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RESEARCH ARTICLE

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# Population-level assessment of genetic diversity and habitat fragmentation in critically endangered Grauer's gorillas

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## Abstract

**Objectives:** The critically endangered Grauer's gorilla (*Gorilla beringei graueri*) has experienced an estimated 77% population decline within a single generation. Although crucial for informed conservation decisions, there is no clear understanding about population structure and distribution of genetic diversity across the species' highly fragmented range. We fill this gap by studying several core and peripheral Grauer's gorilla populations throughout their distribution range.

**Materials and Methods:** We generated genetic profiles for a sampling of an unstudied population of Grauer's gorillas from within the species' core range at 13 autosomal microsatellite loci and combined them with previously published and newly generated data from four other Grauer's gorilla populations, two mountain gorilla populations, and one western lowland gorilla population.

**Results:** In agreement with previous studies, the genetic diversity of Grauer's gorillas is intermediate, falling between western lowland and mountain gorillas. Among Grauer's gorilla populations, we observe lower genetic diversity and high differentiation in peripheral compared with central populations, indicating a strong effect of genetic drift and limited gene flow among small, isolated forest fragments.

**Discussion:** Although genetically less diverse, peripheral populations are frequently essential for the long-term persistence of a species and migration between peripheral and core populations may significantly enrich the overall species genetic diversity. Thus, in addition to central Grauer's gorilla populations from the core of the distribution range that clearly deserve conservation attention, we argue that conservation strategies aiming to ensure long-term species viability should include preserving peripheral populations and enhancing habitat connectivity.

**KEYWORDS**
eastern Democratic Republic of Congo, feces, microsatellites, noninvasive samples, population structure

## 1 | INTRODUCTION

Genetic diversity is broadly considered a pre-requisite for the long-term survival of populations and species (Frankham, Ballou, & Briscoe, 2010). Population fragmentation and reduction in population size are two leading forces behind genetic diversity loss (Méndez, Vögeli, Tella, & Godoy, 2014). Small isolated populations are vulnerable to stochastic processes (Lande, 1993; Melbourne & Hastings, 2008) and drift becomes the dominant evolutionary force, overriding the effect of selection. The genetic consequence of small population size is frequently an increase in slightly deleterious mutations that may become fixed through mating with close relatives. Generally, increased levels of inbreeding and loss of genetic diversity threaten long-term population survival and may lead to population extinction through decreased fertility, reduced ability to adapt to environmental changes, and enhanced susceptibility to infectious disease (Evans & Sheldon, 2008; Keller &

  

Waller, 2002; O'Grady et al., 2006; Smith, Sax, & Lafferty, 2006; Willi, Van Buskirk, & Hoffmann, 2006). Threatened populations are frequently impacted by a multitude of factors starting with small long-term effective population size (Abascal et al., 2016; Xue et al., 2015), low genetic diversity, habitat fragmentation and loss (Bergl, Bradley, Nsubuga, & Vigilant, 2008; Casas-Marce, Soriano, López-Bao, & Godoy, 2013), and other anthropogenic pressures (Brown, Peacock, & Ritchie, 2016; Wang, Qiao, Li, Pan, & Yao, 2017).

Faced with the challenge of limited financial and logistic resources, conservation efforts are frequently directed towards the largest and most genetically diverse populations (Petit, El Mousadik, & Pons, 1998). As a result, smaller populations may be left without formal protection. However, such small, genetically depauperate populations frequently harbor unique genetic variants as a consequence of the combined effects of genetic drift and natural selection in divergent marginal habitats (Lesica & Allendorf, 1995). In recent years, a growing amount of evidence indicates that immigration from inbred peripheral populations can significantly contribute to survival and health of larger central populations (Chen et al., 2016; Robles & Ciudad, 2017). Therefore, for any given conservation decision, it is important to evaluate how genetic diversity is distributed across the species' range. Such understanding may lead to balanced conservation strategies that incorporate small isolated populations in a more sustainable, long-term species conservation approach.

Here we study the distribution of genetic diversity in gorillas, with particular focus on the Grauer's gorillas (*Gorilla beringei graueri*) endemic to the eastern Democratic Republic of Congo (DRC). All four gorilla subspecies are critically endangered (IUCN, 2016) through a combination of threats from bushmeat hunting, habitat loss and degradation, and spread of infectious disease (Bermejo et al., 2006; Caillaud et al., 2006; Junker et al., 2012). The four subspecies differ markedly in population and range sizes, levels of genetic diversity and extend of habitat fragmentation (Fünfstück & Vigilant, 2015), partly as result of biogeographic processes and partly as a consequence of recent anthropogenic activities (Bergl & Vigilant, 2007; Plumptre et al., 2016; Roy et al., 2014a; Xue et al., 2015). Western lowland gorillas (*G. gorilla gorilla*) occupy the largest range of all gorilla taxa, inhabiting largely continuous habitat that allows for high rates of gene flow (Fünfstück & Vigilant, 2015; IUCN, 2016). They also have the largest census population size of all gorilla species, with an estimated 140,000 individuals (Williamson, Maisels, & Groves, 2013). Only a few hundred Cross-River gorillas (*G. g. diehli*) survive in highly fragmented forest across the Nigerian-Cameroon border (Bergl et al., 2012; Dunn et al., 2014). Both eastern gorilla subspecies, mountain gorillas (*G. b. beringei*) and Grauer's gorillas (*G. b. graueri*), went through a pronounced continuous population decline over the past 100,000 years (Roy et al., 2014a; Xue et al., 2015). Both isolated populations of mountain gorillas receive dedicated conservation attention and are increasing in number, currently totaling about 900 individuals (Gray et al., 2013; Roy et al., 2014b). In contrast, Grauer's gorillas experienced a dramatic population decline of 77% in the last 20 years, with population size estimates today of only about 3,800 individuals (Plumptre et al., 2016). Research and conservation activities within the range of the Grauer's gorilla are impeded by political instability in the DRC and therefore Grauer's gorilla is probably the least studied gorilla taxon. Information about the distribution of genetic diversity and the potential for gene flow among the highly fragmented and isolated populations is urgently needed to develop and implement effective conservation strategies for this critically endangered great ape.

Here we perform comparative analyses of genetic diversity and differentiation among several Grauer's gorilla populations from throughout the species range. To this end, we generated novel data from an unstudied core Grauer's gorilla population and additional data for the best-studied population of this taxon from the Kahuzi-Biega National Park (KBNP). After combining the newly generated and published data, our complete Grauer's gorilla dataset contains samples of three peripheral and two central populations. To put the genetic diversity and population structure of Grauer's gorilla into a broader context, we included a sampling of genetic profiles of both mountain gorilla populations and one western lowland gorilla population (Arandjelovic et al., 2010; Guschanski et al., 2009; Roy et al., 2014a). We used microsatellite genotyping (i) to compare genetic diversity among eastern gorilla populations; (ii) to estimate levels of differentiation among five Grauer's gorilla populations and infer any genetic structure; and (iii) to test if peripheral and central populations of this species differ from each other in genetic diversity.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection and study dataset

In 2014, noninvasive fecal samples were collected from the night nests of two Grauer's gorilla social groups in two different locations following the 2-step collection method (Nsubuga et al., 2004). Nineteen samples were collected from the nest of the Chimanuka group in the high-altitude sector of KBNP at 2,390 m above sea level (2° 18′ 42″ S/28° 44′ 24″ E). We refer to this sample set as KB-2014. Thirty-nine samples were collected from an unhabituated group ranging in the Nkuba Research and Conservation Area in the Walikale territory, north Kivu (hereafter referred to as Nkuba), between the low-altitude sector of KBNP and Maïko National Park at 610–640 m above sea level (1° 29′ 35″ S/27° 45′ 47″ E, Figure 1 and Table 1). In this region, samples from the same nest site were collected over two consecutive days due to adverse weather conditions, so that the majority of nests was sampled multiple times.

We complemented these new Grauer's gorilla samples with previously published data from three additional populations that were sampled between 2000 and 2004 in the Itombwe Massif Natural Reserve (IMNR), Mount Tshiaberimu, and Walikale and a gorilla group sampled in the high-altitude sector of KBNP in 2000, referred to as KB-2000 (Roy et al., 2014a). We also included a subset of data from two populations of mountain gorillas from Bwindi Impenetrable NP (BINP, Uganda) (Guschanski et al., 2009) and the Virunga Massif (Bradley et al., 2005). A single western lowland gorilla population from Loango NP (Gabon) (Arandjelovic et al., 2010) was included to provide comparison with a demographically relatively stable gorilla taxon that is

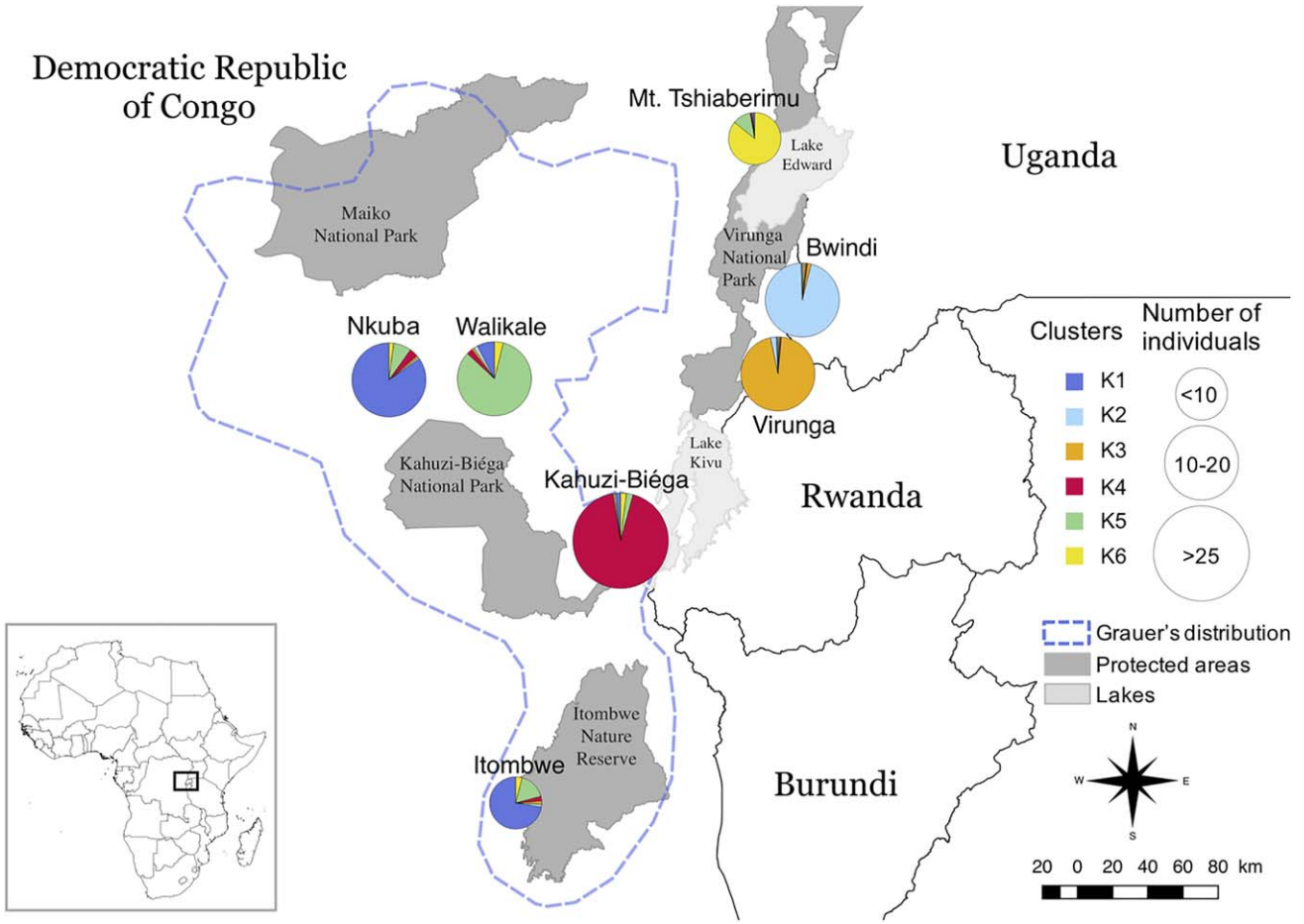


**FIGURE 1** Geographic location and genetic population structure of eastern gorillas. The easternmost Bwindi and Virunga populations are mountain gorillas, whereas all other populations are Grauer's gorillas. The Loango population of western lowland gorillas from Gabon is not shown. The location of the pie-charts represents the approximate sampling location, pie-chart size corresponds to the number of unique individuals (see Table 1), and the color of the pie-chart reflects the proportion of the group's genome membership in one of the six genetic clusters as inferred using Structure

characterized by comparatively large census and long term effective population size (IUCN, 2016; Prado-Martinez et al., 2013; Xue et al., 2015). We selected mountain and western lowland gorilla samples maximizing the completeness of genotyping information for the study loci and aiming for similar sample sizes as our Grauer's gorilla datasets. Furthermore, as newly generated and published Grauer's gorilla datasets likely correspond to a limited number of social groups, we matched the relatedness structure for mountain and western lowland gorillas, selecting samples from several social groups that can be expected to contain relatives (Supporting Information Table S1).

## 2.2 | Microsatellite genotyping

Samples were stored in the field for up to two months at ambient temperature and then transferred to Uppsala University, where they were stored for 8–12 months at 4°C prior to DNA extraction. Noninvasively collected fecal samples ($n = 58$) were extracted with QIAamp® DNA Stool Mini Kit (QIAGEN) following the manufacturer's protocol with slight modifications (Nsubuga et al., 2004). Nearly half ($n = 24$) of these extracts failed to amplify during standard PCR reaction for the amelogenin locus (Bradley, Chambers, & Vigilant, 2001) and, using a dilution test, were shown to contain PCR inhibitors. The corresponding samples were re-extracted with the 2CTAB/PCI method (Vallet, Petit, Gatti, Levréro, & Ménard, 2008) with several modifications: no RNA digestion was carried out and second lysis step was shortened to one hour.

All samples were sexed using the amelogenin assay (Bradley et al., 2001) and genotyped at 13 autosomal microsatellites loci (D1s2130, D1s550, D2s1326, D5s1457, D5s1470, D6s474, D6s1056, D7s817, D8s1106, D10s1432, D14s306, D16s2624, VWF, see Guschanski et al., 2009) using a two-step multiplexing approach (Arandjelovic et al., 2009) with a few modifications. In the singleplex reaction, we increased the amount of $MgCl_2$ to 1.125 mM, decreased the amount of fluorescently labeled forward primers (FAM, VIC, or NED) and the unlabeled nested reverse primer to 0.20 mM, and used 1 μl of the 1:100 diluted multiplex PCR product as template in a 10 μl reaction. All PCR reactions were performed in a 2720 Thermal cycler (Applied Biosystem). Singleplex products were combined according to primer dyes and allele size ranges and electrophoresed on an ABI genetic analyzer 3730XL. Alleles sizes were manually scored relative to an internal size

**TABLE 1** Genetic diversity estimates in the study populations

| Taxon | Population | Site | Individuals | $H_O$ | $H_E$ | NA | $A_E$ | $F_{IS}$ | $A_R$ | $R$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Western gorilla | Loango | Loango NP | 18 | 0.75 | 0.71 | 5.62 | 3.34 | −0.052 | 3.25 | 0.18 |
| Mountain gorilla | Bwindi | BINP | 20 | 0.68 | 0.64 | 4.77 | 2.97 | −0.056 | 2.96 | 0.30 |
| | Virunga | VNP | 19 | 0.49 | 0.55 | 3.77 | 2.34 | 0.098 | 2.51 | 0.36 |
| Grauer's gorilla | Nkuba | Nkuba | 18 | 0.67 | 0.70 | 5 | 3.51 | 0.053 | 3.24 | 0.12 |
| | Walikale | Walikale | 15 | 0.70 | 0.68 | 4.62 | 3.05 | −0.036 | 3.08 | 0.22 |
| | Itombwe | IMNR | 6 | 0.72 | 0.69 | 3.46 | 2.82 | −0.054 | 2.99 | 0.12 |
| | Mt. Tshiaberimu | Mt. Tshiaberimu | 9 | 0.59 | 0.57 | 3.23 | 2.35 | −0.037 | 2.53 | 0.42 |
| | KB-2000 | KBNP | 29 | 0.68 | 0.63 | 4.23 | 2.83 | −0.073 | 2.8 | 0.27 |
| | KB-2014 | KBNP | 14 | 0.61 | 0.58 | 3.62 | 2.43 | −0.057 | 2.56 | 0.32 |
| | KB-2000 + KB-2014 | KBNP | 43 | 0.66 | 0.63 | 4.38 | 2.85 | −0.034 | 2.8 | 0.17 |

Abbreviations ($H_O$ = observed heterozygosity; $H_E$ = expected heterozygosity, NA = mean number of alleles, $A_E$ = effective number of alleles; $F_{IS}$ = inbreeding index; $A_R$ = allelic richness; $R$ = average relatedness values based on Wang's estimator corrected for sample size.

standard (GeneScan GS600LIZ) in GeneMapper version 3.7 (Applied Biosystems).

Each locus was amplified at least four and up to 14 times. Genotypes were accepted as reliable if a heterozygote was observed at least twice in two independent reactions and a homozygote was observed at least three times independently (Frantz et al., 2003). Additional checks were put in place to confirm the newly generated genotypes. For the KB-2014 sample set, we genotyped additional samples collected from individually identified members of the Chimanuka group using the same criteria as above. We could match, without mismatches, 13 of the 14 identified individuals using identity check function in Cervus version 3.0 (Kalinowski, Taper, & Marshall, 2007, see below for details). The single individual without correspondence between the two datasets was collected twice within the KB-2014 sample set and both samples yielded identical genotypes (Supporting Information Table S2). For the Nkuba social group, 13 of the 18 identified individuals were sampled multiple times and all yielded identical genotypes (Supporting Information Table S2). Furthermore, to ensure compatibility of the newly generated genotypes with published data, we also re-genotyped two mountain gorilla samples from the Virunga Massif that were part of the published dataset (Roy et al., 2014a). A translation table was computed based on these samples and applied to the newly generated data.

## 2.3 | Individual identification and parent-offspring dyads

To detect instances of repeated sampling of individuals, we compared genotypes at a minimum of four loci using the identity check function in Cervus version 3.0 (Kalinowski et al., 2007). Genotypes that mismatched at one or two loci were re-examined for possible genotyping errors or allelic dropout. The probability of identity statistics pID and pIDsib (Waits, Luikart, & Taberlet, 2001) for each putative match were calculated in Cervus and used to ensure that our loci could reliably discriminate individuals before merging the data into consensus genotypes.

To identify parent-offspring dyads we also performed parentage analysis in Cervus. The presence of related individuals can lead to reduced estimates of genetic diversity and increased signal of genetic differentiation among populations. We therefore calculated mean relatedness values for each population with Wang's estimator corrected for sample size (Wang, 2002) in the R package *Demerelate* (Kraemer & Gerlach, 2017) and assessed its correlation to measures of genetic diversity using Spearman's rank correlation coefficient $r$.

## 2.4 | Null alleles, linkage, and Hardy-Weinberg equilibrium (HWE)

After collapsing repeatedly sampled individuals into consensus genotypes, the frequency of null alleles at each locus was estimated with FreeNa (Chapuis & Estoup, 2007) using the Expectation Maximization algorithm (Dempster, Laird, & Rubin, 1977). We tested for linkage disequilibrium (LD) between all pairs of loci in Genepop 4.2 (Raymond & Rousset, 1995) and for deviation from HWE in Arlequin version 3.5 (Excoffier & Lischer, 2010). We conducted all tests treating the complete data as a single population and for each population separately. Adjustment for multiple testing was performed in R version 3.3 (Team R Core, 2016) using Bonferroni correction (Rice, 1989).

## 2.5 | Genetic diversity estimates

We assessed the genetic diversity of each gorilla subspecies and each population using standard measures of heterozygosity and allelic diversity. We computed observed ($H_O$) and expected ($H_E$) heterozygosity in Arlequin (Excoffier & Lischer, 2010), using Levene's (1949) correction for small sample size. In addition, we also computed the mean number of alleles per locus (NA) and effective allele number ($A_E$) in PopGene

version 1.32 (Yeh, Yang, Boiley, Ye, & Mao, 1999). FSTAT 2.9 (Goudet, 1995) was used to estimate Wright's inbreeding coefficient ($F_{IS}$) and to calculate allelic richness ($A_R$), a measure that accounts for differences in sample size in our dataset. Statistical significance of differences between subspecies and populations in these estimates was assessed with a Friedman test, followed by a posthoc procedure based on Fisher's least significant difference to identify divergent dyads in the Agricolae R package (de Mendiburu, 2010).

### 2.6 | Population differentiation

As recommended (Meirmans & Hedrick, 2011), we computed two measures of population differentiation, $F_{ST}$ and $F'_{ST}$. Pairwise $F_{ST}$ values were calculated in Arlequin (Excoffier & Lischer, 2010) and their significance was assessed with 110 permutations. We used GenoDive v. 2.0b23 (Meirmans & Van Tienderen, 2004) to compute standardized $F_{ST}$ ($F'_{ST}$), which depicts $F_{ST}$ relative to the maximum value possible given the observed amount of within-population diversity (Meirmans, 2006). Missing values were replaced by a random value picked from the pool of alleles using population-specific allele frequencies.

We conducted a principal component analysis (PCA) for the complete dataset and only Grauer's gorilla populations in Adegenet R package version 1.4 (Jombart & Bateman, 2008). To represent evolutionary relationships between populations, a neighbor-joining tree was constructed in PHYLIP 3.696 (Felsenstein, 1989) from Jost's $D_{EST}$ genetic distance matrix (Jost, 2008) calculated in SMOGD (Crawdord, 2010). Population structure was assessed in Structure 2.3 (Pritchard, Stephens, & Donnelly, 2000) for the eastern gorillas in an unsupervised manner, without including information about population origin. We conducted 10 independent runs for each assumed number of clusters ($K$) from 2 to 8. We used a burn-in of 50,000 iterations and data collection for 100,000 iterations. The independent runs of each cluster were merged in CLUMPP 1.1 (Jakobsson & Rosenberg, 2007) and visualized in Pophelper R package version 1.2 (Francis, 2017). The most likely value of $K$ was established in Pophelper by determining the value of $K$ with the highest probability, $p(X|K)$ (Pritchard et al., 2000), and by calculating the measure of the second rate of change in the likelihood of $K$, $\Delta K$ (Evanno et al., 2005).

## 3 | RESULTS

### 3.1 | Genotyping data

From the 58 fecal samples, six were excluded from further analysis, as microsatellite amplification failed at 12 or more loci. For the remaining 52 samples, identical genotypes at five or more loci were obtained for nine sample pairs, four trios, and one quad (Supporting Information Table S2). In each case, these genotypes had a pIDsib $<0.01$ and were collapsed into a consensus genotype, and hence these 34 samples represented 14 unique individuals. In a single case we combined samples with higher pIDsib (NB_2_3 and NB_2_34, last triad, Supporting Information Table S2), as they were collected as replicates from the same dung pile. The remaining 18 genotyped samples each represented one individual. Thus, the newly generated dataset contained 32 individuals, 28 of which were genotyped at ten or more loci: 14 individuals from the KB-2014 group and 18 individuals from Nkuba. No identical individuals were found between newly generated and published data, so that the final dataset contained between six and 29 individuals per population (Table 1 and Supporting Information Table S1).

Parentage analysis revealed potential parent-offspring dyads in every Grauer's population in our dataset, including published data, as samples were collected from gorilla social groups that frequently contain pre-dispersal immature offspring. It is therefore very likely that other categories of less readily detected related individuals, such as full and half siblings are present in each population. However, removing related individuals would have considerably reduced the sample size of some populations, and we therefore retained all individuals in our dataset. In addition, parentage analysis revealed the presence of 10 potential parent-offspring dyads between two sets of samples collected from the same location (KBNP) 14 years apart: KB-2000 and KB-2014. Therefore, all analyses were carried out twice, treating the two groups separately and combining them into a single unit.

None of the loci exhibited null allele frequency $>0.20$ and we therefore retained all loci in subsequent analyses (Dakin & Avise, 2004). We observed no departures from HWE and no LD when populations were analyzed separately and when KB-2000 and KB-2014 were grouped together.

### 3.2 | Genetic diversity estimates

Estimated average relatedness within samples of Grauer's gorilla populations ranged from 0.12 to 0.42 (Table 1), suggesting that some sample sets contained a large proportion of close relatives. However, mean relatedness was not correlated to observed heterozygosity, mean number of alleles, effective number of alleles, and allelic richness after Bonferroni correction for multiple testing ($p > .07$). This implies that presence of differing proportions of related individuals in population samples of Grauer's gorillas does not explain differences in genetic diversity estimates observed among these populations.

We found significantly higher $H_E$, NA, and $A_R$ in western lowland gorillas compared to mountain and Grauer's gorilla using Friedman tests ($H_E$: $\chi^2 = 9.38$, df = 2, $p < .01$; NA: $\chi^2 = 13.22$, df = 2, $p < .01$; $A_R$: $\chi^2 = 14$, df = 2, $p < .01$), whereas the other measures ($H_O$, $A_E$, $F_{IS}$) were not significant. No significant differences were found between mountain and Grauer's gorillas analyzed at the species level. At the population level, the global Friedman tests were significant for $H_E$, NA, $A_R$, and $A_E$ ($H_E$: $\chi^2 = 26.89$, df = 8, and $p < .01$; NA: $\chi^2 = 43.62$, df = 8, $p < .01$; $A_R$: $\chi^2 = 28.57$, df = 8, $p < .01$; $A_E$: $\chi^2 = 24.33$, df = 8, $p < .01$), suggesting that differences in these measures of genetic diversity existed between at least some population pairs. The post-hoc test consistently revealed significantly higher genetic diversity in Loango compared with the Virunga mountain gorilla population (Table 2). Similarly, Loango was genetically more diverse than most Grauer's gorilla populations for at least one of the genetic diversity measures. The exceptions were the two central populations within the Grauer's gorilla range, Nkuba and Walikale (Table 2 and Figure 1). Bwindi mountain gorillas

**TABLE 2** Differences in measures of genetic diversity among study populations

| | WLG | (Eastern) MG | | (Eastern) GG | | | | |
|---|---|---|---|---|---|---|---|---|
| | Loango | Bwindi | Virunga | Nkuba | Walikale | Itombwe | Tshiabe-rimu | KB-2000 |
| Diversity measures | $H_E$ NA $A_E$ $A_R$ | $H_E$ NA $A_E$ $A_R$ | $H_E$ NA $A_E$ $A_R$ | $H_E$ NA $A_E$ $A_R$ | $H_E$ NA $A_E$ $A_R$ | $H_E$ NA $A_E$ $A_R$ | $H_E$ NA $A_E$ $A_R$ | $H_E$ NA $A_E$ $A_R$ |
| Virunga | ↑ ↑ ↑ ↑ | – – – – | | | | | | |
| Nkuba | – – – – | – – – – | ↓ – – ↓ | | | | | |
| Walikale | – – – – | – – – – | – – – – | – – – – | | | | |
| Itombwe | – ↑ – – | – ↑ – – | – – – – | – ↑ – – | – – – – | | | |
| Tshiaberimu | ↑ ↑ ↑ ↑ | – ↑ – – | – – – – | ↑ ↑ ↑ ↑ | – ↑ – – | – – – – | | |
| KB-2000 | – ↑ – – | – – – – | – – – – | – – – – | – – – – | – – – – | – – – – | |
| KB-2014 | – ↑ – ↑ | – – – – | – – – – | – ↑ – ↑ | – – – – | – – – – | – – – – | – – – – |
| KB-2000 + KB-2014 | – ↑ – – | – – – – | – – – – | – – – – | – – – – | – – – – | – – – – | |

Each cell contains four diversity indices that were inferred to be significant in at least one comparison (see main text for details) in the following order: expected heterozygosity ($H_E$), mean number of alleles (NA), effective number of alleles ($A_E$), and allelic richness ($A_R$). Populations on the top are compared to the population on the left hand size. An upwards-pointing arrow indicates significantly higher value in the top population. Dashes signify no significant difference.
Abbreviations (WLG = western lowland gorilla; MG = mountain gorilla; GG = Grauer's gorilla).

showed a significantly greater mean number of alleles than two peripheral Grauer's populations, Itombwe and Mt. Tshiaberimu. Within Grauer's gorillas, the centrally located population of Nkuba was genetically more diverse than the peripheral populations of Itombwe, Mt. Tshiaberimu, and KB-2014.

## 3.3 | Population differentiation

All populations were significantly differentiated from one another (Table 3, Supporting Information Table S3). However, $F'_{ST}$ values were low between KB-2000 and KB-2014 (0.17), and between Nkuba and Walikale (0.20), reflecting temporal sampling from the same location and close geographic proximity of populations, respectively. High $F'_{ST}$ values between peripheral Grauer's gorilla populations (e.g., KB and Mt. Tshiaberimu, $F'_{ST} > 0.50$) not only reflect absence of gene flow, but are also likely the result of their overall reduced genetic diversity (see above) and differences in allele frequencies as effect of genetic drift.

The PCA conducted for the entire dataset clearly separated the three gorilla taxa along the first and second principle components (Figure 2a). PC3 separated the two mountain gorilla populations, whereas Grauer's gorilla populations remained tightly clustered (Figure 2b). We repeated the PCA for the dataset containing only Grauer's gorilla populations and found clear separation of the populations from KBNP along PC1 and Mt. Tshiaberimu along PC2 (Figure 2c).

A population tree based on $D_{EST}$ genetic distances corroborated the PCA results by providing evidence for clear separation of Grauer's gorillas from other two gorilla taxa (Figure 3). The western lowland Loango population and the two mountain gorilla populations, Bwindi and Virunga, were located on the longest branches, reflecting their strong genetic differentiation from each other and from Grauer's gorillas. All Grauer's gorilla populations shared a common branch, reflecting their common origin. Low genetic differentiation was observed between Itombwe, Nkuba, and Walikale, as reflected by short branch length. In contrast, Mt. Tshiaberimu and the two groups from KBNP

**TABLE 3** Pairwise $F'_{ST}$ (standardized $F_{ST}$)

| | Loango | Bwindi | Virunga | Nkuba | Walikale | Itombwe | Mt. Tshiaberimu | KB-2000 | KB-2014 |
|---|---|---|---|---|---|---|---|---|---|
| Loango | – | | | | | | | | |
| Bwindi | 0.58 | – | | | | | | | |
| Virunga | 0.62 | 0.64 | – | | | | | | |
| Nkuba | 0.50 | 0.57 | 0.64 | – | | | | | |
| Walikale | 0.50 | 0.50 | 0.62 | 0.20 | – | | | | |
| Itombwe | 0.63 | 0.54 | 0.61 | 0.26 | 0.36 | – | | | |
| Mt. Tshiaberimu | 0.64 | 0.53 | 0.77 | 0.29 | 0.30 | 0.41 | – | | |
| KB-2000 | 0.65 | 0.62 | 0.75 | 0.33 | 0.40 | 0.49 | 0.50 | – | |
| KB-2014 | 0.71 | 0.66 | 0.76 | 0.38 | 0.46 | 0.60 | 0.54 | 0.17 | – |

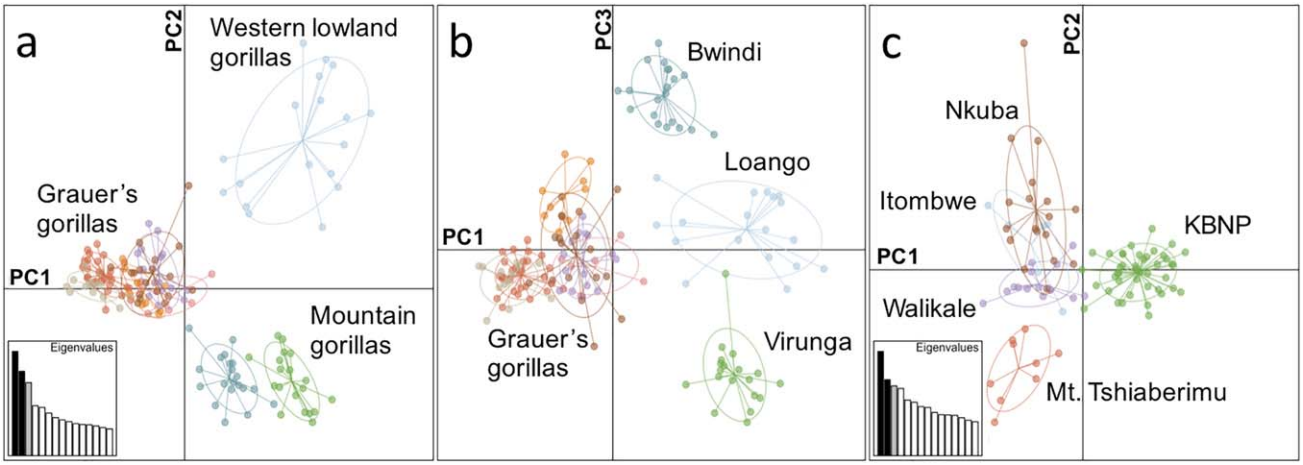


**FIGURE 2** (a and b) PCA of the complete dataset. (c) PCA only for the sample of Grauer's gorilla populations

were located on long branches, indicating their high degree of differentiation from other populations.

Structure analysis of the eastern gorilla dataset first assigned Grauer's and mountain gorillas into separate clusters [optimal $K = 2$ according to $\Delta K$ (Evanno et al., 2005)], reflecting the strongest signal of differentiation (Supporting Information Fig. S1B). With increasing number of $K$, both mountain gorilla populations and subsequently most Grauer's gorilla populations were assigned into separate clusters [Figure 1, Supporting Information Fig. S1a and c, optimal $K = 6$, based on highest $P(X|K)$ (Pritchard et al., 2000)]. As preliminary Structure runs showed no differentiation between KB-2000 and KB-2014, we combined them into a single population. Similar to our population tree analysis and the PCA, Structure indicated the presence of genetic similarities between Itombwe Massif and Nkuba (Supporting Information Fig. S1a).

## 4 | DISCUSSION

### 4.1 | Genetic diversity, population differentiation, and genetic population structure

The relative levels of genetic diversity among gorilla taxa obtained in our study (highest in western lowland gorillas, followed by Grauer's and finally mountain gorillas) are in line with previous work (Fünfstück & Vigilant, 2015; Prado-Martinez et al., 2013; Xue et al., 2015), despite our small sample sizes and inclusion of relatives. Furthermore, although we used microsatellite loci originally developed in western lowland gorillas (Bradley, Boesch, & Vigilant, 2000), which may lead to erroneous inferences in cross-species comparisons (Lachance & Tishkoff, 2013; Rogers & Jorde, 1996), our results are consistent with studies that relied on whole genome and mitochondrial sequencing data, which are free of ascertainment bias (Prado-Martinez et al., 2013; Xue et al., 2015; van der Valk et al, in review). The observed differences among gorilla taxa have been suggested to reflect differences in current effective and census population sizes and are the result of continuous, long-term population decline, limited current distribution range and fragmented nature of the eastern gorilla habitat (Fünfstück & Vigilant, 2015; Plumptre et al., 2016; Roy et al., 2014a; Xue et al., 2015). Among eastern gorillas, Grauer's gorillas tend to show higher values of genetic diversity than mountain gorillas, but the difference is not significant (this study and Fünfstück and Vigilant (2015)). In mountain gorillas, the Bwindi population is generally more genetically diverse than the Virunga population, consistent with previous reports (Fünfstück & Vigilant, 2015). Among Grauer's gorillas, we find that central populations of Walikale and particularly Nkuba are more genetically diverse than populations located at the periphery of the species' range, Mt. Tshiaberimu, Itombwe, and KBNP (Tables 1 and 2).

Different analyses of population differentiation produced highly consistent results. We find that central populations of Nkuba and Walikale show low divergence, whereas peripheral Grauer's gorilla populations of KBNP and Mt. Tshiaberimu are strongly differentiated from the rest (Table 3 and Figures 2 and 3). These results mimic our observations for genetic diversity, highlighting the correspondence between genetic differentiation and genetic diversity estimates that is often found in various study systems (reviewed in Eckert et al., 2008). However, the peripheral Itombwe population showed genetic similarity with

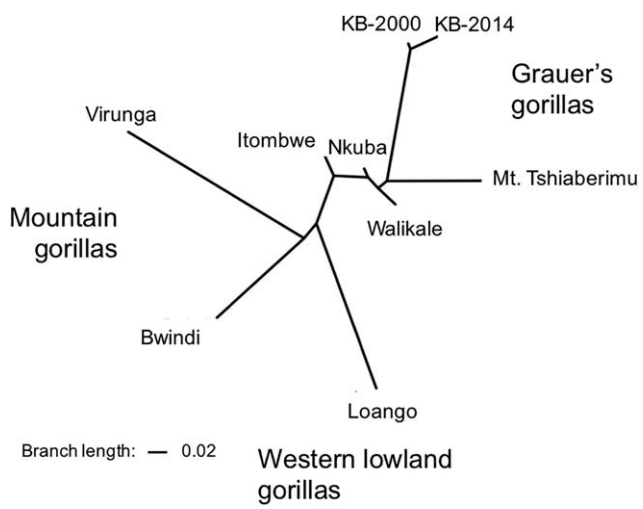


**FIGURE 3** Population tree of the Grauer's, mountain and western lowland gorilla populations based on Jost's $D_{EST}$ measure of genetic differentiation

the central Nkuba population (Table 3, Figures 1–3, and Supporting Information Fig. S1). It is unlikely that gene flow is present between these locations, since they are separated by 250 km of unsuitable habitat, and a high density of human habitation. Rather, the similarity in allele frequencies may be due to the very low sample size for the Itombwe population. Uneven sampling was shown to downwardly bias estimates of population subdivision with Structure (Puechmaille, 2016) and could affect our analyses for Grauer's gorilla populations. In addition, the ability to recover correct population clusters using model-based methods is strongly affected by the presence of missing data and genotyping error, which tend to produce elevated levels of admixture and lead to smaller number of identified clusters (Reeves, Bowker, Fettig, Tembrock, & Richards, 2016). It is thus possible, that stronger genetic differentiation is present in our Grauer's gorilla dataset but remains undetected by Structure due to low sample sizes and the exacerbating effect of unavoidable errors and missing data, typical for non-invasive samples.

## 4.2 | Peripheral versus central populations

Among Grauer's gorillas, we consistently detect higher genetic diversity and lower differentiation in central populations of Walikale and Nkuba compared with populations located at the periphery of the species' range, Mt. Tshiaberimu, Itombwe, and KBNP (Tables 1–3 and Figures 2 and 3). Despite lack of clear population size estimates, the lowland regions from which the two central populations were sampled was found to have the highest gorilla occupancy probability throughout the species' range (Plumptre et al., 2016). However, the effects of poaching in this region have intensified in the last few decades (IUCN, 2016) and therefore low levels of genetic differentiation between Nkuba and Walikale may reflect previous population connectivity, which is not present today.

The peripheral Grauer's gorilla populations from KBNP, Itombwe, and Mt. Tshiaberimu show strongly reduced levels of genetic diversity. The gorilla population in the high-altitude sector of KBNP was found to be slowly recovering from the heavy poaching incidents of the late 1990s, which saw the killing of about 50% of KBNP gorillas (Yamagiwa, 1999). However, it still numbers just over 200 individuals (KBNP directorate, personal communication) and the dispersal corridor into the lowland sector of the national park has been severed by habitat conversion (Amsini et al., 2008). Itombwe represents the southernmost limit of Grauer's gorilla range today, although historically gorillas have occurred even further south, in the bamboo forests of the western rift escarpment (Maldonado et al., 2012). This population is now divided into several reproductively isolated units and has suffered 50% decline since the 1990s, when it numbered ca. 850 individuals (Hall, Saltonstall, Inogwabini, & Omari, 1998; Omari et al., 1999; Plumptre et al., 2016). The entire region is under high anthropogenic pressure and habitat conversion is widespread. Mt. Tshiaberimu is the northern-most habitat of Grauer's gorillas. It is a small forest island of only 60 $km^2$ and is completely isolated from other populations. Whereas 14 individuals inhabited Mt. Tshiaberimu in the mid-1990s (Hall et al., 1998), only six individuals are still present today (Sikubwabo, 2015). The peripheral locations of the KBNP, Itombwe, and Mt. Tshiaberimu populations can partly explain their reduced genetic diversity due to the combined effect of small population size and limited gene flow (Eckert et al., 2008). Thus, we can confirm that peripheral populations in gorillas appear to be genetically less diverse and more highly differentiated from each other than populations from the core range.

## 4.3 | Conservation implications

After an estimated population decline of almost 80% in a single generation (Plumptre et al., 2016), the Red List conservation status of Grauer's gorillas was upgraded to critically endangered (IUCN, 2016). While we cannot quantify genetic diversity loss resulting from this most recent population decline with our data (such inference would require comparisons between modern and historical museum samples that predate the causative events (e.g., Thalmann et al., 2011)), we can speculate about the genetic consequences of this process. In Grauer's gorillas, overall population decline was accompanied by increased fragmentation and extirpation of several, mostly peripheral, populations (Plumptre et al., 2016), likely leading to the loss of rare genetic variants. Core populations have suffered decrease in gorilla abundance. Thus, they will appear relatively stable at first, before the effects of reduced migration from lost populations and increased inbreeding due to lower population size will leave a genetic signature. Population decrease puts the Grauer's gorillas under great risk, as increased inbreeding leads to reduction of genetic diversity, which in turn decreases the species evolutionary potential, by reducing its adaptability, survival and fitness (Frankham et al., 2010). Small populations are also under greater threat from stochastic demographic and environmental processes, which in turn can lead to further reduction in population size. However, the general negative effects of a population bottleneck can be ameliorated if rapid population recovery is possible (Kirkpatrick & Jarne, 2000). Our study demonstrates that compared to mountain gorillas, which are recovering thanks to dedicated conservation efforts, appreciable genetic diversity is still present in Grauer's gorillas, but that it is not evenly distributed across the species range. Specifically, isolated populations are less genetically diverse than those located in the core of the species range. Similar edge effects were observed in peripheral populations of western lowland gorillas (Fünfstück & Vigilant, 2015) and many other species (Eckert et al., 2008).

To assist with recovery of Grauer's gorillas, dedicated conservation measures have to be put in place. Because conservation resources are limited, conservation efforts often prioritize genetically more diverse populations, as these are more likely to persist over time. However, small peripheral populations can be essential for the species survival (Channell, 2004; Leppig & White, 2006; Lesica & Allendorf, 1995). Immigrants from low-diversity, isolated populations into larger central ones can introduce novel variants that help maintain genetic diversity and increase fitness of the central populations (Åkesson et al., 2016; Chen et al., 2016; Hogg, Forbes, Steele, & Luikart, 2006; Vilà et al., 2003). More specifically and directly relevant to our study, negative fitness impact of reduced immigration from genetically less diverse peripheral populations into the central population was shown for

Florida scrub-jays (Chen et al., 2016). Thus, even though peripheral populations may not contain unique genetic variants (as assessed by a limited number of neutral genetic markers, such as microsatellites used here), and show lower genetic diversity and higher inbreeding, they may still introduce important genetic variation that is relevant for the long-term species persistence. Therefore, to be effective in the long run, conservation efforts have to include preservation of small, isolated populations and restoration of connectivity between them and the species' core range. In particular, enabling gene flow among isolated populations is important for the recovery of genetic diversity after a population bottleneck (Jangjoo, Matter, Roland, & Keyghobadi, 2016).

We show that the study site established in 2012 by the Dian Fossey Gorilla Fund and the local community from the village of Nkuba is of particular importance for Grauer's gorilla conservation. It is home to a highly diverse gorilla population and its central location within the Grauer's gorilla distribution range makes it a critical area to ensure gene flow between the protected regions of KBNP and Maiko NP. Low levels of genetic differentiation between Nkuba and Walikale suggest that gene flow between these locations may still be ongoing or was possible until very recently, and calls for particular attention towards maintaining possible dispersal corridors. Our results highlight the importance of establishing community-managed protected areas in the lowland regions between KBNP and Maiko NP to reduce great ape bushmeat hunting, especially around mining sites.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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