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Report

Demographic History and Genetic Differentiation in Apes

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Summary

Comparisons of genetic variation between humans and great apes are hampered by the fact that we still know little about the demographics and evolutionary history of the latter species [1–4]. In addition, characterizing ape genetic variation is important because they are threatened with extinction, and knowledge about genetic differentiation among groups may guide conservation efforts [5]. We sequenced multiple intergenic autosomal regions totaling 22,400 base pairs (bp) in ten individuals each from western, central, and eastern chimpanzee groups and in nine bonobos, and 16,000 bp in ten Bornean and six Sumatran orangutans. These regions are analyzed together with homologous information from three human populations and gorillas. We find that whereas orangutans have the highest diversity, western chimpanzees have the lowest, and that the demographic histories of most groups differ drastically. Special attention should therefore be paid to sampling strategies and the statistics chosen when comparing levels of variation within and among groups. Finally, we find that the extent of genetic differentiation among "subspecies" of chimpanzees and orangutans is comparable to that seen among human populations, calling the validity of the "subspecies" concept in apes into question.

Results and Discussion

Diversity

A total of 22,401 base pairs (bp) (26 independent regions) were amplified in bonobos and chimpanzees, and 16,001 bp (19 independent regions) were amplified in orangutans. The regions collected in orangutans were available from three human populations [6] (regions numbered 1 to 22 in human, see Table S2 in the Supplemental Data available online), as were 16 of the 19 regions, comprising a total length of 14,000 bp, from gorillas (O.T., unpublished data). None of the results described changed qualitatively when we used only sequences orthologous to the gorilla data (results not shown). Thus, we kept all sequence data available for each group, except where noted.

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Table 1 provides two different summaries of nucleotide diversity (π and θ_w), as well as the number of single nucleotide polymorphisms (SNPs) in each group. As seen previously for mtDNA and nuclear minisatellites [7], orangutans have the highest diversity levels among hominoids, with π values of 0.27% and 0.35% for Bornean and Sumatran orangutans, respectively. It has been suggested that this is the result of multiple origins of orangutan populations that repopulated the two islands after successive glacial maxima [8].

Western chimpanzees have the lowest diversity levels among apes, with π values of only 0.08%. The nucleotide diversity of central and eastern chimpanzees (π = 0.19% and 0.16%, respectively) is 1.6 to 2.4 times higher than that of bonobos and western chimpanzees (π = 0.10% and 0.08%, respectively) and is significantly higher with respect to both π and θ_w ($p < 0.05$ after correction for multiple testing, two-tailed Mann-Whitney U test). Central chimpanzees have the highest diversity levels among chimpanzees, in agreement with other studies [9–11]. This is consistent with the view that chimpanzees originated in central Africa [10, 12–16].

Gorillas have diversity levels in the range of eastern chimpanzees (π = 0.15%). With the exceptions of western chimpanzees and bonobos, who have diversity levels close to human ones, all other great apes have diversity levels 1.2 to 5.8 times higher than those of humans, in agreement with previous estimates based on mtDNA, the X chromosome, and autosomal DNA [7, 10, 11, 15, 17, 18].

Demography

We used a multilocus HKA test to assess evidence for differences in selective regimes among the 26 regions in the chimpanzees and bonobos, the 19 regions in orangutans and humans, and the 16 regions in gorillas (see Experimental Procedures). No evidence for a departure from an equilibrium model was detected for any of the groups.

We used the Tajima's *D* statistic to assess the fit of the data to the standard neutral model of a randomly mating population of constant size. Under these conditions, the expectation for Tajima's *D* is roughly zero, whereas a negative value of *D* reflects a relative excess of low-frequency polymorphisms, as is seen after a population expansion. The mean *D* is not significantly different from zero for western and eastern chimpanzees, gorillas, or the three human populations (Table 1). Goldberg and Ruvolo [1] showed that the mismatch distribution among 262 HVR1 mtDNA sequences in eastern chimpanzees is similar in shape to the distribution in humans and indicative of a recent expansion [19, 20]. Given that the mean *D* for eastern chimpanzees is not significantly negative for the 26 nuclear regions ($D = -0.16$, p=0.29) studied here, such an expansion may have taken place recently enough not to be reflected in nuclear DNA sequences. This is similar to the situation in human populations, where none of the three populations considered

Table 1. Summaries of Polymorphism Data in All Groups Included

Species	Population	n	Length	π (%)	θ_w (%)	# SNPs	# Singletons	Tajima's *D*
Bonobos								
		18	22401	0.1	0.12	87	26	−0.42[a]
Chimps								
	All	60	22401	0.19	0.28	286	100	nd[b]
	Central	20	22401	0.2	0.24	182	75	−0.43[a]
	Eastern	20	22401	0.16	0.17	134	45	−0.14
	Western	20	22401	0.08	0.09	70	19	−0.23
Gorillas								
	Western	30	14017	0.15	0.14	74	12	0.13
Orangs								
	All	32	16001	0.36	0.35	210	53	nd
	Bornean	20	16001	0.27	0.27	132	24	0.45[a]
	Sumatran	12	16001	0.35	0.32	148	49	0.37[a]
Humans								
	All	90	16001	0.12	0.14	114	28	nd
	Hausa	30	16001	0.13	0.13	81	16	−0.07
	Italian	30	16001	0.07	0.06	45	5	0.18
	Chinese	30	16001	0.06	0.07	46	13	−0.18

[a] Significant at the 5% level.
[b] nd: not determined.

have a negative Tajima's *D*, although mitochondrial DNA sequences reveal signs of expansions [21]. An alternative formal possibility is that positive selection would have affected the mtDNA in eastern chimpanzees and/or humans. The mean *D* for bonobos and central chimpanzees is significantly negative. The most likely explanations for these negative values are population expansion or fine-scale population subdivision [22–25]. It has been suggested that the latter explanation is most likely in this sample of central chimpanzees [9]. For bonobos, conclusions are probably premature, given that the samples used came from European zoos and may not be representative of the wild population.

The two orangutan populations have a significantly positive Tajima's *D*, because of an excess of intermediate frequency alleles, which is best explained by a recent reduction in population size or by population subdivision. Using 14 microsatellites, Goossens et al. [3] showed that the excess of intermediate allele frequencies in an orangutan population from Borneo can be explained by a very recent decline in population size, mainly as a result of human activity. Because it would take much more time to be able to detect this effect in nuclear DNA, and because our orangutan samples come from different local groups (see Table S1), population structure is a more likely explanation of our observation.

Our results point to different demographic histories in most or all of the groups considered. Thus, this should be taken into account when patterns of genetic variation are compared between homologous regions in humans and apes, for example in order to detect signs of positive selection in human genes.

Differentiation among Groups

Four "subspecies" of chimpanzees have been designated on the basis of geographical barriers [14, 26, 27]: western chimpanzees, Nigerian chimpanzees, central chimpanzees, and eastern chimpanzees. These are separated from one another by the Dahomey gap, the Sanaga River, and the Ubangi River, respectively. Similarly, Bornean and Sumatran orangutans have been regarded as two "subspecies" on the basis of geographical origin [12, 28]. However, morphological differences among the chimpanzee [29, 30] and orangutan "subspecies" are small and hard to define [28, 31–34], and although behavior varies drastically among local chimpanzee and orangutan groups, no consistent differences among "subspecies" have been demonstrated [35, 36]. In the absence of clear morphological and behavioral differentiation, genetic support for chimpanzee and orangutan "subspecies" has often been invoked [2, 7, 15, 27, 37–39], mainly on the basis of mtDNA or microsatellites [1, 15, 37]. Some authors have even suggested that western chimpanzees, as well as Bornean and Sumatran orangutans, should be elevated to the rank of species [15, 39]. These suggestions have led to much debate [2, 27, 37, 40, 41]. Because mitochondrial DNA is maternally inherited, it reflects only the history of females. In addition, mtDNA has a lower effective population size, and both mtDNA and microsatellites have high mutation rates compared to nuclear nonrepetitive DNA sequences. Therefore, they are more likely to show large genetic distance between groups even in the absence of any substantial differentiation across the nuclear genome.

For each pair of closely related groups, we tabulated the proportion of all SNPs that are shared across the two groups, that are unique to one of the two groups, or that are fixed differences between the two groups (see Figure S1). No fixed differences were found between eastern and central chimpanzees, Sumatran and Bornean orangutans, or among the human populations. Overall, 31% and 27% of the SNPs found are

Table 2. F_{st} and π_b Values, above and below the Diagonal, Respectively, for Each Pairwise Comparison

π between (%) \ F_{st}		Bonobos	Chimpanzees			Humans			Gorillas	Orangs	
		Bonobos	Central	Eastern	Western	Hausa	Chinese	Italians	Gorillas	Sumatran	Bornean
Bonobos	Bonobos		0.49	0.54	0.68	0.93			0.93	0.93	
Chimpanzees	Central	0.32		0.09	0.29	0.89			0.89	0.91	
	Eastern	0.31	0.20		0.32						
	Western	0.32	0.21	0.20							
Humans	Hausa	1.12	1.19				0.15	0.14	0.92	0.94	
	Chinese					0.13		0.09			
	Italians					0.14	0.09				
Gorillas	Gorillas	1.55	1.53			1.54				0.93	
Orangs	Sumatran	3.02	3.09			3.19			3.15		0.28
	Bornean									0.41	

shared between eastern and central chimpanzees and between Bornean and Sumatran orangutans, respectively. These estimates are close to those between Hausa and Chinese or Italians (38%), but lower than between Chinese and Italians (57%). Only 7% to 8% of SNPs are shared between western and eastern or central chimpanzees, respectively, whereas more are shared between the other two chimpanzee groups. This is probably the result of more genetic drift due the relatively small effective population size of the western chimpanzee.

To further investigate the amount of differentiation between ape groups, we estimated pairwise F_{st} values and π_b by using the homologous regions available for each pair of groups (Table 2). F_{st} represents the fraction of diversity attributable to between-group differences, and π_b is the mean pairwise sequence difference between two populations. As expected, the F_{st} values between the different species are high. Within the species, F_{st} between eastern and central chimpanzees is 0.09 and thus as low as between Chinese and Italians. Western chimpanzees are more differentiated from other chimpanzee subgroups (F_{st} 0.29 and 0.32), and so are the two subgroups of orangutans (F_{st} = 0.28), whereas human populations have F_{st} between 0.09 and 0.15, in agreement with earlier work [42, 43].

Given that it might be inappropriate to compare the F_{st} values between species because of differences in effective population sizes [44], we performed permutations to test whether the observed F_{st} values are higher than would be expected under random mating by randomly assigning individuals to populations and calculating F_{st} values 500 times. For all three comparisons of chimpanzee groups, the comparison of the two orangutan groups, and the comparison of the human populations, we reject a null model of random mating ($p < 0.05$ after correction for multiple tests).

The values of π_b (Table 2) are in general agreement with published data [45], but yield a different picture from the one based on F_{st} values in that π_b is highest between the Bornean and Sumatran orangutans (0.41%), even higher than between bonobos and chimpanzees (0.31% to 0.32%). The lowest values are between human populations (0.09% to 0.14%), whereas subgroups of chimpanzees are intermediate to these extreme comparisons (0.20% to 0.21%). However, the high π_b seen between orangutans should be viewed in relation to the amount of diversity within orangutan groups (Table 1), which is so high that two randomly chosen individuals from one "subspecies" are likely to be genetically as distant as two individuals belonging to different "subspecies."

With respect to the duration of physical separation, the Dahomey gap that separates western and central chimpanzees was covered with rainforest until about five thousand years ago [46], and Sumatra and Borneo were physically connected until ten to twenty thousand years ago [47]. Thus, the time of separation of the "subspecies" by geographical barriers has certainly been too short for complete lineage sorting by genetic drift and shorter than the separation of many human groups. In addition, migration between the groups may have occurred subsequent to the emergence of these geographical barriers [48]. Indeed, we speculate that a more geographically complete sampling of chimpanzees and orangutans with noninvasive samples from the wild as well as samples from museum specimens in areas where apes are now extinct will eventually demonstrate that the overall picture of genetic variation within chimpanzees and orangutans is one of isolation by distance, as is largely the case among humans [49, 50].

Conclusions

Among ape groups, diversity levels based on DNA sequences from multiple nuclear noncoding regions show that different groups of apes have different demographic histories. Thus, the demographics of ape populations must be carefully considered if their within-group diversity is compared to humans, for example in order to detect signatures of selection in humans. The extent of genetic differentiation among groups of orangutans and chimpanzees lends no support to the notion that "subspecies" are genetically distinct entities. This may be of relevance for the management of wild and captive ape populations.

Experimental Procedures

Samples

A total of 55 individuals were used for this study. Thirty unrelated common chimpanzees were considered, including ten central chimpanzees (*Pan troglodytes troglodytes*) born in Gabon, ten western chimpanzees (*Pan troglodytes verus*) from Sierra Leone, and ten eastern chimpanzees (*Pan troglodytes schweinfurthii*) from the Sweetwater Reserve in Kenya. The nine unrelated bonobos (*Pan paniscus*) considered were sampled from European zoos. Furthermore, we used ten Bornean (*Pongo pygmaeus pygmaeus*) and six Sumatran orangutans (*Pongo pygmaeus abelii*), all of known origin and sampled from most existing populations on the two islands (Table S1). In addition to these samples, the DNA sequences of 15 lowland gorillas (*Gorilla gorilla gorilla*) from Cameroon were used (O.T., unpublished data), and previously published sequence information from 45 human samples were used [6].

Genomic Regions and Characteristics

We sequenced 19 noncoding, autosomal regions, ranging from 650 bp to 1500 bp in length and representing a total of ~16,000 bp, in the orangutan, chimpanzee, and bonobo samples. These regions consist of one side of nine locus pairs previously sequenced in chimpanzee and human populations and were selected to have large-scale recombination rates and GC contents close to the genome average [6, 9, 51]. With the gorillas, the dataset was reduced to 16 regions, because three of the 19 PCR products could not be amplified. Another seven regions were extended to a length of 800 bp from Yu et al. [11] and additionally amplified for the chimpanzee and bonobo samples (Table S2). Thus, for chimpanzees and bonobos, we have a total of 26 regions, corresponding to a total amount of ~22,400 bp per individual.

GC content for each region was determined with Editseq from the DNAstar sequence-analysis package (DNAstar, Madison, Wisconsin). We checked for conserved regions between mouse and human sequences by using the Berkeley genome pipeline (http://pipeline.lbl.gov/).

DNA Extraction

The DNA from the central chimpanzees was taken from the same samples extracted and used in a previous study by Fischer et al. [9]. The DNA from eastern chimpanzees was extracted from blood samplesvia the GuSCN/silica protocol [52]. Two hundred microliters blood were mixed with 4× volumes of guanidinium thiocyanate buffer for 1 hr. The DNA contained in the GuSCN buffer was purified by binding to silica, with 20 μl silica suspension. The mixture was then centrifuged (12,000 rpm), and the supernatant was discarded. Washing of the silica pellet and elution of the DNA were completed as described in Hofreiter et al. [52]. DNA was eluted in a volume of 50 μl. For western chimpanzees and orangutans, the DNA samples were provided after this step by the BPRC (Netherlands) and Stephen O'Brien (USA).

PCR Amplification

Polymerase chain reaction (PCR) primers were designed by using the human and chimpanzee sequences. Two sets of primers were used to independently amplify and sequence each DNA segment taken from each individual in order to minimize the risk of allelic dropout, which may result if a primer fails to amplify one allele in an individual. Amplification reactions were performed in a 96-well microtiter-plate thermal cycler (Applied Biosystem). The PCR reaction mixture (100 μl) contained a standard buffer (10 mM Tris-HCl, 5 mM MgCl2), the four deoxynucleotide triphosphates (0.25 mM each), primers (0.5 pM each), Amplitaq Gold™ (Perkin Elmer), and genomic DNA (30 ng). Thermal cycling was performed with PCR of 30 cycles with an initial denaturation step at 95ºC for 5 min, followed by 16 cycles of denaturation at 94ºC for 45 s; primer annealing for 1 min at 57ºC, 59ºC, and 63ºC, depending on the primer pair; and primer extension at 72ºC for 2 min.

DNA Sequencing

The PCR primers were used for sequencing. Additional internal sequencing primers were designed to anneal approximately every 400 bp, for complete coverage in both orientations for each locus. After DNA amplification, PCR products were purified on Millipore plates, and the amount of purified DNA was estimated via electrophoresis in a 1% agarose gel and measurement with a spectrophotometer. Ten nanograms of the purified sample was used as a sequencing template. Cycle sequencing was performed according to the manufacturer's instructions with the BigDye Terminator Cycle Sequencing kit and the DNA analyzer 3730 (Perkin Elmer Biosystems).

Chromatograms were imported into Seqman (DNAstar) for assembly into contigs and the identification of single nucleotide polymorphisms (SNPs). Diploid sequence was determined by inspecting each nucleotide position in high-quality chromatograms.

Hardy-Weinberg equilibrium was tested for each polymorphic site with Arlequin version 2.0 (http://lgb.unige.ch/arlequin/). Overall, there were no more departures than expected by chance (data not shown).

For insertions/deletions that made direct sequencing difficult, we utilized the following procedure: After the PCR was performed, the products were cloned with the TOPO TA-cloning kit (Invitrogen). Ten clones were then sequenced, following the same procedure as above, for each individual.

Statistical Analyses

We used the program DNAsp [53] to obtain a number of commonly used statistics. To summarize diversity levels, we calculated the average pairwise difference, π [54], as well as θ_w [55], a diversity measurement based on the number of polymorphic sites. We also calculated one summary of allele frequencies, namely Tajima's *D* [56].

We tested the goodness of fit of the data to a standard neutral model of a random-mating population of constant size for each population, excluding regions that were not polymorphic, by asking how often the mean Tajima's *D* across the considered regions are as low or lower than observed in 10,000 simulations. We also assessed whether there was significant heterogeneity in the ratio of polymorphism to divergence across regions, by using a multilocus Hudson-Kreitman-Aguadé (HKA) test [57], in which human was used as the outgroup for all the apes and chimpanzee was the outgroup when testing human populations. These three tests were implemented with the program HKA, available from Jody Hey's home page (http://lifesci.rutgers.edu/~heylab/).

Initially, we attempted a set of coalescent-based Markov chain Monte Carlo (MCMC) analyses designed to estimate effective population sizes, times of divergence, and strength and directionality of migration, as well as mutation rates. We attempted this with two such computer programs, IM [58] and Lamarc (http://evolution.gs.washington.edu/lamarc.html). In no case were we able to obtain convergence of the Markov chains. Although we have collected a large amount of sequence data at many loci, we hypothesize that the combination of very recent divergence and slow mutation rate does not provide enough information to allow the MCMC algorithm to converge on any true population parameters of interest.

Differentiation among Groups

We tabulated the proportion of all polymorphisms that are shared between any two groups, fixed between groups, or found in only one group. We reduced data sets, where needed, so that we had orthologous sequences for both groups. To summarize differences in allele frequencies between the different chimpanzee populations and bonobos, we calculated F_{st} values for each pair of populations. F_{st} is defined as $1 - H_w/H_b$ [59, 60], where H_w is the average pairwise difference between sequences taken from the same population and H_b is the average pairwise difference between sequences taken from different populations. F_{st} values were estimated with DNAsp. We also estimated the diversity levels between groups (π_b).

Supplemental Data

Supplemental Data include one figure and two tables and are available with this article online at: http://www.current-biology.com/cgi/content/full/16/11/1133/DC1/.

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Accession Numbers

The chimpanzee and orangutan sequences have been deposited in GenBank under the accession numbers DQ494880–DQ496097.