



Brief Communication

# Admixture Between Historically Isolated Mitochondrial Lineages in Captive Western Gorillas: Recommendations for Future Management

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## Abstract

Although captive populations of western gorilla have been maintained in the United States for over a century, little is known about the geographic origins and genetic composition of the current zoo population. Furthermore, although previous mitochondrial analyses have shown that free-range gorilla populations exhibit substantial regional differentiation, nothing is known of the extent to which this variation has been preserved in captive populations. To address these questions, we combined 379 pedigree records with data from 52 mitochondrial sequences to infer individual haplogroup affiliations, geographical origin of wild founders and instances of inter-breeding between haplogroups in the United States captive gorilla population. We show that the current captive population contains all major mitochondrial lineages found within wild western lowland gorillas. Levels of haplotype diversity are also comparable to those found in wild populations. However, the majority of captive gorilla matings have occurred between individuals with different haplogroup affiliations. Although restricting crosses to individuals within the same haplogroup would preserve the phylogeographic structure present in the wild, careful management of captive populations is required to minimize the risk of drift and inbreeding. However, when captive animals are released back into the wild, we recommend that efforts should be made to preserve natural phylogeographic structure.

**Subject areas:** Population structure and phylogeography, Conservation genetics and biodiversity

**Key words:** Conservation, Captive gorillas, Great apes, Mitochondrial diversity

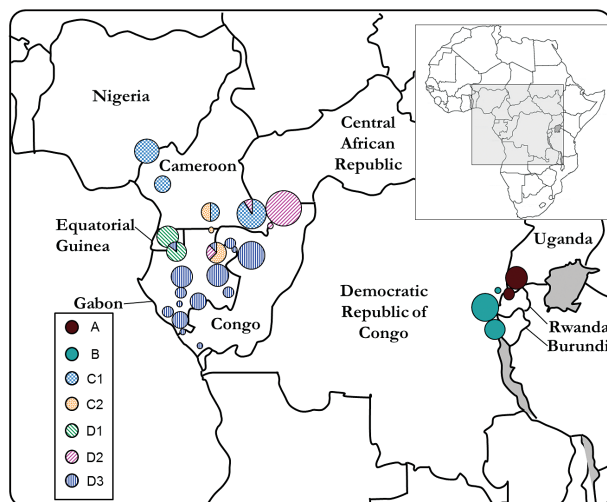
Western lowland gorillas (*Gorilla gorilla gorilla*) face mounting pressure from hunting, habitat loss, and infectious disease and are currently considered by the International Union for the Conservation of Nature (IUCN) as critically endangered (Bermejo et al. 2006; Walsh et al. 2008). As natural populations continue to decline (Walsh et al. 2003; Tutin et al. 2005; Guschanski et al. 2009), the demographic

and genetic management of captive populations is of critical concern (King et al. 2008; King et al. 2013). Challenges to the management of the existing zoo population is also exacerbated by the fact that no new animals have been introduced since the inception of the Convention of International Trade in Endangered Species (CITES), making the loss of genetic variation increasingly likely without

continued careful management of the population. Although western lowland gorillas have been maintained in captivity in the United States for more than 100 years, little is known about the geographic origins of the current zoo population of ~340 animals (Elsner 2012). Furthermore, virtually nothing is known of the extent to which genetic variation in the wild is represented in captive populations.

Previous noninvasive genetic studies of free-living populations have shown that gorillas exhibit pronounced regional genetic structure (Clifford et al. 2004; Anthony et al. 2007b). Within western lowland gorillas, 2 major mitochondrial haplogroups (C and D) have been identified. Each of these haplogroups is composed of several subdivisions that appear to occupy largely non-overlapping geographic distributions (Figure 1). This remarkable genetic structure is thought to reflect diversification due to riverine barriers and past forest fragmentation during the Pleistocene. Morphological and genomic DNA analyses of both western (*G. gorilla*) and congeneric eastern (*G. beringei*) gorilla populations indicate that both species have a complex history of hybridization (Thalmann et al. 2007; Ackermann and Bishop 2009; Prado-Martinez et al. 2013) and confirm the regional genetic structure evident in the mitochondrial dataset (Anthony et al. 2007b; Simons et al. 2012; Prado-Martinez et al. 2013).

Only 2 previous studies have examined genetic variation in captive gorillas, and their analyses were limited to nuclear data in a subset of animals of either unknown or country-wide geographic origin (Nsuguba et al. 2010; Simons et al. 2012). Since the main goal of the current gorilla Species Survival Plan (SSP) is to ensure the long term survival of gorillas in captivity (Leeds 2008), comparing levels of mitochondrial variability in the initial founder and current population is an important step for assessing the genetic status and loss of genetic variation in the captive population. Moreover, evaluating the extent to which the current zoo population has captured the mitochondrial genetic structure of wild populations has not yet been attempted, largely due to difficulties in ascertaining the geographic origin of many founder animals (Nsubuga et al. 2010). Admixture between historically-isolated lineages in the wild has also not been evaluated yet could provide important information for future breeding and release programs.



**Figure 1.** Frequencies and geographic distribution of major gorilla mitochondrial haplogroups across Central Africa. Color/texture patterns and circle areas represent the geographic distribution of different haplogroups and relative sample sizes respectively, using data (modified) from (Anthony et al. 2007a).

This study is the first attempt to understand how the mitochondrial phylogeographic structure and diversity found in wild populations is represented in the current United States captive population. Here we combine zoo gorilla pedigree data with mitochondrial sequence data from a subset ( $n = 52$ ) of captive gorillas to infer individual mitochondrial haplogroup affiliations and assess the degree to which gorillas from historically isolated lineages have been interbred. We also assess the correspondence between the reported country records of a subset of wild gorillas introduced into United States zoos and their likely geographical origins inferred from mitochondrial data. Finally, we make recommendations on the future genetic management of the captive gorilla population and the reintroduction of animals back into the wild.

## Methods

### Amplification and Sequencing of HVI

Using the DNeasy Blood & Tissue Kit (Qiagen), we extracted genomic DNA from peripheral blood samples of 42 western lowland gorillas (*Gorilla gorilla gorilla*) captive in US zoos, kindly donated by participating institutions and collaborators. In order to safeguard against the possibility of amplifying nuclear translocations of mitochondrial DNA or numts, we first amplified a fragment of 6.9 Kb of the mitochondrial region containing the first hyper-variable domain (HVI) through long-range PCR using the primers mt10261\_Fa and mt726\_R as previously described (Soto-Calderón 2012). From this amplification product, we then sequenced a 258 bp fragment of the HVI using primers mt15365\_F and mt15888\_R. In addition to the 42 sequences generated in this study, we also retrieved ten HVI sequences from captive gorillas (*G. g. gorilla*) from GenBank (L76755, L76759, L76757, AF451968, L76756, L76765, AF451971, L76767, L76758, and L76753), yielding a total of 52 sequences of western lowland gorillas from US zoos (Supplementary Table S1 online). We aligned these sequences with 197 reference sequences representing all the major mitochondrial lineages described to date in western (*G. g. gorilla* and *G. g. diehli*) and eastern gorillas (*G. beringei beringei* and *G. b. graueri*) (Anthony et al. 2007a), for a total of 249 HVI sequences analyzed in this study. We aligned all sequences using the ClustalW algorithm implemented in MEGA v5.2.2 (Tamura et al. 2011) and manually edited them to remove a hyper-variable poly-C stretch of ~25 bp, leaving a final alignment of 234 bp.

### Bayesian Phylogenetic Inference

We constructed a Bayesian phylogeny with Beauti/Beast v1.7.5 7 (Drummond et al. 2012) implementing the TPM3uf+G model ( $\alpha = 0.4270$ ) as determined with the Bayesian Information Criterion in jModelTest v2.1.4 (Posada 2008). A Monte Carlo Markov Chain was run for 50 million steps, with a sampling interval every 1000 steps. The first 10% of samples were discarded as burn-in. Convergence on the stationary phase of the posterior distribution was verified for all the parameters using Tracer v1.6 (Drummond et al. 2012). All parameter values had an effective sample size of 200 or greater. A final tree was summarized in TreeAnnotator v1.7.5 and edited with FigTree v1.4.0 (Drummond et al. 2012). The tree was rooted with haplogroups A and B, both unique to eastern gorillas. The mitochondrial haplotype affiliation of the 52 captive gorilla sequences was determined through their placement in the resulting Bayesian phylogeny.

### Molecular Diversity

We also compared the molecular diversity of both free-range eastern and western gorilla species and subspecies to one another and to captive individuals using the population genetic parameter  $\theta_s$

(Watterson 1975) and haplotype diversity estimate  $H$  (Nei 1987) in the program DNAsp v5.10.01 (Librado and Rozas 2009). Of the 249 sequences analyzed here, 6 were excluded from this analysis since they correspond to western gorillas of unknown origin from previous studies (either captive or wild).

### Pedigree Analysis

The genealogical relationships of all known gorillas historically held in the United States, Mexican, and Canadian zoos were previously described in detail in the international gorilla Studbook (Wilms 2010). Using this pedigree, we traced the maternal/mitochondrial lineages of all 52 captive gorillas with known mitochondrial affiliation back to their female founders and then inferred the maternal lineage of all their descendants. In this way, we were able to assign the mitochondrial haplotype affiliation of the individuals that were sequenced as well as intervening individuals in the pedigree. Information on the geographical origin of wild founder gorillas was taken directly from the International Studbook (Wilms 2010) where available and compared to the likely origin of each gorilla as inferred from the geographical distribution of each mitochondrial haplogroup. Finally, we identified animals that were descended from admixture between 2 or more haplogroups. In fulfillment of data archiving guidelines (Baker 2013), we have deposited the primary data underlying these analyses with GenBank (Accession codes KM555059 to KM555099).

### Results

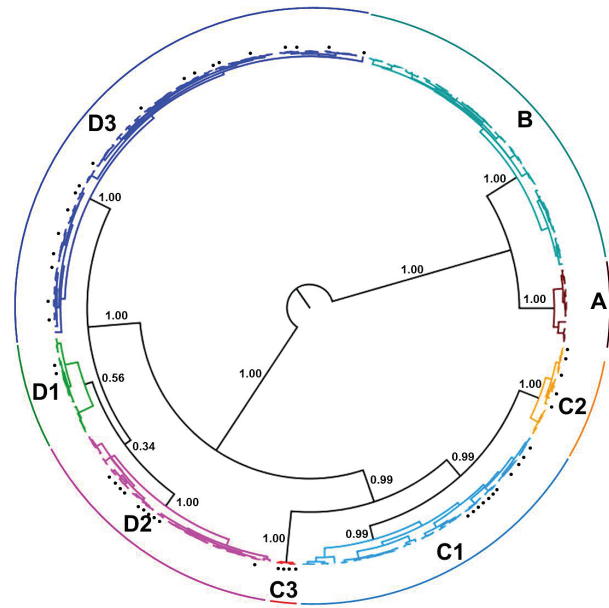
We did not find any evidence of numts in our dataset, either in the form of secondary peaks in chromatograms or presence of numt diagnostic sites (Anthony et al. 2007b; Wilms 2010). The phylogenetic analysis obtained from this study recovered all the previously defined mitochondrial haplogroups with posterior probabilities of 0.99 or higher, except in the case of subhaplogroup D1 (Figure 2). All mitochondrial lineages found within wild western lowland gorillas were also recovered in the 52 sequenced captive gorillas. Phylogenetic analysis also revealed a well-defined group of sequences present in captive gorillas, hereafter named C3, that to date has only been identified in wild populations in Cameroon (Soto-Calderón et al., unpublished data). This lineage is closely related to lineages C1 and C2 and was introduced to the United States by at least 3 wild gorillas.

Forty-six variable sites were identified in the HVI alignment and are depicted in Supplementary Table S2 online. Nineteen of these sites allow unambiguous diagnosis of the lineages A, B, C1, C2, C3, D2, and D3. Lineage D1 does not exhibit unique variants but nevertheless shares synapomorphic variants with the other lineages within the group D. Although haplotype diversity ( $H$ ) between wild and captive gorillas did not differ, nucleotide diversity ( $\theta_s$ ) was significantly lower in the captive population. Wild eastern gorillas also have a significantly low  $\theta_s$  and  $H$  relative to either wild and captive western gorillas (Tables 1 and 2).

The haplogroup affiliation of 394 gorillas was inferred from pedigree and mitochondrial sequence analysis. This number is comprised

of 379 gorillas historically held in US zoos, including 228 gorillas of the contemporary captive population (67.5%) and 151 dead animals (31.3%) (Supplementary Table S1 online). The remaining 15 gorillas represent individuals historically held elsewhere in North American zoos (i.e., Canada or Mexico).

The haplogroup composition of 39 wild founders of the US zoo population was very similar to that found in the current US captive population (Figure 3). Of the 39 wild founders whose mitochondrial



**Figure 2.** Bayesian phylogenetic tree of the first hyper-variable region (HVI) from 197 reference gorillas (*Gorilla gorilla* and *G. beringei*) and 52 gorillas (*G. gorilla*) captive in US zoos. Haplogroups A (*G. beringei beringei*) and B (*G. b. graueri*) were used to root the tree. Numbers indicate the posterior probability support for a given branch in the tree and bullets (·) represent captive gorilla sequences.

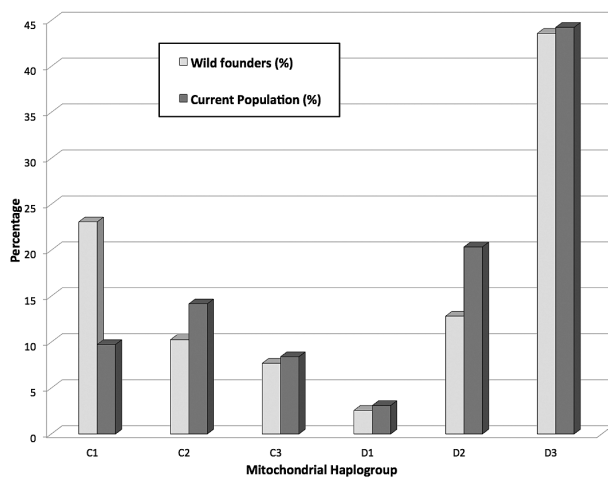
**Table 1.** Nucleotide diversity of the HVI (234 bp) in eastern (*Gorilla beringei*) and western (*G. gorilla*) gorilla subspecies.

	$n$	$S$	$\theta_s$	$H$
<i>G. beringei</i>	59	29	0.0286 ± 0.0023	0.859 ± 0.010
<i>G. b. beringei</i>	12	7	0.0102 ± 0.0030	0.788 ± 0.051
<i>G. b. graueri</i>	47	17	0.0173 ± 0.0018	0.789 ± 0.017
<i>G. gorilla</i> (free-range)	132	43	0.0487 ± 0.0226	0.878 ± 0.004
<i>G. g. gorilla</i>	123	44	0.0492 ± 0.0024	0.878 ± 0.004
<i>G. g. dielhi</i>	9	3	0.0048 ± 0.0021	0.694 ± 0.096
<i>G. gorilla</i> (captive)	52	40	0.0417 ± 0.0358	0.885 ± 0.010
Total	243			

Only western gorillas of known geographic origin were considered for this analysis.  $n$ : Sample size;  $S$ : number of segregating sites;  $\theta_s$ : Watterson's estimate of theta based on  $S$ ;  $H$ : Haplotype diversity.

**Table 2.** Pairwise student  $t$  tests of differences in theta based on the number of segregating sites ( $\theta_s$ ) (below diagonal) and haplotype diversity ( $H$ ) (above diagonal) between eastern, free-range western and captive western gorillas.

	Eastern	Free-range western	Captive western
Eastern	—	$t_{189} = 4.12; P < 0.0001$	$t_{108} = 3.56; P = 0.0006$
Free-range western	$t_{189} = 10.53; P < 0.0001$	—	$t_{181} = 1.56; P = 0.1206$
Captive western	$t_{108} = 6.17; P < 0.0001$	$t_{181} = 3.18; P = 0.0017$	—



**Figure 3.** Percentage of mitochondrial haplogroups in 39 wild founders and 228 captive gorillas in US zoos (Wilms 2010).

affiliation was identified, the reported country of origin was available in 14 cases (Wilms 2010). In 12 of these cases, the reported origin overlaps with the known geographical distribution of the assigned haplogroup (Table 3). However, 2 gorillas allegedly from Cameroon have haplogroup D3, which to date has only been found in gorillas from Equatorial Guinea, Gabon, and the Republic of Congo.

An analysis of the breeding history and haplotype affiliations of descendent individuals sampled in this study also revealed that 88 out of 105 crosses (84%) were between individuals with different haplogroup affiliations (Supplementary Table S3 online). Of the potential crosses that could occur, C2/D3 and C1/C2 lineages account for 43% of all hybrids and sometimes occurred repeatedly between the same individuals. One of the most outstanding examples of this was 12 recorded matings between 2 C2 females and 2 D3 males within the same zoo (Supplementary Tables S1 and S3 online).

## Discussion

Our findings show that US zoo gorillas possess all of the major mitochondrial lineages found across the entire range of western gorillas. This includes the novel lineage C3, which has recently been identified in gorillas from Cameroon (Soto-Calderon, unpublished data). Our results indicate that the 42 HVI sequences obtained in this study are of mitochondrial origin because the amplicons used as templates for HVI sequencing were ~7 Kbp, a length that is larger than most numts reported in the gorilla genome (Soto-Calderón 2012). We also did not find any evidence of numts in our dataset, such as consistent secondary peaks in sequence chromatograms or presence of numt diagnostic sites in these sequences (Anthony et al. 2007b). Although our findings are based on a small fragment of mitochondrial DNA that may not necessarily reflect genome-wide patterns of genetic variation, our study nevertheless demonstrates that mitochondrial data can provide important information on historical population structure and breeding history of gorillas and other endangered taxa in captivity.

The new haplotype information obtained in this study allowed us to infer haplogroup affiliation and approximate geographic origin of two-thirds of the current gorilla population in US zoos. This substantial sample is likely to be broadly representative of the captive gorilla population as a whole. The observed levels of diversity in captive animals shows no indication of a population bottleneck associated with the establishment of the captive population, which

**Table 3.** Thirty-nine wild founder gorillas and their haplogroup affiliation based on sequences analyzed in this study.

Studbook #	Haplogroup	Phylogeographic origin <sup>a</sup>	Studbook reported origin <sup>b</sup>		
12	C1	Nigeria or Cameroon (1)	Cameroon <sup>Y</sup>		
537			Cameroon <sup>Y</sup>		
548			Cameroon <sup>Y</sup>		
191			—		
192			—		
52	C2	Cameroon or Gabon (2)	—		
115			—		
224			—		
226			—		
336			Cameroon <sup>Y</sup>		
175	C3	Cameroon (3)	—		
210			—		
221			—		
379			Cameroon <sup>Y</sup>		
180			—		
393	D1	Equatorial Guinea or Gabon (4)	—		
359			—		
140			D2	CAR or Congo (5)	—
710					Congo <sup>Y</sup>
195					Congo <sup>Y</sup>
159	—				
160	—				
23	D3	Gabon or Congo (6)	—		
344			Cameroon <sup>N</sup>		
43			Cameroon <sup>N</sup>		
63			Congo <sup>Y</sup>		
80			Congo <sup>Y</sup>		
423			Congo <sup>Y</sup>		
524			Congo <sup>Y</sup>		
64			Gabon <sup>Y</sup>		
96			—		
150			—		
168			—		
271	—				
292	—				
389	—				
390	—				
398	—				
506	—				

The reported country of origin in the SSP gorilla Studbook is also listed alongside the most likely origin as inferred from previous mitochondrial phylogeographic studies.

<sup>a</sup>Geographic distribution of mitochondrial lineages in Central Africa (updated from Anthony et al. 2007a): (1) The lineage C1 is mainly distributed in Cameroon from the Cross River area in Nigeria eastwards to southeastern Cameroon. (2) C2 has been sampled in the Dja region in central Cameroon, the Minkébé region in northern Gabon and the west bank of the Ivindo/Ayala River in central Gabon. (3) C3 is distributed in central Cameroon on the south bank of the Sanaga River. (4) D1 is highly restricted to the montane regions of Equatorial Guinea and the adjacent Monts de Cristal in northwestern Gabon. (5) D2 is present in the Dzanga-Sangha region of Central African Republic (CAR), next to the border with Congo. (6) D3 is widely distributed in Gabon and adjacent Republic of the Congo. <sup>b</sup>Congruence (Y) or lack of congruence (N) between the reported origin in the Studbook and the likely country of origin according to the distribution of mitochondrial haplogroups in Central Africa.

is also consistent with results from autosomal loci (Nsubuga et al. 2010; Simons et al. 2012). Although mitochondrial diversity in the captive population encompasses much of the range-wide variation



found in western gorillas, our findings nevertheless point to substantial hybridization between individuals with different mitochondrial lineages and geographic origins. Since major mitochondrial haplogroups would have historically remained either largely separated or exchanged individuals in well-defined hybrid zones in nature (Anthony et al. 2007a), the current breeding history of the United States is therefore unlikely to reflect patterns in the wild.

With regards to the future management of the United States population, it is clear that current breeding programs are consistent with a longstanding goal in conservation biology, that is, to maximize genetic diversity at the species level (Maguire and Lacy 1990). Given this mandate and the limited number of animals in captivity, it would seem prudent to continue to allow admixture between lineages when necessary. Although subdividing the existing gorilla population into a number of smaller subgroups would preserve the phylogeographic structure present in the wild, it could also lead to a further erosion of genetic variation and increase the risk of inbreeding. However, in the case of planned introductions, such as those being undertaken in Central Africa (King et al. 2012), we recommend that in order to prevent hybridization and to preserve the phylogeographic structure present in the wild gorillas should only be released into areas where their haplogroups are present, and gorillas with history of haplogroup hybridization should not be released into wild populations. Furthermore, we also recommend that the mitochondrial haplotypes of all remaining captive gorillas be determined in order to provide an additional metric of genetic variation and to help guide current and future breeding efforts.

## Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>.

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