

RESEARCH ARTICLE

Effects of Habitat Fragmentation, Population Size and Demographic History on Genetic Diversity: The Cross River Gorilla in a Comparative Context

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In small and fragmented populations, genetic diversity may be reduced owing to increased levels of drift and inbreeding. This reduced diversity is often associated with decreased fitness and a higher threat of extinction. However, it is difficult to determine when a population has low diversity except in a comparative context. We assessed genetic variability in the critically endangered Cross River gorilla (*Gorilla gorilla diehli*), a small and fragmented population, using 11 autosomal microsatellite loci. We show that levels of diversity in the Cross River population are not evenly distributed across the three genetically identified subpopulations, and that one centrally located subpopulation has higher levels of variability than the others. All measures of genetic variability in the Cross River population were comparable to those of the similarly small mountain gorilla (*G. beringei beringei*) populations (Bwindi and Virunga). However, for some measures both the Cross River and mountain gorilla populations show lower levels of diversity than a sample from a large, continuous western gorilla population (Mondika, *G. gorilla gorilla*). Finally, we tested for the genetic signature of a bottleneck in each of the four populations. Only Cross River showed strong evidence of a reduction in population size, suggesting that the reduction in size of this population was more recent or abrupt than in the two mountain gorilla populations. These results emphasize the need for maintaining connectivity in fragmented populations and highlight the importance of allowing small populations to expand. *Am. J. Primatol.* 70:848–859, 2008. © 2008 Wiley-Liss, Inc.

Key words: genetic diversity; heterozygosity; fragmentation; bottleneck; conservation genetics; *Gorilla gorilla diehli*

INTRODUCTION

Many living primate populations are small [reviewed in Eudey, 1987; Mittermeier et al., 1992], have undergone bottlenecks [e.g., Bornean orangutans, Goossens et al., 2006; Delacour's langur, the Cat Ba langur, Mittermeier et al., 2005] or exist in fragmented habitats [Anderson et al., 2007; Miller et al., 2004; Pope, 1996]. Until recently, it had been generally accepted that demographic and environmental effects are likely to push such small populations to extinction before genetic effects [Caro & Laurenson, 1994; Caughley, 1994; Harcourt, 1995; Lande, 1988]. Though genetic diversity has long been recognized as an important component of fitness and population viability [Frankel, 1974; Wright, 1977], deterministic factors (hunting, habitat loss, disease, etc.), in combination with demographic and environmental stochasticity were considered more immediate threats to population persistence. However, recent evidence suggests that the genetic consequences of small population size may pose a more significant threat to survival than previously recognized [Amos & Balmford, 2001; Frankham, 2005;

Reed & Frankham, 2003; Srikwan & Woodruff, 2000; Vucetich & Waite, 1999]. Populations that are small will lose genetic diversity more rapidly through genetic drift and inbreeding [Lande & Barrowclough, 1987; Nei, 1987; Wright, 1931], and these effects will be intensified when such populations are also fragmented [Dudash & Fenster, 2000]. Experimental

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data have shown that less genetically diverse populations and those that are inbred have lower fitness and are more susceptible to extinction [Bjilma et al., 2000; England et al., 2003; Reed & Frankham, 2003; Spielman et al., 2004]. Recent studies of wild populations also document the importance of genetic variability and inbreeding avoidance for fitness and survival [Charpentier et al., 2007; Crnokrak & Roff, 1999; Da Silva et al., 2005; Keller & Waller, 2002; Keller et al., 1994].

The genus *Gorilla* offers the opportunity to investigate how population history, size and habitat fragmentation affect patterns of genetic diversity and examine the implications of this diversity for conservation. Two species of gorilla (*Gorilla gorilla* and *G. beringei*) are currently recognized and divided into four subspecies based primarily on morphological differences (Fig. 1) [Groves, 2001; Grubb et al., 2003; Stumpf et al., 2003]. All forms of gorilla are endangered [IUCN, 2007], but occur in populations that vary considerably in size and subdivision. The Cross River gorilla (*G. gorilla diehli*) in particular

may be under threat from genetic factors as its population is small, fragmented and may have undergone a considerable reduction in size [Bergl, 2006; Oates et al., 2003], numbering only an estimated 250–300 individuals today. Determining how genetic diversity is partitioned within this population could help guide conservation efforts by suggesting management strategies that would maximize variability. It is also possible to assess genetic diversity in the Cross River population from a comparative perspective, as equivalent genetic data have recently become available for populations from both mountain (*G. beringei beringei*) and western lowland (*G. gorilla gorilla*) gorillas [Bradley et al., 2004, 2005; Nsubuga et al., 2008].

We examined patterns of genetic diversity in the Cross River gorilla at both the intra- and inter-population level using data from autosomal micro-satellite loci. We compared the genetically defined subpopulations of Cross River gorillas [Bergl & Vigilant, 2007] using both heterozygosity-based and allelic measures. We also compared the Cross River

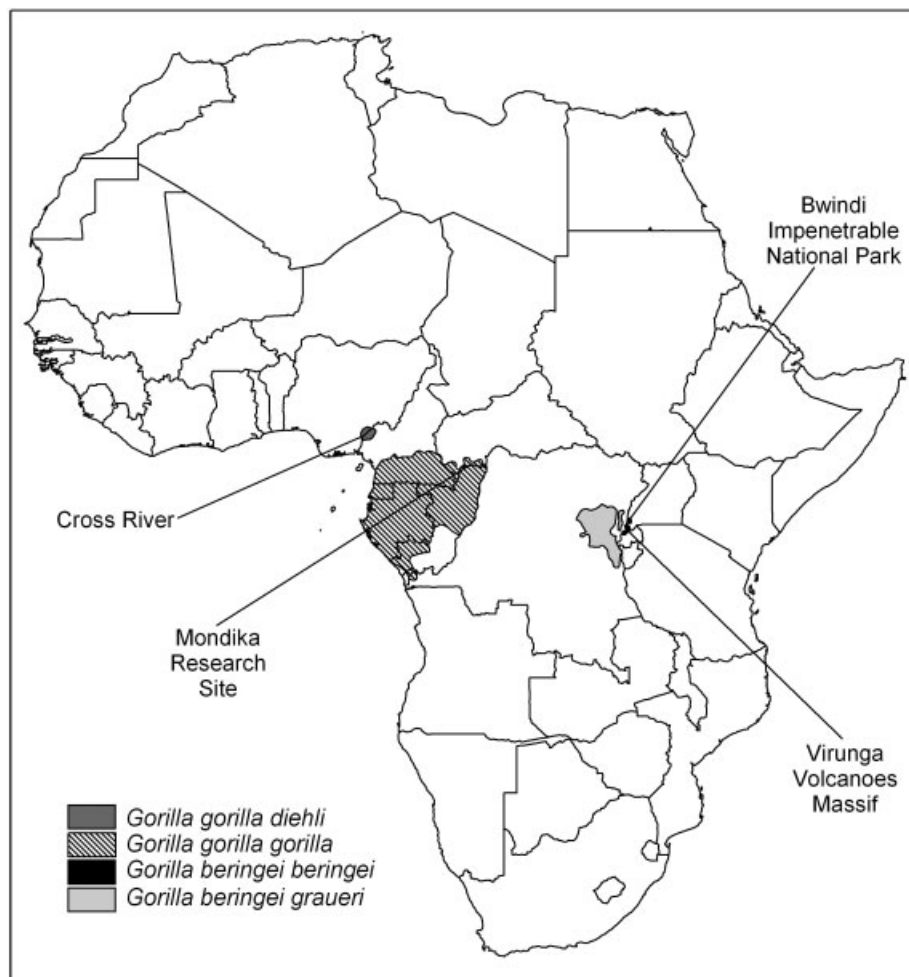


Fig. 1. Distribution of the genus *Gorilla* and approximate locations of populations included in the analysis.

population as a whole to three other gorilla populations (Bwindi, the Virungas and Mondika) using the same microsatellite metrics. The genetic data from the four gorilla populations were also examined for evidence of demographic bottlenecks.

MATERIALS AND METHODS

Study Sites, Sources of Data and Analysis of Genetic Equilibrium

Field work for this study was conducted between December 2002 and September 2004 in Cross River State, Nigeria and Southwest Province, Cameroon (Fig. 1). The Cross River gorilla occupies approximately 11 primarily highland sites dispersed across a larger forest landscape in this region. Autosomal microsatellite data for the Cross River population were generated from non-invasively collected fecal samples. The Cross River study site and the acquisition of genotypic data from these gorillas are described in detail in Bergl [2006] and Bergl and Vigilant [2007]. Comparative data came from a single western lowland gorilla (*G. gorilla gorilla*) population (Mondika, Central African Republic and Republic of Congo), and the two mountain gorilla (*G. beringei beringei*) populations (Virunga mountains, Volcanoes National Park, Rwanda and Bwindi Impenetrable National Park, Uganda) [Bradley et al., 2004, 2005; Nsubuga et al., 2008] (Fig. 1). The Bwindi and Virunga gorillas represent small, isolated populations, whereas the Mondika gorillas are members of a large, continuous and presumably relatively undisturbed population of animals [sites described in Doran et al., 2002; Robbins & McNeilage, 2003; Stewart et al., 2001] (Table I). These data sets are comparable given the close phylogenetic affinity between populations, similar patterns of group composition and breeding structure, and equivalent sample collection regimes employed. All data sets were tested independently for deviations from Hardy-Weinberg equilibrium and linkage equilibrium using GENEPOP 3.4 [Raymond & Rousset, 1995].

Analysis of Genetic Diversity

Genetic variability was quantified both within the Cross River population and for each separate gorilla population using measures of heterozygosity and allelic diversity. Previous genetic analysis of the Cross River population identified three subpopulations, referred to herein as Western (20–30 individuals), Central (160–230 individuals) and Eastern (20–30 individuals) [Bergl, 2006; Bergl & Vigilant, 2007], which were used as the units for comparison in intra-population analyses. Intra-population analyses of the Cross River gorillas were conducted using data from 11 microsatellite loci. A sub-sample of eight microsatellite loci common among all the gorilla data sets was used for inter-population comparisons [D1s550, D2s1326, D4s1627, D7s817, D7s2204, D10s1432, D16s2624, vWF; Bradley et al., 2000]. These loci were originally selected because (i) they amplified well using the degraded DNA of non-invasive samples [Bradley et al., 2000] and (ii) they were known to be variable in other primate species, particularly humans and baboons [Morin et al., 1998]. As the markers were screened and selected for variability in these non-gorilla taxa, they are expected to be free of ascertainment bias across the gorilla populations we considered. We calculated Nei's unbiased estimate of expected heterozygosity [H_E , Nei, 1973] in POPGENE 1.31 [Yeh et al., 1999]. We also calculated individual heterozygosity (H_I), the proportion of heterozygous loci per individual as a measure of variability at the individual level [Slate et al., 2000]. Within the Cross River population, differences in heterozygosities between subpopulations were tested for significance using analysis of variance (ANOVA) under the assumption that heterozygosity data are normally distributed [Archie, 1985; Nei, 1987]. Post hoc comparisons were made using Fisher's least significant difference test. Heterozygosity values for each of the small populations (Cross River, Bwindi and Virunga) were compared with heterozygosities in the large population (Mondika) to check for reduced levels of diversity

TABLE I. Conservation Status of Gorilla Populations Included in the Analysis

Taxon	Site	Geographical region	IUCN threat category	Estimated population size	Individual genotypes analyzed
<i>Gorilla gorilla diehli</i>	Cross River	Nigeria-Cameroon border	Critically endangered	204–292 ^a	71
<i>Gorilla beringei beringei</i>	Virungas	Rwanda, Uganda and DRC	Endangered	380 ^b	92
<i>Gorilla beringei beringei</i>	Bwindi	Southwestern Uganda	Endangered	320 ^c	77
<i>Gorilla gorilla gorilla</i>	Mondika	Central African Republic and Rep. of Congo	Endangered	Several thousand	45

^aOates et al. [2003]; Sunderland-Groves et al. [2003]; Bergl [2006].

^bGray et al. [2007].

^cMcNeilage et al. [2006].

using *t*-tests [Archie, 1985; Nei, 1987]. Differences in heterozygosity between the small populations were tested for significance using ANOVA.

We calculated the mean number of alleles per locus, *NA*, in POPGENE and allelic richness (*AR*), a measure that corrects for differences in sample size in FSTAT 2.9.3.2 [Goudet, 2001]. Using POPGENE we also calculated the effective allele number [*AE*, Kimura & Crow, 1964], a measure that incorporates the evenness of an allele frequency distribution and is not biased by the presence of rare alleles. Within the Cross River population, differences in allelic measures between subpopulations were tested using a Friedman's test and post hoc testing followed Bortz et al. [2000]. Lower levels of allelic diversity in the smaller populations (Cross River, Bwindi and Virunga) were compared with allelic diversities in the larger Mondika population using Wilcoxon's sign-rank tests. Differences in allelic measures between the three small populations were tested with a Friedman's test. The number of private alleles (*AP*) in the Cross River population (those present in only one of the three subpopulations) were identified via pairwise comparisons in Convert [Glaubitz, 2004].

In order to compare levels of inbreeding we used the coalescent-based simulation method employed in 2MOD [Ciofi et al., 1999] to estimate the inbreeding coefficient (*F*), the probability that any two alleles are identical by descent. We ran 100,000 iterations of the simulation and discarded the first 10% of values to avoid dependence on starting conditions. Estimates and ranges of *F* were calculated using density estimation.

We utilized the method of Petit et al. [1998] to quantify the contribution of each subpopulation to overall genetic diversity within the Cross River population. This method partitions a given subpopulation's total contribution to overall diversity into the fraction of contribution owing to its own diversity and the fraction owing to its differentiation from the other subpopulations. These contributions are expressed as percentages of the total diversity and can be either positive or negative. Positive contributions indicate that the value of the diversity index (for the population as a whole) is higher when the subpopulation is included than when it is not. This approach can help determine which subpopulations are more important for the overall diversity of the population and allow subpopulation diversity and divergence to be considered separately [Petit et al., 1998].

Analysis of Past Demographic Events

We tested for the genetic signal of a reduction in size in each of the gorilla populations using the BOTTLENECK program [Piry et al., 1999]. We used a two-phase mutation model (TPM) for estimating

H_{eq} , as microsatellite loci are unlikely to strictly follow either the infinite alleles (IAM) or step-wise mutation models (SMM) [Di Rienzo et al., 1994; Piry et al., 1999]. In order to accommodate the uncertain mode of mutation in microsatellites and to control for variation between loci we followed the approach of Weckworth et al. [2005] and conducted analyses using 70, 75, 80, 85 and 90% step-wise changes. Bottleneck signals were identified by testing for a difference between heterozygosity present in the population (H_E) and the heterozygosity expected assuming mutation-drift equilibrium (H_{eq} , a coalescent-based estimate calculated from the observed number of alleles and sample size) using Wilcoxon signed-rank tests [Piry et al., 1999].

We calculated the *long-term* effective population size of each of the gorilla populations using H_E and the mutation rate [Nei, 1987; Ohta & Kimura, 1973] as demographic data or temporally spaced genetic samples [Frankham, 1995; Nei & Tajima, 1981; Waples, 1989] were not available to allow calculation of more precise N_e estimates. Long-term N_e estimates reflect historical changes in population size [Avice, 2000] and were calculated according to both the IAM:

$$N_e = \frac{H_E}{4(1 - H_E)}$$

and the SMM:

$$N_e = \left(\frac{1}{(1 - H_E)^2 - 1} \right) \frac{1}{8}$$

where μ is the mutation rate. We estimated N_e assuming mutation rates ranging from 10^{-3} to 10^{-4} , which are average mutation rates for autosomal microsatellite loci [Schlotterer, 2000].

All methods complied with ASP principles for the ethical treatment of non-human primates and relevant IACUC regulations. All research was conducted in accordance with the laws of Nigeria, Cameroon, Germany and the United States of America.

RESULTS

Tests for Equilibrium

Deviations from the Hardy-Weinberg equilibrium were observed at two loci in the Cross River population (D5s1470 and D8s1106) and at single loci in the Virunga, Bwindi and Mondika populations (D4s1627, D1s550 and D3s2459, respectively). Two different pairs of loci showed evidence of linkage disequilibrium in each of the Bwindi and Mondika populations. The observed mild deviations from equilibrium are most likely the result of including related individuals in the sample and by population subdivision in the case of the Cross River gorilla. Prior analyses of the Virunga, Bwindi and Mondika

data sets have shown that when known parent-offspring pairs are removed from the analysis no deviations from equilibrium are observed [Bradley et al., 2005; Lukas et al., 2004]. Though it was not possible to reanalyze the Cross River data in this manner, given the low level of disequilibrium and previous evidence of the effect of related individuals and parent-offspring pairs, we treated all loci in each population as if they were in equilibrium and independent. At both the subpopulation and population level average H_I and H_O values were similar, demonstrating that heterozygosity values were not dominated by one or a few loci and that there was no serious among-locus sampling error in estimates of H_E .

Diversity Within the Cross River Gorilla Population

There was a trend toward differences in average H_E in the Cross River gorilla subpopulations (Table II; $F = 3.41$, $P = 0.053$), with H_E highest in the Central subpopulation for 8 of 11 loci. Differences in H_I between subpopulations were significant ($F = 7.25$, $P = 0.001$) and followed the same pattern as was observed for H_E . Post hoc tests revealed that the Central subpopulation had significantly higher H_I than the Western subpopulation ($P = 0.0003$) and that differences between the Central and Eastern subpopulations approached significance ($P = 0.069$). H_I was not significantly different between the Western and Eastern subpopulations ($P = 0.181$).

Differences in allelic diversity were less marked, but suggest that the Central subpopulation may be more variable than the other two Cross River gorilla subpopulations. The mean number of alleles (NA) observed in the Central subpopulation was greater than NA in the Eastern subpopulation ($\chi^2 = 10.864$, $df = 2$, $P = 0.002$). NA can be affected by unequal sample sizes, but levels of AR (allelic richness corrected for sample size) also tended to differ ($\chi^2 = 5.07$, $df = 2$, $P = 0.084$), as did AE ($\chi^2 = 5.091$, $df = 2$, $P = 0.087$). Private alleles were observed approximately three times as frequently in the Central subpopulation.

The contribution of each subpopulation to overall genetic diversity in Cross River was uneven (Table III). The Central subpopulation contributes positively to H_E , AR and AE owing largely to its own high levels of diversity for each measure. The Eastern and Western subpopulations have slightly negative contributions to overall AR , reflecting their low allelic diversity. The Eastern subpopulation has a positive effect on both H_E and AE owing to this subpopulation's marked differentiation. The Western subpopulation has positive contributions owing to differentiation, but equivalent negative contributions owing to diversity.

TABLE II. Heterozygosity and Allelic Diversity Values for Cross River Gorilla Subpopulations

Locus	H_O	H_E	H_I	NA	AE	AR
Eastern ($N = 23$)						
D16s2624	0.43	0.39	–	2	1.63	2.00
D10s1432	0.68	0.55	–	3	2.23	3.00
D8s1106	0.17	0.23	–	3	1.30	2.60
D7s2204	0.74	0.62	–	5	2.61	4.22
D7s817	0.77	0.70	–	5	3.30	4.62
D5s1470	0.83	0.77	–	7	4.27	6.52
D5s1457	0.61	0.67	–	5	3.07	4.58
D4s1627	0.55	0.65	–	4	2.86	3.99
D2s1326	0.65	0.70	–	4	3.32	4.00
D1s550	0.52	0.64	–	4	2.76	3.85
VWF	0.36	0.50	–	4	1.99	3.59
Mean	0.57	0.58	0.58	4.18	2.67	3.91
Central ($N = 33$)						
D16s2624	0.61	0.56	–	3	2.29	2.94
D10s1432	0.85	0.80	–	8	4.97	6.94
D8s1106	0.61	0.56	–	4	2.26	3.70
D7s2204	0.73	0.71	–	5	3.44	4.71
D7s817	0.77	0.63	–	5	2.67	4.17
D5s1470	0.81	0.76	–	8	4.15	6.89
D5s1457	0.74	0.62	–	5	2.66	4.39
D4s1627	0.77	0.68	–	5	3.10	4.40
D2s1326	0.79	0.77	–	7	4.30	6.46
D1s550	0.67	0.65	–	5	2.89	3.93
VWF	0.58	0.68	–	5	3.17	4.29
Mean	0.72	0.67	0.73	5.45	3.26	4.80
Western ($N = 15$)						
D16s2624	0.40	0.32	–	2	1.47	2.00
D10s1432	0.93	0.80	–	7	4.90	7.00
D8s1106	0.33	0.28	–	2	1.38	2.00
D7s2204	0.80	0.60	–	4	2.53	3.93
D7s817	0.60	0.52	–	3	2.10	3.00
D5s1470	0.47	0.36	–	2	1.56	2.00
D5s1457	0.80	0.75	–	5	3.98	4.93
D4s1627	0.87	0.57	–	3	2.33	3.00
D2s1326	0.73	0.68	–	6	3.08	5.93
D1s550	1.00	0.77	–	6	4.40	6.00
VWF	0.50	0.52	–	3	2.10	3.00
Mean	0.68	0.56	0.67	3.91	2.71	3.89

H_O , observed heterozygosity; H_E , Nei's expected heterozygosity; H_I , individual heterozygosity; NA , number of alleles; AE , effective allele number; AR , allelic richness.

Diversity Comparisons With Other Gorilla Populations

Despite the considerably larger size of the population of which the Mondika gorillas are a part, no difference in average H_E was detected between Mondika and the Cross River or Bwindi populations (Table IV; $t = -1.370$, $P = 0.213$; $t = -1.125$, $P = 0.298$, respectively), but H_E in the Virungas was lower than in Mondika ($t = -2.619$, $P = 0.034$). No difference in H_E was detected among the

TABLE III. Contributions of Each Cross River Gorilla Subpopulation to Overall Population Diversity as Estimated by Heterozygosity (H_E), Allelic Richness (AR), and Effective Allele Number (AE)

	H_E			AR			AE		
	Western	Central	Eastern	Western	Central	Eastern	Western	Central	Eastern
Differentiation contribution	2.95	-0.84	8.44	2.87	2.14	-4.70	1.49	4.65	11.86
Diversity contribution	-2.74	3.97	-4.27	-3.81	7.83	-4.02	-4.46	7.95	-3.49
Total contribution	0.21	3.13	4.17	-0.94	9.97	-8.73	-2.97	12.59	8.38

TABLE IV. Heterozygosity and Allelic Diversity Values for Cross River, Virunga, Bwindi and Mondika Populations

Locus	H_O	H_E	H_i	NA	AE	AR
Cross River ($N = 71$)						
D16s2624	0.50	0.53	-	3	2.12	2.73
D10s1432	0.81	0.76	-	8	4.21	6.89
D7s817	0.73	0.69	-	5	3.24	4.32
D7s2204	0.75	0.67	-	7	3.05	4.96
D4s1627	0.72	0.67	-	5	3.08	4.25
D2s1326	0.73	0.78	-	7	4.58	6.36
D1s550	0.69	0.70	-	8	3.36	5.61
VWF	0.49	0.63	-	5	2.72	4.12
Mean	0.68	0.68	0.68	6.00	3.30	4.90
Virungas ($N = 92$)						
D16s2624	0.72	0.60	-	5	2.52	3.93
D10s1432	0.61	0.53	-	4	2.11	3.48
D7s817	0.61	0.50	-	5	2.01	4.03
D7s2204	0.64	0.63	-	5	2.72	4.32
D4s1627	0.80	0.66	-	5	2.98	4.20
D2s1326	0.67	0.69	-	6	3.25	4.70
D1s550	0.73	0.69	-	6	3.20	4.61
VWF	0.65	0.63	-	5	2.69	4.47
Mean	0.68	0.62	0.71	5.13	2.69	4.22
Bwindi ($N = 77$)						
D16s2624	0.68	0.51	-	5	2.03	2.99
D10s1432	0.82	0.77	-	6	4.30	5.65
D7s817	0.78	0.78	-	6	4.60	5.57
D7s2204	0.60	0.63	-	6	2.68	5.36
D4s1627	0.75	0.71	-	5	3.40	4.81
D2s1326	0.61	0.64	-	6	2.78	4.90
D1s550	0.69	0.70	-	8	3.30	4.86
VWF	0.72	0.69	-	7	3.19	6.31
Mean	0.70	0.68	0.71	6.13	3.28	5.05
Mondika ($N = 45$)						
D16s2624	0.50	0.49	-	4	1.95	3.38
D10s1432	0.86	0.80	-	7	4.90	6.29
D7s817	0.68	0.62	-	6	2.61	4.87
D7s2204	1.00	0.75	-	5	4.02	5.00
D4s1627	0.91	0.73	-	6	3.75	5.35
D2s1326	0.84	0.75	-	8	4.05	7.64
D1s550	0.86	0.78	-	7	4.59	6.80
VWF	0.89	0.75	-	7	4.05	6.34
Mean	0.82	0.71	0.81	6.25	3.74	5.71

H_O , observed heterozygosity; H_E , Nei's expected heterozygosity; H_i , individual heterozygosity; NA , number of alleles; AE , effective allele number; AR , allelic richness.

similarly small Cross River, Mondika and Virunga populations ($F = 1.627$, $P = 0.22$). Differences in H_I between the Cross River gorillas and the two mountain gorilla populations were also not significant ($F = 0.422$, $P = 0.656$). However, H_I was significantly lower in the Cross River, Bwindi and Virunga gorilla populations when compared with the Mondika sample ($t = -3.748$, $P < 0.001$; $t = -2.917$, $P = 0.004$; $t = -3.191$, $P = 0.002$, respectively).

Mondika had the highest NA of the four populations despite having the smallest sample size. As with the comparison of H_E , the Virungas had lower NA than Mondika ($Z = -1.983$, $P = 0.047$), whereas NA for the Cross River and Bwindi populations were not significantly different ($Z = -0.513$, $P = 0.608$; $Z = -0.333$, $P = 0.739$, respectively). Differences in NA between the small gorilla populations were not significant ($\chi^2 = 4.261$, $df = 2$, $P = 0.119$). Allelic diversity corrected for sample size (AR) was significantly lower in the Cross River and Virunga populations than in Mondika ($Z = -2.1$, $P = 0.036$; $Z = -2.38$, $P = 0.017$, respectively), but not in Bwindi ($Z = -1.4$, $P = 0.161$). This suggests that the smaller sample size from Mondika (45 vs. at least 71 individuals) caused an underestimate of allelic diversity for this population using NA . Differences in AR between Cross River and the two mountain gorilla populations tended to differ, but were not significant ($\chi^2 = 5.25$, $df = 2$, $P = 0.072$). Of these small gorilla populations, only the Virungas had a lower AE when compared with Mondika ($Z = -2.38$, $P = 0.017$). Mean AE did not differ among the small gorilla populations ($\chi^2 = 3.25$, $df = 2$, $P = 0.197$). Estimates of AE were uniformly lower than AR in all of the gorilla populations, suggesting that NA and AR are somewhat inflated by the presence of infrequent alleles.

Estimates of F showed a similar pattern. Each of the small gorilla populations had similar estimates of F . Cross River was lowest of the three ($F = 0.18$, 95% highest posterior density (HPD) range: 0.12–0.26), with the Virungas ($F = 0.24$, 95% HPD range: 0.19–0.35) and Bwindi ($F = 0.2$, 95% HPD range: 0.15–0.27) slightly higher. The estimate of F for Mondika was considerably lower and more precise than estimates for any of the small gorilla populations ($F = 0.11$, 95% HPD range: 0.07–0.16). These values suggest that rates of inbreeding are higher in

each of the small populations than in the large, continuous population. Though these estimates of F offer insights into inbreeding levels in the comparative context of the four gorilla populations we examined, they cannot be interpreted as absolute indices of inbreeding. As multiple generations of gorillas were included in each population sample, F will be positively biased. Thus, these F estimates cannot be directly compared with estimates of inbreeding calculated from data sets that are not similarly biased.

Demographic History

The history of each of the gorilla populations considered is poorly known, but the small size of the Cross River population, combined with the presumed recent loss of habitat for the Virunga and Bwindi populations suggests that they have gone through a reduction in population size. However, comparison of H_E and H_{eq} provides strong evidence of a recent population bottleneck in only the Cross River population (Table V). Running the BOTTLENECK analysis on only the large central Cross River subpopulation also revealed a bottleneck signal (data not shown), suggesting that it is a reduction in population size and not population structure causing the signal [see Excoffier & Heckel, 2006]. A bottleneck signal was also observed in the Virunga and Bwindi populations (one-tailed $P < 0.05$), though the strength of the signal was lower than in Cross River. No evidence of a reduction in population size was detected in the Mondika population.

Long-term effective population size (N_e) estimates for the Cross River gorilla population ranged from approximately 500 to 11,000, depending on the mutation rate and model of microsatellite evolution assumed. Similar estimates were obtained for each of the mountain gorilla populations. These values of N_e are considerably larger than the estimated census population size for these three populations (ca. 250–400). The N_e estimates for the Mondika gorillas were greater than for any of the small populations (approximately 600–13,500), but potentially less than the census size for this area, which numbers at least in the thousands [Tutin et al., 2005].

DISCUSSION

Conservation research on gorillas has, to date, focused on demographic factors [e.g., Harcourt, 1995; Plumptre and Williamson, 2001; Werikhe et al., 1998], human impacts [e.g., Hall et al. 1998; McNeilage et al., 2008; Plumptre & Williamson, 2001; Remis, 2000] and the influence of disease [e.g., Walsh et al., 2003]. Genetic data have been primarily used to examine questions related to social structure, relatedness and mating strategies [Bradley et al., 2004, 2005], and phylogenetics [Clifford et al., 2004; Garner & Ryder, 1996; Jensen-Seaman, 2000; Jensen-Seaman and Kidd, 2001; Jensen-Seaman et al., 2003; Ruvolo et al., 1994;]. Yet when populations are small, genetic diversity and its distribution within a population may be as important as other factors for assessing the overall conservation status of a group of organisms [Frankham, 2005; Srikwan & Woodruff, 2000; Vucetich & Waite, 1999]. However, in the absence of historical genetic data, defining “reduced diversity” is difficult; there is no absolute value below which a population should be considered genetically depauperate.

Distribution of Diversity Within the Cross River Gorilla Population

Genetic diversity is not evenly distributed within the Cross River gorilla population. The largest Central subpopulation exhibited higher levels of genetic variability than either of the peripheral subpopulations in terms of both heterozygosity and allelic diversity. Indeed, the smaller subpopulations may consist primarily of single social groups (Bergl unpublished data). In these smaller subpopulations the loss of diversity owing to drift and inbreeding will be considerably greater, and both of these subpopulations exhibit higher inbreeding coefficients [Bergl & Vigilant, 2007]. This reduction of diversity will also have been exacerbated by isolation in the form of low levels of immigration from the Central subpopulation [Bergl, 2006; Bergl & Vigilant, 2007]. In contrast, the Central subpopulation will have been less affected by drift given its relatively greater size, and less susceptible to inbreeding owing to the presence of gene flow between localities.

TABLE V. Genetic Evidence of Population Bottlenecks in the Four Gorilla Populations

	Number of loci used	Percent stepwise changes				
		70	75	80	85	90
Cross river	11	0.0012	0.0012	0.0034	0.0337	0.0874
Virungas	8	0.0195	0.0371	0.0977	0.0977	0.1914
Bwindi	12	0.0320	0.0757	0.1018	0.1697	0.3667
Mondika	9	0.0645	0.0645	0.1016	0.1797	0.2852

P values from the program BOTTLENECK using Wilcoxon one-tailed test for heterozygosity excess. Significant values ($P < 0.05$) are shown in bold.

The higher level of diversity present in the Central subpopulation is also evident in its positive contributions to overall population variability. For each of the three measures we considered, the Central subpopulation contributes a large amount to the diversity of the population as a whole. As would be predicted given its larger size and as suggested by its greater variability, these positive contributions are due primarily to the inherent diversity of the Central subpopulation. Perhaps surprisingly, given its lower levels of heterozygosity and allelic diversity, the Eastern subpopulation makes a positive contribution to both heterozygosity and the number of effective alleles of the overall population. This contribution is not because of particularly high diversity in the Eastern subpopulation, but rather to its differentiation from the population as a whole. Similarly, the Western subpopulation, though having a generally negative total contribution to diversity, has a uniformly positive contribution owing to differentiation.

The differentiation of the peripheral subpopulations and their associated contributions to overall diversity emphasizes the need for a unified approach to preserving and maximizing genetic variability in the Cross River gorilla population. Though the Central subpopulation has higher levels of diversity, preserving only this subpopulation would result in losses of overall diversity. Loss of either peripheral subpopulation would cause the loss of unique alleles, and loss of the Eastern subpopulation would result in a reduction in heterozygosity and a decline in the effective allele number. Nevertheless, the Central subpopulation is both the largest Cross River subpopulation and the repository of the greatest portion of genetic diversity. Protection of all subpopulations is important, but the Central subpopulation is integral to the long-term survival of these gorillas.

Comparative Diversity of the Cross River Gorilla

Contrary to what might have been expected, the Cross River gorilla population did not exhibit uniformly lower genetic diversity than either a large, undisturbed gorilla population (Mondika) or small, yet continuous populations (Virungas and Bwindi). Our comparisons between the Cross River and mountain gorilla populations revealed no evidence that compared with these similarly sized populations, the Cross River gorillas are genetically depauperate. In fact, the Cross River population shows slightly greater diversity for some parameters. H_E for each of the gorilla populations considered was broadly comparable to H_E reported for other apes including *Pan troglodytes verus* [$H_E = 0.79$, Bradley et al., 2000], *P.t. schweinfurthii* [$H_E = 0.65$, Morin et al., 1994; Reinartz et al., 2000] and *Pongo pygmaeus* [$H_E = 0.71$, Goossens et al., 2005].

However, while the Cross River population compares favorably to the mountain gorilla and other primate populations, it did show reduced diversity for some measures when compared with the larger Mondika population. Indeed, all the small gorilla populations had lower levels of H_I and Cross River and Virunga had lower levels of AR . A similar pattern was previously observed in a specific comparison between the Mondika and Bwindi gorillas [Lukas et al., 2004]. Lower average H_I compared with H_E may also indicate that structure within the populations is contributing to increased levels of H_E . Taken together, our results suggest that the Cross River population (along with the Virunga and Bwindi populations) shows some evidence for reduced variability when compared with a relatively undisturbed gorilla population. Though similar, albeit more severe, losses of diversity have been reported in other bottlenecked populations [e.g., koalas, Houlden et al., 1996; ibex, Maudet et al., 2002; elephants, Whitehouse & Harley, 2001], a key difference is the statistically equivalent levels of H_E in the Cross River and Mondika populations. This pattern of high expected heterozygosity combined with lower H_I and allelic diversity appears to be the result of a *recent* population reduction (see also below). An analogous situation has recently been reported for orangutans [Goossens et al., 2005, 2006].

Genetic Evidence for Population Bottlenecks

Using genetic data, we were able to detect a significant signal of a reduction in population size in the Cross River, Virunga and Bwindi gorilla populations. The bottleneck signal was much stronger in the Cross River gorillas. This is perhaps surprising given that each of the populations is similarly small. However, the genetic signal for a bottleneck created by a reduction in allelic diversity is transient, and can be lost after a relatively small number of generations [Luikart & Cornuet, 1998]. Additionally, situations in which the reduction in population size is gradual, as opposed to a classic rapid bottleneck, will not leave an easily detectable genetic signature [Beaumont, 1999; 2003; Luikart & Cornuet, 1998]. Using P values as a guide [Spencer et al., 2000], our data suggest that Cross River population reduction was quite recent and/or severe (perhaps within the last 100–200 years), whereas reductions in the mountain gorilla populations were either older (and the signal has been lost over time) or more gradual (the disparity between H_{eq} and H_E is not as marked).

Estimates of long-term N_e support the hypothesis that the Cross River, Virunga and Bwindi populations have been characterized by larger population size over their long-term history. Regardless of mutation rate or model applied, N_e estimates are considerably larger than census size for each of the populations. These large N_e values suggest that

the H_E observed is characteristic of populations considerably larger than those seen today.

Overall, it appears that though the Cross River, Virunga, and Bwindi populations are equally small, the ways in which they reached their current sizes are different. Two contrasting elements of the Cross River and mountain gorillas' habitat may have influenced the differing bottleneck signals we observed. First, the Cross River gorillas inhabit a large forested area [over 2,000 km², Oates et al., 2003]. Much of this area, while currently unoccupied by gorillas, may represent habitat from which they have been recently extirpated [Bergl, 2006; Bucknell and Groves, 2002; Fay, 1987]. In contrast, the two mountain gorilla populations are limited to two relatively small forest areas, each of which is approximately 350 km². The land surrounding the mountain gorilla habitat is the most densely populated area of Africa, and has been cultivated and used for cattle grazing for at least the last 400 years [Spinage, 1972]. Second, the Cross River region has a long history of bushmeat hunting [Oates et al., 2004], which likely intensified in the 19th century with the introduction of firearms. Conversely, the hunting of primates for meat in the range of the mountain gorillas is rare, though hunting for trophies and conflict-related mortality have occurred sporadically [Plumptre & Williamson, 2001].

An explanation consistent with these observations and our genetic data is that the Cross River population was recently larger, and the current population size is the result of hunting during the last two hundred years. This decline may have accelerated as guns became more common and hunting of larger, potentially dangerous mammals more common. A similar situation has been observed recently in Central Africa where the introduction of large bore shotgun shells greatly increased hunting off-take of large mammals [Wilkie et al., 1992]. In contrast, the current sizes of the Virunga and Bwindi gorillas appear to be the result of a more gradual decline, mediated by the increasing habitat loss owing to farming. We cannot rule out the alternate explanation that size reductions in all three populations are much older than we have suggested. However, the heterozygote excess used to detect a bottleneck is temporary; hence, the signal we detected could not be extremely ancient.

Implications for Conservation

Our analysis of genetic diversity in the Cross River gorillas has important implications for the conservation and management of this population. At the within-population level, diversity is unequally distributed between subpopulations. While the peripheral subpopulations contribute to the diversity of the population as a whole, their lower levels of diversity may reduce their prospects of long-term

survival. All the Cross River subpopulations can be considered small by mammal standards, and very small populations such as these can be highly susceptible to inbreeding in the short term [Keller & Waller, 2002] and suffer from a limited future evolutionary potential [Lacy, 1997]. Increasing the variability of these smaller subpopulations must be part of any effort to preserve them.

Two complementary strategies could be applied to maximizing diversity in these cases. First, population expansion, beyond its obvious benefits of increasing census size, will also promote greater diversity. Second, gene flow into small, genetically depauperate subpopulations can drastically increase variability [Maudet et al., 2002]. A single additional migrant per generation could significantly improve levels of diversity, particularly if from a divergent and more variable subpopulation (i.e., the Central subpopulation). Such outcrossing of divergent populations has been demonstrated to stimulate recovery of genetic diversity in a wide range of species [e.g., adders, Madsen et al., 2004; gray wolves, Vilá et al., 2003; reviewed in Frankham, 2005; greater prairie chickens, Westemeier et al., 1998]. As migration from the peripheral subpopulations to the larger Central subpopulation has been discovered [Bergl & Vigilant, 2007], natural migration in the opposite direction may be possible. Management efforts should foster movement of individuals between subpopulations by maintaining habitat corridors and controlling hunting in lowland areas. Alternatively, the possibility of translocating animals between subpopulations could be explored.

Similar levels of H_E for both the small Cross River and large Mondika populations are encouraging. Studies have shown that heterozygosity is important for short-term evolutionary potential [England et al., 2003] and is more representative of the relationship between genetic diversity and fitness than other measures [Da Silva et al., 2005; Keller & Waller, 2002]. Similarly, the lack of difference in diversity indices between Cross River, the Virungas and Bwindi is promising, as the mountain gorillas (though endangered) are generally considered to be demographically stable [McNeilage et al., 2001; Werikhe et al., 1998]. Together these results suggest that the Cross River population is not in immediate danger of extinction because of genetic factors.

However, levels of diversity in the Cross River population must be viewed with caution. The bottleneck signature and low H_I and AR compared with Mondika, in combination with high estimates of N_e , raise the possibility that the relatively robust levels of H_E in the Cross River gorillas are an artifact of a historically larger population. In such a case, this diversity would be transient and may be lost quickly if the population is maintained at its current size. The same situation has recently been reported in the Kinabatangan orangutan population [Goossens

et al., 2005, 2006] and will undoubtedly become more common as threatened wildlife populations become smaller and more fragmented.

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References

- Amos W, Balmford A. 2001. When does conservation genetics matter? *Heredity* 87:257–265.
- Anderson J, Rowcliffe JM, Cowlshaw G. 2007. The Angola black-and-white colobus (*Colobus angolensis palliatus*) in Kenya: historical range contraction and current conservation status. *Am J Primatol* 69:664–680.
- Archie JW. 1985. Statistical analysis of heterozygosity data: independent sample comparisons. *Evolution* 39:623–637.
- Avice JC. 2000. *Phylogeography: the history and formation of species*. Cambridge, MA: Harvard University Press.
- Beaumont MA. 1999. Detecting population expansion and decline using microsatellites. *Genetics* 153:2013–2029.
- Beaumont M. 2003. Estimation of population growth or decline in genetically monitored populations. *Genetics* 164:1139–1160.
- Bergl RA. 2006. Conservation biology of the Cross River gorilla (*Gorilla gorilla diehli*). Ph.D. Thesis, New York: City University of New York.
- Bergl RA, Vigilant L. 2007. Genetic analysis reveals population structure and recent migration within the highly fragmented range of the Cross River gorilla (*Gorilla gorilla diehli*). *Mol Ecol* 16:501–516.
- Bjilmsa R, Bundgaard J, Boerema A. 2000. Does inbreeding affect the extinction risk of small population? Predictions from *Drosophila*. *J Evol Biol* 13:502–514.
- Bortz J, Lienert GA, Boehnke K. 2000. *Verteilungsfreie Methoden in der Biostatistik. Korrigierte und Aktualisierte Auflage, Vol. 2*. Berlin: Springer.
- Bradley BJ, Boesch C, Vigilant L. 2000. Identification and redesign of human microsatellite markers for genotyping wild chimpanzee (*Pan troglodytes verus*) and gorilla (*Gorilla gorilla gorilla*) DNA from feces. *Conservation Genet* 1:289–292.
- Bradley BJ, Doran-Sheehy DM, Lukas D, Boesch C, Vigilant L. 2004. Dispersed male networks in western gorillas. *Curr Biol* 14:510–513.
- Bradley BJ, Robbins MM, Williamson EA, Steklis HD, Steklis NG, Eckhardt N, Boesch C, Vigilant L. 2005. Mountain gorilla tug-of-war: silverbacks have limited control over reproduction in multimale groups. *Proc Nat Acad Sci* 102:9418–9423.
- Bucknell D, Groves JL. 2002. Local perception of the population size, distribution and ranging behaviour of the Cross River Gorilla within the Takamanda and Mone Forest Reserves and the Mbulu Forest, Cameroon. New York: Wildlife Conservation Society Report.
- Caro TM, Laurenson MK. 1994. Ecological and genetic factors in conservation: a cautionary tale. *Science* 263:485–486.
- Caughley G. 1994. Directions in conservation biology. *J Anim Ecol* 63:215–244.
- Charpentier MJE, Widdig A, Alberts SC. 2007. Inbreeding depression in non-human primates: a historical review of methods used and empirical data. *Am J Primatol* 69:1370–1386.
- Ciofi C, Beaumont MA, Swingland IR, Bruford MW. 1999. Genetic divergence and units for conservation in the Komodo dragon *Varanus komodoensis*. *Proc Roy Soc Lond Ser B—Biol Sci* 266:2269–2274.
- Clifford SL, Anthony NM, Bawe-Johnson M, Abernethy KA, Tutin CEG, White LJT, Bermejo M, Goldsmith ML, McFarland K, Jeffery KJ, Bruford MN, Wickings EJ. 2004. Mitochondrial DNA phylogeography of western lowland gorillas (*Gorilla gorilla gorilla*). *Mol Ecol* 13:1551–1565.
- Crnokrak P, Roff DA. 1999. Inbreeding depression in the wild. *Heredity* 75:530–540.
- Da Silva A, Luikart G, Yoccoz NG, Cohas A, Allaine D. 2005. Genetic diversity-fitness correlation revealed by microsatellite analyses in European alpine marmots (*Marmota marmota*). *Conservation Genet* 7:371–382.
- Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB. 1994. Mutational processes of simple-sequence repeat loci in human populations. *Proc Nat Acad Sci USA* 91:3166–3170.
- Doran DM, McNeilage A, Greer D, Bocian C, Mehlman P, Shah N. 2002. Western lowland gorilla diet and resource availability: new evidence, cross-site comparisons, and reflections on indirect sampling methods. *Am J Primatol* 58:91–116.
- Dudash M, Fenster C. 2000. Inbreeding and outbreeding depression in fragmented populations. In: Young AJ, Clarke G, editors. *Genetics, demography and viability of fragmented populations*. New York: Cambridge University Press.
- England PR, Osler GHR, Woodworth LM, Montgomery ME, Briscoe DA, Frankham R. 2003. Effects of intense versus diffuse population bottlenecks on microsatellite genetic diversity and evolutionary potential. *Conservation Genet* 4:595–604.
- Eudey AA. 1987. Action plan for Asian primate conservation: 1987–1991. Gland, Switzerland: IUCN.
- Excoffier L, Heckel G. 2006. Computer programs for population genetics data analysis: a survival guide. *Nat Rev Genet* 7:745–758.
- Fay JM. 1987. Report on the participation of J. Michael Fay in the Takamanda Gorilla survey project (May 1–May 20 1987). Washington, DC: World Wildlife Fund—US.
- Frankel OH. 1974. Genetic conservation: our evolutionary responsibility. *Genetics* 78:53–65.
- Frankham R. 1995. Effective population size/adult population size ratios in wildlife: a review. *Genet Res* 66:95–107.
- Frankham R. 2005. Genetics and extinction. *Biol Conservation* 126:131–140.
- Garner KJ, Ryder OA. 1996. Mitochondrial DNA diversity in gorillas. *Mol Phylogenet Evol* 6:39–48.
- Glaubitz JC. 2004. CONVERT: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Mol Ecol Notes* 4:309–310.
- Goossens B, Chikhi L, Ancrenaz M, Lackman-Ancrenaz I, Mohamed M, Andau P, Bruford M. 2005. Patterns of genetic diversity and migration in increasingly fragmented and

- declining orang-utan (*Pongo pygmaeus*) populations from Sabah, Malaysia. *Mol Ecol* 14:441–456.
- Goossens B, Chikhi L, Ancrenaz M, Lackman-Ancrenaz I, Andau P, Bruford M. 2006. Genetic signature of anthropogenic population collapse in orang-utans. *PLoS Biol* 4:285–291.
- Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices. Version 2.9.3.2: Department of Ecology & Evolution, Lausanne University, Switzerland.
- Groves CP. 2001. Primate taxonomy. Washington, DC: Smithsonian Institution Press.
- Grubb P, Butynski TM, Oates JF, Bearder SK, Disotell TR, Groves CP, Struhsaker TT. 2003. Assessment of the diversity of African primates. *Int J Primatol* 24:1301–1357.
- Hall JS, Saltonstall K, Inogwabini B-I, Omari I. 1998. Distribution, abundance and conservation status of Grauer's gorilla. *Oryx* 32:122–130.
- Harcourt AH. 1995. Population viability estimates: theory and practice for a wild gorilla population. *Conservation Biol* 9:134–142.
- Houlden BA, England PR, Taylor AC, Greville WD. 1996. Low genetic variability of koala *Phascolarctos cinereus* in south-eastern Australia following a severe population bottleneck. *Mol Ecol* 5:269–281.
- IUCN. 2007. 2007 IUCN red list of threatened species.
- Jensen-Seaman M. 2000. Evolutionary genetics of gorillas. Ph.D. Thesis, New Haven: Yale University.
- Jensen-Seaman M, Kidd K. 2001. Mitochondrial DNA variation and biogeography of eastern gorillas. *Mol Ecol* 10:2241–2247.
- Jensen-Seaman M, Deinard A, Kidd K. 2003. Mitochondrial and nuclear DNA estimates of divergence between western and eastern gorillas. In: Taylor A, Goldsmith M, editors. *Gorilla biology: a multidisciplinary perspective*. Cambridge: Cambridge University Press. p 247–268.
- Keller LF, Waller DM. 2002. Inbreeding effects in wild populations. *Trends Ecol Evol* 17:230–241.
- Keller L, Arcese P, Smith J, Hochachka WM, Stearns SC. 1994. Selection against inbred song sparrows during a natural population bottleneck. *Nature* 372:356–357.
- Kimura M, Crow JF. 1964. The number of alleles that can be maintained in a finite population. *Genetics* 49:725–738.
- Lacy RC. 1997. The importance of genetic variation to the viability of mammalian populations. *J Mammal* 78:320–335.
- Lande R. 1988. Genetics and demography in biological conservation. *Science* 241:1455–1460.
- Lande R, Barrowclough G. 1987. Effective population size, genetic variation and their use in population management. In: Soulé ME, editor. *Viable populations for conservation*. New York: Cambridge University Press.
- Luikart G, Cornuet JM. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biol* 12:228–237.
- Lukas D, Bradley BJ, Nsubuga AM, Doran-Sheehy D, Robbins MM, Vigilant L. 2004. Major histocompatibility complex and microsatellite variation in two populations of wild gorillas. *Mol Ecol* 13:3389–3404.
- Madsen T, Ujvari B, Olsson M. 2004. Novel genes continue to enhance population growth in adders (*Vipera berus*). *Biol Conservation* 120:145–147.
- Maudet C, Miller C, Bassano B, Breitenmoser-Wursten C, Gauthier D, Obexer-Ruff G, Michallet J, Taberlet P, Luikart G. 2002. Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in Alpine ibex [*Capra ibex (ibex)*]. *Mol Ecol* 11:421–436.
- McNeillage A, Plumtre AJ, Brock-Doyle A, Vedder A. 2001. Bwindi impenetrable National Park, Uganda: gorilla census 1997. *Oryx* 35:39–47.
- McNeillage A, Robbins MM, Gray M, Olupot W, Babaasa D, Bitariho R, Kasangaki A, Rainer H, Asuma S, Mugiri G, Baker J. 2006. Census of the mountain gorilla (*Gorilla beringei beringei*) population in Bwindi Impenetrable National Park. *Oryx* 40:419–427.
- Miller L, Savage A, Giraldo H. 2004. Quantifying remaining forested habitat within the historic distribution of the cotton-top tamarin (*Saguinus oedipus*) in Columbia: implications for long-term conservation. *Am J Primatol* 64:451–457.
- Mittermeier RA, Konstant WR, Nicoll ME, Langrand O. 1992. Lemurs of Madagascar: an action plan for their conservation: 1993–1999. Gland, Switzerland: IUCN.
- Mittermeier RA, Valladares-Padua C, Rylands AB, Eudey AA, Butynski T, Ganzhorn JU, Kormos R, Aguiar JM, Walker S. 2005. Primates in peril: the world's 25 most endangered primates 2004–2006. Washington, DC: IUCN/SSC Primate Specialist Group, Conservation International.
- Morin PA, Moore JJ, Chakraborty LJ, Goodall J, Woodruff DS. 1994. Kin selection, social structure, gene flow, and the evolution of chimpanzees. *Science* 265:1193–1201.
- Morin PA, Mahboubi P, Wedel S, Rogers J. 1998. Rapid screening and comparison of human microsatellite markers in baboons: allele size is conserved, but allele number is not. *Genomics* 53:12–20.
- Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proc Nat Acad Sci USA* 70:3321–3323.
- Nei M. 1987. Molecular evolutionary genetics. New York: Columbia University Press.
- Nei M, Tajima F. 1981. Genetic drift and estimation of effective population size. *Genetics* 98:625–640.
- Nsubuga AM, Robbins MM, Boesch C, Vigilant L. 2008. Patterns of paternity and group fission in wild multimale mountain gorilla groups. *American Journal of Physical Anthropology* 135:263–274.
- Oates J, McFarland KL, Groves JL, Bergl RA, Linder JM, Disotell TR. 2003. The Cross River gorilla: natural history and status of a neglected and critically endangered subspecies. In: Taylor A, Goldsmith ML, editors. *Gorilla biology*. Cambridge: Cambridge University Press.
- Oates JF, Bergl RA, Linder JM. 2004. Africa's Gulf of Guinea forests: biodiversity patterns and conservation priorities. Washington, DC: Conservation International Center for Applied Biodiversity Science.
- Ohta T, Kimura M. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genet Res* 22:201–204.
- Petit RJ, El Mousadik A, Pons AO. 1998. Identifying populations for conservation on the basis of genetic markers. *Conservation Biol* 12:844–855.
- Piry S, Luikart G, Cornuet JM. 1999. BOTTLENECK: a computer program for detecting recent reductions in effective population size from allele frequency data. *J Heredity* 90:502–503.
- Plumtre AJ, Williamson EA. 2001. Conservation-oriented research in the Virunga region. In: Robbins MM, Sicotte P, Stewart KJ, editors. *Mountain gorillas: three decades of research at Karisoke*. Cambridge: Cambridge University Press.
- Pope TR. 1996. Socioecology, population fragmentation, and patterns of genetic loss in endangered primates. In: Avise JC, Hamrick JL, editors. *Conservation genetics: case histories from nature*. New York: Chapman & Hall. p. 119–159.
- Raymond M, Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Heredity* 86:248–249.
- Reed DH, Frankham R. 2003. Correlation between fitness and genetic diversity. *Conservation Biol* 17:230–237.
- Reinartz GE, Karron JD, Phillips RB, Weber JL. 2000. Patterns of microsatellite polymorphism in the range-restricted bonobo (*Pan paniscus*): considerations for inter-specific comparison with chimpanzees (*P. troglodytes*). *Mol Ecol* 9:315–328.

- Remis MJ. 2000. Preliminary assessment of the impacts of human activities on gorillas *Gorilla gorilla gorilla* and other wildlife at Dzanga-Sangha Reserve, Central African Republic. *Oryx* 34:56–65.
- Robbins MM, McNeilage A. 2003. Home range and frugivory patterns of mountain gorillas in Bwindi Impenetrable National Park, Uganda. *Int J Primatol* 24:467–491.
- Ruvolo M, Pan D, Zehr S, Goldberg T, Disotell TR, von Dornum M. 1994. Gene trees and hominoid phylogeny. *Proc Nat Acad Sci USA* 91:8900–8904.
- Schlotterer C. 2000. Evolutionary dynamics of microsatellite DNA. *Chromosoma* 109:365–371.
- Slate J, Kruuk LEB, Marshall TC, Pemberton JM, Clutton-Brock TH. 2000. Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). *Proc R Soc Lond* 267:1657–1662.
- Spencer CC, Neigel JE, Leberg PL. 2000. Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. *Mol Ecol* 9:1517–1528.
- Spielman D, Brook BW, Briscoe DA, Frankham R. 2004. Does inbreeding and loss of genetic diversity decrease disease resistance? *Conservation Genet* 5:439–448.
- Spinage CA. 1972. The ecology and problems of the Volcano National Park, Rwanda. *Biol Conservation* 4:194–204.
- Srikwan S, Woodruff DS. 2000. Genetic erosion in isolated small mammal populations. In: Young AJ, Clarke G, editors. *Genetics, demography and viability of fragmented populations*. New York: Cambridge University Press.
- Stewart KJ, Sicotte P, Robbins MM. 2001. Mountain gorillas of the Virungas: a short history. In: Robbins MM, Sicotte P, Stewart KJ, editors. *Mountain gorillas: three decades of research at Karisoke*. Cambridge: Cambridge University Press.
- Stumpf R, Polk J, Oates J, Jungers W, Heesy C, Groves C, Fleagle J. 2003. Patterns of diversity in gorilla cranial morphology. In: Taylor A, Goldsmith M, editors. *Gorilla biology: a multidisciplinary perspective*. Cambridge: Cambridge University Press.
- Tutin C, Stokes E, Boesch C, Morgan D, Sanz C, Reed T, Blom A, Walsh P, Blake S, Kormos R. 2005. Regional action plan for the conservation of chimpanzees and gorillas in Western Equatorial Africa. Washington, DC: Conservation International.
- Vilá C, Sundqvist AK, Flagstad Ö, Seddon J, Björnerfeldt S, Kojola I, Casulli A, Sand H, Wabakken P, Ellegren H. 2003. Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proc R Soc Lond Ser B—Biol Sci* 270:91–97.
- Vucetich J, Waite T. 1999. Erosion of heterozygosity in fluctuating populations. *Conservation Biol* 13:860–868.
- Walsh PD, Abernethy KA, Bermejo M, Beyers R, De Wachter P, Akou ME, Huijbregts B, Mambounga DI, Toham AK, Kilbourne AM, Lahm SA, Latour S, Maisels F, Mbina C, Mihindou Y, Obiang SN, Effa EN, Stakey MP, Telfer P, Thibault M, Tutin CEG, White LJT, Wilkie DS. 2003. Catastrophic ape decline in Western Equatorial Africa. *Nature* 422:611.
- Waples RS. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* 121:379–391.
- Weckworth BV, Talbot S, Sage GK, Person DK, Cook J. 2005. A signal for independent coastal and continental histories among North American wolves. *Mol Ecol* 14:917–931.
- Werikhe S, Macfie L, Rosen N, Miller P. 1998. Can the mountain gorilla survive? Population and habitat viability assessment for *Gorilla gorilla beringei*. Apple Valley, MN: IUCN SSC Conservation Breeding Specialist Group.
- Westemeier RL, Brawn JD, Simpson SA, Esker TL, Jansen RW, Walk JW, Kershner EL, Bouzat JL, Paige KN. 1998. Tracking the long-term decline and recovery of an isolated population. *Science* 282:1695–1698.
- Whitehouse AM, Harley EH. 2001. Post-bottleneck genetic diversity of elephant populations in South Africa, revealed using microsatellite analysis. *Mol Ecol* 10:2139–2149.
- Wilkie DS, Sidle JG, Boundzanga GC. 1992. Mechanized logging, market hunting, and a bank loan in Congo. *Conservation Biol* 6:570–580.
- Wright S. 1931. Evolution in Mendelian populations. *Genetics* 16:97–159.
- Wright S. 1977. Evolution and the genetics of populations, experimental results and evolutionary deductions, Vol. 3. Chicago: University of Chicago Press.
- Yeh FC, Yang R-C, Boyle TB, Ye Z-H, Mao JX. 1999. POPGENE, user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.