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RESEARCH ARTICLE

The Geographic Distribution of Genetic Diversity within Gorillas

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Gorillas, like all non-human great apes, are endangered. Understanding the distribution of genetic diversity across their range is important because low diversity may arise in small populations through increased inbreeding, and, by reducing reproductive fitness, may lead to decreased chances of persistence of a given population. Previous studies found higher genetic diversity in the western (*Gorilla gorilla*) than in the eastern gorillas (*Gorilla beringei*), but rarely employed individuals of known geographic origin to investigate the distribution of diversity across multiple populations. The present study fills that gap by analyzing 1,161 individuals from nine sites across all four currently recognized *Gorilla* subspecies. Genetic diversity at each site was estimated using published data from seven highly-variable microsatellite loci. We found that the small and fragmented populations of Cross River gorillas, eastern lowland gorillas and mountain gorillas were less diverse than any of the five analyzed western lowland gorilla populations. The higher levels of genetic variation within the western lowland gorillas might be best explained by the facts that they (i) exhibit larger present and past effective population sizes than the other subspecies and (ii) maintain higher rates of gene flow through the existence of largely continuous habitat within their range. With regard to conservation, the high genetic diversity within western lowland gorillas is encouraging and retention of dispersal corridors between already protected areas is essential. Am. J. Primatol. 77:974–985, 2015.

Key words: ***gorilla gorilla*; *gorilla beringei*; great apes; population fragments; conservation genetics**

INTRODUCTION

Genetic diversity is one of the three forms of biodiversity recognized by the International Union for Conservation of Nature (IUCN) as deserving global conservation priority [McNeely et al., 1990]. Loss of genetic diversity is connected with inbreeding and inbreeding decreases reproductive fitness [Reed & Frankham, 2003]. Moreover, demographic and environmental stochasticities are more likely to lead to serious consequences in populations with lower genetic variation, as those possess reduced potential for responding to environmental changes [Chang et al., 2012; Willi et al., 2006]. The more genetically diverse a population is, the higher its capacity for adaption to changing environments [Lande & Shannon, 1996]. Accordingly, both experimental [Bijlsma et al., 2000; Frankham, 1995a] and empirical data [e.g. Saccheri et al., 1998; Westemeier, 1998; reviewed in Keller & Waller, 2002], have shown that less genetically diverse or more inbred populations face an increased risk of extinction. Therefore, determination of the genetic diversity of a population may aid in assessing its conservation potential and its degree of isolation [Frankel, 1974; Frankham, 1995a; Keller & Waller, 2002].

Establishing baseline estimates of current day diversity of endangered populations is important for monitoring and detecting of future losses in diversity. This approach may facilitate recognition of extinction vortices, which are characterized by declining census sizes accompanied by loss of fitness and genetic variation with increasing speed [Fagan & Holmes, 2006; Gilpin & Soulé, 1986]. One of the first studies that found empirical evidence for the existence of an extinction vortex in a vertebrate species, the Iberian lynx (*Lynx pardinus*), used repeated measurements to detect decreasing genetic diversity and

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deteriorating demographic traits [Palomares et al., 2012]. Populations that are recognized to be threatened by an extinction vortex may have higher chances of evading final disappearance, because conservation measures such as improving connectivity with outside populations, introduction of individuals [Griffith et al., 1989; Johnson et al., 2010; Madsen et al., 1999] or, more speculatively, genetic engineering [Thomas et al., 2013] might then be applied in time.

Because there are no absolute values that render a population diverse enough to be considered as "fit," spatial comparisons between different populations have been applied more often than monitoring temporal changes within the same population. These studies found across many taxa such as plants [Cruzan, 2001; Jacquemyn et al., 2009], amphibians [Wang et al., 2014], birds [Méndez et al., 2014], and mammals [Sato et al., 2014] that large populations often have higher diversity than small populations [reviewed in Frankham, 1996]. This is due to the fact that populations with a large census size N mostly also have a large effective population size N_e [Frankham, 1995b], a measure indicating the number of individuals in a theoretical idealized population possessing the same diversity characteristics as the study population [Wright, 1931]

It has further been shown that genetic diversity is often lower in fragmented versus continuous landscapes, because fragmented populations experience increased genetic drift and restricted gene flow [e.g. Caizergues et al., 2003; Kyle & Strobeck, 2001]. Interestingly, some studies investigated different species occupying the same fragmented landscapes and found variation in their response to fragmentation. In certain species of rodents [Mech & Hallett, 2001], beetles [Brouat et al., 2003], and spiders [Vandergast et al., 2004], habitat generalists were less susceptible to fragmentation effects than specialists [reviewed in Keyghobadi, 2007].

Many mammalian populations are declining in size and increasing in fragmentation [e.g. daSilva & Mendes, 2008; Mortelliti et al., 2010]. Also the abundance of gorillas is highly threatened due to habitat destruction, fragmentation, poaching and diseases [Le Gouar et al., 2009; Walsh et al., 2003]. Gorillas occur across equatorial Africa from Nigeria in the west to Uganda in the east and from Cameroon in the north to Angola in the south (Fig. 1). The current taxonomy distinguishes two species, the critically endangered western gorilla (*G. gorilla*) and the endangered eastern gorilla (*G. beringei*) [Groves, 2001; Grubb et al., 2003; IUCN, 2014; Stumpf et al., 2003]. Both species are further divided into two subspecies. The western gorillas are represented by the western lowland gorillas (WLGs) (*G.g. gorilla*) and Cross River gorillas

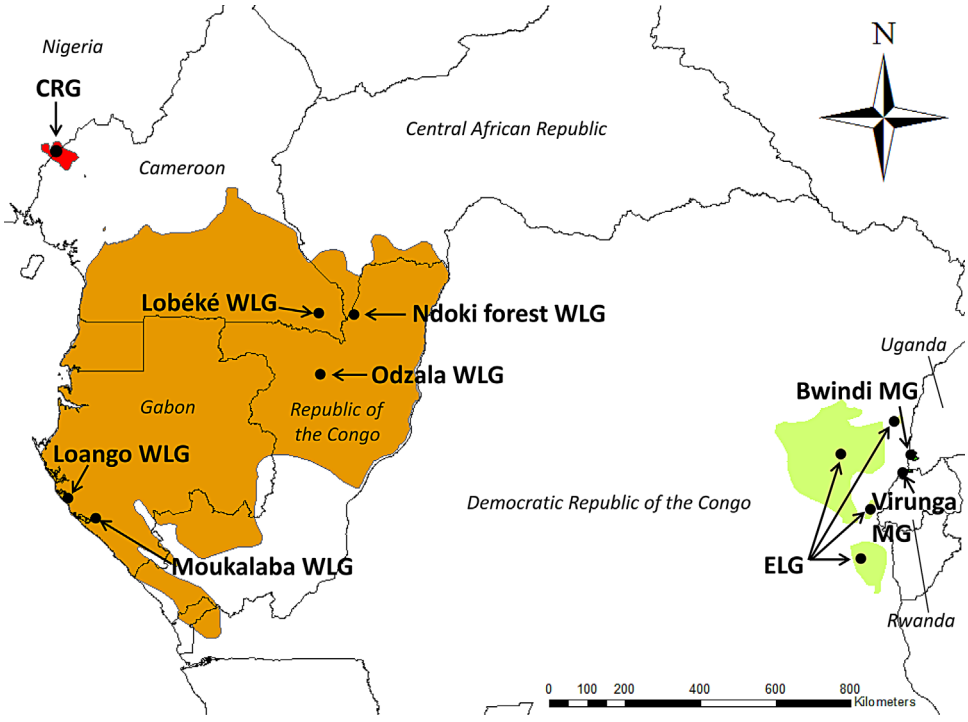

Fig. 1. Map of Central Africa with approximate ranges of Cross River gorillas (CRG) in red, western lowland gorillas (WLG) in orange, eastern lowland gorillas (ELG) in light green and mountain gorillas (MG) in dark green. The locations of the nine gorilla populations analyzed in the present study are indicated by black dots. Notes: (i) the ELG range is currently revised and might actually be smaller and more fragmented; (ii) the analyzed sample of ELGs consists of animals from four different locations; (iii) nearly the entire range of MGs is hidden behind the dots of the Virunga and Bwindi populations.

(CRGs) (*G.g. diehli*) and the eastern gorillas consist of the eastern lowland gorillas (ELGs) (*G.b. graueri*) and the mountain gorillas (MGs) (*G.b. beringei*).

The smallest gorilla populations number only several hundred individuals, but the two ranges of the mountain gorillas are each continuous, highly protected and the populations show increasing census sizes [Gray et al., 2013; Roy et al., 2014b] (Table I). In contrast, the few hundred Cross River gorillas occur in highly fragmented patches and some of these patches are above all located within unprotected areas [Bergl & Vigilant, 2007; Imong et al., 2014a], although potential gorilla habitat still connects the various fragments within the CRG range [Oates et al., 2007]. The total number of ELGs and WLGs, although only poorly known, is higher, and might be on the order of 5,000 and 100,000 individuals, respectively [Butynski 2001; Harcourt, 1996; Maldonado et al., 2012] (Table I). WLGs occupy the largest area of all gorilla subspecies and several protected areas exist within their range. ELGs are the subspecies that occurs across the broadest altitudinal scale, but their territorial range is considerably smaller and more fragmented than those of the WLGs (Fig. 1 and Table I).

As closed-canopy specialists whose distribution does not extend across the savannah–forest boundary, gorillas are expected to be notably susceptible to fragmentation effects and thus constitute a suitable taxon to test the prediction that genetic diversity will be lower in fragmented versus continuous landscapes. Further, because of their markedly varied distribution across Africa, ranging from small scattered populations in the east to a widespread contiguous population in west central Africa, gorillas present a good opportunity for investigation of the relationship between population size and genetic diversity. Although it has already been shown that the genetic diversity of the western gorilla is about twice that of eastern gorillas [Prado-Martinez et al., 2013; Scally et al., 2012,2013; Thalmann et al., 2007], estimating genetic diversity of geographically specified populations may allow the detection of populations with exceptional conservation value. Some surveys from various parts of the western lowland range have found notably different levels of genetic variation [Anthony et al., 2007; Clifford et al., 2004; Douadi et al., 2007; Garner & Ryder, 1996]. Furthermore, the CRGs seem to have lower genetic diversity than other western gorillas [Bergl et al., 2008; Nsubuga et al., 2009; Simons et al., 2013]. Comparisons of nuclear diversity within eastern gorillas have been limited, although recent analysis of eastern gorilla genotypes inferred roughly similar effective population sizes of the two MG populations, whereas the ELG effective population size was approximately three times larger, arguing for higher genetic variation in the ELGs [Roy et al., 2014c].

Here we use published data to assess variation at seven autosomal microsatellite loci from 1,161 individuals from nine wild populations (Fig. 1, Table II) in order to obtain a comprehensive comparative assessment of genetic diversity in gorillas. Autosomal microsatellite loci evolve rapidly, are commonly selectively neutral and biparentally inherited, leading to their widespread use in surveys of genetic variation in wildlife, particularly in species which are sampled noninvasively. Our goals here are to (i) extend the previous finding of higher genetic diversity of western gorillas as compared to eastern gorillas by including many more individuals of known geographic origins; (ii) assess if fragmented and/or small populations exhibit a decreased genetic diversity; (iii) determine the areas of highest and lowest genetic diversity within WLGs; (iv) highlight which populations and/or subspecies of gorillas may face an increased risk of extinction due to low genetic diversity and increased rates of inbreeding. The last point allows estimation of the conservation value of the populations and to speculate about their viability. Due to the limited resources available in conservation such a valuation might be used for effective protection of key populations within both gorilla species.

TABLE I. Approximate Number of Individuals and Size of Ranges of the Four *Gorilla* Subspecies

	Species	Number	Geographical range	Occupancy area
Cross River gorillas (*Gorilla gorilla diehli*)	Western gorilla (*Gorilla gorilla*)	<300 [Oates et al., 2007]	~120,000 km^2 [Oates et al., 2007]	~600 km^2 [Imong et al., 2014b]
Western lowland gorillas (*Gorilla gorilla gorilla*)		Not exactly known, probably ~100,000 [Harcourt, 1996; Butynski, 2001]	~709,000 km^2 [Butynski, 2001]	Not exactly known
Eastern lowland gorillas (*Gorilla beringei graueri*)	Eastern gorilla (*Gorilla beringei*)	Not exactly known, between 2,000 and 10,000 [Maldonado et al., 2012]	~52,000 km^2 [Mehlman, 2008]	21,600 km^2 [Mehlman, 2008]
Mountain gorillas (*Gorilla beringei beringei*)		>880 [Roy et al., 2014b]	~780 km^2 [Mehlman, 2008]	~680 km^2 [Mehlman, 2008]

TABLE II. Genetic Diversity Estimates From Nine Gorilla Populations. Estimates Were Calculated Using Data From Seven Microsatellite Loci That Were Typed in Each of the Population Samples

Species	Subspecies	Population	*Inds*	*Ho*	*He*	*NA*	*Ne*	*I*	*AR*	*F*	Reference
Western gorillas	Western lowland gorillas	Odzala	42	0.75	0.78	7.71	5.14	1.7	6.47	0.03	Fünfstück et al. [2014]
		Lobéké	24	0.75	0.75	6.57	4.36	1.57	6.05	−0.03	
		Ndoki forest	258	0.78	0.74	8.57	4.22	1.6	5.77	−0.05	Fünfstück et al. [2014]; Bradley et al. [2004]
		Moukalaba	38	0.76	0.75	6.57	3.93	1.51	5.38	−0.04	Inoue et al. [2013]
		Loango	85	0.73	0.72	6.86	3.76	1.48	5.21	−0.02	Arandjelovic et al. [2010]
	Cross River gorillas	Cross River gorillas	71	0.65	0.68	6	3.45	1.33	4.57	0.05	Bergl & Vigilant [2007]
Eastern gorillas	Eastern lowland gorillas	Eastern lowland gorillas	64	0.64	0.71	6.14	3.48	1.4	4.87	0.09	Roy et al. [2014c]
	Mountain gorillas	Bwindi	324	0.64	0.64	5.29	2.94	1.19	3.99	−0.01	Guschanski et al. [2008]; Roy et al. [2014b]
		Virunga	255	0.59	0.61	6	2.64	1.14	3.83	0.03	Roy et al. [2014a]

Inds, number of genotyped individuals; *Ho*, observed heterozygosity; *He*, unbiased expected heterozygosity; *NA*, number of alleles; *Ne*, effective number of alleles; *I*, information index; *AR*, allelic richness; *F*, fixation index. All measures are given as means across loci.

METHODS

Samples and Data

We compared the genetic diversity of samples of nine different gorilla (*Gorilla*) populations (Fig. 1, Table II). We based our delineation of populations on geographic distance and barriers to movement between them. We also could have used an exploratory population structure analysis such as STRUCTURE (Pritchard et al., 2000) in order to define populations. However, there is evidence for a geographic patterning of genetic variation across WLGs [Fünfstück et al., 2014]. Furthermore, Fünfstück et al. (2014) showed that there is a strong pattern of IBD across large parts of the WLG range. This can lead to problems for STRUCTURE to infer the correct amount of clusters [Frantz et al., 2009; Fünfstück et al., 2015; Schwartz and McKelvey 2009]. Six of the nine populations that were defined for the present study were western gorillas, including five populations from Odzala, Lobéké, Ndoki forest, Moukalaba and Loango as well as the other western gorilla subspecies, the Cross River gorillas. Although the WLGs from Odzala, Lobéké and Ndoki forest do range in relatively continuous rain forest and are located not very far from each other (~100 to 180 km, Fig. 1) they were considered discrete populations because they are separated from each other by rivers and have been shown to form different genetic clusters [Fünfstück et al., 2014]. Similarly, WLGs from Moukalaba and Loango are separated by only around 100 km, but were considered as separate populations because Loango is isolated by the Atlantic Ocean to the west and a large lagoon to the east [Arandjelovic et al., 2011; Boesch et al., 2007]. Moreover, when we grouped together the individuals from Odzala, Lobéké and Ndoki forest into one and the individuals from Moukalaba and Loango into another population, neither of these two populations were in HWE (results not shown), whereas all five populations were in HWE when analyzed separately (see below), which further strengthens the supposition that there are allele frequency differences between these independently-evolving populations.

Eastern gorillas occur in small fragmentary populations. We sampled the ELG subspecies from four different locations (Itombwe Massif, $n = 6$; Kahuzi-Biega highland sector, $n = 29$; Walikale, $n = 12$; Mount Tshiaberimu, $n = 9$; unknown origin, $n = 8$), and analyzed them together due to the low sample sizes which made testing of allele frequency differences problematic. The samples of the MG subspecies consisted of the two discrete populations from Bwindi Impenetrable National Forest and the Virunga Massif.

To compare the genetic diversity of the nine gorilla populations we used seven loci (D1s550, D2s1326, D4s1627, D5s1470, D7s817, D8s1106, D16s2624) that were typed in all datasets. In cases where different studies used different primers for the amplification of the same loci (e.g., nested primers vs. unnested primers) we translated all genotypes to be consistent with the primers used in [Fünfstück et al., 2014].

For those populations represented by genotypes from more than one publication (WLGs from Ndoki forest [Bradley et al., 2004; Fünfstück et al., 2014] and MGs from Bwindi [Guschanski et al., 2008; Roy et al., 2014b]) we applied CERVUS 3.0 [Kalinowski et al., 2007] to the combined datasets to calculate P_{IDsib} [Waits et al., 2001], the probability

that samples with matching genotypes come from siblings rather than from the same individual. Usually, genotypes are combined into a consensus genotype when the P_{IDsib} is <0.01, whereas in cases in which two genotypes are matching but the P_{IDsib} is >0.01 the less complete genotype is removed from further analyses [Fünfstück et al., 2014, 2015]. However, in order to be consistent with the original published genotypes we did not generate consensus genotypes, but removed the matching genotype that was typed at fewer loci from the dataset even if the P_{IDsib} was <0.01.

We used CERVUS 3.0 to test each dataset for the presence of null alleles. GENEPOP 4.2 [Raymond & Rousset, 1995] was used to perform the exact tests of Guo & Thompson [1992] for deviations from HWE within each population. Linkage disequilibrium (LD) was not examined as it is unlikely that true LD (synteny of loci) could be detected in our sample. The detection of LD may be influenced by factors such as genetic drift, bottlenecks and inbreeding [Hedrick, 2011]. At least some of our populations contain related individuals which can lead to an erroneous detection of LD, because large portions of the genome are identical among related individuals [Myles et al., 2009; Tenesa et al., 2003]. There is also evidence from human studies that linkage might be mistakenly inferred if correcting for background relatedness among individuals was not conducted [Allen-Brady et al., 2003; Weir et al., 2006]. The occurrence of LD between nonsyntenic loci is a common phenomenon that can be attributed to population structure and mating system [Farnir, 2000; McRae et al., 2002; Slate & Pemberton, 2007].

Data Analysis

Using GenAlEx 6.5 [Peakall & Smouse, 2006, 2012] we calculated for each population, as averaged across all loci, the observed heterozygosity *Ho*; the unbiased expected heterozygosity *He*; the number of alleles *NA*; the effective number of alleles *Ne* (incorporates the evenness of an allele frequency distribution and is not biased by the presence of rare alleles); the information index *I* (unlike *He* this is unbounded by 1 and may therefore be a better measure of allelic and genetic diversity [Peakall & Smouse, 2012]) and the fixation index *F* (where values close to zero are expected under random mating, positive values indicate inbreeding or undetected null alleles and negative values indicate excess of heterozygosity [Peakall & Smouse, 2012]). Fstat2.9.3.2 [Goudet, 1995] was used for calculations of allelic richness *AR* (a measure of alleles that controls for differences in sample size). We used numerous measures of diversity due to their differing characteristics and to facilitate comparisons with data from the literature or future studies.

Within the nine-population dataset we tested if the means of *Ho*, *He*, *NA*, *Ne*, *I*, *AR*, and *F* were significantly different between populations by using the following approach. First, we generated for each estimator a matrix including the values of that estimator for each population at each locus. Then we performed for each matrix a global Friedman test to ask whether there were significant differences between the means of a given estimator in at least one pair of populations. Due to the non-independence of the different estimators, we were not able to use family wise error rate (FWER) or false discovery rate (FDR) procedures to account for the problem of multiple testing. Instead, we used an approach suggested by Potter and Griffiths [2006] where we combined the *P*-values derived from the global Friedman tests for each matrix into a χ^2-value as done in Fisher's Omnibus test [Haccou & Meelis, 1992]. We then permuted the measures between populations and within loci (simultaneously in all seven matrices, due to the non-independence of the matrices) 1,000 times, including the original data as one permutation. For each permutation we performed seven Friedman tests (one for each matrix) and then combined their *P*-values into one χ^2-value as for the original data. After that we determined how many of the χ^2 values derived from the permutations were at least as large as the χ^2-value derived from the original data. If that value was smaller than 0.05 we considered that there were significant differences between at least one pair of populations in at least one estimator of genetic diversity. The *P*-values obtained from the global Friedman tests for the original data were then used to determine at which estimator there were significant differences between pairs of populations. To assess which of the 36 pairs of populations were significantly different from each other, individual post hoc Wilcoxon tests were performed for each estimator at which the global Friedman test revealed a *P*-value <0.05. Such post hoc tests are assumed to not need corrections for multiple testing if the global Friedman tests already were significant [Zar, 1999].

We also divided the dataset into five populations (CRGs, WLGs, ELGs, Bwindi MGs, Virunga MGs) for which we could obtain estimates of effective population sizes and census population sizes from the literature [Butynski, 2001; Harcourt, 1996; Maldonado et al., 2012; Oates et al., 2007; Roy et al., 2014b,c; Thalmann et al., 2011]. We determined the correlation coefficients between effective and estimated population size. In order to assess the relationship of population size and genetic diversity we estimated *AR* for each of these populations, using this diversity estimator because it takes into account sample sizes. We determined the correlation coefficients between *AR* and estimated population size as well as between *AR* and effective population size.

Because our study did not involve animal testing we did not violate any regulations of the Deutsches Tierschutzgesetz and the US Public Health Service Policy on Humane Care and Use of Laboratory Animal. The study complied with ASP principles for the ethical treatment of non-human primates. All research was conducted in accordance with the laws of Germany.

RESULTS

Number of Individuals, Null Alleles, Hardy–Weinberg Equilibrium (HWE)

In those populations that were represented by genotypes from more than one publication, no matching genotypes were detected in case of the WLGs from Ndoki forest [Bradley et al., 2004; Fünfstück et al., 2014], whereas 74 pairs of genotypes were matching in the two studies of MGs from Bwindi [Guschanski et al., 2008; Roy et al., 2014b]. We thus removed the less complete genotype from each of the 74 pairs of matching genotypes from the Bwindi MG dataset, leaving us with 24 to 324 genotyped individuals per population (Table II).

Null allele frequency estimates were >0.05 in Odzala WLGs, Lobéké WLGs, Loango WLGs CRGs, and ELGs and reached a maximum of 0.14 for D8s1106 in the CRGs. It is, however, possible that the null allele frequency is overestimated when sample size is small, which was the case for these mentioned populations (between 24 and 85 individuals). The two populations with the highest null allele frequencies (CRGs, ELGs) are also the only two populations out of HWE over all loci. However, the original study reporting the CRG genotypes found that the null alleles were apparently the result of genetic structure in the population, not systematic nonamplification of an allele [Bergl & Vigilant, 2007; Bergl et al., 2008]. Given the similarly fragmented nature of the CRG and ELG populations, we expect that this explanation is also true for the ELGs. In addition, these two populations appear as distinct clusters in a STRUCTURE analysis (results not shown) which groups individuals into clusters by maximizing the HWE within those clusters. Although we cannot ascertain the reason for the presence of a different single locus with null allele frequency estimates greater than 0.05 in each of the Odzala WLGs, Lobéké WLGs, and Loango WLGs, we note that inclusion of these loci in the analyses is inherently conservative by making it more difficult to find high levels of genetic diversity in these populations. Hence, we did not remove any loci from our analyses and treated them within each population as if they were in equilibrium.

Genetic Diversity

For each of the nine gorilla populations we calculated the observed heterozygosity *Ho*, the unbiased expected heterozygosity *He*, the number of alleles *NA*, the effective number of alleles *Ne*, the information index *I*, the allelic richness *AR* and the fixation index *F* (Table II). For these measures higher values are associated with higher diversity with the exception of the fixation index where positive values indicate inbreeding or undetected null alleles and negative values indicate an excess of heterozygosity.

The global Friedman tests indicated that there were significant differences between populations for each measure tested (observed heterozygosity *Ho*: $\chi^2 = 21.03$, $df = 8$, $P = 0.0071$; unbiased expected heterozygosity *He*: $\chi 2 = 20.19$, $df = 8$, $P = 0.0097$; number of alleles *NA*: $\chi^2 = 19.57$, $df = 8$, $P = 0.0121$; effective number of alleles *Ne*: $\chi^2 = 18.48$, $df = 8$, $P = 0.0179$; information index *I*: $\chi^2 = 19.89$, $df = 8$, $P = 0.0108$; allelic richness *AR*: $\chi^2 = 24.72$, $df = 8$, $P = 0.0017$; fixation index *F*: $\chi^2 = 17.40$, $df = 8$, $P = 0.0262$). The permutation test conducted to control for multiple testing revealed that there were significant differences between at least one pair of populations in at least one measure (χ^2 of original data = 65.12, $P = 0.001$), meaning that we could trust the findings of significance from the global Friedman tests. Using post hoc Wilcoxon tests we determined that of the 36 pairwise comparisons between populations possible at each of the seven diversity estimators, five (number of alleles) to 11 (allelic richness) were significantly different from one other ($P < 0.05$) (Table III).

Considering our results by population, each population was compared at each of the seven measures to eight other populations, for a total of 56 comparisons per population. For 25 of these 56 comparisons the Virunga MGs showed a signal of significantly lower diversity than other populations, followed by the CRGs (14 times) and the Bwindi MGs (11 times). Thus, the populations that were genetically least diverse were also the smallest and most isolated ones. The populations that were most often significantly more diverse than other populations were WLGs from Ndoki forest, Moukalaba and Odzala (all 13 times). These populations harbor more individuals and are much less fragmented, ranging in continuous rain forest.

If we were to focus on one diversity measure, the most relevant might be allelic richness, which corrects for differences in sample size and is thus considered to be the most informative means to compare genetic diversity between different populations [Simons et al., 2013]. We found that the Virunga MGs ($AR = 3.83$) were significantly less diverse than ELGs ($AR = 4.87$) as well as WLGs from Loango ($AR = 5.21$), Moukalaba ($AR = 5.38$), Lobéké ($AR = 6.05$), and Odzala ($AR = 6.47$). The

TABLE III. A Matrix Depicting Significant Differences Between Populations

	Odzala	Lobéké	Ndoki forest	Moukalaba	Loango	ELG	CRG	Bwindi
Lobéké	—— -							
Ndoki forest	—— ↑	—↓— -						
Moukalaba	—— -	—— -	—↑— -					
Loango	—— -	—— -	—— -	—— -				
ELGs	—— -	—— -	↑—— ↓	—↑—— -	—— ↓			
CRGs	—↑-↑↑↑ -	—-↑-↑ -	↑-↑↑↑↑ ↓	—↑↑—- -	—— -	—— -		
Bwindi	—↑-↑↑↑ -	—-—↑ -	—↑—- ↓	↑↑-↑-↑ -	—— -	—— -	—— -	
Virunga	—↑-↑↑↑ -	—-↑-↑ -	↑↑↑↑↑- ↓	↑↑-↑↑↑ -	↑—↑↑ -	—↑-↑↑↑ -	—— -	—— ↓

Note that each cell consists of seven entries that are always in the same order: (i) observed heterozygosity *Ho*, (ii) unbiased expected heterozygosity *He*, (iii) number of alleles *NA*, (iv) effective number of alleles *Ne*, (v) information index *I*, (vi) allelic richness *AR*, (vii) fixation index *F*. An upward oriented arrow indicates that the population at the top of the table has a significantly higher value at that estimate than the population on the left side. The opposite case is indicated by a downward oriented arrow. Dashes indicate that no significant differences were found. The fixation index *F* is the only estimate in which higher values are not associated with higher genetic diversity, but rather indicate inbreeding or undetected null alleles. In order to avoid confusion the results for that measure are separated from the six other measures by a space.

Bwindi MGs ($AR = 3.99$) exhibited significantly lower values of allelic richness than WLGs from Moukalaba, Lobéké and Odzala, whereas the CRGs ($AR = 4.57$) exhibited significantly lower values of allelic richness than WLGs from Ndoki forest ($AR = 5.77$), Lobéké and Odzala. Generally, higher values of allelic richness were observed in the WLGs. The Loango population, which exhibited the lowest allelic richness of all WLGs, still showed a greater allelic richness than any population from the three other gorilla subspecies, although that difference was only significant in case of the MGs from Virunga.

Estimates of census and effective population sizes of five populations were available in the literature and highly correlated with each other ($r = 1$). We also compared these sizes with allelic richness, because it accounts for sample size and produced results consistent with the other measures. Both estimated ($r = 0.96$) and effective population size ($r = 0.95$) were highly correlated with allelic richness. Whereas the ELGs, CRGs, and MGs constitute fragmented populations, the WLGs form a largely continuous population and exhibit both the highest estimated and effective population size. Accordingly they also showed the highest allelic richness (Fig. 2).

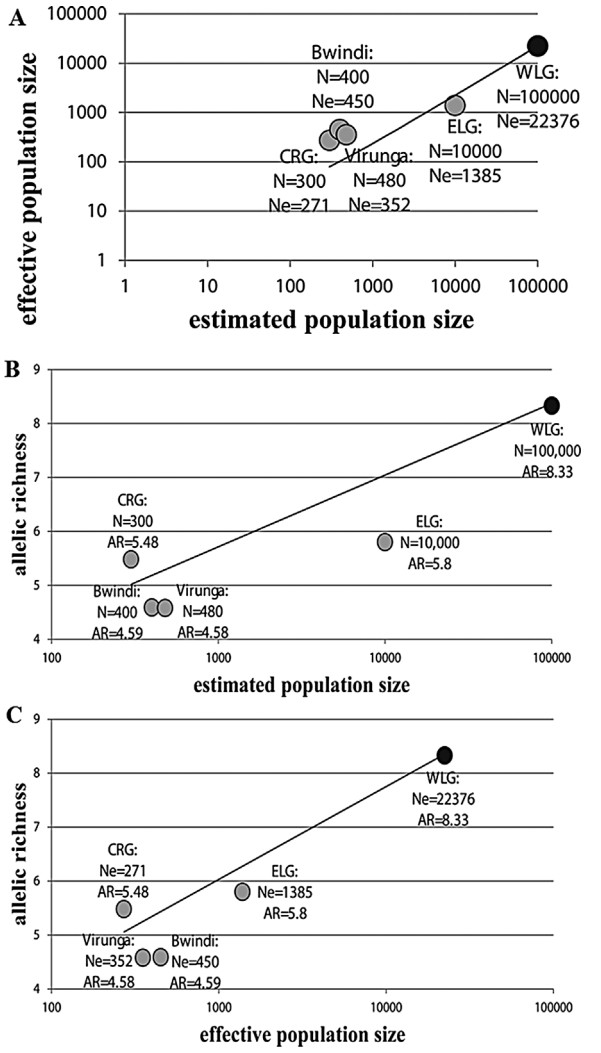

Fig. 2. Effective population size (Ne) plotted against estimated population size (N) (A) as well as allelic richness (AR) plotted against estimated population size (B) and effective population size (C), respectively. Note that estimated and effective population sizes are always in logarithmic scale and that grey circles refer to fragmented and black circles to continuous populations.

DISCUSSION

Comparison Between Eastern and Western Gorillas

All previous studies of the mitochondrial as well as the nuclear genome found lower levels of genetic variation in eastern than in western gorillas [Anthony et al., 2007; Clifford et al., 2004; Garner & Ryder, 1996; Jensen-Seaman & Kidd 2001; McManus et al., 2014; Prado-Martinez et al., 2013; Scally et al., 2012, 2013; Thalmann et al., 2007]. The present work, analyzing autosomal microsatellite genotypes from an unprecedented amount of individuals,

generally corroborates this observation. However, CRGs were not more diverse than the eastern gorillas and no significant differences between CRGs and the eastern populations were present (Table II and III). Thus, instead of a profound difference between the two gorilla species we rather found that the WLG subspecies was significantly more diverse than all other subspecies. Our findings are in accordance with the fact that CRGs, ELGs, and MGs exhibit much smaller current effective population sizes [McManus et al., 2014; Roy et al., 2014c; Thalmann et al., 2011] and additionally inhabit a much more fragmented range than WLGs.

The low variation in CRGs, on the other hand, is more difficult to reconcile with analyses of Prado-Martinez et al. [2013] and McManus et al. [2014] in which their single CRG sample was found to be roughly one and a half times as heterozygous as their ELGs. Prado-Martinez et al. [2013] described different demographic histories for gorillas over the last 200,000 years. While western gorillas (including CRGs) retained their effective population sizes for a long period of time after cessation of gene flow with eastern gorillas, ELGs experienced a considerable decrease of their effective population size, possibly explaining their lower genetic variation. Reasons why Prado-Martinez et al. [2013] and McManus et al. [2014] found lower variation in ELGs than CRGs, but we did not, might be that they considered only a very low sample size of CRGs (one individual) and ELGs (three and two individuals, respectively) and/or the fact that the present study analyzed microsatellite markers, whereas the other studies investigated whole-genome sequence data. In microsatellites mutations occur via replication slippage which happens orders of magnitude more often than point mutations in non-repetitive sequences studies [Jarne & Lagoda, 1996].

Distribution of Genetic Diversity Within Western Gorillas

Six of the nine populations analyzed in the present study belonged to the western gorilla species. While five of them were from the large range of WLGs, we also analyzed the isolated population of CRGs. That enabled us to not only compare the genetic diversity between the two gorilla species as a whole, but to also describe the distribution of genetic diversity within western gorillas in more detail.

As expected based upon its small census number and its fragmentation into approximately 14 forest patches totaling $\sim$600 km^2 scattered over a landscape of $\sim$12,000 km^2 [Bergl et al., 2012; Imong et al., 2014b; Oates et al., 2007], the CRG population exhibited lower values for all estimates of genetic diversity than any of the five WLG populations. These differences were, however, not always significant. Ndoki forest and Odzala had the most significant differences to CRGs, followed by Lobéké and Moukalaba. Interestingly, WLGs from Loango, a site bordered on two sides by the Atlantic Ocean and a lagoon, respectively [Arandjelovic et al., 2011; Boesch et al., 2007], were not significantly more diverse than the CRGs. While this might suggest that the Loango gorillas have limited genetic diversity through impeded gene flow, we note that the nearby Moukalouba gorillas are not more diverse than those at Loango. This suggests that these populations located near the south-western limits of the western lowland gorilla distribution may have relatively lower diversity due to an edge effect, whereby populations peripheral to the main distribution of a species show lower diversity as a consequence of smaller effective population sizes and greater geographical isolation [reviewed in Eckert et al., 2008].

Our results are consistent with an origin of WLG variation in the north-central part of their current range, based upon our findings of highest diversity in three populations from that approximate area. This inference is widely in accordance with mtDNA sequence studies which found highest nucleotide diversity at the center and at the northern end of the WLG range, whereas the lowest genetic variation was detected at the southern range of the subspecies [Anthony et al., 2007; Clifford et al., 2004; Douadi et al., 2007].

Distribution of Genetic Diversity Within Eastern Gorillas

As with the western gorillas, the comparisons among the three eastern gorilla populations revealed rather similar diversity levels. While the ELGs exhibited higher values for all estimates of genetic diversity than either of the MG populations, the only significant differences occurred between ELGs and Virunga MGs, whereas the differences between Bwindi MGs and ELGs never reached significance. These results are in accordance with our expectations based upon the approximately ten-fold higher census sizes of eastern lowland as compared to mountain gorillas [Maldonado et al., 2012; Roy et al., 2014b,c].

How Is the Genetic Diversity of a Population Influenced by Its Size, Fragmentation and Position?

In sum our results are consistent with the expectation that small and fragmented as well as peripheral populations should exhibit decreased genetic variation [Eckert et al., 2008; Frankham, 1996]. For a subset of five populations we obtained estimated and effective population sizes from the literature. Both measures correlated highly with allelic richness and fragmented populations

generally had smaller population sizes and lower genetic variation (Fig. 2). Within the original nine-population dataset, degree of fragmentation and size of a given population had a larger impact on genetic diversity than its position, as the peripheral populations of Loango and Moukalaba, although they have been the least diverse ones within the largely continuous range of WLGs, were still more diverse than any of the fragmented and comparatively small populations of the three other gorilla subspecies. The pronounced effect of fragmentation may be due to the fact that gorillas are habitat specialists that only range in closed-canopy rain forest. Thus, they are not able to maintain long-term gene flow across fragmented landscapes in which patches of rain forest alternate with patches of agriculture, urban settlements or savannah. Our study is in agreement with previous observations that habitat specialists are especially prone to fragmentation effects [Brouat et al., 2003; Mech & Hallett, 2001; Vandergast et al., 2004; reviewed in Keyghobadi, 2007].

Implications for Conservation

Due to increasing pressure through poaching for bushmeat trade, timber harvesting, diseases and political instabilities, gorillas are listed as endangered (ELGs) and critically endangered (CRGs, WLGs, MGs), respectively [IUCN, 2014]. Populations with declining census sizes that eventually approach the verge of extinction are assumed to lose fitness and genetic diversity with increasing speed, a phenomenon known as extinction vortex [Fagan & Holmes, 2006; Gilpin & Soulé, 1986]. The present work established baseline levels of current day genetic diversity in key populations of gorillas across their entire range. Furthermore, demographic data such as population size [e.g. Gray et al., 2013; Roy et al., 2014b] and reproductive success [Robbins et al., 2007, 2011, 2014] do exist, at least for some gorilla populations. In addition to an effective protection, continued monitoring of demographic and genetic variables is necessary to distinguish stochastic variation from indications of a population potentially entering a terminal decline [Fagan & Holmes, 2006; Palomares et al., 2011, 2012]. Fortunately, at present no single population appears uniquely threatened by sudden loss of genetic diversity and levels of diversity were broadly similar, although significant differences sometimes occurred.

As conservation resources are limited, the difficult decision how to spend them most effectively has to be undertaken. Genetically more diverse populations are expected to exhibit a reduced risk of extinction due to lower rates of inbreeding and accordingly higher reproductive fitness [Reed & Frankham, 2003]. All WLG populations exhibited especially high genetic diversity and differences between populations were mainly insignificant, whereas CRGs were less diverse, probably because of a severe reduction in their effective population size [Thalmann et al., 2011] along with high rates of fragmentation [Bergl & Vigilant, 2007]. Thus, the key for maintaining high levels of genetic diversity in WLGs lies in keeping fragmentation of the WLG range as minimal as feasible. All WLG sites that were analyzed in the present study are already under protection as they all have the status of a national park. Maintaining extensive corridors of suitable gorilla habitat between already protected areas together with effective law-enforcement within these areas is necessary to preserve current WLG genetic diversity.

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