

Supporting Information

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SI Methods

Dataset Preparation. DNA from chimpanzees housed at the Limbe Wildlife Centre (LWC) in Limbe, Cameroon was isolated from whole blood samples; and DNA extract yields were quantified as previously reported (1). DNA samples were transported from Cameroon to the United States in full compliance with Convention on International Trade in Endangered Species and Centers for Disease Control export and import regulations. This research was carried out with Institutional Animal Care and Use Committee approval from the University at Albany, State University of New York.

We produced microsatellite genotype profiles of 45 chimpanzees drawn from a subset of 310 microsatellite loci (2). This subset of autosomal loci ($n = 27$) we analyzed here has been shown previously (2) to have considerable power for: (i) distinguishing bonobos from chimpanzees, (ii) classifying individual chimpanzees into geographically disjunct populations (western, central, and eastern) that correspond to three of the recognized chimpanzee subspecies, and (iii) reliably detecting hybrid individuals. Details about the loci included in this study are given in Table S1.

PCR reactions were performed using the Qiagen Multiplex PCR kit (Qiagen, Valencia, CA) in Eppendorf Mastercycler (Eppendorf, Westbury, NY), and were carried out using 1 ng of DNA for each reaction following the manufacturer's protocol. Microsatellite genotyping was carried out in four multiplex PCR reactions using the ABI G5 dye set (Applied Biosystems, Foster City, CA). Each multiplex PCR product was analyzed on an ABI 3130 capillary array genetic analyzer (Applied Biosystems). Fragment sizes were determined against Genescan 600 Liz size standard (Applied Biosystems). Allele sizes were determined using GENEMAPPER ID version 2.7 software (Applied Biosystems). Heterozygous and homozygous loci were scored a minimum of two and a maximum of four times by independent PCRs for each individual.

Dataset Integration. These allele size data were integrated with allele size data from individuals previously genotyped for the same loci reported by Becquet et al. (2). We corrected for allele size differences due to apparatus and protocol discrepancies (3) by retyping a subset of individuals ($n = 10$) reported previously (2). Table S1 lists marker and allele size adjustments made for each locus included in this study. Previous studies in humans have shown that such integration yields datasets suitable for making inferences about population history (4). Of the original 130 individuals genotyped by us and those reported by Becquet et al. (2), we removed the following individuals from subsequent analyses: 27 captive-born chimpanzees, two chimpanzees listed as wild-born but with untraceable/unreliable International Species Information System (ISIS) records, and one LWC individual that was missing alleles for >15% of the loci (despite repeated attempts to produce allele sizes suitable for scoring). The combined dataset contains genotype profiles for six bonobos and 94 wild-born chimpanzees with estimated origins from the following locations: Cameroon ($n = 45$) (1), western Africa ($n = 31$), central Africa ($n = 12$), and eastern Africa ($n = 6$) (2). Allele sizes newly generated for 45 LWC chimpanzees with estimated origins in Cameroon (1) are listed in Table S2.

Data Analysis. Cluster analysis. Population structure and individual ancestry were examined using a Bayesian clustering approach implemented in the STRUCTURE Version 2.3 software package

(5). This program estimates the shared population history of individuals based solely on their genotypes under a model of Hardy-Weinberg equilibrium and linkage equilibrium in the ancestral populations, thereby making no a priori assumptions regarding population classifications. STRUCTURE estimates individual proportions of ancestry into K clusters, where K is specified for the program in advance across independent runs and corresponds to the number of putative ancestral populations. The program then assigns admixture estimates for each individual (Q) from each inferred ancestral population cluster. STRUCTURE runs were performed: (i) with a model that allows individuals to have ancestry in multiple populations ("admixture mode"); (ii) with correlated allele frequencies; and (iii) blinded to a priori population labels. Runs were performed with a burn-in step of 500,000 Markov Chain Monte Carlo (MCMC) iterations and 1,000,000 MCMC iterations. Fifty runs each for $K = 1$ to $K = 10$ were performed for all datasets. STRUCTURE outputs were processed with CLUMPP (6); and a G-statistic >99% was used to assign groups of runs to a common clustering pattern. CLUMPP output for each K value was plotted with DISTRUCT (7). We used a combination of methods to infer a maximum number of chimpanzee populations (K_{MAX}) including, (i) the K value at which the posterior probability distribution (PPD) values reached an apex before decreasing (5), (ii) high stability of clustering patterns between runs, (iii) the K_{MAX} value at which $K_{MAX} + 1$ no longer split the cluster distinguished by K_{MAX} (4), (iv) correspondence between maximum PPD values from STRUCTURE runs and significant eigenvectors recovered by PCA, and (v) calculating an adhoc statistic, ΔK (8), as estimated by the STRUCTURE HARVESTER software package version 0.56.4 (9).

Principal components analysis (PCA). The EIGENSOFT software package (10) was used to perform PCA on individual genotypes to identify significantly different populations. We developed a script in MATLAB (The MathWorks, Natick, MA) that converted the microsatellite data into a false SNP format by scoring the presence or absence of each of $n - 1$ alleles (where n is the number of alleles in the sample). This file was processed in *SmartPCA*, which produced eigenvectors and eigenvalues. The statistical significance of each eigenvector was tested by Tracy-Widom statistics. Each significant eigenvector recovered by this PCA approach separates the samples in such a way that the first and subsequent eigenvectors distinguish, in order, the most to least differentiated populations in the sample (10). All analyses using EIGENSOFT were performed blinded to a priori population labels.

Allele frequency differentiation. Three measures of population genetic differentiation were calculated using the ARLEQUIN 3.5 software package (11): D^2 , R_{ST} , and $(\delta\mu)^2$. The D^2 (12) genetic distance is based on a model in which genetic drift is the only force influencing allele frequency differences across populations and is sensitive to recent differentiation events. R_{ST} (13) and $(\delta\mu)^2$ (14) are similar to D^2 , but both assume a stepwise mutation model (SMM). Consequently, R_{ST} and $(\delta\mu)^2$ are more likely to capture whether differences in the mutation processes are important in driving population differentiation and are also more sensitive for detecting ancient population separations (4). These latter models differ in that R_{ST} is based on the fraction of the total variance in allele size between populations and is analogous to F_{ST} (13), whereas $(\delta\mu)^2$ is based on differences in the means of microsatellite allele sizes (14). Recent work has shown convincing evidence that the loci typed for this study appear to follow the SMM in both chimpanzees and bonobos (2, 15).

D^2 calculations were completed on untransformed allele size calls. Because R_{ST} and $(\delta\mu)^2$ assume the SMM, allele sizes were transformed to repeat size units before analysis in ARLEQUIN (11). Allele sizes were transformed such that the smallest allele for each locus was scored as n and each subsequent allele was scored as $n + 1$. In infrequent cases where repeat unit sizes did not follow the $n + 1$ model, and instead repeat units skipped x repeat(s), the next allele in the data were scored as $(n + x + 1)$. Individuals with $\geq 25\%$ membership ($n = 7$) in more than one ancestral cluster from the STRUCTURE analysis (Fig. 2A) were treated as potential hybrids and excluded from population pairwise genetic distance calculations. Each pairwise genetic distance calculation was determined by 100,000 replications in ARLEQUIN. The significance of these pairwise population genetic distance calculations were evaluated by a significance test at $P < 0.05$.

Recent work has shown that microsatellite loci can be used to build robust phylogenies (16). We constructed phylogenetic trees using three measures of population genetic differentiation D^2 (12), R_{ST} (13), and $(\delta\mu)^2$ (14) described above. Trees based on D^2 were included here, because D^2 gives more reliable phylogenetic results compared with R_{ST} and $(\delta\mu)^2$ (16, 17), in cases where microsatellite alleles do not follow a stepwise mutation process, where other evolutionary forces such as genetic drift and/or gene flow have stronger influence on shaping diversity than mutation, and when the number of loci is relatively small, as is the case in this study (16).

For the D^2 analysis, we resampled the dataset 10,000 times to generate multiple distance matrices. We constructed unrooted neighbor joining phylograms for these matrices using the PHYLIP software package, version 3.5 (18) with the *Neighbor* program. *Consense* was used to obtain a consensus tree that was then used by *Contml* to generate branch lengths from allele frequency data using a maximum likelihood algorithm. For the R_{ST} and $(\delta\mu)^2$ analyses, single trees with branch lengths were produced using *Neighbor* from population pairwise differentiation values calculated with ARELEQUIN. *Consense* calculated bootstrap values. Trees were plotted using the GENEIOUS software package (Version 4.8; ref. 19), and branches with at least 70% support were labeled.

Population divergence times. Calculating population divergence times is challenging using microsatellites, especially when the time to most recent common ancestor ($T_{MRC A}$) might be quite ancient (16). However, the microsatellite loci included in this study have been shown to be accurate molecular clocks for *Pan* compared with autosomal resequencing data (15). We calculated population divergence times based on $(\delta\mu)^2$ (14) assuming a mutation rate (μ) of 1.6×10^{-4} , the median μ for these loci reported by Wegemann and Excoffier (20). We further assumed a 20-y generation time (g), which is consistent with studies from the wild (21) and has been used in recent studies (22, 23). Population splitting times were calculated using the method described by Goldstein et al. (14): $(\delta\mu)^2 \times g/2\mu$.

Dataset validation. We evaluated the reliability of the analyses for the dataset reported here using three methods. First, our analyses were based on a dataset containing only 9% of the loci reported by Becquet et al. (2), and unlike that study, this dataset included only wild-born chimpanzees. We examined how well this reduced dataset captured the genetic structure reported by Becquet et al. (2), including bonobos and chimpanzees from Upper Guinea (western), central Africa, and eastern Africa, but excluding those from Cameroon. Second, our analyses are also based on unequal sample sizes for chimpanzees from different regions of Africa. In particular, the samples size of chimpanzees reported to originate from eastern Africa ($n = 6$) was much smaller than for the other three regions: Upper Guinea ($n = 31$), Cameroon ($n = 45$), and central Africa ($n = 12$). Unequal population sample sizes can greatly bias estimates of genetic differentiation, as well as the

numbers of distinct and private alleles found per locus in different populations (24, 25). We used two rarefaction procedures to explore the possibility that our results were the result of unequal sample sizes instead of real population structure. First, we constrained sample sizes to be equal in all populations identified in the full dataset by creating randomized data subsets ($n = 10$) that included six each of chimpanzees from Upper Guinea, the Gulf of Guinea region, southern Cameroon, central Africa, eastern Africa, and bonobos. We carried out cluster analyses including, generating STRUCTURE (5) and PCA plots (10), along with reevaluating K_{MAX} for each randomized dataset. Finally, we applied a rarefaction procedure developed by Kalinowski (25) for counting alleles private to combinations of populations corrected for unequal sample sizes between populations as implemented in the ADZE software package (26).

SI Results and Discussion

The PCA (Fig. S3) for the 27-locus genotype profiles including only wild-born Upper Guinea (western), central, and eastern chimpanzees recovered three significant principal components (PCs). These axes recapitulate the major population clusters reported previously by Becquet et al. (2). PC 1 separated bonobos from chimpanzees, extracting 36.7% of the observed variation. PC 2 separated Upper Guinea chimpanzees from chimpanzees occupying equatorial Africa, extracting for 44.1% of the genetic variation. PC 3 separated the central and eastern populations, accounting for 19.2% of the variation. We did not detect the fourth axis of variation reported by Becquet et al. (2), possibly due to the lack of captive-born individuals reported as “hybrids” by these authors. Alternatively, the reduced number of loci may lack the power to resolve subtle population differences at higher values of K . Table S3 compares R_{ST} and F_{ST} values between the full dataset including 310 loci as reported by Becquet et al. (2) compared with these including only bonobos along with the Upper Guinea (western), central, and eastern populations for the 27-locus dataset. Allele frequency differentiation values for the 27-locus dataset (this study) versus the 310-locus dataset reported by Becquet et al. (2) were highly correlated ($R_{ST} r^2 = 0.96$, $P < 0.5$; $F_{ST} r^2 = 0.96$, $P < 0.5$). Based on these findings, we concluded that the suite of microsatellite loci included in this study adequately captured the population structure reported by Becquet et al. (2) for Upper Guinea (western), central, and eastern chimpanzees. Consequently, the 27-locus dataset should yield a reliable picture regarding how chimpanzees from Cameroon contribute to the population structure of this species.

The population structure inferred for the ten randomized datasets of equal population size was highly consistent across runs. Each dataset returned identical K_{MAX} values as well as the same number of significant PCs by PCA. Fig. S4 A–C shows results for the inferred population structure for one of these randomized datasets composed of equal sized populations. The cluster analysis in STRUCTURE (Fig. S4B) revealed that K_{MAX} was 5 using both the PPD and ΔK criteria (Fig. S4C), instead of $K_{MAX} = 4$ or $K_{MAX} = 6$ for the full dataset including all 100 individuals. Also in contrast to the full dataset, chimpanzees originating in eastern Africa were distinguished from the others at lower values of K ($K = 4$) by STRUCTURE analysis than for the full dataset, and those from southern Cameroon clustered with chimpanzees from other parts of central Africa at $K_{MAX} = 5$. The PCA (Fig. S4A) captured four significant PCs that distinguished five significantly different populations of chimpanzees, whereas the PCA for the full dataset recovered six significantly different populations. The difference between the datasets containing equal population sizes compared with the full dataset was that the equal population size datasets did not recover a statistically significant PC that distinguished chimpanzees originating from southern Cameroon from chimpanzees

originating from other areas of central Africa, as was found by PCA of the full dataset. We concluded based on these results that oversampling of chimpanzees from Cameroon did not greatly bias the results we obtained from the full dataset. However, unequal population sample sizes appear to have influenced discerning subtle population structure across equatorial Africa. In particular, the full dataset may be an underestimate of the allele frequency differentiation that separates central and east African chimpanzees, or alternatively, may be an overestimate of the subtle distinction that separates chimpanzees originating from southern Cameroon from those originating elsewhere in central Africa.

Fig. S5 A and B shows allele richness by region and private alleles found in each population corrected for unequal population sample size. Allele richness did not vary considerably between regions, whereas private alleles occurred more frequently among chimpanzees originating in central and eastern Africa compared with Upper Guinea, the Gulf of Guinea region, or southern Cameroon. Fig. S6 shows shared private alleles be-

tween various population pairs extrapolated to equal population size. Intriguingly, more shared private alleles were found among the central-eastern population pair and among the Gulf of Guinea/southern Cameroon population pair than any other population combinations. The relatively high number of shared private alleles between central and eastern chimpanzees taken together with their low allele frequency differentiation values (Table 1) suggests that these populations probably share much of their recent genetic history and might be characterized by ongoing gene flow as suggested by some previous studies (27). In contrast, the relatively high number of shared private alleles between the Gulf of Guinea/southern Cameroon chimpanzee population pair considered jointly with their higher allele frequency differentiation (Table 1) suggests the possibility that these two lineages may be characterized by a pattern of recent introgression between lineages, as expected based limited evidence reported in previous studies (1, 28, 29).

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Table S3. Comparison of genetic differentiation among populations

Location*	Western	Eastern	Central	Bonobo
Western		0.44 (0.26)	0.42 (0.19)	0.82 (0.34)
Eastern	0.31 (0.32)		0.025 (0.07)	0.70 (0.26)
Central	0.31 (0.32)	0.05 (0.09)		0.70 (0.21)
Bonobo	0.68 (0.68)	0.57 (0.54)	0.51 (0.49)	

*Microsatellite genotypes for western, central, and eastern chimpanzees were reported by Becquet et al. (1). Pairwise R_{ST} (versus F_{ST}) is shown. Numbers in bold above the diagonal were calculated from the subset of 27 autosomal microsatellite loci from Becquet et al. (1). Numbers below the diagonal appear in Becquet et al. (1).

1. Becquet C, Patterson N, Stone AC, Przeworski M, Reich D (2007) Genetic structure of chimpanzee populations. *PLoS Genet* 3:e66.