ORIGINAL RESEARCH

Title:

Genotyping contemporary captive and historical wild western lowland gorillas indicates captive breeding is maintaining genetic diversity in a critically endangered primate

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Short title: Captive breeding maintains genetic diversity in gorillas

Abstract

Genetic diversity is a fundamental component of biodiversity and a conservation priority. Genetic studies on captive populations, vulnerable to genetic erosion, are therefore needed for long-term conservation of threatened species. The western lowland gorilla (*Gorilla gorilla gorilla*) is considered Critically Endangered, but no major conservation genetic studies have been published on the captive gorilla population in the European Ex situ Programme (EEP). Here, using 10 microsatellite loci and mitochondrial DNA (mtDNA), we investigated the genetic diversity, genetic structure, signatures of inbreeding, and taxonomic affinities of captive gorillas at Howletts and Port Lympne (HPL), conservation zoos holding ~10% of the EEP population, and compared this with historical wild gorillas from the Powell-Cotton Museum. High heterozygosity values and similar allelic richness were found in contemporary captive and historical wild populations, with no evidence of nuclear genetic diversity loss or inbreeding. However, contemporary captive gorillas had lower effective population size and mtDNA diversity than the historical wild population, and weak genetic differentiation from historical wild gorillas. All HPL gorillas showed expected taxonomic affinities to the subspecies *G. g. gorilla*. Our results demonstrate that captive breeding has successfully maintained heterozygosity and allelic richness (i.e., genetic diversity) as compared with a historical wild population, crucial for the evolutionary potential of this endangered primate. However, genetic differentiation between contemporary captive and historical wild gorillas likely reflects ~50 years of captive breeding starting from a small founder population sourced from various locations. The HPL population is genetically well placed to sustain future generations in captivity and the wild through conservation translocations, contributing to biodiversity goals.

KEYWORDS: Conservation genetics, *Gorilla gorilla gorilla*, Historical DNA, Microsatellites, Threatened species.

1. Introduction

Genetic diversity is essential for the long-term persistence of species as it provides the foundation for natural selection, adaptation and evolution of populations to changing environments (Reed and Frankham 2003; Frankham et al. 2017; Hoban et al. 2022). Alongside ecosystem and species diversity, the International Union for Conservation of Nature (IUCN) recognises the preservation and monitoring of genetic diversity as one of three conservation priorities (Hvilsom et al. 2022), and genetic diversity is considered by the Convention on Biological Diversity's (CBD) Kunming-Montreal Global Biodiversity Framework (GBF) as one of three fundamental components of biodiversity (CBD 2022; Frankham 2022). Furthermore, the GBF specifies the maintenance and restoration of genetic diversity through *in situ* and *ex situ* conservation and sustainable management practices as a global target for 2030 and as a goal for 2050 (CBD 2022).

Small, fragmented natural populations, as well as captive populations, are vulnerable to reduced genetic diversity and inbreeding depression through the accumulation of deleterious alleles (Frankham 2005; Frankham 2010; Frankham et al. 2017). Evidence shows that inbreeding and loss of genetic variation, in concert with other threats, contribute to extinction risk in small or fragmented populations (Frankham 2005; Frankham et al. 2017). Genetic erosion, with its known influence on population persistence, needs to be quantitatively monitored broadly for improved risk assessment and conservation of threatened species (Frankham 2022; Hoban et al. 2022). Genetic management is essential in captive populations to minimise inbreeding and genetic erosion, making genetic analyses a critical component of conservation initiatives (Witzenberger and Hochkirch 2011). While species-level conservation remains the primary objective of captive breeding and reintroduction programmes, maintaining genetic diversity within captive populations is vital for achieving this goal.

Nonhuman primates play an essential role in tropical biodiversity, facilitating ecosystem health and contributing to forest regeneration (Estrada et al. 2017). Currently, 75% of primate species show decreasing population trends due to anthropogenic pressures and 60% are considered threatened with extinction (Cotton et al. 2016; Estrada et al. 2017). The western lowland gorilla

(*Gorilla gorilla gorilla*) is the most widespread and numerous of the gorilla subspecies (Figure 1a) with most recent surveys estimating about 360,000 individuals in the wild; but with populations predicted to sustain a reduction in excess of 80% over three generations, it is classified as critically endangered (Maisels, Bergl, and Williamson 2018; Strindberg et al. 2018). Habitat loss and fragmentation, infectious diseases and illegal hunting are the main causes of gorilla population declines (Estrada et al. 2017; Maisels et al. 2018; Strindberg et al. 2018).

Western lowland gorillas are a popular species in zoos globally, with approximately 800 registered in Zoological Information Management Software (ZIMS) at 131 institutions, including over 400 in Europe and over 300 in North America (https://species360.org/). Previous population genetic studies using microsatellites revealed higher levels of heterozygosity and allelic diversity in the North American western gorilla Species Survival Plan (SSP) captive population than in wild populations, no evidence of population bottlenecks for the SSP population, but loss of allelic diversity in captivity compared with the SSP captive population founders (Nsubuga et al. 2010; Simons, Wagner, and Lorenz 2013). Cryptic population structure was detected, with captive gorillas in North America showing at least two genetic clusters and admixed individuals, suggesting distinct geographic origins of the founders and evidence of genetic exchange in the wild before animals were taken into captivity (Nsubuga et al. 2010). Comparable levels of mitochondrial DNA (mtDNA) nucleotide and haplotypic diversity were found in captive western lowland gorillas from North America and wild populations, and the captive population contained all mtDNA lineages found in wild animals (Soto-Calderón et al. 2015). However, despite the significant advances in the genetic study of contemporary wild and captive gorillas in North America, no major conservation genetic studies have been published for the captive population in Europe.

With over 40 captive gorillas, Howletts and Port Lympne (HPL) in Kent, UK, hold the largest number of captive gorillas in the world, and approximately 10% of the total captive population across 77 institutions in the European Ex situ Programme (EEP). HPL gorillas descend from at least 34 wild-sourced founders, including 18 from Cameroon (collected between 1948-1980), four from

Congo (1984-1987), three from Gabon (1972-1982) and nine from unspecified origin (1950s to 1970s). HPL have strong links to The Aspinall Foundation, a UK-registered charity which undertakes several global conservation projects, including a western lowland gorilla rescue, rehabilitation and reintroduction programme in the Republics of Congo and Gabon (King and Courage 2008; King, Chamberlan, and Courage 2012; King et al. 2014). The EEP captive gorillas, and the HPL captive gorillas in particular, currently play an important role in the reinforcement of the reintroduced gorilla populations, for the retention of genetic diversity and for their long-term population viability (King 2013; King et al. 2014). One important question that remains unanswered is whether captive breeding programmes of EEP gorillas are effective at maintaining genetic diversity.

Museum specimens provide a window into the past by offering a valuable source of material for conservation genetics (Nakahama 2020; Raxworthy and Smith 2021) and allowing the study of anthropogenic impacts through time on wild populations (van der Valk et al. 2019). Historical DNA can evidence changes in heterozygosity and allelic richness through time, the genetic structure of past populations, and detect signatures of genetic bottlenecks, useful for monitoring the genetics of populations retrospectively for conservation and management (Nakahama 2020; Raxworthy and Smith 2021). For example, microsatellite genotyping museum samples collected from 1904-1907 and contemporary Cross River gorillas (*G. g. diehli*) revealed a decline in population size, and lower heterozygosity and allelic richness (albeit these were not significantly different) between historical and contemporary samples (Thalmann et al. 2011); and mitochondrial genome sequencing of museum samples spanning 110 years showed a significant decrease in haplotype and nucleotide diversities through time in eastern lowland gorillas (*G. beringei graueri*), and historically low genetic diversity values in mountain gorillas (*G. b. beringei*) (van der Valk et al. 2019). The Powell-Cotton Museum (PCM) houses an internationally significant primate collection, including many specimens of western lowland gorillas of known provenance. This collection represents a largely untapped and valuable source of biological material, useful for evaluating the genetic diversity in a historical wild gorilla population at a time of trophy hunting and colonial expansion during the late 19th and early

130 20th centuries, and as a point of comparison with contemporary captive gorilla populations for
131 conservation genetics and phylogeographic studies. However, further work is needed to understand
132 to what extent the contemporary captive gorilla population in the EEP reflects past genetic diversity
133 and structure in wild populations, and to confirm their taxonomic affinities and geographic origins.
134 Using a combination of microsatellite markers and mtDNA, we studied the population
135 genetics of contemporary EEP captive (from HPL) and historical wild (from PCM) western lowland
136 gorillas. The specific objectives were: 1) to investigate the nuclear (heterozygosity levels and allelic
137 richness) and mitochondrial (haplotypic and nucleotide) genetic diversity for the contemporary
138 captive and historical wild populations, and to confirm the taxonomic identity and geographic
139 provenance of the captive population, 2) to determine presence of private alleles and genetic
140 signatures of inbreeding and population bottlenecks, 3) to estimate the genetic structure of the
141 historical wild population and the temporal genetic differentiation between the contemporary
142 captive and historical wild populations, and 4) to evaluate the parental relationships and relatedness
143 levels in the contemporary captive and historical wild populations. This study presents genetic
144 diversity and structure data from contemporary captive and historical wild western lowland gorillas,
145 offering insights that may inform conservation planning and long-term management efforts for this
146 critically endangered species.
147
148 **2. Materials and methods**
149 **2.1. Contemporary and historical gorilla samples**
150 The total sample included 140 gorillas, considered to be *G. g. gorilla* based on morphological
151 features and reported geographic origins. The contemporary captive population consisted of 64
152 biobanked blood samples (in Whatman FTA cards) from HPL, including samples from several
153 members (sires, dams and offspring) belonging to various family groups (Table S1). The historical
154 wild population consisted of 76 skin samples, including 74 gorilla specimens at the PCM and two

specimens at the Royal College of Surgeons (RCS) (suspected mother and offspring), originally from PCM, with all specimens hunted between 1927 and 1936 in western equatorial Africa (Table S1).

For the captive population, blood DNA was extracted using an in-house method. For the museum samples, DNA was extracted using the QIAamp Fast DNA Tissue Kit (Qiagen, UK) with minor modifications from manufacturer's protocol. Negative controls were included in every DNA extraction session. Detailed protocols for DNA extraction, visualisation and quantification are available in Supporting Information.

2.2. Microsatellite genotyping

Multilocus microsatellite genotypes were produced for all western lowland gorillas. A panel of 10 pairs of primers were used to amplify polymorphic autosomal microsatellite loci D2s1326, D10s1432, D16s2624, D4s1627, D7s817, D5s1470, vWF, D7s2204, D1s550 and D8s1106 (Bradley, Boesch, and Vigilant 2000). There are several studies on gorillas using microsatellite markers, allowing a comparison of results with previous work. Negative controls were included in all PCRs. To check for reproducibility and to avoid genotyping errors, 25% of the total sample was re-amplified and genotyped. Microsatellite genotyping was performed by DBS Genomics (Durham University). Detailed microsatellite genotyping methods are provided in Supporting Information.

2.3. Microsatellite data analysis

Genotype data was divided into two groups, namely: contemporary captive (from HPL) and historical wild (from PCM) populations. COANCESTRY (Wang 2010) and FRIENDS & FAMILY (de Jager et al. 2017) were used for the identification and retention of unrelated individuals. Removing close relatives from genetic analyses can help produce more accurate estimates of population parameters, but the best approach varies case-by-case and removing all close relatives may introduce bias. Using a cut-off relatedness value of 0.5 (removing 1st degree relatives: parent-offspring, full-siblings) (de Jager et al. 2017), 106 western lowland gorillas were retained for downstream analyses, 41 in the

181 contemporary captive and 65 in the historical wild populations. All analyses were carried out using
182 this reduced data set unless specifically stated. The historical wild population was further divided
183 into three geographical groups named: Eastern (n = 35, eastern Cameroon), Western (n = 25,
184 western and central Cameroon) and Southern (n = 5, Congo) populations. Groupings were based on
185 50 km radius buffer zones for each sampling locality (Cipolletta 2003) and merging overlapping
186 buffer zones using QGIS version 3.32 (http://www.qgis.org) (Figure 1b). MICRO-CHECKER version
187 2.2.3 (van Oosterhout et al. 2004) was used to test for presence of null alleles, scoring errors due to
188 stuttering, and large allele dropout for every locus per population. No evidence of linkage
189 disequilibrium, null alleles, scoring errors due to stuttering, or large allele dropout was detected.
190 Populations were tested independently for deviations from Hardy-Weinberg equilibrium and linkage
191 disequilibrium using GENEPOP version 4.7.5 (Rousset 2008) adjusting for multiple testing with
192 standard Bonferroni correction. A preliminary analysis revealed deviations from Hardy-Weinberg
193 equilibrium in one locus in the Western group due to heterozygote deficit. Considering that
194 deviations from equilibrium are not uncommon in microsatellite studies of gorillas (Bergl and
195 Vigilant 2007; Bergl et al. 2008; Thalmann et al. 2011; Simons et al. 2013; Roy et al. 2014), and that
196 no linkage disequilibrium, null alleles, scoring errors or allele dropout were detected, all loci and
197 individuals were retained for analysis.

198 To explore the levels of genetic diversity within the contemporary captive and historical wild
199 populations, the number of different alleles averaged across loci (N_A), number of effective alleles
200 (A_E), levels of observed heterozygosity (H_O) and unbiased expected heterozygosity (H_E), inbreeding
201 coefficient (F_{IS}), and private alleles (A_P) were calculated in GENALEX version 6.5 (Peakall and Smouse
202 2012). Allelic richness (A_R) was calculated in FSTAT version 2.9.4 (Goudet 1995) using the rarefaction
203 method. Differences in genetic diversity values per locus (N_A, A_E, A_R, H_O and H_E) between
204 contemporary captive and (pooled) historic wild populations were tested using analysis of variance
205 (ANOVA) in R version 4.3.1 (https://www.R-project.org/). Using the full data set, heterozygosity

values for each locality of the historical sample were calculated in GENALEX and the interpolation function in QGIS was used to visualise the spatial distribution of genetic diversity across the region.

A Bayesian clustering approach using STRUCTURE version 2.3.4 (Pritchard, Stephens, and Donnelly 2000) was implemented to detect population genetic structure under the assumption of *k* clusters, and to assign individuals to clusters based on their likelihood-based individual assignment due to mixed ancestry. To determine the optimal number of *k*, 10 independent replicates were run for values of $k = 1 – 10$, evaluated after 1 million MCMC replications and sampling every 1000 steps (first 10% discarded as burn-in). The admixture model with correlated allele frequencies and Locprior (recommended when the data is suspected to have weak genetic structure, Hubisz et al. 2009) options were selected. The analyses were performed in GALAXY web platform (https://usegalaxy.eu/). The selection of the optimal number of *k* was done using STRUCTURESELECTOR (Li and Liu 2018) based on Puechmaille's method by MaxMean*k* recommended for unequal sample sizes (Puechmaille 2016). Log likelihood probability [LnP(*k*)] and Evanno's Δ*k* method (Evanno, Regnaut, and Goudet 2005) are also reported. Individual probability plots were generated using STRUCTUREPLOT version 2.0 (Ramasamy et al. 2014). The population genetic structure was also assessed with pairwise F_{ST}, with hierarchical Analysis of Molecular Variance (AMOVA), and with Principal Coordinates Analysis (PCoA) using GENALEX. For the historical population, we plotted the individual-by-individual pairwise genetic distances versus geographic distances (in km) followed by a Mantel test (10,000 permutations) to identify isolation-by-distance. A spatial autocorrelation analysis (10,000 permutations) with 50 km distance classes was used to assess the spatial signal of genetic structure. When significant positive structure is present, the value of the correlation coefficient (*r*) decreases with increasing distance class, and when *r* does not significantly differ from zero gives an approximate distance where population structure can be detected. Mantel test and spatial autocorrelation analysis were performed in GENALEX.

Effective population size (Ne) was estimated for the contemporary captive and the historical wild populations using the co-ancestry method as implemented in NEESTIMATOR version 2.0 (Do et

al. 2014). Signatures of recent genetic bottlenecks for the captive and historical populations were tested using BOTTLENECK version 1.2 (Piry, Luikart, and Cornuet 1999). As recommended for low sample sizes and less than 20 loci (Piry et al. 1999), we pooled the historical samples into one group, selected the parameters Two-Phase Model (TPM) (95% stepwise mutation model and 5% infinite alleles model), variance of 12 (among multiple steps) and 10,000 simulations, and used the Wilcoxon signed-rank test to test for significant differences between observed and expected heterozygosity values.

Relatedness values obtained using COANCESTRY allowed us to obtain the number of parent/offspring relationships in the captive and historical populations, and to estimate R for common relationship categories (R = 1 for identical twins, 0.5 for parent/offspring and full siblings, 0.25 for 2nd degree or half siblings, 0.125 for 3rd degree or first cousins, and 0 for unrelated individuals). For the captive population, we assessed the mean pairwise relatedness within established family groups (sires, dams and offspring), to explore the relatedness among sires and dams, and to confirm the relationship between sires and offspring (Table S1). For the historical groups, we established possible parent/offspring relationships and estimated levels of relatedness among males and females.

2.4. Mitochondrial DNA sequencing

We used nested primers to amplify a 258 base-pair fragment of the mitochondrial DNA D-loop hypervariable region I (HVI) (Garner and Ryder 1996; Clifford et al. 2004). This mtDNA region has been used in several studies on gorillas, allowing comparison with a broader DNA data set. Negative controls were included to check for potential PCR contamination. Detailed protocols for PCR purification, visualisation and sequencing are available in Supporting Information. Forward and reverse sequences were inspected by eye and were then aligned and made into consensus sequences in BIOEDIT version 7.2.5 (Hall 1999).

A total of 110 sequences were obtained (59 contemporary captive gorillas and 51 historical wild gorillas). We used BLAST to check for nuclear inserts of mtDNA (numts), common in great apes (Clifford et al. 2004), and to confirm species identity. This resulted in 29 new numt-free HVI sequences (eight from HPL and 21 from PCM) (GenBank accession numbers PV540398-PV540426). In addition, 117 HVI sequences were obtained from GenBank, including: captive western lowland gorillas in American zoos, contemporary wild western lowland gorillas from Central African Republic, Republic of Congo, Cameroon, Gabon, Nigeria and Equatorial Guinea, and contemporary wild eastern mountain gorillas and eastern lowland gorillas. Downstream analyses were carried out using a total of 146 HVI sequences (Table S1).

2.5. Mitochondrial DNA data analysis

Multiple pairwise DNA sequence alignment was performed for all numt-free HVI sequences using MEGA version 11.0.8 (Tamura, Stecher, and Kumar 2021) to identify the main gorilla haplogroups (Soto-Calderón et al. 2015). A 24-27 bp polymorphic C region of HVI was removed from all samples prior to analysis (Garner and Ryder 1996; Clifford et al. 2004; Anthony et al. 2007), and the 5' and 3' ends of DNA sequences were trimmed resulting in a total sequence length of 185 bp.

MRBAYES version 3.2.5 (Ronquist and Huelsenbeck 2003) was used to construct a Bayesian phylogenetic tree using two independent runs with 2 million MCMC steps, four independent chains and sampling every 1000 steps (first 10% discarded as burn-in). TRACER version 1.7.1 (Rambaut et al. 2018) was used to check for convergence, and FIGTREE version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) was used to build the tree. Median-joining networks were made in POPART version 1.7 (Leigh and Bryant 2015) using the greedy algorithm. DNASP version 6 (Rozas 2009) was used to calculate number of polymorphic sites (P), number of haplotypes (H), haplotypic diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (k).

3. Results

3.1. Microsatellite genetic diversity and structure

We found high and similar heterozygosity levels and no signatures of inbreeding in the contemporary captive and historical wild populations (Table 1). There were no significant differences in genetic diversity between the contemporary captive and historical wild populations (for N_A, A_E, A_R, H_O and H_E Levene's tests $p > 0.05$, ANOVAs $p > 0.05$). There were 4-7 alleles per locus (N_A), with only 3-5 effective alleles (A_E), and all populations having 3-4 alleles on average per locus whilst controlling for sample size (A_R) (Table 1). In total, there were 12 private alleles (Table 1) (out of 81 observed alleles; 14.8%), two in the contemporary captive population (in two individuals: Tamidol and Emba) and 10 in the historical wild population (in 10 individuals, four gorillas from the Eastern, five from the Western and one from the Southern group). For the historical wild population, there was overall greater heterozygosity towards the eastern and central parts of the sampling distribution than to the west and south (Figure 1c).

There were low but significant levels of genetic differentiation among populations (AMOVA $F_{ST} = 0.02$, $p < 0.05$) and pairwise F_{ST} values ranging from 0.01 to 0.05), with the Southern group showing the greatest pairwise F_{ST} values followed by the contemporary captive population (Table S2). The low level of genetic differentiation was also apparent in the PCoA with only 18.14% of total variability explained by the first three principal coordinates, with the contemporary captive population mostly overlapping the historical wild groups (Figure S1). Bayesian population genetic structure based on Puechmaille's MaxMean*k*, Evanno's Δ*k* and LnP(*k*) resulted in $k = 3$ clusters (Figure 2; Figure S2a-d). In the historical wild population, there was no clear geographical differentiation (Eastern, Western and Southern regions) and only eight individuals showed Q-values > 0.8 (Cluster 1). In the contemporary captive population, five individuals showed affinity for Cluster 2 and eight individuals showed affinity to Cluster 3 (Q-values > 0.8). There was a genetic shift in estimated membership coefficients from the historical wild to the contemporary captive populations, with the historical wild population having more representation of Clusters 1 and 2 than

309 Cluster 3, and the contemporary captive population having more representation of Clusters 2 and 3
310 than Cluster 1. There was a weak but significant correlation between genetic and geographic
311 distances (coefficient of correlation $r = 0.12$, $p < 0.001$) and a significant local spatial structure for the
312 first distance class (50 km; $r = 0.021$, $p = 0.03$) (Figure S3a-b).
313 The contemporary captive population showed nearly half the effective population size than
314 the historical wild population, with estimated $Ne_{Captive} = 23.7$ (95% CI = 9.1-45.1) versus $Ne_{Historical} =$
315 44.9 individuals (95% CI = 1.1-165.6). Although the effective population size for the historical wild
316 population was low, there was no signature of recent population bottleneck (one tailed Wilcoxon
317 signed-rank test for heterozygosity excess, $p > 0.05$); however, there was a significant signature of
318 recent population bottleneck for the contemporary captive population (one tailed Wilcoxon signed-
319 rank test for heterozygosity excess, $p = 0.001$).
320 The contemporary captive population had an average R = 0.1 (equivalent to 3rd degree
321 relationship to unrelated individuals). All known parent/offspring and full sibling relationships in the
322 captive population were confirmed based on genotypes. Family groups 3 and 6 showed 1st degree
323 relationships (R = 0.34 and 0.33, respectively) (but no sire or dam were genotyped in either family
324 group, likely resulting in an inflated relatedness values), while for other family groups ranged
325 between 2nd and 3rd degree (R = 0.12 – 0.25). Sire-Dam relatedness values were also low (equivalent
326 to unrelated individuals). One case of extra-pair paternity was detected (Kisane rather than
327 Djanghou was identified as the biological father of Kwimba's two sampled infants). For the historical
328 wild population, there was an average R = 0.1, also corresponding to 3rd degree relationship to
329 unrelated individuals (only the Southern group reached 3rd degree relationship with R = 0.2). For
330 males and females, there were low relatedness values ($R_{Males} = 0.08$, $R_{Females} = 0.1$). There were 14
331 estimated parent/offspring relationships, including RCS-PA62 and RCS-PA63 (previously thought to
332 be mother and offspring, respectively).
333
334 *3.2. Mitochondrial DNA diversity and structure*

335 The phylogenetic analysis and network recovered four haplogroups, with haplogroups A and B
336 restricted to mountain and eastern lowland gorillas, respectively, and haplogroups C and D
337 (geographically widespread) corresponding to western gorillas (Figure 3; Figure S4). All numt-free
338 HPL and PCM HVI sequences clustered within the subspecies *G. g. gorilla* confirming their taxonomic
339 identity. HPL sequences clustered in sub-haplogroup D2 (with samples found in Cameroon, Congo
340 and Central African Republic), whereas PCM and RCS sequences primarily clustered in sub-
341 haplogroup C2 (16 sequences) (with samples found in Cameroon, Congo and Gabon), three in sub-
342 haplogroup C1 (with samples from Cameroon and Nigeria) and two in sub-haplogroup D2. Sub-
343 haplogroup C3 only included captive animals from North America (with origins in Cameroon), sub-
344 haplogroup D1 included captive animals from North America and wild animals from Equatorial
345 Guinea, and sub-haplogroup D3 included captive animals from North America and wild animals from
346 Congo and Gabon. For western lowland gorilla sub-haplogroups C1, C2, D1, D2 and D3, the most
347 central haplotype was geographically widespread.
348 There was lower mtDNA genetic diversity in the HPL population than in the historical sample
349 and in the captive western gorilla population of North American zoos (Table 2); however, results
350 need to be taken with caution due to the number of numts detected. Haplotype and nucleotide
351 diversity values of the HPL population were comparable to those found in the sub-haplogroups.
352 Among haplogroups, mountain and eastern lowland gorillas (haplogroups A and B) had lower
353 diversity than western gorillas (haplogroups C and D).
354
355 **4. Discussion**
356 The results obtained here represent the first published genetic study of a large proportion of the
357 captive gorilla population in Europe, and specifically of gorillas held at Howletts and Port Lympne
358 (HPL), with important conservation implications for ongoing captive breeding and reintroduction
359 programmes. This is also the first broad scale genetic study of the Powell-Cotton Museum (PCM)
360 gorilla collection, with ~100-year-old specimens, providing a glimpse at past genetic diversity and

structure of this primate and demonstrating the value of museum specimens for conservation genetics (Nakahama 2020; Raxworthy and Smith 2021). Our study provides a temporal perspective for the conservation genetics of this critically endangered and ecologically important flagship primate, and it reflects the value and roles of conservation zoos for meeting global conservation goals (Spooner et al. 2023).

The high and similar levels of heterozygosity found in the contemporary captive and historical wild populations mirrored previous microsatellite-based studies on wild and/or North American captive western lowland gorillas (Nsubuga et al. 2010; Simons et al. 2013; Soto-Calderón et al. 2015). Although newer techniques are now available, such as whole-genome sequencing and single nucleotide polymorphisms from reduced representation libraries, microsatellites are still cost-effective in conservation genetic studies (Hauser, Athrey, and Leberg 2021) and there are several microsatellite and mtDNA studies in gorillas useful for comparison purposes. Whole-genome sequencing has also uncovered higher genetic diversity (fewer runs of homozygosity) in wild western lowland gorillas than in other gorilla subspecies (Scally et al. 2014; van der Valk et al. 2019, 2024; Alvarez-Estape et al. 2023). However, the similar levels of allelic richness between our sampled populations contrast with the significant loss of allelic diversity observed in the North American captive population when compared with captive population founders (Simons et al. 2013). Nonetheless, the low effective population size and genetic signature of population bottleneck detected in the contemporary captive HPL population highlight the pervasive effects of long-term captive breeding. This was also reflected in the lower mtDNA diversity of captive gorillas at HPL than in the historical sample from PCM, which was also lower than those reported for the North American captive population (although this was likely due to other studies sampling from various zoos and matrilines, or due to numts in our data set). Interestingly, our historical sample showed similar mtDNA diversity to contemporary wild populations (Clifford et al. 2004; Anthony et al. 2007; Soto-Calderón et al. 2015).

386 Although there has been a total net loss in forest extent of about 22% in tropical Africa
387 during the last century, the Congolese forest extent has increased slightly on average and in recent
388 decades forest progression has been documented in southern Congo (Aleman, Jarzyna, and Staver
389 2018). This means that wild populations of western lowland gorillas could have maintained high
390 genetic diversity through time and avoided a genetic bottleneck, and that historical levels of nuclear
391 genetic diversity may still be reflected in the captive populations in the EEP and the SSP.
392 Nonetheless, attention should be paid to the small effective population size of the HPL captive
393 population in comparison with the historical wild population. With predicted current trends of
394 population decrease of more than 80% over three generations (Maisels et al. 2018), maintaining
395 high genetic diversity levels in the captive population of gorillas may be beneficial for the long-term
396 conservation of the species.
397 Complex group membership dynamics, daily movements, ranging behaviours and long-
398 distance dispersal of western gorillas (Cipolletta 2003; Douadi et al. 2007; Hagemann et al. 2018;
399 Morrison et al. 2020) may explain the observed general genetic homogeneity in the historical wild
400 population and the weak genetic structure and isolation-by-distance driven by spatial variation at
401 local scales. For the historical specimens, field notes state that the animals originated from different
402 family groups, even if several gorillas were taken from the same location; however, the hunting trips
403 lasted for several months and hunters returned to the same region over several years. Overall, this
404 may explain the level of relatedness observed among individuals in the historical sample. The effects
405 of rivers and Pleistocene refugia may explain the low but significant genetic differentiation observed
406 in the historical sample in an evolutionary time scale (Anthony et al. 2007, Fünfstück et al. 2014).
407 This evolutionary diversification with long-distance movements may explain the mixed ancestry of
408 the historical wild population, and that of the contemporary captive population with founders
409 originating from Cameroon, Congo and Gabon. Moreover, the shift in cluster membership values and
410 mixed ancestry observed between the contemporary captive and historical wild populations may be
411 the effect of ~50 years of captive breeding starting from a small founder population obtained from

various geographical locations. Similarly, Nsubuga et al. (2010) found two genetic clusters in the North American captive gorilla population, one having originated from Cameroon or Congo and the other one from Gabon or Cameroon, and some captive gorillas showing mixed ancestry. Fünfstück et al. (2014) showed two to four genetic clusters in a contemporary wild sample; however, their sample was obtained further east than our historical data set. In contrast, contemporary wild Cross River and mountain gorillas, only found in small and fragmented populations, show comparatively lower genetic diversity than western lowland gorillas and significant genetic structure (Anthony et al. 2007; Nsubuga et al. 2010; Simons et al. 2013; Soto-Calderón et al. 2015).

The phylogenetic analyses showed the expected deep divergences of western lowland gorillas from eastern and mountain gorillas (Clifford et al. 2004; Anthony et al. 2007; Soto-Calderón et al. 2015) and placed gorillas from HPL and PCM within western lowland gorilla haplogroups. This is an important taxonomic aspect for ongoing reintroduction programmes which has not been previously evidenced via genetic analysis. The lower mtDNA diversity of gorillas at HPL than in the historical population reflected the low number of female founders sampled from the captive population; however, the prevalence of numts could have affected these results. Based on PCM HVI sequences, the D2 sub-haplogroup is now recorded in Congo (specimen FC_114) and further west in Cameroon (specimen CAM_I_14) than previously reported, and the C1 sub-haplogroup is now recorded in Congo (specimen FC_147). This indicates that haplogroups were more geographically widespread than has been previously recorded, highlighting the importance of using museum specimens for current conservation research.

The management of captive populations can be important for the long-term conservation of threatened species (Svengren et al. 2017; Ogden et al. 2020; Farré et al. 2022). However, the risks of inbreeding and loss of genetic diversity in captive populations, coupled with issues such as low genetic diversity of founder individuals, and taxonomic, disease and behavioural aspects, threatens the value of these populations for the conservation of the species (Shen et al. 2009; Hvilsom et al. 2013; Ogden et al. 2018; Vega et al. 2023). Therefore, studying genetic variation and inbreeding has

become increasingly important for the careful management of captive populations. This study showed promising results for the long-term management of the western lowland gorilla captive population due to the high heterozygosity values, similar to and genotypically representative of the historical wild population, and no evidence of inbreeding, but we did find lower effective population size in comparison to a historical wild population and signature of genetic bottleneck, as well as weak genetic differentiation that may be the result of captive breeding. For the contemporary captive population, only one case of extra-pair paternity was detected, and all other parent-offspring relationships were confirmed, highlighting the importance of incorporating genetic analyses for captive management. Based on our results, the aim of conserving 90% of heterozygosity over the next 100 years in the captive-bred population, while minimizing inbreeding and mean kinship, may be achievable (Frankham 2010; Frankham 2022). This study contributes to the broader efforts of HPL as conservation zoos in aligning with international biodiversity frameworks, including Sustainable Development Goal 15 (Life of Land), GBF 2030 Target 1 (decreasing biodiversity loss) and Target 4 (maintaining and restoring the genetic diversity of species *in situ* and *ex situ*), and GBF 2050 Goal A (maintaining genetic diversity within populations to safeguard their adaptive potential) (CBD 2022). The findings also illustrate how conservation zoos engage in research-based conservation practice for averting the biodiversity crisis (IUCN Species Survival Commission 2023).

In conclusion, our results represent a positive outcome in terms of conservation and highlight that contemporary strategic captive breeding efforts have been so far successful in maintaining genetic diversity (heterozygosity and allelic richness) compared with that of a historical wild population and preventing the loss of the evolutionary potential of this critically endangered primate. The captive population may be considered as genetically 'healthy', showing low levels of inbreeding, high heterozygosity values and allelic richness similar to the presumably larger historical population, and with taxonomic affinities to the western lowland gorilla subspecies. Albeit showing some genetic differentiation from a historical wild population and signature of recent genetic bottleneck, likely due to the reduced number of founders, the historical genetic diversity of wild

464 animals is still reflected in the contemporary captive population. Although further work is needed to
465 characterise genetically the rest of the EEP gorillas, the captive population of western lowland
466 gorillas in HPL is genetically well placed to sustain future generations in captivity, has the genetic
467 potential to make real contributions to reintroductions and other conservation translocation
468 programmes, and can help achieve global biodiversity targets and goals in the face of current
469 anthropogenic threats to biodiversity.
470

471 **Author contributions**

472 J.M., S.H., T.K. and R.V. conceived the research idea and designed the methodology; J.Ho. and J.M.
473 collected the samples; J.Hi. did the GIS analyses; J.M. and R.V. did the genetic analyses and led the
474 writing of the manuscript; all co-authors participated in the discussion of the results and critically
475 reviewed the manuscript.
476

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483 captive management programme.
484

485 **Ethics Statement**

486 The blood samples from contemporary captive gorillas were collected by veterinarians and under
487 ethical approval by Howletts and Port Lympne ethics committee, and their use for research at
488 Canterbury Christ Church University was authorised by the university's ethics committee.
489

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data generated or analysed during this study are provided in full within the published article and its Supporting Information.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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Figure captions

FIGURE 1. a) Geographical distribution of gorilla subspecies. b) Geographical distribution of historical wild samples of western lowland gorilla (*Gorilla gorilla gorilla*) from Powell-Cotton Museum (PCM) divided into three regional groups (Eastern, Western and Southern) inferred by 50 km radius buffer zones. c) Interpolation of heterozygosity values (H_e) for each locality based on microsatellite genotypes (full data set).

FIGURE 2. Individual probability plot showing *k* = 3 (Puechmaille's MaxMean*k*) genetic clusters of historical wild (Powell-Cotton Museum, PCM) and contemporary captive (Howletts and Port Lympne, HPL) western lowland gorillas (*Gorilla gorilla gorilla*) based on a Bayesian analysis of genetic structure using microsatellite data. Individuals were sorted by population and by Q-values (for Cluster 1).

FIGURE 3. Phylogenetic haplotype network showing the evolutionary relationships among western and eastern gorillas (*Gorilla gorilla* and *G. beringei*, respectively) based on mtDNA D-loop hypervariable region I (HVI). Samples include contemporary captive western lowland gorillas (*G. g. gorilla*) from Howletts and Port Lympne (HPL) and historical wild western lowland gorillas from Powell-Cotton Museum (PCM), newly obtained here, and GenBank samples including contemporary captive and wild western gorillas (*G. gorilla*), eastern lowland gorillas (*G. b. graueri*) and mountain gorillas (*G. b. beringei*). Notches on internodes indicate mutational steps among haplotypes; black circles indicate hypothetical haplotypes (not observed).

741 **Tables**

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TABLE 1. Genetic diversity values based on microsatellite genotypes from contemporary captive and historical wild populations of western lowland gorilla (*Gorilla gorilla gorilla*).

Population	N	N_A	A_E	A_R	A_P	H_O	H_E	F_{IS}
Contemporary captive	41 (64)	6.8 (7.0)	4.7 (4.6)	4.2 (4.1)	2 (2)	0.79 (0.78)	0.79 (0.78)	-0.023 (-0.007)
Historical wild	65 (76)	6.0 (8.0)	4.1 (4.8)	4.1 (4.1)	10 (10)	0.74 (0.75)	0.77 (0.78)	- 0.023 (0.035)
Eastern	35 (44)	6.8 (6.9)	4.6 (4.6)	4.2 (4.2)	3 (4)	0.79 (0.76)	0.78 (0.78)	-0.023 (0.009)
Western	25 (27)	7.2 (7.2)	4.7 (4.6)	4.3 (4.3)	6 (5)	0.7 (0.73)	0.79 (0.78)	0.061 (0.043)
Southern	5 (5)	4.1 (4.1)	3.1 (3.1)	3.8 (3.8)	1 (1)	0.72 (0.72)	0.73 (0.73)	-0.017 (-0.107)

Full data set between parentheses. N = population sample size; N_A = number of different alleles averaged across loci; A_E = number of effective alleles; A_R = allelic richness; A_P = private alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; F_{IS} = inbreeding coefficient.

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TABLE 2. MtDNA diversity of the first hypervariable region (HVI) in western gorillas (*Gorilla gorilla*) and eastern gorillas (*G. beringei*) belonging to different haplogroups and sub-haplogroups, and from western lowland gorillas (*G. g. gorilla*) from a contemporary captive population (Howletts and Port Lympne) and historical wild population (Powell-Cotton Museum).

	N	P	H	Hd	π	k
Contemporary captive	8	2	2	0.250	0.003	0.500
Historical wild	21	32	11	0.910	0.045	7.790
NA-CAP*	51	35	18	0.872	0.059	9.416
Haplogroups						
A	6	2	3	0.080	0.006	1.067
B	10	6	6	0.867	0.010	1.800
C	59	43	21	0.933	0.054	9.382
C1	26	12	10	0.898	0.014	2.597
C2	29	11	9	0.810	0.009	1.704
C3	4	0	1	0.000	0.000	0.000
D	71	23	23	0.837	0.038	5.737
D1	7	4	2	0.286	0.006	1.143
D2	31	11	14	0.895	0.012	1.957
D3	33	7	8	0.384	0.003	0.481

N = sample size; P = polymorphic sites; H = number of haplotypes; Hd = haplotypic diversity; π = nucleotide diversity; k = average number of nucleotide differences. *NA-CAP = Captive gorillas in North American zoos.

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752 **Figures**

753 FIGURE 1.

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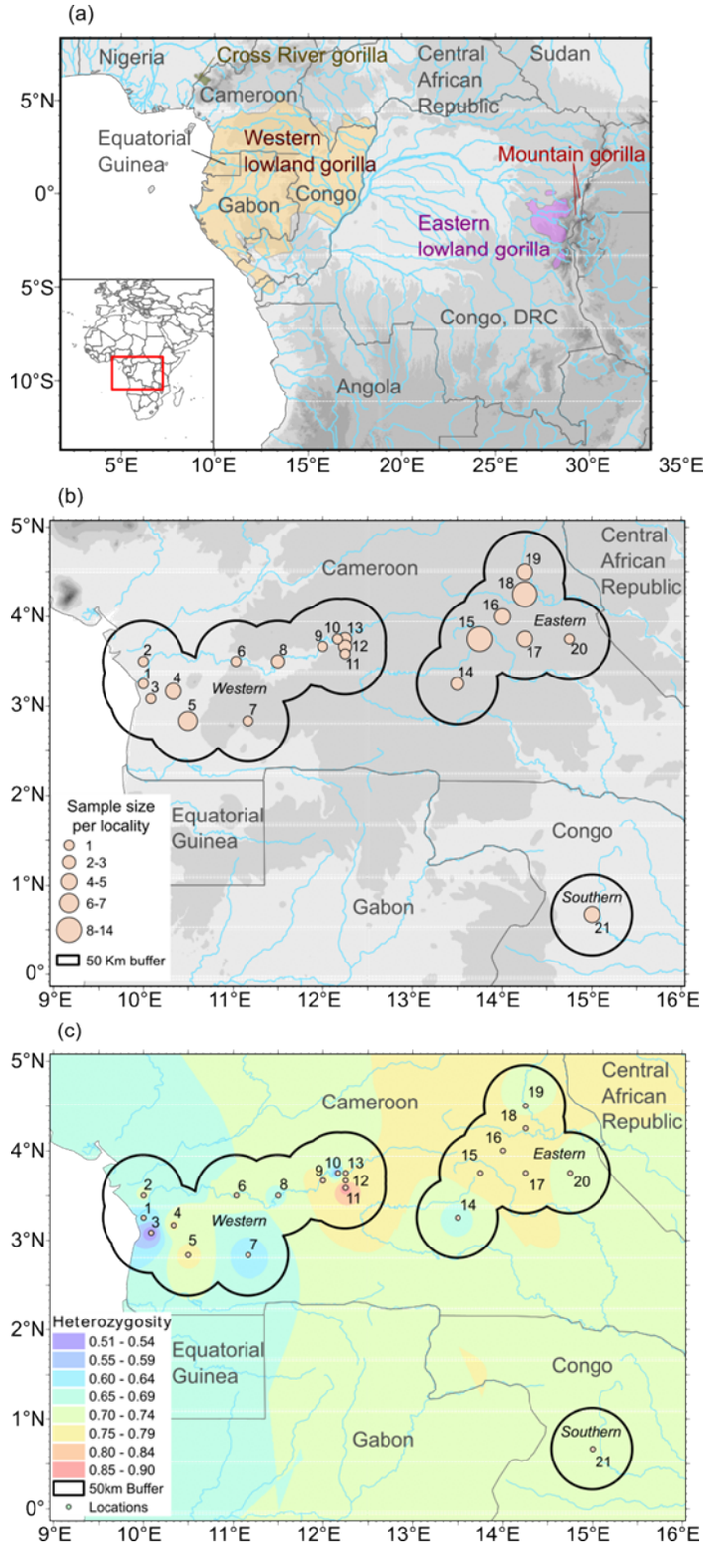
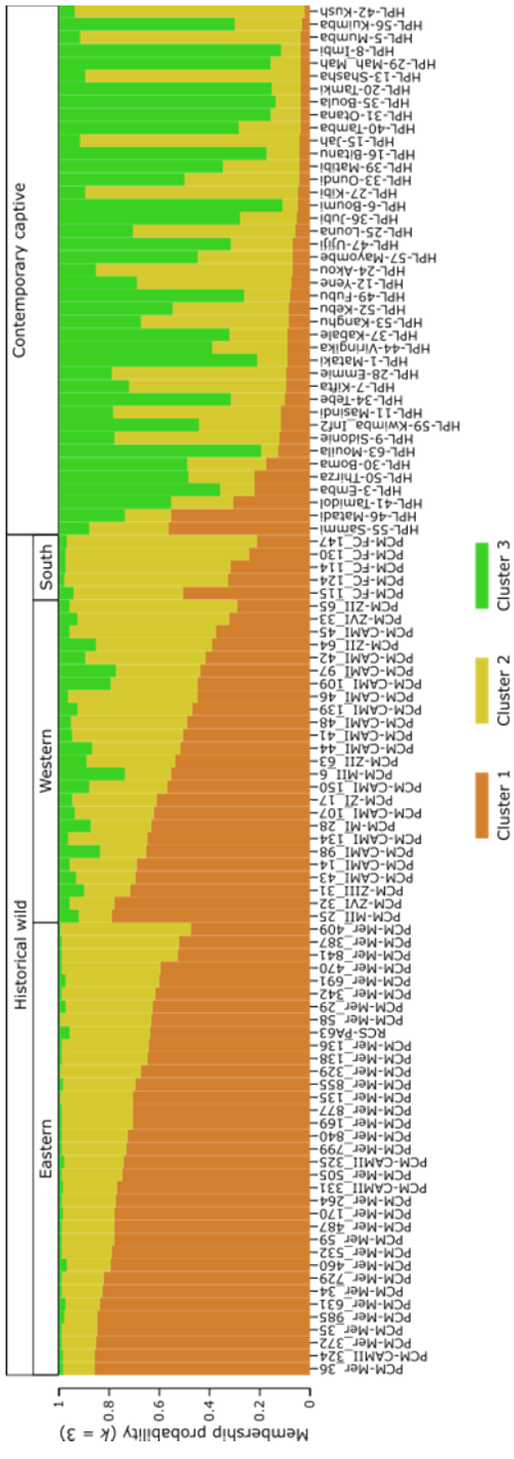


755

756 FIGURE 2.



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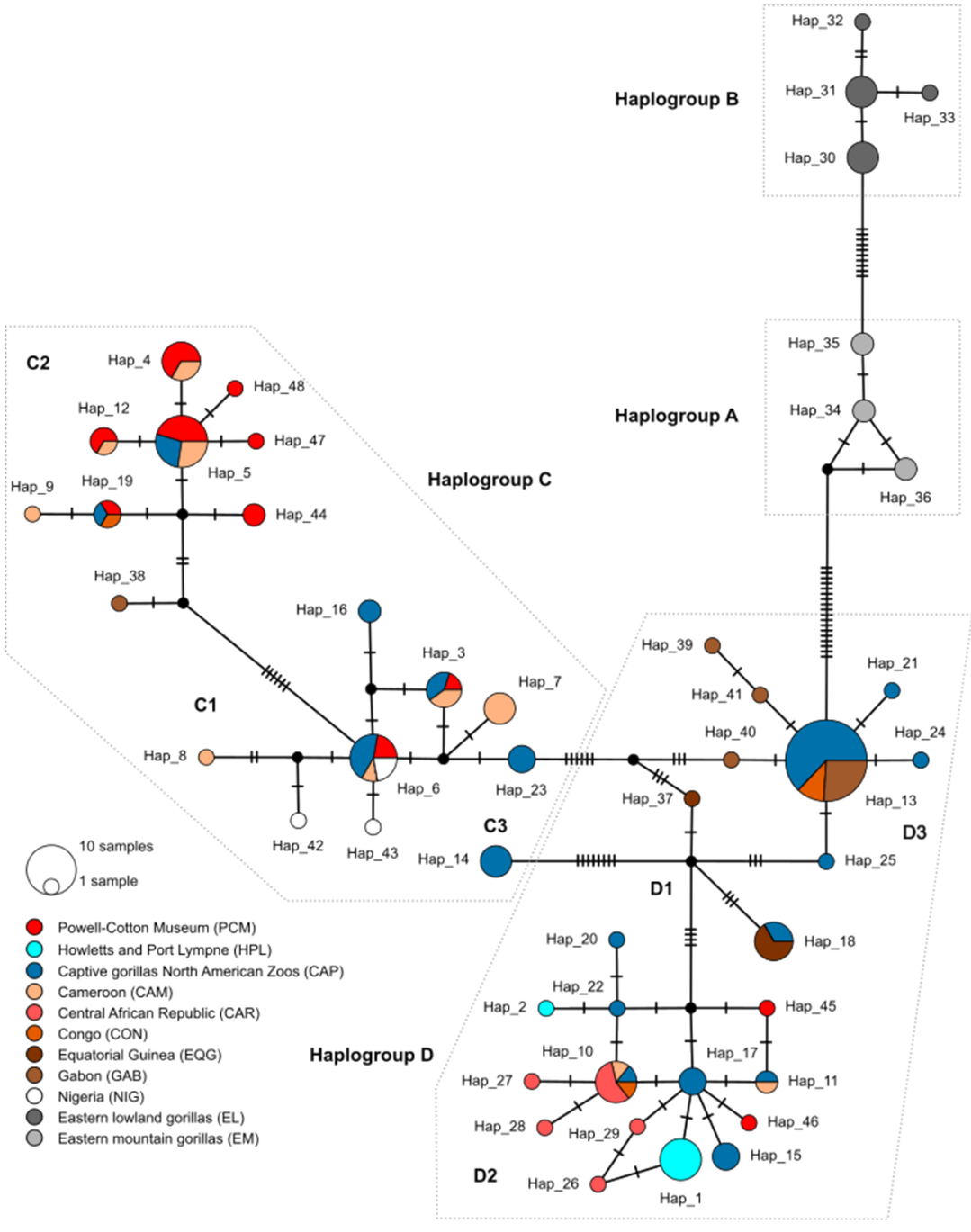
758 FIGURE 3.

759



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762 **Supporting Information**

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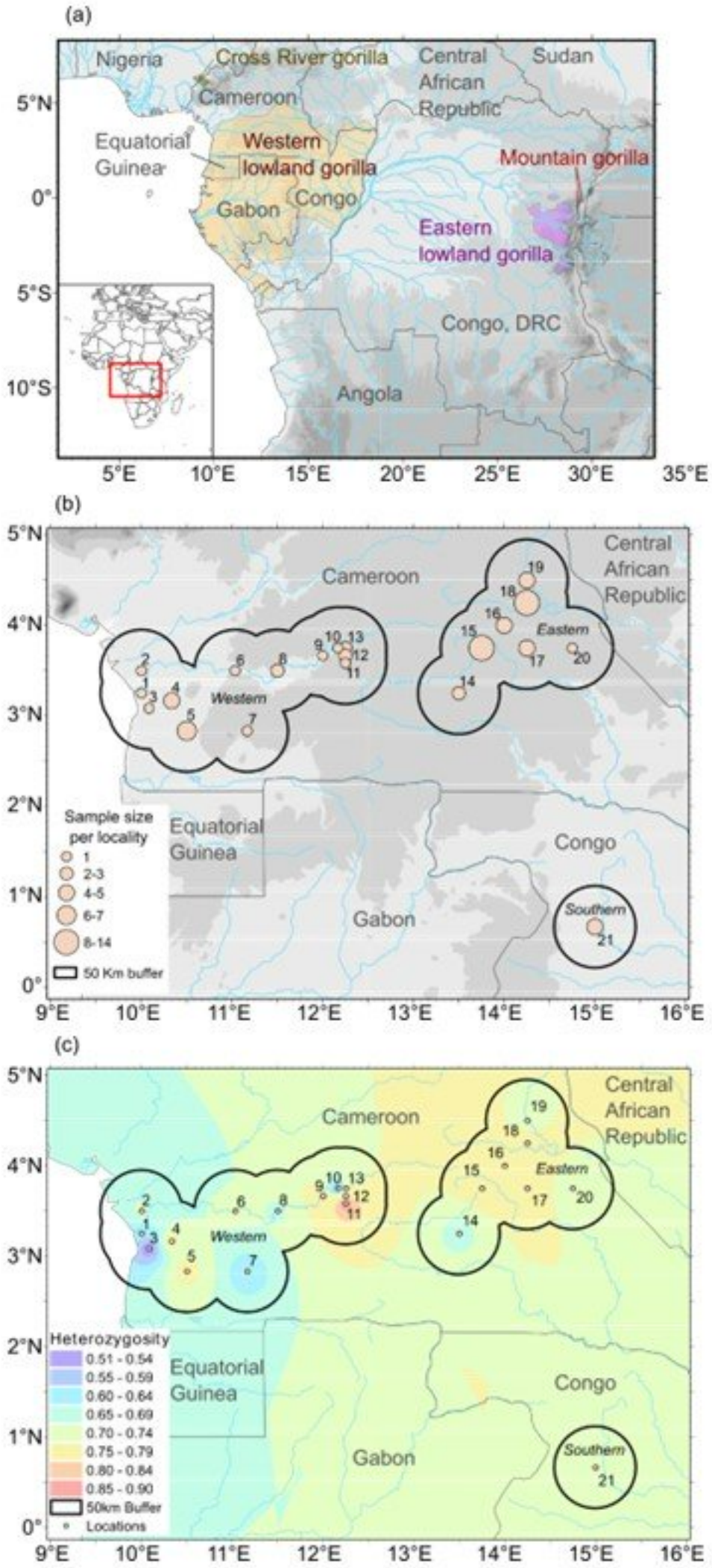
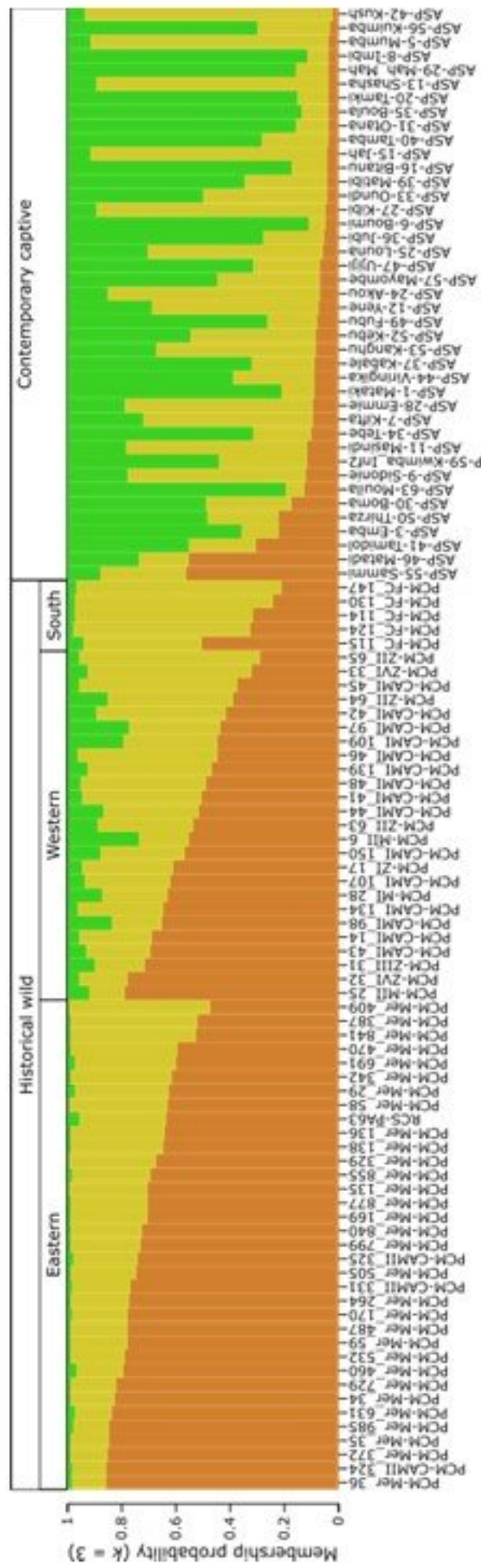
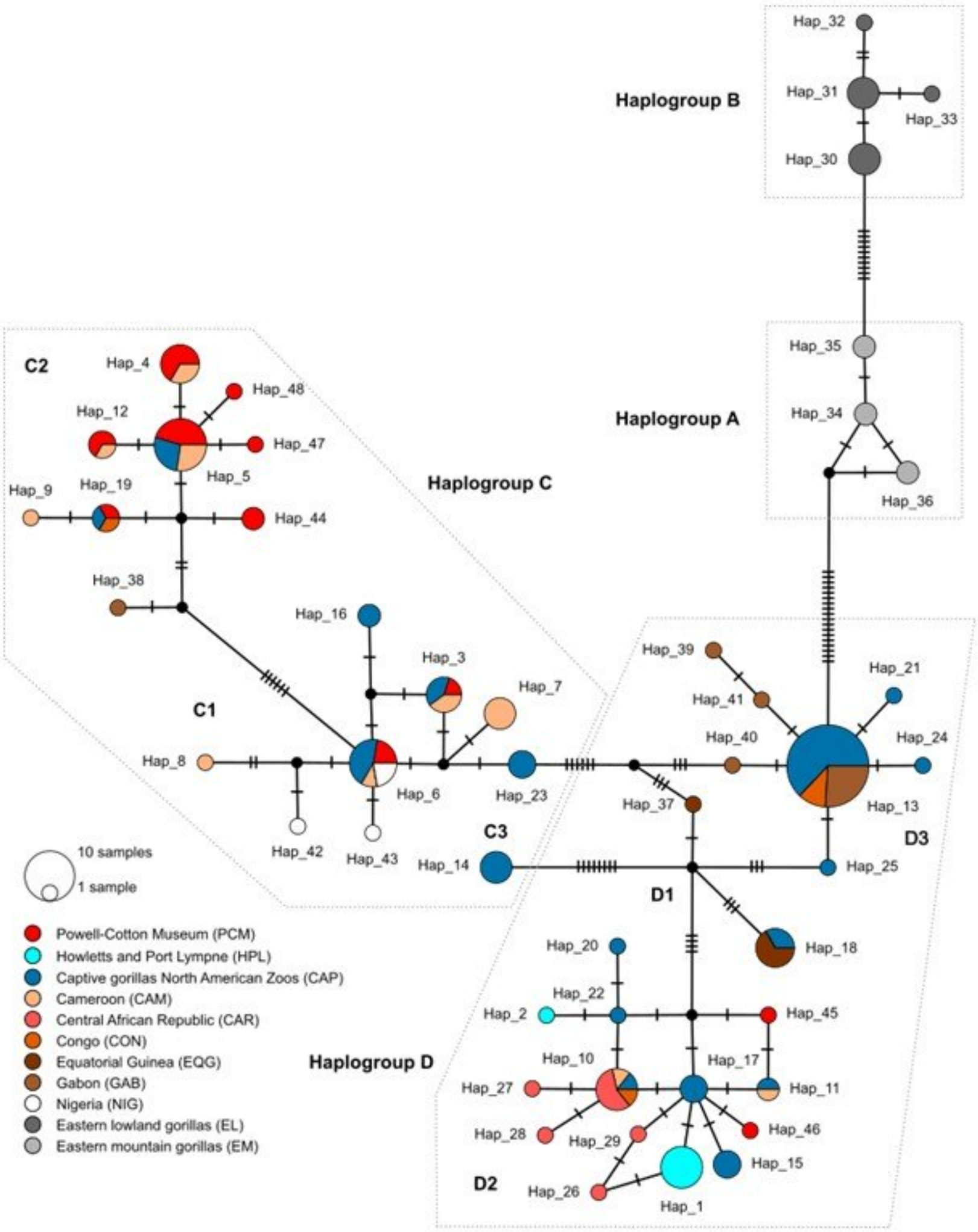

(a)
Nigeria
Cross River gorilla
Central African Republic
Sudan
Cameroon
Equatorial Guinea
Western lowland gorilla
Mountain gorilla
Gabon
Congo
Eastern lowland gorilla
Congo, DRC
Angola
5°N
0°
5°S
10°S
5°E
10°E
15°E
20°E
25°E
30°E
35°E
(b)
Cameroon
Central African Republic
Western
Eastern
Equatorial Guinea
Gabon
Congo
Southern
Sample size per locality
1
2-3
4-5
6-7
8-14
50 Km buffer
5°N
4°N
3°N
2°N
1°N
0°
9°E
10°E
11°E
12°E
13°E
14°E
15°E
16°E
(c)
Heterozygosity
0.51 - 0.54
0.55 - 0.59
0.60 - 0.64
0.65 - 0.69
0.70 - 0.74
0.75 - 0.79
0.80 - 0.84
0.85 - 0.90
50km Buffer
Locations


Contemporary captive
Historical wild
South
Western
Eastern
Cluster 1
Cluster 2
Cluster 3
Membership probability (k = 3)
0
0.2
0.4
0.6
0.8
1


Haplogroup B
Hap_32
Hap_31
Hap_33
Hap_30
Haplogroup A
Hap_35
Hap_34
Hap_36
C2
Hap_4
Hap_48
Hap_12
Hap_47
Hap_5
Hap_9
Hap_19
Hap_44
Hap_38
Haplogroup C
Hap_16
Hap_3
Hap_7
C1
Hap_8
Hap_6
Hap_23
Hap_42
Hap_43
C3
Hap_14
Hap_39
Hap_21
Hap_41
Hap_40
Hap_24
Hap_13
D3
Hap_37
Hap_25
D1
Hap_20
Hap_18
Hap_22
Hap_2
Hap_45
Hap_10
Hap_17
Hap_27
Hap_11
Hap_28
Hap_29
Hap_46
Hap_15
D2
Hap_26
Hap_1
Haplogroup D
10 samples
1 sample
Powell-Cotton Museum (PCM)
Howletts and Port Lympne (HPL)
Captive gorillas North American Zoos (CAP)
Cameroon (CAM)
Central African Republic (CAR)
Congo (CON)
Equatorial Guinea (EQG)
Gabon (GAB)
Nigeria (NIG)
Eastern lowland gorillas (EL)
Eastern mountain gorillas (EM)

Supporting Information

Title:

Genotyping contemporary captive and historical wild western lowland gorillas indicates captive breeding is maintaining genetic diversity in a critically endangered primate

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Short title: Captive breeding maintains genetic diversity in gorillas

Extended materials and methods

Contemporary and historic gorilla samples

The total sample included 140 individuals considered to be *G. g. gorilla* based on morphological features and reported geographic origins. The contemporary captive population consisted of 64 samples and the historical wild population consisted of 76 samples.

The EEP for western lowland gorillas was established in 1985 and seven years later, institutions from the British Isles and Ireland joined the programme. At the end of 2023, there were ~100 founders of the EEP (specifically, there were 101 founders of the EEP although eight had no living descendants, and if they die without descendants they would no longer be considered founders). HPL began in 1957 as a private collection and opened to the public in 1975. HPL is not a closed population and has regular gorilla exchanges with other institutions in the EEP. For the captive gorillas, we sampled six founders directly and 28 founders indirectly (through their offspring) (based on summary data provided by the EAZA gorilla EEP co-ordinator; Tjerk ter Meulen, pers. comm.). Of the six wild founders we sampled directly, five have living descendants in the EEP.

For the historical wild population obtained from Powell-Cotton Museum (PCM), the animals were hunted over a duration of nine years from 1927 to 1936 in Western Equatorial Africa, and geographical coordinates and location were recorded at the time of capture by Major Percy Horace Gordon Powell-Cotton and/or Frederick George Merfield. The two specimens from the Royal College of Surgeons (RCS) do not have geographical data recorded, but archival evidence indicates that those specimens were originally part of the PCM collection and were donated by Powell-Cotton to the RCS.

For the contemporary captive animals, blood DNA from FTA cards (previously collected and biobanked by zoo veterinarians) was extracted by placing 3 – 4 mm^2 of FTA card in 100 µL sterile H_2O, heating at 96 °C for 15 min, removing all water, and then adding 100 µL sterile H_2O, heating at 96 °C for an extra 20 min. The water containing DNA was stored at -20 °C until use. For museum specimens, skin tissue DNA was extracted using the QIAamp Fast DNA Tissue Kit (Qiagen, UK) with

the incubation period increased to 50 minutes, 50 µl of buffer ATE, and incubation at room temperature increased to 5 minutes. Negative controls were included in every DNA extraction session. Agarose gel electrophoresis was used to check for the presence and integrity of DNA [1% Agarose in 1X TAE buffer pH 8.0, with 5 µl SYBR Safe DNA stain (Invitrogen, UK)], and Qubit 2.0 fluorometer (Thermo Fisher Scientific, UK) was used for DNA quantification using the 1X dsDNA BR Assay Kit (Invitrogen, UK) following manufacturer's protocol.

Microsatellite genotyping

For microsatellite genotyping, the forward primers were fluorescently labelled at the 5' end for downstream fragment analysis of microsatellites. All the loci selected [D2s1326, D10s1432, D16s2624, D4s1627, D7s817, D5s1470, vWF, D7s2204, D1s550 and D8s1106 (Bradley et al. 2000)] are tetranucleotide repeats (4 bp). Locus D5s1470 is a tetranucleotide repeat with a 2 bp indel (insertion/deletion). Singleplex PCR reactions were carried out in a final volume of 13 µl containing: 6.25 µl DreamTaq Green Hot Start Green DNA Polymerase master mix (Thermo Scientific, UK), 1.25 µl of forward Primer and reverse primers (10 µM), 3.25 µl of dH_2O, and 1 µl of DNA. The microsatellite genotype dataset is available upon request.

Mitochondrial DNA sequencing

We used nested primers to amplify a short 258 base-pair fragment of the mitochondrial DNA D-loop hypervariable region I (HVI) (Garner & Ryder 1996; Clifford et al. 2004). First-round amplification with primers PDPF1 (5'-CACCATCAGCACCCAAAGCTAATAT-3') and PDPR2 (5'-TTGTGCGGGATATTGATTTCACGGA-3') and the second-round amplification with primers L91-115 and H402-27 (Garner & Ryder 1996; Clifford et al. 2004) followed the same PCR cycle conditions: denaturation at 94°C for 3 minutes, followed by 50 cycles of 94°C for 30 seconds, annealing at 50-68°C (depending on the sample) for 30 seconds and extension at 72°C for 30 seconds, with a final extension at 72°C for 10 minutes. Negative controls were included to control for potential PCR

78 contamination. Each first-round PCR contained a final volume of 13 µl consisting of 6.25 µl of
79 DreamTaq Green PCR Master Mix 2x (Thermo Scientific), 1.25 µl of PDPF1 and PDPR2 (10 µM), 3.25
80 µl of H_2O, and 1 µl of DNA. The nested PCRs with the second-round primers consisted of the same
81 volumes, but with 1 µl of first-round PCR product. PCR products were purified using a GeneJET PCR
82 Purification Kit (Thermo Fisher Scientific), visualised under UV light after agarose gel electrophoresis,
83 and sequenced with the second-round primers L91-115 and H402-27 by DBS Genomics (Durham
84 University). Approximately 25% of samples were re-sequenced to ensure consistency of results.
85 A total of 110 sequences were obtained (59 contemporary captive gorillas and 51 historical
86 wild gorillas). We used BLAST to check for nuclear inserts of mtDNA (numts), common in great apes
87 (Clifford et al. 2004), and to confirm species identity based on sequence homology. This resulted in
88 29 new numt-free HVI sequences (eight from HPL and 21 from PCM) (GenBank accession numbers
89 PV540398-PV540426; Table S1). A total of 29 new gorilla HVI sequences were obtained. In addition,
90 117 HVI sequences were obtained from GenBank (Table S1).
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Table S1. Samples of gorillas (*Gorilla* spp.) used in this study.

Howletts and Port Lympne (HPL; contemporary captive) – Western lowland gorillas

Sample ID	Population/ Region	Haplotype/ Haplogroup	Sex	Sire	Dam	Matriline founder	Family group (by known sire or genotyped*)	Lifespan
HPL-63-Mouilla	Captive		F	Wild	Wild	H61001-Mouila (Cameroon)	Fam5	1960-2014
HPL-61-Babydoll	Captive		F	Wild	Wild	H61000-Baby Doll (Cameroon)	Fam5	1961-2018
HPL-51-Shumba	Captive		F	Mumbah	Mushie	H69000-Mushie (Cameroon)	Fam3	1980-2024
HPL-34-Tebe	Captive		F	Wild	Wild	H82000-Tebe (Gabon)	Fam2	1980-2025
HPL-4-Djala	Captive		M	Wild	Wild	Djala mother (Congo)	Fam1*	1982-2024
HPL-5-Mumba	Captive		F	Kijo	Shumba	H69000-Mushie (Cameroon)	Fam3	1987-2014
HPL-26-Kishi	Captive		F	Kijo	Mushie	H69000-Mushie (Cameroon)	Fam1/Fam3	1988-2014
HPL-1-Mataki	Captive		M	Bitam	Killa Killa	H61001-Mouila (Cameroon)	Fam5	1988-2023
HPL-6-Boumi	Captive		M	Bitam	Mouila	H61001-Mouila (Cameroon)	Fam5	1989-2011
HPL-20-Tamki	Captive		F	Bitam	Killa Killa	H61001-Mouila (Cameroon)	Fam5	1989-2014
HPL-10-Timbou	Captive		M	Bitam	Mouila	H61001-Mouila (Cameroon)	Fam5	1990-2021
HPL-21-Fou_Fou	Captive		F	Kijo	Founa	H72001-Founa (Gabon)	Fam1/Fam3	1992-2014
HPL-27-Kibi	Captive		F	Kijo	Shumba	H69000-Mushie (Cameroon)	Fam1/Fam3	1992-2014
HPL-42-Kush	Captive		M	Kijo	Mushie	H69000-Mushie (Cameroon)	Fam1/Fam3	1992-2022
HPL-47-Ujiji	Captive		M	Bitam	JuJu	H62000-JuJu (Cameroon)	Fam5	1994-2022
HPL-30-Boma	Captive		F	Tam Tam	Hyasmina	S91066/-Hyasmina (Cameroon)		1996-2016
HPL-41-Tamidol	Captive		F	Bitam	Baby Doll	H61000-Baby Doll (Cameroon)	Fam5	1998-2024

HPL-7-Kifta	Captive		F	Kifu	Tambabi	H61000-Baby Doll (Cameroon)	Fam2	2000-2013
HPL-46-Matadi	Captive		M	Sekondi (Twycross)	Ozala (Twycross)	28/TWY-Biddy (unknown)		2003-2023
HPL-49-Fubu	Captive		M	Kifu	Bamilla	H61001-Mouila (Cameroon)	Fam2	2003-2024
HPL-50-Thirza	Captive		F	Bokito (Rotterdam)	Tamani (Rotterdam)	285-Mintha (Cameroon)		2007-2016
HPL-25-Louna	Captive		M	Djala	FouFou	H72001-Founa (Gabon)	Fam1	2008-2015
HPL-56-Kuimba	Captive		F	Asato (Beauval)	Tamarilla	H61001-Mouila (Cameroon)		2010-2019
HPL-24-Akou	Captive		F	Djala	Kishi	H69000-Mushie (Cameroon)	Fam1	2011-2014
HPL-2-Kwimba_Inf1	Captive	Hap_1/D2	infant		Kwimba	H60000-Shamba (Cameroon)	Fam8	2015-2015
HPL-59-Kwimba_Inf2	Captive	Hap_1/D2			Kwimba	H60000-Shamba (Cameroon)	Fam8	2018-2018
HPL-58-Masindi_Inf	Captive		F	Sammi	Masindi	H69000-Mushie (Cameroon)	Fam7	2018-2018
HPL-62-Viringika_Inf	Captive				Viringika	26FRAN-Dorret (unknown)	Fam4	2019-2019
HPL-53-Kangu	Captive	Hap_2/D2	M	Kifu	Sangha	P21320-Sangha (Congo)	Fam2	1999-?
HPL-48-Kouyou	Captive		M	Kifu	Sounda	H87005-Sounda (Congo)	Fam2	2002-?
HPL-22-Mbwambe	Captive		F	Djala	Kishi	H69000-Mushie (Cameroon)	Fam1	2006-?
HPL-9-Sidonie	Captive		F	Wild	Wild	Sidonie (Cameroons)		1972-
HPL-60-Kouillou	Captive		M	Wild	Wild	Kouillou mother (Congo)	Fam4*	1984-
HPL-40-Tamba	Captive	Hap_1/D2	F	Bitam	Shumba	H60000-Shamba (Cameroon)	Fam4/Fam5	1986-
HPL-43-Tambabi	Captive		F	Bitam	Baby Doll	H61000-Baby Doll (Cameroon)	Fam2/Fam5	1986-
HPL-39-Matibi	Captive		F	Bitam	Kaja	?H62000-JuJu	Fam5	1988-
HPL-36-Jubi	Captive		F	Bitam	JuJu	H62000-JuJu (Cameroon)	Fam5	1990-
HPL-38-Mambi	Captive		F	Bitam	Baby Doll	H61000-Baby Doll (Cameroon)	Fam4/Fam5	1990-
HPL-28-Emmie	Captive		F	Kibobo (Palmyre)	Aline/Sabrina (Palmyre)	Martha (Palmyre)		1991-
HPL-3-Emba	Captive		F	Bitam	Bamenda	M2/JERSEY (Cameroon)	Fam4/Fam5	1991-

HPL-64-Djanghou	Captive		M	Djala	Sangha	P21320-Sangha (Congo)	Fam1/Fam6*	1993-
HPL-44-Viringika	Captive		F	Ngola (Durrell)	Inge (Frankfurt)	26FRAN-Dorret (unknown)	Fam4	1995-
HPL-45-Kwimba	Captive	Hap_1/D2	F	Kouillou	Tamba	H60000-Shamba (Cameroon)	Fam4/Fam8	1997-
HPL-52-Kebu	Captive		M	Kifu	Tebe	H82000-Tebe (Gabon)	Fam2	2000-
HPL-8-Imbi	Captive		F	Kouillou	Mambi	H61000-Baby Doll (Cameroon)	Fam4	2000-
HPL-12-Yene	Captive		F	Djala	Foufou	H72001-Founa (Gabon)	Fam1	2001-
HPL-13-Shasha	Captive		F	Kijo	Shumba	H69000-Mushie (Cameroon)	Fam3	2001-
HPL-14-Bitono	Captive		M	Kijo	Mushie	H69000-Mushie (Cameroon)	Fam3	2001-
HPL-31-Otana	Captive	Hap_1/D2	M	Kouillou	Tamba	H60000-Shamba (Cameroon)	Fam4	2001-
HPL-15-Jah	Captive		M	Kijo	Dihi	285-Mintha (Cameroon)	Fam3	2002-
HPL-29-Mah_Mah	Captive		F	Kouillou	Mambi	H61000-Baby Doll (Cameroon)	Fam4	2002-
HPL-16-Bitanu	Captive		M	Bitam	JuJu	H62000-JuJu (Cameroon)	Fam5	2003-
HPL-17-Popa	Captive		M	Kijo	Mushie	H69000-Mushie (Cameroon)	Fam3	2004-
HPL-18-Baloo	Captive	Hap_1/D2	M	Kouillou	Tamba	H60000-Shamba (Cameroon)	Fam4	2004-
HPL-32-Lou_Lou	Captive		F	Kijo	Shumba	H69000-Mushie (Cameroon)	Fam3	2004-
HPL-19-Imbizo	Captive		M	Kouillou	Emba	M2/JERSEY (Cameroon)	Fam4	2005-
HPL-55-Sammi	Captive		M	Samson (Wild)	Minnie	Martha (Palmyre)	Fam7*	2005-
HPL-11-Masindi	Captive		F	Djanghou	Kimba	H69000-Mushie (Cameroon)	Fam6/Fam7	2006-
HPL-23-Djongo	Captive		M	Djala	Kibi	H69000-Mushie (Cameroon)	Fam1	2006-
HPL-33-Oundi	Captive		F	Kifu	Sounda	H87005-Sounda (Congo)	Fam2	2006-
HPL-54-Kisane	Captive		M	Djanghou	Sanki	H87005-Sounda (Congo)	Fam6/Fam8*	2006-
HPL-35-Boula	Captive		F	Kouillou	Mambi	H61000-Baby Doll (Cameroon)	Fam4	2007-
HPL-57-Mayombe	Captive		F	Asato (Beauval)	Inge (Beauval)	26FRAN-Dorret (unknown)		2007-
HPL-37-Kabale	Captive	Hap_1/D2	M	Kouillou	Tamba	H60000-Shamba (Cameroon)	Fam4	2011-

Powell-Cotton Museum (PCM; historical wild) – Western lowland gorillas								
Specimen ID	**Population/ Region**	**Haplotype/ Haplogroup**	**Sex**	**Locality**	**Locality Num.**	**Latitude (N)**	**Longitude (E)**	**Year Killed**
PCM-CAMI_107	Western		M	Akonolinga/Cameroon	12	3.6667	12.25	1929
PCM-CAMI_109	Western		F	Akonolinga/Cameroon	12	3.6667	12.25	1929
PCM-CAMI_134	Western		M	Olangina/Cameroons	13	3.6875	12.25	1929
PCM-CAMI_139	Western		F	Beycar/Cameroon	9	3.6667	12	1929
PCM-CAMI_14	Western	Hap_45/D2	F	Belar/Cameroon	3	3.0833	10.0833	1929
PCM-CAMI_149	Western		F	S Yaounde/N'Yong/Cameroon	8	3.5	11.5	1929
PCM-CAMI_150	Western		F	S Yaounde/N'Yong/Cameroon	8	3.5	11.5	1929
PCM-CAMI_224	Western		M	Olangina/Cameroons	13	3.6875	12.25	1929
PCM-CAMI_41	Western		M	SE Kribi/Cameroon	5	2.8333	10.5	1929
PCM-CAMI_42	Western		F	SE Kribi/Cameroon	5	2.8333	10.5	1929
PCM-CAMI_43	Western		F	SE Kribi/Cameroon	5	2.8333	10.5	1929
PCM-CAMI_44	Western		F	SE Kribi/Cameroon	5	2.8333	10.5	1929
PCM-CAMI_45	Western		M	SE Kribi/Cameroon	5	2.8333	10.5	1929
PCM-CAMI_46	Western		M	SE Kribi/Cameroon	5	2.8333	10.5	1929
PCM-CAMI_48	Western		M	SE Kribi/Cameroon	5	2.8333	10.5	1929
PCM-CAMI_97	Western		F	Olangina/Cameroons	11	3.5833	12.25	1929
PCM-CAMI_98	Western		F	Olangina/Cameroons	13	3.6875	12.25	1929
PCM-CAMII_323	Eastern		M	Beri/Batouri District/Cameroon	19	4.5	14.25	1932
PCM-CAMII_324	Eastern	Hap_44/C2	F	Beri/Batouri District/Cameroon	19	4.5	14.25	1932
PCM-CAMII_325	Eastern	Hap_44/C2	F	Beri/Batouri District/Cameroon	19	4.5	14.25	1932
PCM-CAMII_331	Eastern		M	Beri/Batouri District/Cameroon	19	4.5	14.25	1932

PCM-FC_114	Southern	Hap_46/D2	F	Mambili/French Congo	21	0.6667	15	1927
PCM-FC_115	Southern		M	Mambili/French Congo	21	0.6667	15	1927
PCM-FC_124	Southern		F	Keba/French Congo	21	0.6667	15	1927
PCM-FC_130	Southern		M	Mambili/French Congo	21	0.6667	15	1927
PCM-FC_147	Southern	Hap_6/C1	F	Keba/French Congo	21	0.6667	15	1927
PCM-Mer_135	Eastern		M	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_136	Eastern	Hap_4/C2	F	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_137	Eastern	Hap_4/C2	M	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_138	Eastern		F	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_139	Eastern		F	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_169	Eastern		M	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_170	Eastern		F	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_184	Eastern		M	Gadji/SW of Batouri/Cameroon	16	4	14	1935
PCM-Mer_264	Eastern	Hap_4/C2	M	Lomie District/Cameroon	14	3.25	13.5	1936
PCM-Mer_29	Eastern	Hap_5/C2	F	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_329	Eastern		F	Meyoss/Batouri District/Cameroon	16	4	14	1932
PCM-Mer_34	Eastern	Hap_5/C2	M	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_342	Eastern		M	Lomie District/Cameroon	14	3.25	13.5	1932
PCM-Mer_35	Eastern		F	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_36	Eastern	Hap_19/C2	F	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_372	Eastern		M	Meyoss/Batouri District/Cameroon	16	4	14	1932
PCM-Mer_387	Eastern		F	Meyoss/Batouri District/Cameroon	16	4	14	1932
PCM-Mer_409	Eastern		F	Obala/Batouri District/Cameroon	17	3.75	14.25	1933
PCM-Mer_460	Eastern		F	Obala/Batouri District/Cameroon	20	3.6875	14.25	1933

PCM-Mer_470	Eastern	Hap_47/C2	F	Obala/Batouri District/Cameroon	17	3.75	14.25	1933
PCM-Mer_471	Eastern	Hap_5/C2	M	Obala/Batouri District/Cameroon	18	4.25	14.25	1933
PCM-Mer_487	Eastern	Hap_48/C2	M	Lelo/Batouri District/Cameroon	18	4.25	14.25	1932
PCM-Mer_505	Eastern		M	Obala/Batouri District/Cameroon	18	4.25	14.25	1933
PCM-Mer_532	Eastern		F	Lelo/Batouri District/Cameroon	18	4.25	14.25	1933
PCM-Mer_58	Eastern	Hap_12/C2	F	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_59	Eastern	Hap_5/C2	M	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_631	Eastern		M	Obala/Batouri District/Cameroon	17	3.75	14.25	1932
PCM-Mer_691	Eastern		F	Obala/Batouri District/Cameroon	17	3.75	14.25	1933
PCM-Mer_720	Eastern	Hap_5/C2	M	Obala/Batouri District/Cameroon	18	4.25	14.25	1932
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PCM-Mer_799	Eastern		F	Batouri District/Cameroon	18	4.25	14.25	1934
PCM-Mer_840	Eastern	Hap_4/C2	F	Obala/Batouri District/Cameroon	18	4.25	14.25	1932
PCM-Mer_841	Eastern		F	Obala/Batouri District/Cameroon	18	4.25	14.25	1932
PCM-Mer_855	Eastern		F	Batouri District/Cameroon	18	4.25	14.25	1934
PCM-Mer_865	Eastern		F	Batouri District/Cameroon	18	4.25	14.25	1934
PCM-Mer_877	Eastern		F	Batouri District/Cameroon	18	4.25	14.25	1934
PCM-Mer_95	Eastern		F	Batouri and Lomie/Cameroon	15	3.75	13.75	1935
PCM-Mer_985	Eastern		F	Meyoss/Batouri District/Cameroon	16	4	14	1932
PCM-MI_28	Western	Hap_6/C1	M	Yaounde-Kribi Rd/Cameroon	6	3.5	11.2667	1930
PCM-MII_25	Western		F	Yaounde District/Cameroon?	6	3.5	11.2667	1930
PCM-MII_6	Western		M	Akonolinga District/Cameroon	10	3.6875	12.2292	1930
PCM-ZI_17	Western		M	Bakoko/N'Jong/Cameroon	2	3.5	10	1929

PCM-ZII_63	Western		F	River Mlonking/Bulu Bush/Cameroon	4	3.1667	10.3333	1930
PCM-ZII_64	Western		M	River Bikiango/Bulu Bush/Cameroon	4	3.1667	10.3333	1930
PCM-ZII_65	Western		M	Azija Bakoko/Cameroon	1	3.25	10	1930
PCM-ZIII_31	Western		M	Bipindi/Cameroon	4	3.1667	10.3333	1930
PCM-ZVI_32	Western	Hap_3/C1	M	Ebodonka Rd/Ebolowa/Cameroon	7	2.8333	11.1667	1933
PCM-ZVI_33	Western		F	Bikiango Rd/Bipindi/Cameroon	4	3.1667	10.3333	1933
RCS-PA62	Eastern	Hap_12/C2		Royal College of Surgeons				1935
RCS-PA63	Eastern			Royal College of Surgeons				1935

GenBank samples – Western lowland gorillas (unless specified)

Sample ID	Population/ Region	Haplotype/ Haplogroup	Accession Number	
CAM_AM392409	Cameroon	Hap_3/C1	AM392409	
CAM_AM392415	Cameroon	Hap_4/C2	AM392415	
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CAM_AM392422	Cameroon	Hap_5/C2	AM392422	
CAM_AM392424	Cameroon	Hap_6/C1	AM392424	
CAM_AY530113	Cameroon	Hap_7/C1	AY530113	
CAM_AY530114	Cameroon	Hap_7/C1	AY530114	
CAM_AY530115	Cameroon	Hap_7/C1	AY530115	
CAM_AY530116	Cameroon	Hap_7/C1	AY530116	
CAM_AY530117	Cameroon	Hap_3/C1	AY530117	
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CAM_AY530119	Cameroon	Hap_5/C2	AY530119	

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CAM_AY530127	Cameroon	Hap_10/D2	AY530127	
CAM_AY530132	Cameroon	Hap_11/D2	AY530132	
CAM_KF029423	Cameroon	Hap_4/C2	KF029423	
CAM_KF029427	Cameroon	Hap_12/C2	KF029427	
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CAP_AF451971	Captive	Hap_6/C1	AF451971	
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CAP_KM555060	Captive	Hap_15/D2	KM555060	
CAP_KM555061	Captive	Hap_15/D2	KM555061	
CAP_KM555062	Captive	Hap_16/C1	KM555062	
CAP_KM555063	Captive	Hap_15/D2	KM555063	
CAP_KM555064	Captive	Hap_13/D3	KM555064	
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CAP_KM555098	Captive	Hap_13/D3	KM555098	
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CAR_AY079510	Central African Republic	Hap_27/D2	AY079510	
CAR_AY530128	Central African Republic	Hap_10/D2	AY530128	
CAR_AY530130	Central African Republic	Hap_10/D2	AY530130	
CAR_AY530131	Central African Republic	Hap_28/D2	AY530131	
CAR_AY530133	Central African Republic	Hap_29/D2	AY530133	
CAR_L76761	Central African Republic	Hap_10/D2	L76761	
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CON_AY530129	Congo	Hap_10/D2	AY530129	
CON_AY530135	Congo	Hap_13/D3	AY530135	
CON_AY530141	Congo	Hap_13/D3	AY530141	

CON_L76763	Congo	Hap_13/D3	L76763	
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EL_AF187549	Eastern lowland gorilla	Hap_31/B	AF187549	
EL_AY530104	Eastern lowland gorilla	Hap_30/B	AY530104	
EL_AY530105	Eastern lowland gorilla	Hap_30/B	AY530105	
EL_AY530106	Eastern lowland gorilla	Hap_31/B	AY530106	
EL_AY530107	Eastern lowland gorilla	Hap_31/B	AY530107	
EL_AY530108	Eastern lowland gorilla	Hap_31/B	AY530108	
EL_L76771	Eastern lowland gorilla	Hap_32/B	L76771	
EL_L76772	Eastern lowland gorilla	Hap_33/B	L76772	
EL_L76773	Eastern lowland gorilla	Hap_30/B	L76773	
EM_AY530102	Mountain gorilla	Hap_34/A	AY530102	
EM_AY530103	Mountain gorilla	Hap_35/A	AY530103	
EM_L76749	Mountain gorilla	Hap_35/A	L76749	
EM_L76750	Mountain gorilla	Hap_36/A	L76750	
EM_L76751	Mountain gorilla	Hap_36/A	L76751	
EM_L76752	Mountain gorilla	Hap_34/A	L76752	
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EQG_AY530123	Equatorial Guinea	Hap_18/D1	AY530123	
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EQG_AY530125	Equatorial Guinea	Hap_18/D1	AY530125	
EQG_AY530126	Equatorial Guinea	Hap_37/D1	AY530126	
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GAB_AY530134	Gabon	Hap_13/ D3	AY530134	
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GAB_AY530139	Gabon	Hap_13/ D3	AY530139	
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NIG_AY530110	Nigeria	Hap_6/ C1	AY530110	
NIG_AY530111	Nigeria	Hap_42/ C1	AY530111	
NIG_AY530112	Nigeria	Hap_43/C1	AY530112	

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TABLE S2. Pairwise genetic structure (F_{ST}) based on microsatellite genotypes of western lowland gorillas (*Gorilla gorilla gorilla*) from a contemporary captive population from Howletts and Port Lympne (HPL) and from historical wild samples from Powell-Cotton Museum divided into three regional groups.

	HPL	Eastern	Western	Southern
HPL	-			
Eastern	0.018 (**0.019**)	-		
Western	**0.015** (**0.018**)	0.012 (0.011)	-	
Southern	**0.044** (**0.029**)	0.039 (0.017)	0.049 (**0.029**)	-

Full data set results between parentheses. Values in bold significant after Bonferroni correction.

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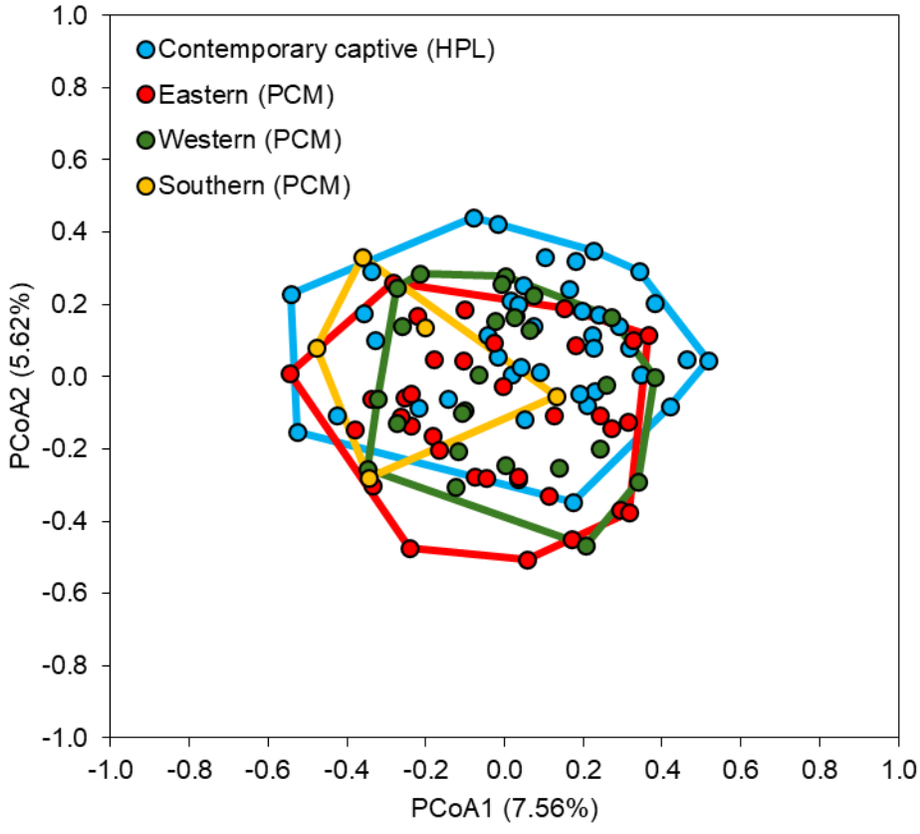
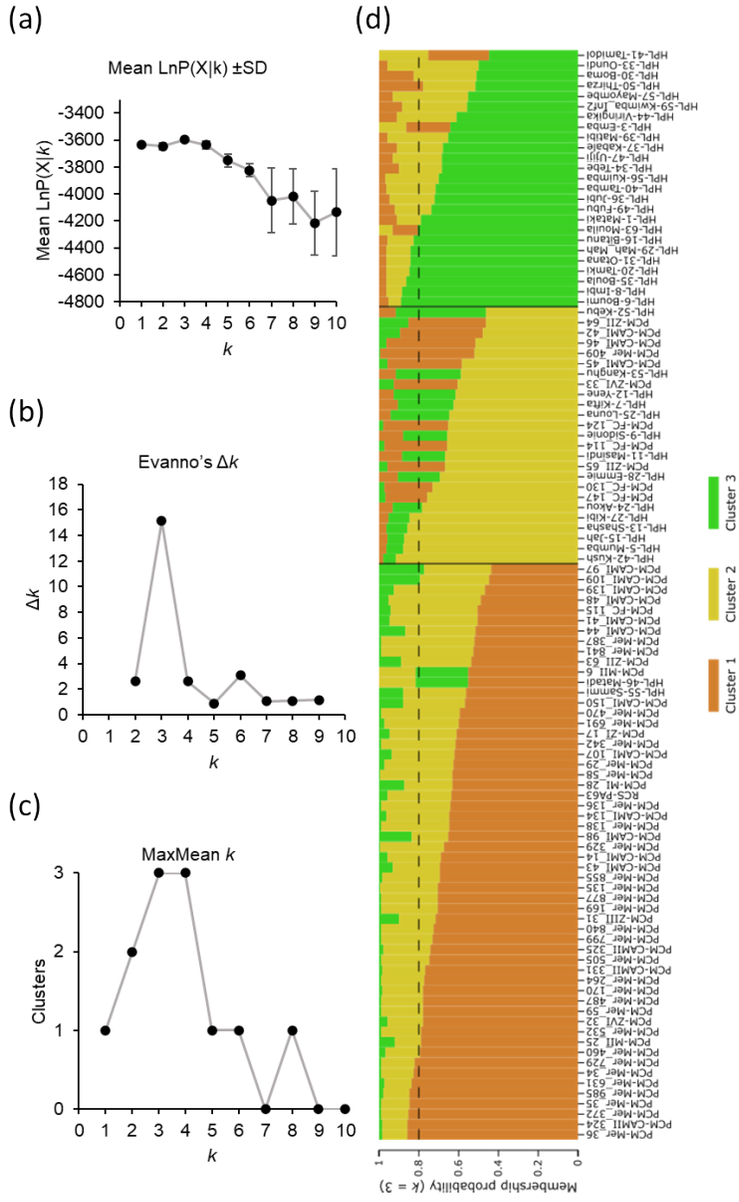
100



101 **Figure S1.** Principal coordinates analysis (PCoA) based on microsatellite genotypes of western
102 lowland gorillas (*Gorilla gorilla gorilla*) from a contemporary captive population (Howletts and Port
103 Lympne; HPL) and from a historical wild population (Powell-Cotton Museum; PCM) divided into
104 three regional groups (Eastern, Western and Southern). Convex hulls illustrate the outline of the
105 individuals from each group.

106

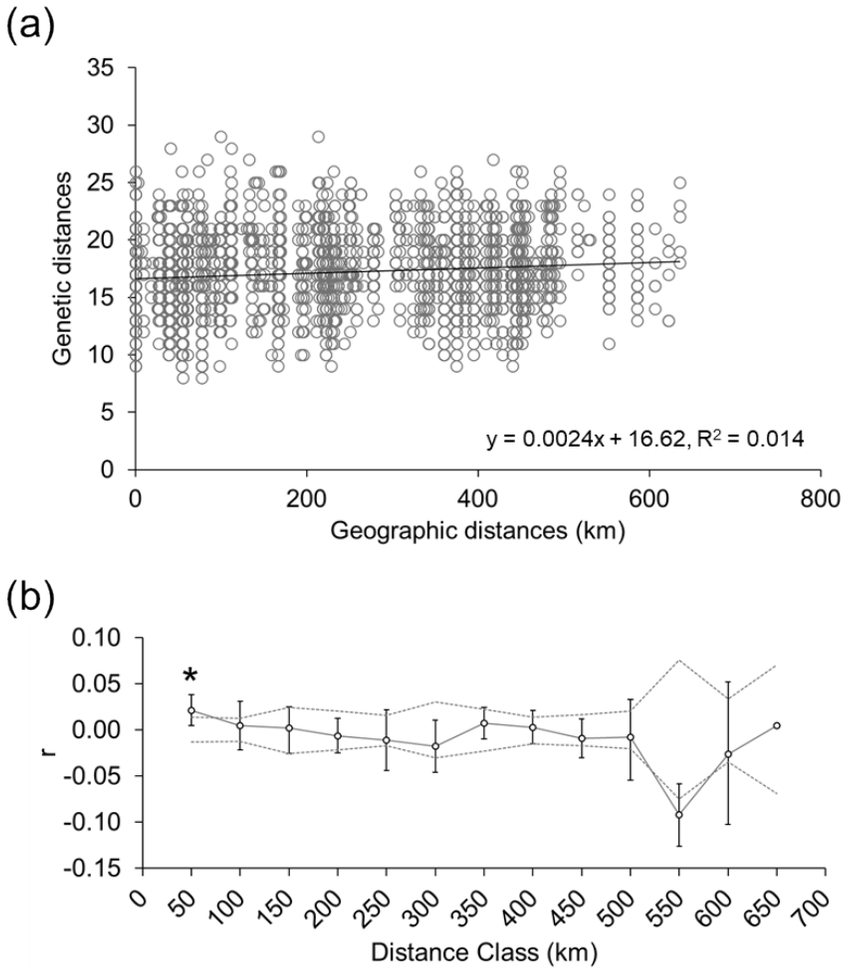
107



108 **Figure S2.** Bayesian analysis of population structure. Identification of *k* clusters of historical wild
109 (Powell-Cottom Museum, PCM) and contemporary captive (Howletts and Port Lympne, HPL)
110 western lowland gorillas using microsatellite data: a) LnPr(X|*k*), b) Evanno's Δ*k* method, and c)
111 Puechmaille's method by MaxMean*k*. d) Individual probability plot for *k* = 3 clusters (arranged by
112 clusters for Q > 0.5). Dashed line shows Q-value = 0.8 (for PCM, eight individuals had Q-values > 0.8,
113 all found in Cluster 1; for HPL, five individuals had Q-values > 0.8 in Cluster 2 and eight individuals
114 had Q-values > 0.8 in Cluster 3).

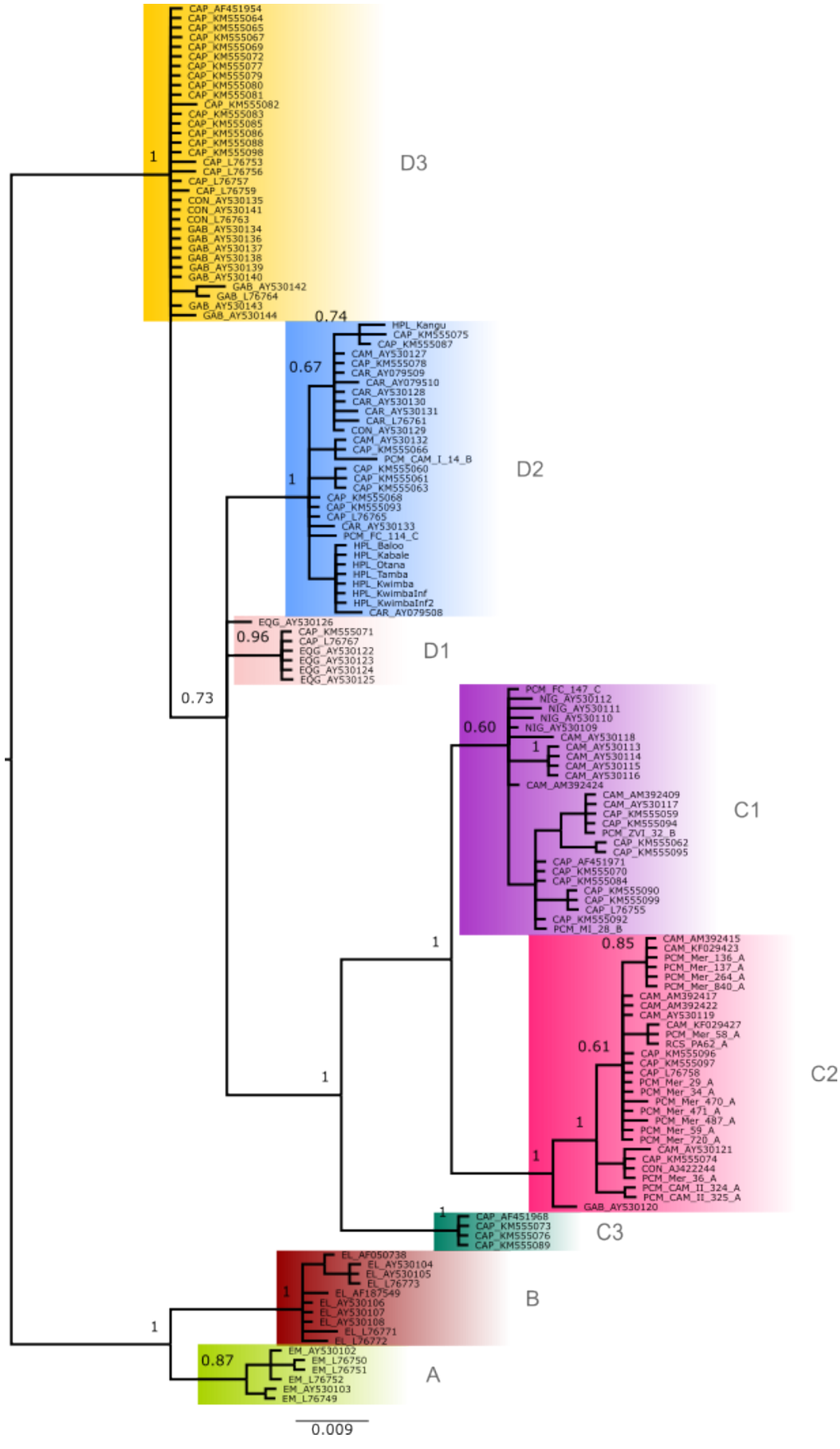
115

116



117 **Figure S3.** Isolation-by-distance test among historical wild western lowland gorillas (from Powell-
118 Cotton Museum, PCM). a) Pairwise individual-by-individual genetic and geographic distances
119 showing a weak but significant signal of isolation-by-distance (coefficient of correlation $r = 0.12$, $p <$
120 0.001). b) Correlogram plot showing the spatial autocorrelation coefficient (r) decreasing with
121 increasing distance class and positive structure at small spatial scale (50 km distance class). Error
122 bars for r determined by bootstrap resampling (10,000 permutations), dashed lines showing upper
123 and lower bounds of a 95% confidence interval for r under the null hypothesis of random
124 distribution of genotypes (10,000 permutations), and asterisk indicates significant autocorrelation
125 within the distance class (50 km; $r = 0.021$, $p = 0.03$).

126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CAP_L76759
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CON_AY530141
CON_L76763
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GAB_AY530137
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GAB_AY530140
GAB_AY530142
GAB_L76764
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GAB_AY530144
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D3
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CAP_KM555087
CAM_AY530127
CAP_KM555078
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CAR_AY079510
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CAR_AY530130
CAR_AY530131
CAR_L76761
CON_AY530129
CAM_AY530132
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CAP_KM555068
CAP_KM555093
CAP_L76765
CAR_AY530133
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HPL_Kabale
HPL_Otana
HPL_Tamba
HPL_Kwimba
HPL_KwimbaInf
HPL_KwimbaInf2
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D1
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A
0.009

Figure S4. Bayesian phylogenetic tree of the first hyper-variable region (HVI) from 117 reference gorillas (*Gorilla gorilla* and *G. beringei*) obtained from GenBank, and eight contemporary captive gorillas in Howletts and Port Lympne (HPL), 19 historical wild gorillas obtained from Powell-Cotton Museum (PCM) and two historical wild gorillas obtained from Royal College of Surgeons (RCS) (all belonging to *G. g. gorilla*). GenBank samples included: captive western lowland gorillas in American zoos (CAP), contemporary wild western lowland gorillas from Central African Republic (CAR), Republic of Congo (CON), Cameroon (CAM), Gabon (GAB), Nigeria (NIG) and Equatorial Guinea (EG), and contemporary wild eastern mountain gorillas (EM) and eastern lowland gorillas (EL). Haplogroups A (*G. b. beringei*) and B (*G. b. graueri*) were used to root the tree. Numbers on branches (≥0.60) indicate posterior probability support (only shown for most relevant branches).