable

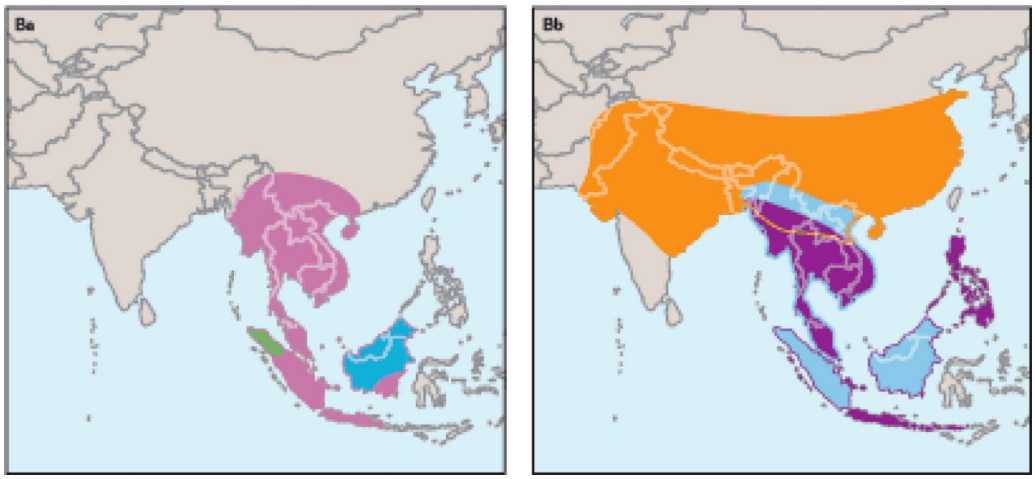


Figure 2. Geographic distribution and genetic variation in selected primates

Despite having modest or small current population sizes, and in most cases being either endangered or critically endangered (www.iucnredlist.org), most nonhuman primate species investigated to date have substantial levels of within-species genetic variability. Panel A: The approximate geographic distributions of African ape species, although actual distributions are generally discontinuous isolated populations within these areas. Hatching indicates the level of genetic variation estimated through the Great Ape Genome Project[36]. The IUCN conservation status is indicated by legend font. Panel B: Approximate geographic

distributions for gibbons, orangutans and selected macaque species. As for the African species, conservation status is indicated by the legend font and the estimated level of genetic variation is shown by hatching. Information on genetic variation from references #33, 34 and 66.

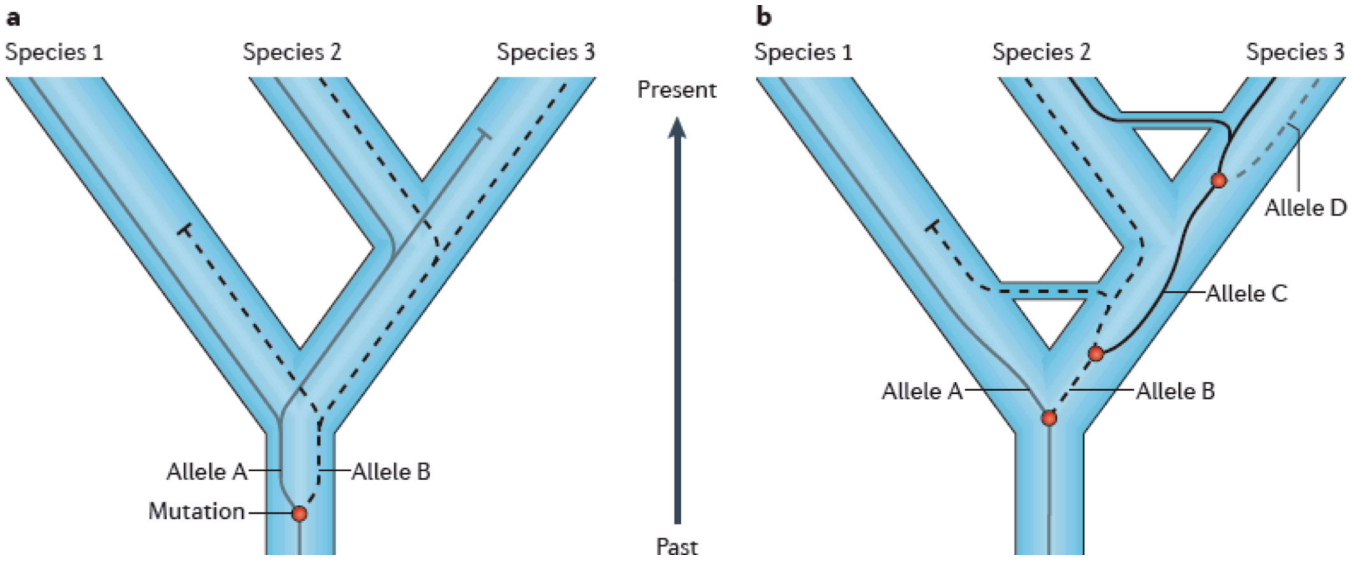


Figure 3. Incomplete lineage sorting

Incomplete lineage sorting can produce discrepancy between the phylogenetic tree for a specific gene or genomic segment and the overall species-level phylogenetic tree. If an ancestral species is polymorphic, segregating alleles A and B, and divides into two descendent lineages, both alleles can be retained in both daughter lineages. If one of those lineages then divides again relatively soon, all three species lineages may carry both alleles. Over time, each lineage will lose one or the other allele due to drift or selection. In this case, assume that Species 1 retains allele A and Species 3 retains B. Species 2 will, for this genomic segment, appear more closely related to either Species 1 or Species 3 depending on whether it retains allele A or B. Retention of allele B would mean this genomic segment matches the overall species-level phylogenetic tree, but retention of allele A would lead to discrepancy. Analysis of whole genome sequences for human, chimpanzee and gorilla indicate that “gene trees” for a significant fraction of the genome do not match the overall species-level phylogeny, which places chimpanzees are more closely related to humans than to gorillas[19].

Table 1

Published primate genome sequences

Common Name	Species Name	Bases in contigs	Contig N50	Scaffold N50	Ref.
Draft Genome Assemblies					
Chimpanzee	*Pan troglodytes*	2.7 Gb	15.7 kb	8.6 Mb	1
Bonobo	*Pan paniscus*	2.7 Gb	67 kb	9.6 Mb	64
Gorilla	*Gorilla gorilla*	2.8 Gb	11.8 kb	914 kb	5
Orangutan	*Pongo abelli*	3.1 Gb	15.5 kb	739 kb	13
Indian rhesus macaque	*Macaca mulatta*	2.9 Gb	25.7 kb	24.3 Mb	2
Chinese rhesus macaque	*Macaca mulatta*	2.8 Gb	12.0 kb	891 kb	91
Vietnamese cynomolgus macaque	*Macaca fascicularis*	2.9 Gb	12.5 kb	652 kb	91
Aye-aye	*Daubentonia madagascarensis*	3.0 Gb	na	13.6 kb	9
2× Sanger Genome Sequences*					
Mouse lemur Bushbaby Tarsier	*Microcebus murinus Otolemur garnetti Tarsier syrichta*	na	na	Na	11
Whole genome re-sequencing studies without assembly					
Indian rhesus macaque	*Macaca mulatta*	na	na	Na	33
Chinese rhesus macaque	*Macaca mulatta*	na	na	Na	112
Mauritian cynomolgus macaque	*Macaca fascicularis*	na	na	Na	91
Malaysian cynomolgus macaque	*Macaca fascicularis*	na	na	Na	114

* 2× whole genome coverage.

na, not applicable. N50, weighted median statistic such that 50% of the entire assembly is contained in contigs or scaffolds equal to or larger than this value.
YES, OK.