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The complex Y-chromosomal history of gorillas

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Abstract

Studies of the evolutionary relationships among gorilla populations using autosomal and mitochondrial sequences suggest that male-mediated gene flow may have been important in the past, but data on the Y-chromosomal relationships among the gorilla subspecies are limited. Here, we genotyped blood and noninvasively collected fecal samples from 12 captives and 257 wild male gorillas of known origin

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representing all four subspecies (*Gorilla gorilla gorilla*, *G. g. diehli*, *G. beringei beringei*, and *G. b. graueri*) at 10 Y-linked microsatellite loci resulting in 102 unique Y-haplotypes for 224 individuals. We found that western lowland gorilla (*G. g. gorilla*) haplotypes were consistently more diverse than any other subspecies for all measures of diversity and comprised several genetically distinct groups. However, these did not correspond to geographical proximity and some closely related haplotypes were found several hundred kilometers apart. Similarly, our broad sampling of eastern gorillas revealed that mountain (*G. b. beringei*) and Grauer's (*G. b. graueri*) gorilla Y-chromosomal haplotypes did not form distinct clusters. These observations suggest structure in the ancestral population with subsequent mixing of differentiated haplotypes by male dispersal for western lowland gorillas, and postisolation migration or incomplete lineage sorting due to short divergence times for eastern gorillas.

KEYWORDS
diversity, genetic distance, microsatellite, phylogeny, short tandem repeat

1 | INTRODUCTION

The evolutionary histories of recently diverged populations can be effectively revealed by the analysis of rapidly evolving genetic markers (Chistiakov et al., 2006; Goldstein & Pollock, 1997). Comparison of insights from analyses of biparentally inherited markers to uniparentally inherited markers such as mitochondrial DNA (mtDNA) and the Y-chromosome is particularly interesting, as discrepant inferences may reflect differences in sex ratio, reproductive skew, rate and distance of natal dispersal, or differences in migration among the sexes (Wilson Sayres, 2018). For example, sequence analysis of the nonrecombining region of the Y-chromosome in humans and other great apes reveals particularly low diversity in humans and gorillas as compared to chimpanzees and bonobos, which has been attributed to high variance in male reproductive success (Hallast et al., 2016; Heyer et al., 2011). However, nuanced insights into the geographic distribution of Y-chromosome variation within a species, and consequent inferences regarding male gene flow among populations, requires numerous samples of known geographical provenance.

In the wild, great apes are typically sampled by means of noninvasive samples, such as feces (Arandjelovic & Vigilant, 2018). DNA extracts from these samples are often composed of less than 1% endogenous DNA, rendering downstream analyses such as high-throughput sequencing or large-scale single nucleotide polymorphism (SNP) analysis both challenging and expensive (Fontsere et al., 2020; Hernandez-Rodriguez et al., 2018; White et al., 2019). Accordingly, microsatellite analysis targeting short, highly variable repetitive DNA segments has long been used to gain insights into the relationships of individuals within groups, dispersal patterns across groups, or the evolutionary relationships among populations. For example, amplification of Y-linked microsatellites in wild chimpanzees has revealed the absence of male-mediated gene flow in Eastern chimpanzees (*Pan troglodytes schweinfurthii*) (Langergraber et al., 2014) but a limited amount in Western chimpanzees (*P. t. verus*) and bonobos (*Pan paniscus*) (Schubert et al., 2011); analysis of autosomal microsatellite data has identified the split times between Grauer's (*Gorilla beringei graueri*) and mountain gorillas (*G. b. beringei*), and among the two populations of mountain gorillas (Roy, Arandjelovic, et al., 2014).

However, the fast mutation rate of microsatellite loci makes them less suitable markers for phylogenetic analyses which aim at identifying relationships among taxa that may have diverged hundreds of thousands of years ago. Allele sizes are constrained by an increased probability of repeat loss with increasing microsatellite length as well as by point mutations that break up repeats and reduce mutation rates. These processes, along with nonconformation of loci to the stepwise mutation model (SMM, Ohta & Kimura, 1973; infinite allele model, Kimura & Crow, 1964) can create size homoplasies in divergent populations, meaning that individuals of distantly related taxa can share the same number of repeats (identity-by-state) (Calabrese et al., 2001; Estoup et al., 2002; Xu et al., 2000). Thus, genetic distance, when calculated from microsatellite data, eventually loses its linearity over time so that distantly related taxa may seem more genetically similar than they actually are. In addition, a low number of loci, variable numbers of repeat additions and subtractions, genotyping error, differences in mutation rates among the loci, or changes in population size over time may increase the noise in the dataset (Estoup et al., 2002; Feldmann et al., 1997; Nauta & Weissing, 1996; Pollock et al., 1998).

Despite these drawbacks, microsatellites have been used successfully in the reconstruction of phylogenetic relationships among plant and animal taxa that diverged up to several million years ago. A set of eight microsatellite loci retained enough phylogenetic signal to distinguish eucalypt genera (*Myrtaceae*) consistent with other genetic and morphological datasets (Ochieng et al., 2007). Microsatellite

phylogenies of Darwin's finches were in agreement with morphological and sequence data independent of the genetic distance and tree-building method used (Petren et al., 1999), and trees based on five loci supported the primary divisions among different populations of the Canary Island Lizard (*Gallotia galloti*) identified by mtDNA and likely colonization scenarios (Richard & Thorpe, 2001).

Unlike the male-philopatric chimpanzees and bonobos, gorillas live in groups featuring dispersal by both males and females (Harcourt & Stewart, 2007; Robbins & Robbins, 2018). In addition, reproduction in the group is dominated by one or very few males (Breuer et al., 2010; Masi et al., 2021; Vigilant et al., 2015). This means that the phylogeography of gorilla Y-chromosomes is of particular interest because it is likely shaped by two opposing forces: the potential for long-distance male dispersal which increases diversity and reduces differentiation among populations (Douadi et al., 2007; Masi et al., 2021; Roy, Gray, et al., 2014) and high male reproductive skew which reduces diversity and leads to rapid differentiation of isolated populations (Bradley et al., 2005; Breuer et al., 2010).

Western lowland gorillas (*G. gorilla gorilla*) have the largest geographical distribution of all gorilla taxa, occurring in western central Sub-Saharan Africa with an estimated population size of 362,000 (Strindberg et al., 2018), whereas the Cross River (*G. g. diehli*) population is the smallest (~200–300 individuals; Dunn et al., 2014), highly fragmented and confined to an area straddling the border between Cameroon and Nigeria (Figure 1). In the east, Grauer's gorillas have the second-largest spatial distribution with a population estimate of ~6800 (Plumptre et al., 2021), and occur in the eastern Democratic Republic of the Congo (Figure 1). Mountain gorillas are found in two small discrete populations in Uganda, Rwanda, and the Democratic Republic of the Congo (~1000 individuals total in the Virunga Massif and Bwindi Impenetrable National Park) (Granjon et al., 2020). Geographic distances between the subspecies ranges vary widely (Figure 1).

A myriad of studies have investigated gorilla demographic history from a mitochondrial (Ackermann & Bishop, 2010; Anthony et al., 2007; Clifford et al., 2004; Das et al., 2014; Garner & Ryder, 1996; Hallast et al., 2016; Jensen-Seaman & Kidd, 2001; van der Valk et al., 2018; Xue et al., 2015) and autosomal perspective (Becquet & Przeworski, 2007; Mailund et al., 2012; McManus et al., 2014; Prado-Martinez et al., 2013; Scally et al., 2012; Thalmann et al., 2007, 2011; van der Valk et al., 2019; Xue et al., 2015). The current view supports a scenario in which eastern and western gorillas split more than 100 kya with gene flow continuing until possibly as recently as 20 kya (Becquet & Przeworski, 2007; Mailund et al., 2012; McManus et al., 2014; Scally et al., 2012; Thalmann et al., 2007; Xue et al., 2015). This was followed by a split of Cross River and western lowland gorillas 17–68 kya with gene flow continuing until very recently (McManus et al., 2014; Prado-Martinez et al., 2013; Thalmann et al., 2011), and a split of Grauer's gorillas and mountain gorillas 10–20 kya (Jensen-Seaman & Kidd, 2001; Roy, Arandjelovic, et al., 2014). These data also indicate a history of drastic population size changes including a reduction in size for the ancestral eastern gorilla population followed by a population expansion of the Grauer's gorilla (Scally et al., 2012; van der Valk et al., 2018; Xue et al., 2015) and the more recent decline of the Cross River population (Bergl et al., 2008; Thalmann et al., 2011).

Two studies have constructed Y-chromosomal phylogenies using a small sample of gorillas representing three of the subspecies. In one study, Y-chromosomal sequence data of three male mountain gorillas and one Grauer's gorilla sample suggested that mountain gorillas are monophyletic (Xue et al., 2015). In contrast, Hallast et al. (2016) found evidence for male-mediated gene flow from the Virunga mountain gorilla population into Grauer's gorillas; one of the three investigated Grauer's gorilla Y-chromosomal sequences in this study was more closely related to the three Virunga mountain gorilla sequences than to the other two Grauer's gorilla sequences. These authors also identified deep structure among eight western lowland gorilla Y-chromosomal sequences. Both studies notably were also limited by samples from only one mountain gorilla population (no samples from Bwindi Impenetrable National Park).

Here, we analyze Y-chromosome microsatellite locus variation in gorillas using a large number of samples from individuals of known origin and representing opposite ends of the distribution of western lowland gorillas, the small Cross River gorilla population, several localities within the Grauer's gorilla range, and both remaining mountain gorilla populations (Virunga and Bwindi). The aim of this study is to reconstruct a male-specific phylogeny of gorillas, assess diversity for the subspecies and place the results in the context of male behavior.

2 | METHODS

2.1 | Ethical statement

As our study did not involve animal testing, and most samples were collected noninvasively, we did not violate any regulations of the Deutsches Tierschutzgesetz or the US Public Health Service Policy on Humane Care and Use of Laboratory Animals. All research was conducted in accordance with the laws of the Republic of the Congo, the Democratic Republic of the Congo, Cameroon, Nigeria, the Central African Republic, Rwanda, Uganda, and Germany. The study complied with the American Society of Primatologist's principles for the ethical treatment of nonhuman primates and followed the American Society of Primatologists Code of Best Practices for Field Primatology.

2.2 | Sampling

The study is based on 245 noninvasively collected fecal samples from individual wild male gorillas (13 Cross River, 167 western lowland, 29 mountain, and 36 Grauer's gorillas) and blood or tissue samples from 12 captive male gorillas (two Grauer's and 10 western lowland gorillas) described in previously published studies (Arandjelovic et al., 2010; Bergl & Vigilant, 2007; Fünfstück et al., 2014; Roy,

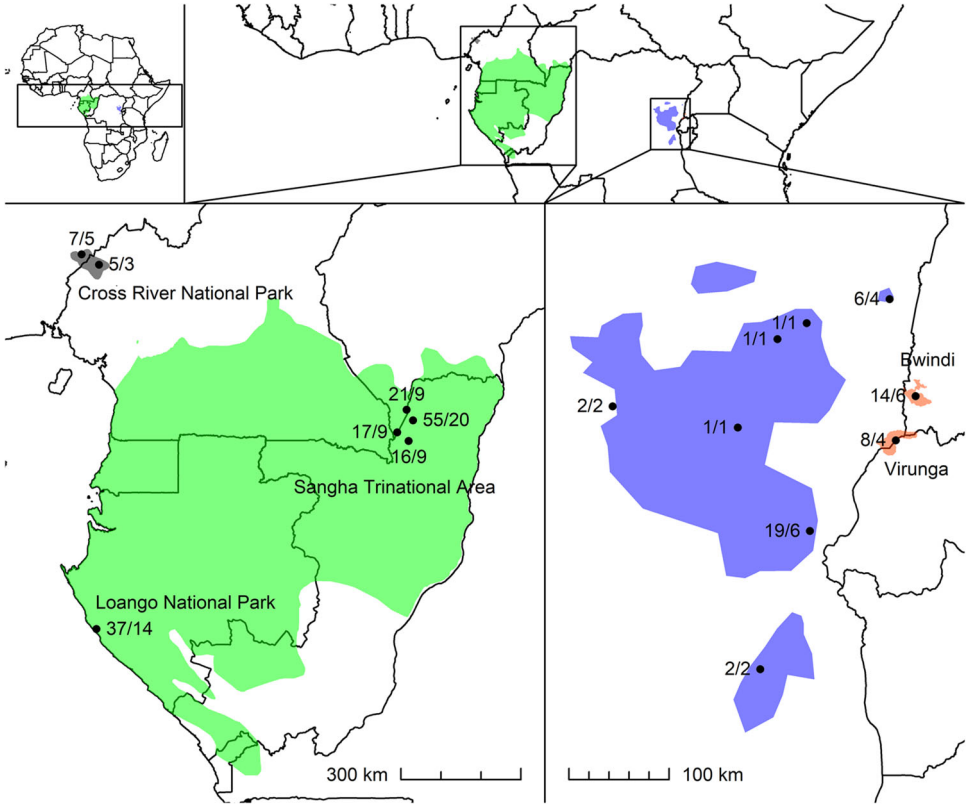

FIGURE 1 Map of sampling locations of gorilla fecal samples used for genetic analysis. Colored areas represent the distribution of the four gorilla subspecies according to the NatureServe and International Union for Conservation of Nature (IUCN) (2016) database (gray: *Gorilla gorilla diehli*, green: *G. g. gorilla*, orange: *G. beringei beringei*, and blue: *G. b. graueri*). Sampling locations are shown as black dots. The first number next to a sampling location indicates the number of genotypes that could be assigned a haplotype, and the second number indicates the number of unique Y-chromosomal haplotypes at a site. In this map, haplotype assignment is based on 10 loci

Arandjelovic, et al., 2014; Thalmann et al., 2007). The capture location of the two Grauer's gorillas was known. The 10 captive western lowland individuals were either born in captivity or their exact capture location was unknown when born in the wild; however, six of them have likely origins in Cameroon (see Table S3 for suspected origins). Wild western lowland gorillas were sampled from Loango National Park (Gabon) and the Sangha Trinational area (Mondika, the Goualougou Triangle, Mbeli Bai [Republic of the Congo] and Bai Hokou [Central African Republic]). Cross River gorilla samples were collected from the Cross River Region (Nigeria and Cameroon). Grauer's gorilla samples were collected from Mt. Tshiaberimu, Kahuzi-Biega National Park, Walikale, and Itombwe Nature Reserve (Democratic Republic of the Congo) (Table S3). Mountain gorilla samples were collected in Bwindi Impenetrable National Park (Uganda) and the Virunga Massif (Rwanda) (Table S3). The majority of fecal samples were obtained from unhabituated populations, and, thus, fecal samples were genotyped at 11–16 autosomal microsatellite loci and one sexing locus to identify samples originating from the same individuals.

In addition to the data generated from these 257 previously published samples, we newly analyzed 61 fecal samples of wild Grauer's gorillas collected between April 2013 and June 2015 at nest sites in Kahuzi-Biega National Park, highland sector (10 samples), Mukingiti & Kingombe Gorilla Reserve (Regomuki, 37 samples), the Usala region (six samples), and Tayna Gorilla Reserve (eight samples).

2.3 | Genotyping

We genotyped the newly collected 61 Grauer's gorilla samples at 16 autosomal loci and identified samples stemming from the same individual according to procedures described in (Hagemann et al., 2018). We then proceeded to generate Y-haplotypes for all-male samples at 10 microsatellite loci (DYs495, DYs622, DYs549, DYs533, DYs543, DYs468, DYs473, DYs546, DYs556, and DYs469) adhering to procedures described in Erler et al. (2004). All genotypes were generated in the same laboratory. Autosomal genotyping was conducted for other projects and those data enabled us to be sure we were not repeatedly typing the same individual from a given site. Previously published Y-chromosomal haplotypes were generated by Mimi Arandjelovic. Y-haplotypes for the set of more recently collected samples were generated by Veronika Städele and were consistent with the earlier genotypes. Specifically, the common Y-haplotype of the two newly collected Kahuzi-Biega samples was identical to the most common haplotype found among the previously genotyped

samples in this same area, thus making it unlikely that assignment of Y-chromosomal alleles was inconsistent due to differences in observers or laboratory machines.

2.4 | Diversity

If not indicated otherwise we performed analyses in R 3.4.0. (R Core Team, 2020).

To facilitate a comparison of diversity among the subspecies despite different samples sizes, we conducted a rarefaction analysis for each diversity measure. Samples of each of the three subspecies with the larger sample sizes were downsampled to the sample size of the subspecies with the smallest sample size 1000 times by randomly drawing subsamples of that size without replacement and calculating the corresponding statistic for the subsample. We present the original value for each statistic, as well as the rarefied mean and its standard deviation and distribution. Analyses were conducted on haplotypes that were either complete at 10 loci or incomplete but unique.

We calculated the number of haplotypes (expected number of haplotypes after downsampling), haplotype diversity (Nei & Tajima, 1981), the allelic diversity, and the modified Garza–Williamson index (G-W index; Garza & Williamson, 2001) for each subspecies to describe current diversity and identify past reductions in population size with the latter estimator. We calculated the modified G-W index as follows:

$$\text{G} - \text{W\_modified} = \frac{k}{R},$$

where k is the number of alleles in the population (here subspecies) and R is the allelic range of all populations in the sample (here the genus as a whole) (Excoffier et al., 2005). R is the difference between the largest and the smallest allele value plus one. While the standard version of the G-W index is sensitive to more recent reductions in population size (Garza & Williamson, 2001), the modified G-W index relates the number of alleles of a subspecies to the range of alleles in all gorillas, thereby capturing how much of the total diversity is retained in the subspecies. Allelic diversity was calculated using the function *poppr* in the "poppr" R package v2.8.5. (Kamvar et al., 2015, 2014). We included all 10 loci in the calculation of estimates of diversity, as these metrics are not, or in the case of the G-W index only moderately (Garza & Williamson, 2001), sensitive to the nonadherence of loci to the strict SMM.

2.5 | The fit of loci to the SMM

We translated the length of the microsatellite alleles into repeat numbers by assigning a length of one repeat to the shortest allele observed for a locus. Haplotypes can be found in the Supporting Information (Table S3).

To identify whether the 10 Y-chromosomal short tandem repeat loci adhered to the SMM across all populations, we performed a quality assessment using the formula published in Bird et al. (2012):

$$q = \frac{|K| S}{r^2},$$

where K is the kurtosis in excess of three (a measure as to what extent the data exhibit outliers relative to a normal distribution, where a normal distribution has a kurtosis of three), S is the skewness of the allele distribution, and r is the allele range size (largest minus smallest allele value). A locus, which conforms perfectly to the SMM, has a quality score of zero. For all following phylogenetic analyses which require adherence of loci to the SMM, we excluded loci with a quality score larger than the recommended threshold of 0.07 (Bird et al., 2012), and loci for which the 95% bootstrap confidence interval, based on 1000 resamplings of the allele distribution, included 0.07.

2.6 | Phylogenetic reconstruction

Phylogenetic inference using microsatellites is impacted by the measure of genetic distance chosen. $(\delta\mu)^2$ is a modification of the average squared distance (ASD) and was designed to overcome problems associated with intra-population variance (Goldstein et al., 1995a, 1995b). Both distance measures have been shown to increase linearly over time and should thus be adequate for estimating the divergence of distantly related taxa (up to several 1000 generations) even when homoplasies due to microsatellite range constraints are considered (Goldstein et al., 1995a, 1995b; Pollock et al., 1998; Feldmann et al., 1997, but see Takezaki & Nei, 2008). One study, comparing the D_A distance (Tateno et al., 1982) to $(\delta\mu)^2$ and three other distance measures, found the D_A distance to most reliably retrieve the correct branching pattern (Takezaki & Nei, 2008). We, therefore, created neighbor-joining trees in POPTREE2 (Takezaki et al., 2010) based on the $(\delta\mu)^2$ and D_A distances. For these analyses, we split western lowland gorillas into haplotypes from the Trinational area and haplotypes from Loango National Park due to their large geographic separation, and mountain gorillas into haplotypes from the Virunga and Bwindi National Parks due to the known lack of present-day gene flow among these populations. For all further analyses requiring genetic distances, we calculated the ASD because it can be applied to calculate distances between single haplotypes.

Noise in microsatellite datasets may lead to conflicting phylogenetic signals. We, therefore, used three alternative methods to infer the phylogenetic relationships among the complete Y-haplotypes and evaluated how consistent our results were across the different methods. We conducted a multidimensional scaling (MDS) analysis and constructed a NeighborNet network (Bryant & Moulton, 2004) and a median-joining network. MDS of the distance matrix over two dimensions was performed using the function *cmdscale* in R 3.4.0. (R Core Team, 2020). The NeighborNet network

was created in the program SplitsTree 4.14.6. (Huson & Bryant, 2006). The NeighborNet network visually represents incompatible signals in the data by highlighting possible, alternative splits as parallel lines, and hence conflicting signals appear as boxes. Thus, the more tree-like the representation, the more certain these parts of the phylogeny are. The median-joining network was created in Network 5.0.0.1 (Bandelt et al., 1999) using default settings.

3 | RESULTS

3.1 | Genotyping

Fifty of the 61 newly collected Grauer's gorilla samples produced autosomal genotypes that could be assigned to 29 unique individuals, 15 of which were male. When genotyped at the Y-chromosomal loci, 12 of these amplified at five loci or more and were thus included in the final analysis (Kahuzi-Biega: 2, Usala: 3, Regomuki: 5, and Tayna: 2).

Overall, we obtained Y-chromosomal genotypes from 269 gorillas. Of these, 207 were complete at all 10 loci while an additional 62 were genotyped at five to nine loci. Overall, genotypes were 96% complete (Cross River: 96%, western lowland: 97%, mountain: 95%, Grauer's gorillas: 89%). The 10 loci had an average of 6.6 ± 2.2 alleles (range: 4–11), and all allele lengths represented multiples of their recognized repeat units (Table S4). We only assigned a genotype to a specific haplotype if it was either complete at 10 loci or incomplete but unique. Thus, 45 of the 62 incomplete genotypes were not attributed to a specific haplotype. In sum, we identified 102 unique haplotypes for 224 individuals (12 captive, 112 wild) (Table S3).

3.2 | Diversity

No haplotypes were shared between subspecies. Within western lowland gorillas, two haplotypes were shared among Bai Hokou, Goualougo and Mbeli Bai. One haplotype was shared among these three locations and Mondika.

At the level of the haplotype (expected number of haplotypes, haplotype diversity), the western lowland gorilla sample showed consistently higher diversity than the other three subspecies (Figure 2, Table S2). Grauer's and Cross River gorillas were more diverse than mountain gorillas. Mountain gorillas showed the lowest diversity at the haplotype level. Similarly, at the locus level, western lowland gorillas had much higher allelic diversity values (mean ± SD: 0.68 ± 0.04; Figure 2, Table S2) than the other three subspecies that had similar allelic diversity values (mean ± SD: Cross River: 0.47; Mountain: 0.50 ± 0.03; Grauer's: 0.46 ± 0.06; Figure 2, Table S2).

For the modified G-W index, the value for western lowland gorillas (mean ± SD: 0.70 ± 0.04) was around two-thirds higher than the lowest values of Cross River (mean: 0.39) and mountain gorillas (mean ± SD: 0.43 ± 0.03), (Figure 2, Table S2). For Grauer's gorillas, the modified G-W index (mean ± SD: 0.50 ± 0.05) was ~15%–30% higher than for Cross River and mountain gorillas but considerably lower than the value for western lowland gorillas (Figure 2, Table S2).

3.3 | The fit of loci to SSM model

The quality scores for eight of the loci ranged from 0.001 to 0.01 (Table S1). The quality scores for DYs546 and DYs473 were above the suggested threshold of 0.07 (0.088 and 0.106; Table S1) and they were thus removed from the dataset for the phylogenetic analyses. After removal of DYs546 and DYs473, 77 unique and complete haplotypes for 211 individuals (11 captives, 200 wild) remained (Table S3).

3.4 | Phylogenetic analyses

In the unrooted neighbor-joining tree based on $(\delta\mu)^2$, western and eastern gorillas formed separate clusters (Figure 3). The western lowland gorillas from the two different geographical locations (the Sangha Trinational area and Loango) clustered together with Cross River gorillas being the next closest taxon (Figure 3). The mountain gorillas from the Bwindi population clustered with the Grauer's gorillas, although very weakly supported, to the exclusion of the mountain gorillas from the Virunga population (Figure 3). The neighbor-joining tree based on the additionally analyzed D_A distance indicated that this distance measure maintained linearity less well than $(\delta\mu)^2$ (Figure S1).

In the MDS plot, most gorillas fell into one of four clusters, with individuals falling outside of these clusters being mostly western gorillas of uncertain geographic origin (Figure 4). Eastern gorillas formed one of the four clusters, within which Grauer's and mountain gorilla haplotypes were not clearly separated from each other (Figure 4, IV). Two Bwindi mountain gorilla haplotypes were more similar to Grauer's gorilla haplotypes than to other mountain gorilla haplotypes. The two mountain gorilla haplotypes from the Virunga Massif were more similar to each other than to any other haplotype.

All but four western gorilla haplotypes (93%) fell into three main clusters (Figure 4, I–III). This clustering did not follow the pattern of geographic origin, with western lowland gorillas from the western Loango population appearing in two of the clusters (I, II) and western lowland gorillas from the eastern Sangha Trinational area falling into all three clusters. Cross River gorilla haplotypes appeared in only one of the three western gorilla clusters (I), but many were as similar to each other as to the western lowland gorilla haplotypes in that cluster (Figure 4).

The median-joining and NeighbourNet networks largely mirrored these patterns: western and eastern gorilla haplotypes clearly clustered by species; subspecies haplotypes also clustered, but to a lesser degree (Figure 5, Figure S2). In both of these analyses, the placement of one Cross River gorilla haplotype that was more similar to eastern gorilla haplotypes than to any other western gorilla haplotypes was not consistent with the MDS plot. Large boxes in the NeigborNet

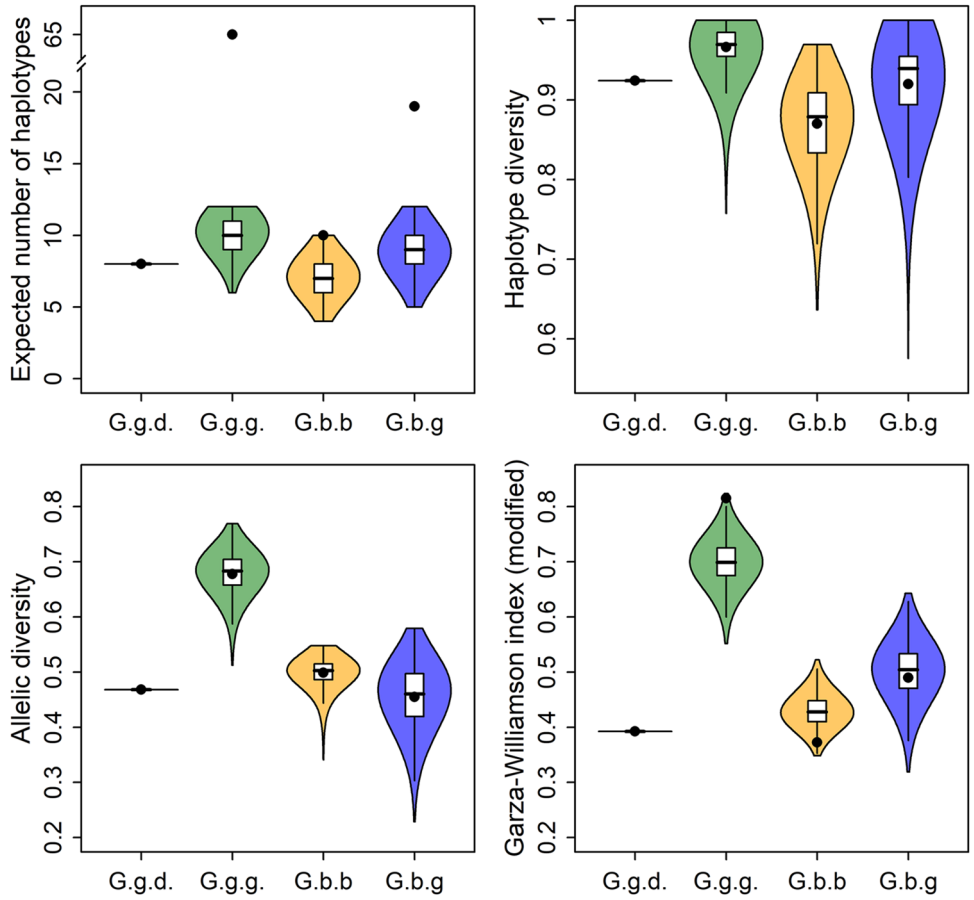

FIGURE 2 Measures of Y-haplotype diversity for *Gorilla sp*. ssp. based on 10-loci haplotypes for Cross River gorillas (*Gorilla gorilla diehli*, $n = 12$), western lowland gorillas (*G. g. gorilla*, $n = 156$), mountain gorillas (*G. beringei beringei*, $n = 22$), and Grauer's gorillas (*G. b. graueri*, $n = 34$). Samples were downsampled 1000 times to the sample size of *G. g. diehli* to enable comparison among the subspecies independent of sample size. The distributions of values generated from these iterations are shown in violin plots. Violins contain boxplots. The tops and bottoms of the violins are minimum and maximum values. Observed values are shown as filled circles

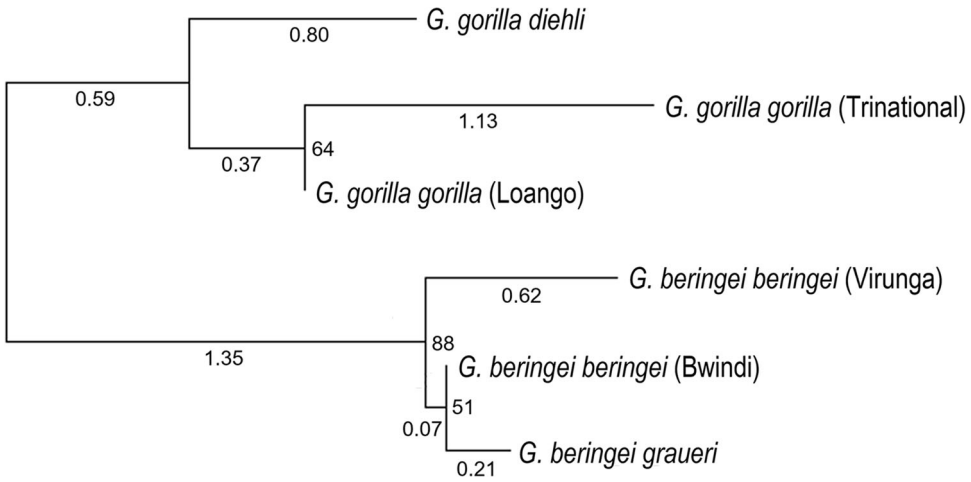

FIGURE 3 Unrooted neighbor-joining tree of eight-loci Y-chromosomal haplotypes. Numbers at nodes are bootstrap support values (1000 bootstraps). Numbers below branches are $(\delta\mu)^2$ distances among population Y-haplotypes.

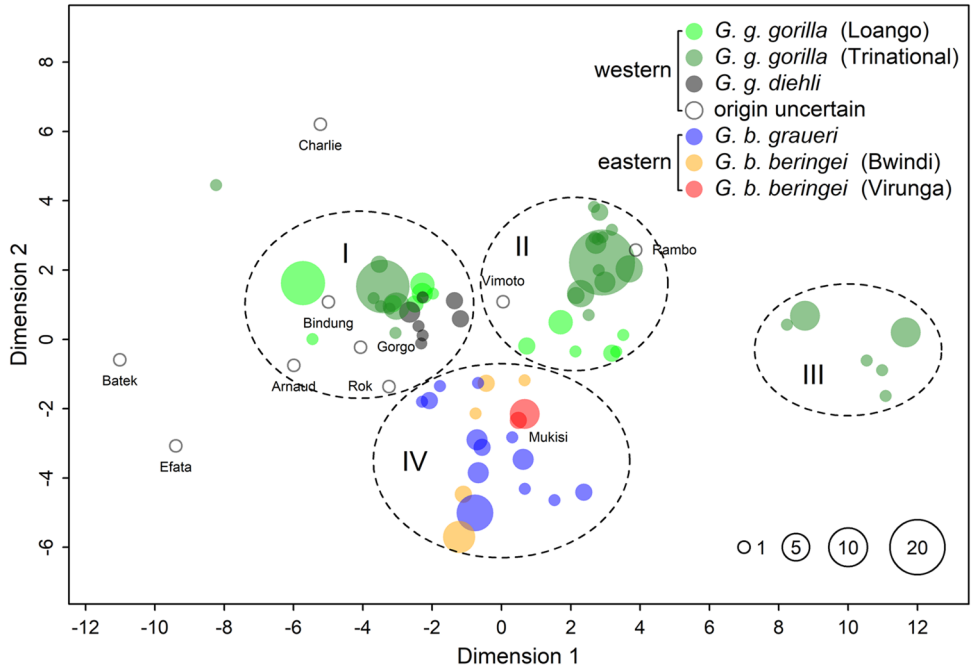

FIGURE 4 Multidimensional scaling plot of eight-loci Y-chromosomal microsatellite haplotypes of all gorilla subspecies. The plot is based on the average squared distance among eight-locus haplotypes. Each circle represents a haplotype. The size of the circles indicates the number of samples sharing the specific haplotype. Open circles represent captive *Gorilla gorilla gorilla* individuals whose exact origins are uncertain. Circles are drawn around three main clusters of western gorilla haplotypes (I–III) as well as all eastern gorilla samples (IV).

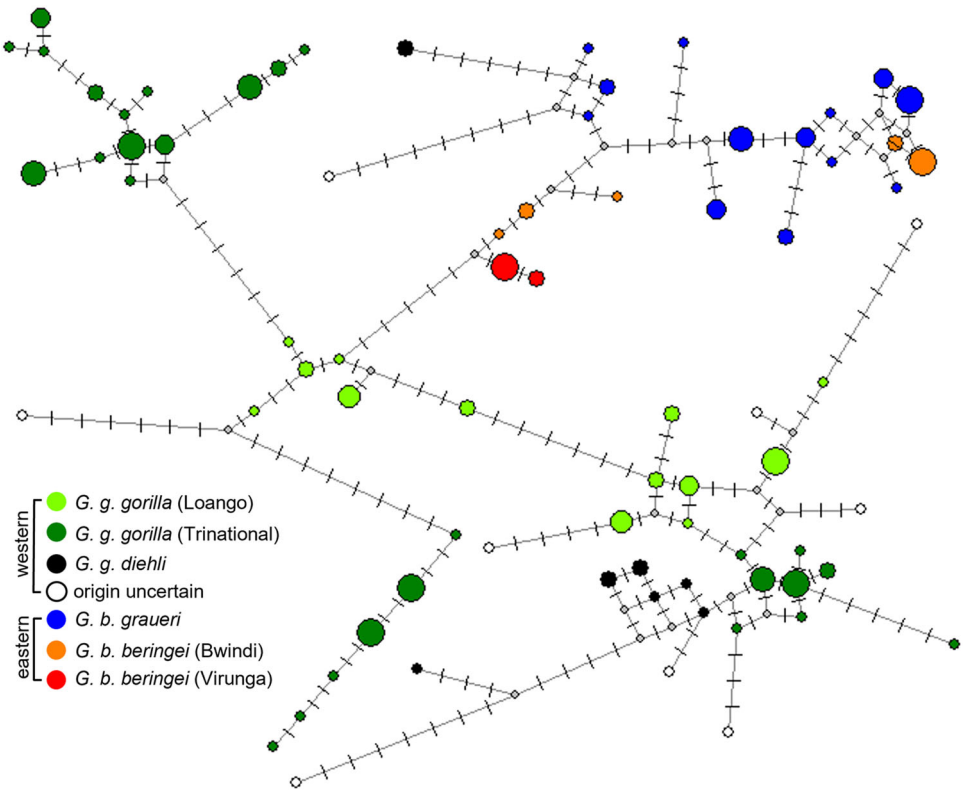

FIGURE 5 Median-joining network of gorilla Y-haplotypes. Each circle represents an eight-locus haplotype. The size of the circles indicates the number of samples sharing this haplotype with the smallest circle representing one haplotype. Open circles represent captive *Gorilla gorilla gorilla* individuals whose exact origins are uncertain. The color of filled circles indicates the subspecies and geographic location of the samples. Gray diamonds represent median vectors. The network was created with Network 5.0.0.3 (Bandelt et al., 1999).

network (Figure S2) indicated large uncertainty about the placement of several haplotypes, in particular about the placement of most of the captive western lowland gorilla haplotypes, which also appeared as genetically distant from other samples of known origin in the MDS plot (Figure 4) and the median-joining network (Figure 5).

The distribution of average genetic distances showed signatures of differing population histories (Figure S3). The distribution for Grauer's gorillas was unimodal pointing towards a population expansion. However, the distribution was slightly right-skewed, and the median-joining network did not show the star-like pattern typical for very recent population expansions (Figure 5). The bimodal distributions for mountain and Cross River gorillas as well as the trimodal distribution for western lowland gorillas indicate more complex population histories. In all haplotype-level analyses, we found haplotypes that were more similar to those of other subspecies than haplotypes of their own subspecies (Figures 4 and 5, Figure S2).

4 | CONCLUSION

In general, analyses separated western from eastern gorillas. Among the four gorilla subspecies, the western lowland gorilla sample was the most diverse for all measures of diversity. We also found several western lowland gorilla haplotypes, which were dissimilar to all other haplotypes and did not fall into any of the main clusters. These were mostly identified in captive individuals with likely origins in Cameroon, an area otherwise not sampled in this study, altogether indicating an even larger Y-haplotype diversity in western lowland gorillas than represented here. We identified three major clusters of Y-haplotypes within western lowland gorillas. The divergence among the haplotypes of these different clusters exceeded the divergence among most Y-haplotypes in any of the other subspecies. This points towards the deep structure in the ancestral western lowland gorilla population and is in accordance with autosomal and mitochondrial data (Anthony et al., 2007; Clifford et al., 2004; Das et al., 2014; Prado-Martinez et al., 2013; Scally et al., 2013). Similarly, STRUCTURE analysis of autosomal data identified structure within western lowland gorillas before separating Grauer's from mountain gorillas (Xue et al., 2015).

However, the Y-haplotype clusters did not correspond to the geographical origin of the samples included in this study. Very dissimilar Y-haplotypes were found within the Sangha Trinational area and very similar Y-haplotypes were found in both Loango National Park in Gabon and the Sangha Trinational area, regions, which are hundreds of kilometers apart. This pattern has, to some extent, been observed for mtDNA and has been attributed to contractions of forests in sub-Saharan Africa during the last glacial maximum that led to the isolation of populations into refugia. Subsequently, recolonization of the expanding forest could have facilitated a mixing of the then diverged haplotypes (Anthony et al., 2007; Clifford et al., 2004; Jensen-Seaman & Kidd, 2001; Thalmann et al., 2007; Tocheri et al., 2016). However, in contrast to the Y-chromosomal data, the geographic distribution of mitochondrial haplotypes and some autosomal data exhibit a pattern of isolation-by-distance (Anthony et al., 2007; Clifford et al., 2004; McManus et al., 2014). The extreme dissociation of Y-chromosomal diversity from a geographic distance may be a consequence of the greater dispersal ranges of males as compared to female gorillas (Douadi et al., 2007; Masi et al., 2021; Roy, Gray, et al., 2014) in combination with the low effective population size of the Y-chromosome due to uniparental inheritance, and higher male reproductive skew brought about by a high degree of polygyny (Bradley et al., 2005; Breuer et al., 2010).

The formation of forest refugia in the Cross River area during the last glacial maximum may have led to the initial divergence of the ancestral Cross River population (Thalmann et al., 2011). Cross River haplotypes were comparatively similar to each other, showed low levels of diversity, and were similar to only one cluster of the three main clusters of western lowland gorilla haplotypes. This is consistent with a recent bottleneck, as detected by autosomal DNA, in which part of the ancestral western gorilla Y-chromosomal variation could have been lost in Cross River gorillas (Thalmann et al., 2011). Within this western cluster, many Cross River haplotypes were not more similar to other Cross River gorilla haplotypes than to western lowland gorilla haplotypes and the node in the neighbor-joining tree separating Cross River from other western gorillas was not well supported (Figure 3). Similarly, only 42% of trees supported Cross River gorillas as the outgroup to western lowland gorillas in a whole-genome study using a single female Cross River gorilla genome (Prado-Martinez et al., 2013). MtDNA also places Cross River gorillas within the variation of western lowland gorillas (Clifford et al., 2004; Prado-Martinez et al., 2013).

In contrast to the majority of previous studies of eastern gorillas based on autosomes or mtDNA, we did not find that Grauer's and mountain gorilla Y-haplotypes formed distinct clusters. This could be due to broader sampling and the larger sample size of this study: Previous studies have often considered only mountain gorillas from the Virunga population or were based on very few samples. The observed pattern is consistent with diversity that was shared among mountain and Grauer's gorillas and randomly reduced by drift in both mountain gorilla populations during their short separation, that is, incomplete lineage sorting. Interestingly, two haplotypes from the Bwindi population were distant from a cluster comprising all other mountain gorilla haplotypes and much more similar to some Grauer's gorilla haplotypes. Assuming that mountain gorilla diversity was reduced to the main clusters of Bwindi and Virunga haplotypes following the separation of the two subspecies, the observed pattern could alternatively be interpreted as an indication of gene flow from Grauer's gorillas into the Bwindi mountain gorilla population. A lack of evidence for gene flow from Grauer's gorillas into the Virunga population is not surprising as our Virunga mountain gorilla sample comprised only two eight-loci Y-haplotypes and the Virunga population underwent a drastic decline in the 70s which may have led to the very recent loss of ancestral variants due to anthropogenic pressures (Weber & Vedder, 1983). Yet, analysis of whole genomes and mtDNA of historical samples indicates that this population may have been characterized by low diversity for much longer (van der

Valk et al., 2019, 2018). Morphological evidence indicates trait similarities between Bwindi mountain and Grauer's gorilla skulls from highland areas of Mt. Tshiaberimu and the highland sector of Kahuzi-Biega, which hampers the taxonomic affiliation of these populations with either *G. b. graueri* or *G. b. beringei* based on morphological traits alone and can be explained by either gene flow between these two populations or parallel adaptations to highland habitats (Groves, 1970; Groves & Stott, 1979).

For Grauer's gorillas, we found a unimodal distribution of genetic distances consistent with a recent population expansion. Yet, allelic diversity, which increases only slowly after reductions of population size (Garza & Williamson, 2001), was low suggesting that the expansion was likely preceded by a reduction in size. This interpretation is in accordance with autosomal and mitochondrial data on Grauer's gorillas (Anthony et al., 2007; Clifford et al., 2004; Jensen-Seaman & Kidd, 2001; van der Valk et al., 2019). However, the loci in this study lie in the nonrecombining region of the Y-chromosome and could thus be linked to loci under selection potentially influencing the observed diversity estimates.

Even though eastern and western gorilla haplotypes were overall clearly separated, many western gorilla haplotypes were not more similar or were even less similar to other western gorilla haplotypes than they were to many eastern gorilla haplotypes. This indicates that, due to the time of separation of the two species that challenges the use of microsatellites for phylogenetic analyses, the linearity of the ASD is starting to decay. In addition, population size changes in either species may have contributed to the loss of linearity (Goldstein et al., 1995a). This effect observed for the haplotype-level analyses based on the ASD disappeared in the population-level tree based on $(\delta\mu)^2$, which placed the subspecies closer in the phylogenetic tree than the species.

Overall, our results derived from eight-loci microsatellite Y-haplotypes are largely consistent with previously suggested scenarios derived from autosomal and mitochondrial sequence data in which western gorilla Y-haplotypes diverged during Pleistocene contractions of forest and highly divergent haplotypes mixed during the subsequent expansion resulting in the most diverse gorilla subspecies. Our study highlights the extent of this Y-chromosomal mixing, finding very similar haplotypes in populations hundreds of kilometers apart. Results from previous studies also indicate that the split of Cross River gorillas from western lowland gorillas was recent (~18 kya), possibly followed by postdivergence gene flow and an eventual reduction in size (Bergl et al., 2008; Thalmann et al., 2011). The population size of the eastern species declined drastically, followed by a population expansion of the Grauer's gorilla (Tocheri et al., 2016; van der Valk et al., 2018; Xue et al., 2015). Despite our efforts to account for the occurrence of homoplasy in our dataset, it is possible that some spurious associations of haplotypes occur. One such association could be the one Cross River haplotype being more similar to all Grauer's gorilla haplotypes than to any other Cross River haplotype in the median-joining tree. However, whole-genome sequencing of three Grauer's and one Cross River gorilla indeed found evidence of historical gene flow between Grauer's and Cross River gorillas (Prado-Martinez et al., 2013). Morphological evidence also suggests historical gene flow among the species (Ackermann & Bishop, 2010). Recent efforts to produce the first gorilla Y-chromosome assembly, despite the Y-chromosome's highly repetitive nature and degradation, should in the future enable time and cost-effective sequencing of further individuals from high-quality DNA and facilitate the identification of SNP panels with higher power for phylogenetic analyses (Cechova et al., 2020; Tomaszkiewicz et al., 2016).

Despite evidence for a population expansion of Grauer's gorillas as well as a relatively large and highly diverse population of western lowland gorillas, it is imperative to point out that Cross River, western, and Grauer's gorillas are currently classified as Critically Endangered and mountain gorillas are considered Endangered (Maisels et al., 2018; Plumptre et al., 2019). Human encroachment on forests for agriculture and logging reduces and fragments gorilla habitat and hunting for bushmeat and Ebola virus outbreaks have drastically reduced present-day population sizes and threaten their long-term viability (Plumptre et al., 2016; Strindberg et al., 2018; Walsh et al., 2003). These reductions in population size in some cases appear to have reduced genetic diversity to the point where it may affect individual fitness. It is estimated that 34% of the mountain gorilla genome and 39% of Grauer's gorilla genome is homozygous indicating excessive inbreeding (van der Valk et al., 2019; Xue et al., 2015).

Moreover, evidence exists for a 77% decline in the size of the Grauer's gorilla population in a single generation, which has led to erosion of genetic diversity and an increase in the frequency of deleterious mutations (Plumptre et al., 2016; van der Valk et al., 2019). While these examples highlight the evolutionary fate of specific populations, the gorilla genus as a whole is still highly diverse harboring great adaptive potential; thus, conservation measures should be taken to maintain viable populations of each subspecies.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

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DATA AVAILABILITY STATEMENT

The data in this study are available in the Supporting Information of this article.

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