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Genetic census reveals increased but uneven growth of a critically endangered mountain gorilla population

Maryke Gray [a,*], Justin Roy [b], Linda Vigilant [b], Katie Fawcett [c], Augustin Basabose [a], Mike Cranfield [d], Prosper Uwingeli [e], Innocent Mburanumwe [f], Edwin Kagoda [g,1], Martha M. Robbins [b]

[a] International Gorilla Conservation Program, P.O. Box 931, Kigali, Rwanda
[b] Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, Leipzig 04103, Germany
[c] Dian Fossey Gorilla Fund International, 800 Cherokee Ave SE, Atlanta, GA 30315-1440, USA
[d] Mountain Gorilla Veterinary Project, School of Veterinary Medicine, University of California Davis, Davis, CA, USA
[e] Parc National des Volcans, Rwanda Development Board, P.O. Box 6239, Gishushu, Kigali, Rwanda
[f] Parc National des Virunga-sud, Institut Congolais pour la Conservation de la Nature, c/o IGCP-DRC, B.P. 137, Gisenyi, Rwanda
[g] Bwindi Mgahinga Conservation Area, Uganda Wildlife Authority, P.O. Box 3530, Kampala, Uganda

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ABSTRACT

Monitoring changes in the population dynamics of endangered species is crucial to effective conservation strategies. The mountain gorilla population of the Virunga Massif has been the subject of intensive conservation efforts, research and several censuses over the last 40 years, but the region has also been affected by political instability and war. Here we present results from the 2010 census, which was the first to utilize genetic analyses of fecal samples for the entire population. The genetic analyses improved the accuracy of the population estimate by identifying several instances in which gorillas otherwise would have been undercounted or double-counted. The population was estimated to be 480 individuals; including 349 individuals found in 24 groups that were habituated for research and tourism, 101 individuals found in 12 unhabituated groups, fourteen solitary males, and a correction factor of sixteen for undetected infants. The population has increased by 26% since 2003 (an annual rate of 3.7%) and it has almost doubled since 1981. Nearly all of the increase can be attributed to a relatively higher growth rate in the habituated groups from 2003 to 2010, and in all five of the previous intervals between consecutive censuses. Nonetheless, it would be imprudent to habituate additional groups due to the concomitant risks of disease transmission from humans, behavioral disturbance and potential vulnerability to poaching. The results show that it is possible for conservation efforts to succeed even under difficult conditions, while highlighting the continuing challenges of managing a wild population of both habituated and unhabituated gorillas.

1. Introduction

Routine monitoring of population size for endangered species is a key element to conservation strategies (Nichols and Williams, 2006). Such monitoring can serve as a measure of both the intensity of threats to the population and the effectiveness of various conservation strategies being implemented (Ferraro and Pattanayak, 2006). Population dynamics are unlikely to be static over time due to variation in environmental conditions and the animals' responses to such changes. Furthermore, growth rates may not be the same for all portions of a population, particularly in cases where threats vary spatially and/or certain management strategies target specific individuals or groups of the population (Rachlow and Berger, 1998). However, in practice, conducting routine monitoring is difficult because of the time, effort and expense involved in obtaining accurate estimates of population size especially when using indirect signs or targeting wary or cryptic animals.

Mountain gorillas (*Gorilla beringei beringei*) are critically endangered and found in only two small island populations: the Virunga Massif and the Bwindi Impenetrable National Park. The Virunga mountain gorilla population is found in the Virunga Massif, which spans the borders of north western Rwanda, south west Uganda and eastern Democratic Republic of Congo (Fig. 1). The population has been the subject of intensive conservation efforts, research and several censuses over the last 40 years, however during the past 20 years the region has also been frequently affected by political instability and war (Robbins et al., 2011). The last census of the

* Corresponding author. Tel.: +250 580465; fax: +250 580466.
E-mail addresses: mgray@igcp.org (M. Gray), roy@eva.mpg.de (J. Roy), vigilant@eva.mpg.de (L. Vigilant), fawcettkatie@gmail.com (K. Fawcett), abasabose@igcp.org (A. Basabose), cranfield.mike@gmail.com (M. Cranfield), prosper.uwingeli@rdb.rw (P. Uwingeli), innocent@gorilla.cd (I. Mburanumwe), robbins@eva.mpg.de (M.M. Robbins).
[1] Deceased.

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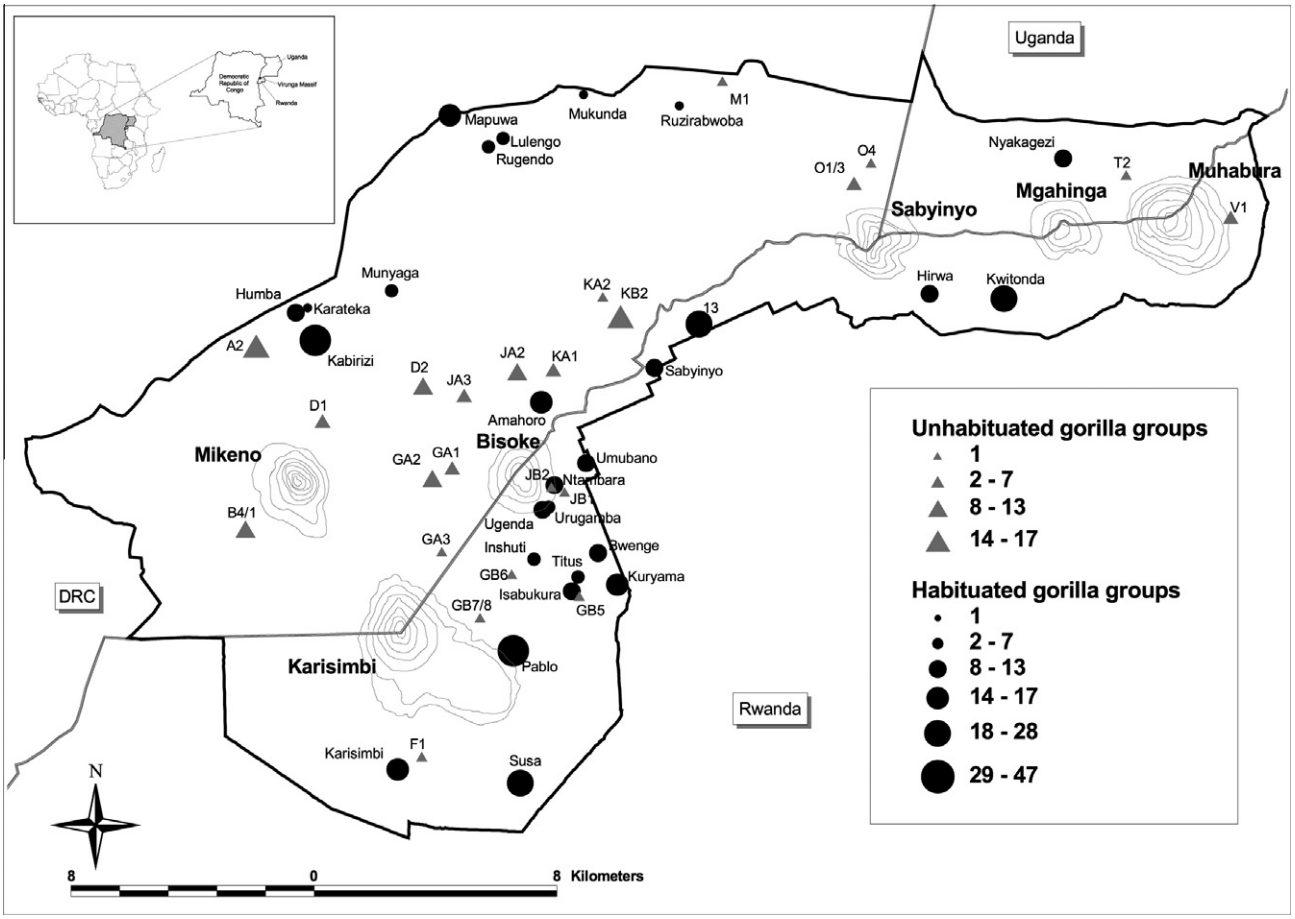

Fig. 1. Distribution of gorilla groups during the 2010 census. Triangles represent unhabituated groups, circles represent habituated groups. The size of the symbol reflects group size. Unhabituated groups were named based on the sector in which they were located. Group names correspond to those found in Table 3.

entire Virunga mountain gorilla population was conducted in 2003 (Gray et al., 2009) when the population was estimated to contain 380 individuals, a 17% increase since the previous census in 1989. These results indicate a steady increase in the population size since the low point of 250 gorillas in 1981. However, an analysis of the population dynamics spanning four decades indicated that the growth occurred unevenly across the population (Robbins et al., 2011). In particular, groups habituated to human presence for either research or tourism apparently grew at a higher rate than unhabituated groups (4.1% annual growth versus a 0.7% annual decline from the 1970s to the mid-2000s). While the entire population has received intensive levels of conventional protection including ranger patrolling for illegal activities, this difference in growth rate was attributed to the benefits of routine, nearly daily protection and veterinary interventions that were possible only for the habituated gorillas. Because the most recent population-wide census was conducted in 2003, the full impact of the subsequent political instability that resulted in intermittent encroachment of armed combatants and civilian populations into the park, sometimes poor security situation limiting the amount of monitoring that could be done, and recent poaching events on the gorilla population (with respect to size, distribution, composition and rate of growth or decline of the population) is unknown. It is known that in 2007 at least ten habituated gorillas were killed in Parc National des Virunga, DRC, but the status of the unhabituated groups is only possible to assess through indirect censusing. In particular, it is important to analyse the population dynamics of the habituated and unhabituated groups separately in order to estimate the impact of differing conservation efforts.

Studies of many species of rare and cryptic animals have shown that censusing methods that rely solely on indirect methods often suffer from limitations that can lead to inaccurate estimates of population size (Norris et al., 2011; Kühl et al., 2008). Incorporating genetic analysis into survey designs can greatly improve the precision of population estimates (Arrendal et al., 2007; Zhan et al., 2006; Arandjelovic et al., 2010, 2011; Katzner et al., 2011). The previous censuses of the Virunga gorilla population have used what is referred to as the 'sweep' method to estimate the number of unhabituated gorillas (all individuals of the habituated groups are known, providing an accurate number for those groups), in which several teams systematically walk throughout the forest looking for fresh signs of gorillas and estimate the population size based upon the number of night nests found (Gray et al., 2009). Based on the high density of reconnaissance teams walking simultaneously through the forest, this method assumes that all, or nearly all, gorillas are found and also assumes that each individual is counted only once. However, the sweep method relies on a number of assumptions that, if not met, may lead to inaccuracies in the population size estimate (Guschanski et al., 2009). First, it is known that the number of nests found from the same group can vary, because gorillas may on occasion make more than one nest per night and that not all nests may be found, so the number of gorillas assigned to a group may in fact be more or less than estimated from the nest counts. Second, the sweep method is also susceptible to the possibility of counting a particular group twice (if they are found in different locations with differing number of nests) or considering two unique groups to be the same group (if they are found in the same area and have similar numbers of nests). Additionally,

since all areas of the Virunga Massif are traversed only once, the sweep method does not enable us to put an estimate of variance around the total population size. Given some of the limitations of an indirect sweep census, genetic analysis of fecal samples collected during the census can be used to validate the results from the sweep census and enable us to determine a more accurate population estimate (Guschanski et al., 2009). Specifically, the genetic analysis enables us to genetically identify nearly all the gorillas found during the census and greatly reduces the problems of possibly under or over counting gorillas.

The goals of this study were to (a) utilize genetic analysis of fecal samples collected during a systematic sweep of the Virunga Massif to provide a more accurate estimate of the population size and (b) assess changes in the population size and structure over a 7-year period, with particular attention to the habituated and unhabituated portions of the population. The results of this study not only provide information concerning the impacts of conflict and conservation activities over a 7-year period and direct future conservation strategies for this population, but have broader implications for refining monitoring methods and understanding the effectiveness of various conservation approaches.

2. Methods

2.1. Census procedure and sample collection

The method to census the unhabituated gorillas in the field was based on those previously used in the Virunga Massif and Bwindi Impenetrable National Park (Sholley, 1991; McNeilage et al., 2001; Gray et al., 2009; Guschanski et al., 2009). The Virunga Massif was divided into sectors ranging in area from 10.5 to 34 km^2. The bare open and rocky areas above approximately 3600 m altitude were not surveyed since gorillas are not known to nest in them (approximately 17 km^2). Each sector was searched by walking an irregular network of reconnaissance routes across the area, taking GPS readings every 250 m. Six teams traversed the Virunga Massif systematically from west to east, proceeding such that no more than 3 days were left between the completion of work in one sector and the beginning of work in the next contiguous sector. When recent gorilla trail (less than 5–7 days old) was found, it was followed until nest sites less than 3 days old were located. The actual direction of reconnaissance trails walked was determined largely by the terrain and the availability of existing trails. To ensure that the routes were sufficiently dense to minimize the possibility that groups were not found, the distance between adjacent trails was never greater than 500–700 m. Distance walked on each reconnaissance trail recorded was measured using the tracklog function on the GPS and corrected for the vertical changes in terrain using GIS (ArcGIS 9.2). Approximately 1143 km of reconnaissance trail were surveyed over an 8 week period in February and March 2010.

At each nest site, we counted nests, measured dung size to estimate the age/sex class of individual gorillas, and recorded the presence of silver hairs presumably shed by silverback males. Smaller dung found within the nest of an older individual, presumably the mother, was recorded as that of an unweaned infant. Fecal samples were collected from all nest sites of habituated and unhabituated groups and lone silverbacks for genetic analysis using the previously described two-step collection method (Nsubuga et al., 2004). Teams aimed to locate and sample three nest sites for each group.

The habituated groups were located during the sweep survey and fecal samples were collected from their nests as well. However, for the final population estimate we used the composition of these groups known from the routine observations and demographic monitoring. Gorillas are considered immature until they reach age eight, and groups are considered multimale when they contain more than one male above age 12 (Robbins et al., 2011). Starting with the 2003 census, assessment of whether groups contain more than one silverback as well as counts of individuals of specific age classes such as immature have been limited to habituated groups due to inaccuracies obtaining such information from nest site data alone (Guschanski et al., 2009). Thus those results are not directly comparable with earlier censuses.

2.2. Genotyping from gorilla feces

A total of 307 fecal samples were collected from the unhabituated gorilla groups in the Virunga Massif. Samples were collected from multiple nesting sites (two sites each for two groups, three sites each for nine groups). DNA was extracted from 236 samples using the QIAamp DNA Stool Kit (QIAGEN) with slight modifications (Nsubuga et al., 2004). These samples were chosen to represent all putative unhabituated gorilla groups and corresponded, for each group, to all samples found at the nesting site with the highest number of nests. In order to confirm that groups were consistently identified, a minimum of three samples were also extracted from each of the other two nesting sites. Extracted samples were estimated to be 1–4 days old upon collection. DNA quality of each extract was then assessed by the PCR amplification of a sex-specific region of the amelogenin locus as described (Bradley et al., 2001).

DNA extracts which yielded products at the amelogenin locus were then amplified at 11 microsatellite loci using primers tested in a previous study (Arandjelovic et al., 2009): D6s1056-D14s306 (Morin et al., 2001), and D1s550-D2s1326-D4s1627-D5s1470-D6s474-D7s817-D8s1106-D16s2624-vWf (Bradley et al., 2000). These loci were chosen based upon their ability to discriminate with statistical significance even genotypes from siblings from the Karisoke research gorilla groups, also located in the Virunga Massif (combined non-exclusion probability for sib identity (7.467×10^{-4}) calculated as in Waits et al., 2001) (data not shown). Genotypes were produced using the two-step multiplexing approach (Arandjelovic et al., 2009). In the initial multiplexing step, all microsatellite loci were amplified in a single reaction containing a final volume of 20 μL:2.0 μL of 10× reaction buffer, 1.4 μL of $MgCl_2$ (25 mM), 1.0 μL of dNTP (2.5 mM), 0.8 μL of bovine serum albumin (BSA, 20 mg/mL), 0.96 μL of primer mix (3.125 mM for each primer), 0.1 μL of 0.5 U Super*Taq* (HT Biotechnology) premixed 2:1 with *Taq*Start Antibody (BD Biosciences), and 5 μL of template DNA. PCR thermocycling was performed in a PTC-200 thermocycler (MJ Research) and included an initial denaturation step of 9 min at 94 °C, followed by 30 cycles of 20 s at 94 °C, 30 s at 57 °C and 30 s at 72 °C, completed by a 4-min elongation step at 72 °C. In the following singleplex step, 5 μL of 1:100 diluted multiplex PCR product was used as template, and all reactions were independently performed in a 20-μL reaction volume containing 2.0 μL of 10× reaction buffer, 0.7 μL of $MgCl_2$ (25 mM), 1.0 μL of dNTP (2.5 mM), 0.8 μL of bovine serum albumin (BSA, 20 mg/mL), 0.5 μL of each forward (FAM-, HEX-, or NED-labeled) and reverse primer (10.0 mM for each primer), 0.08 μL of 0.5 U Super*Taq* (HT Biotechnology) premixed 2:1 with *Taq*Start Antibody (BD Biosciences). The thermocycling conditions were as described above, except that primer-specific annealing temperatures were used for each singleplex PCR and varied from 55 °C and 60 °C as detailed in Arandjelovic et al., 2009. Up to four different PCR products were then pooled in each of three different sets of loci, and electrophoresed on an ABI PRISM 3100 Genetic Analyser. Results were analyzed with GeneMapper Software version 3.7 (Applied Biosystems).

Three to four independent replicates of each sample were initially amplified in 96-well plates, and five negative PCR controls (H_2O) were used during the whole process. For all microsatellite loci, an allele was recorded in the final (consensus) genotype only if it was seen in at least two independent positive PCRs. Up to nine additional replicate PCRs were performed to resolve the ambiguous genotypes. Similar research on mountain gorillas also using DNA from noninvasive samples found that three replicate PCRs for each extract were sufficient to achieve 99% certainty that an apparent homozygote is indeed such at a given locus (Guschanski et al., 2009). For this reason, an individual was assigned as homozygote at any microsatellite locus if the same allele was exclusively seen in at least three replicate PCRs. For the gender identification, an individual was assigned as female if the 104-bp band was exclusively seen in the first four positive PCRs at the amelogenin locus, while the status of male was assigned if the 110-bp band was also seen in at least two positive PCRs.

2.3. Genetic data analysis

The program CERVUS 3.0.3 (Kalinowski et al., 2007) was used to compare results from extracts with a minimum of four fully genotyped loci in order to identify multiple samples from the same individual. Genotypes matching exactly at eight or more loci, without mismatching at any other locus, were then combined into a consensus genotype after checking for consistent sex identification. CERVUS 3.0.3 was then launched a second time and all genotypes matching at a minimum of six completed loci but mismatching at up to two loci were then checked for data entry errors. For genotypes mismatching at one or two loci, we used dung size, date of nest site, group of residence and sex identification to exclude the possibility of them originating from the same individual.

In addition to identity analyses, CERVUS 3.0.3 provided the following information when only distinct genotypes were considered in the analyses: number of alleles, observed and expected heterozygosities (Nei, 1978), Hardy–Weinberg equilibrium test with significance values adjusted by Bonferroni correction for multiple testing, and the non-exclusion probability for sib identity (PI_{sib}, Waits et al., 2001).

In order to identify potential mother-offspring relationships and subsequently infer the number of infants that might not have been sampled, we compared genotypes from medium-sized dung samples genetically identified as female ('mothers') with genotypes derived from small-sized dung ('offspring') from the same social group. We were able to assign each offspring to only one mother in each group, and other potential mothers did not share an allele at each locus with the offspring.

2.4. Growth rate calculations

We used time-series calculations to quantify the growth rate for habituated and unhabituated groups and for the overall population (see Robbins et al., 2011 for similar calculations). The growth rate for habituated groups was determined by starting with an initial number of gorillas and using Eq. (1) to calculate the number of gorillas in each subsequent month:

$$N_i = [N_{i-1} * (1 + r_m)] + A_i \quad (1)$$

In that equation, N_i represents the number of gorillas in month "i", N_{i-1} is the number of gorillas in the previous month, r_m is the monthly growth rate. The adjustment factor "A_i" equaled the number of gorillas that joined the specified groups during each month (e.g. through immigration or additional habituation), minus the number of gorillas that left those groups (e.g. through emigration and death). The time series analyses include both males and females, but the values for A_i do not account for the age or sex in which each adjustment occurred. We used iterative calculations with the bisection method to find the value of r_m that enabled us to match the observed size of the habituated groups at the end of the study period. The monthly growth rate was converted into an annual growth rate (r_a) using Eq. (2), to account for monthly compounding.

$$(1 + r_a) = (1 + r_m)^{12} \quad (2)$$

3. Results

3.1. Genotyping success and individual identification

Of the 236 DNA-extracted samples collected from the unhabituated gorilla groups, we found that 23 samples did not yield any amplifiable DNA while five samples could only be genotyped at fewer than four loci. For the remaining 208 samples, the genotypes were on average 85.9% complete, with the majority of them (188/208, or 90.4%) fully genotyped at eight or more loci. After the genotypes matching exactly at a minimum of eight loci (no mismatch, same sex) were combined, the 208 samples resulted in 107 unique individuals and their genotypes were on average 92.4% complete. In fact, 105 out of the 107 unique individuals (98.1%) were genotyped at eight or more loci. One sample came from a solitary male in DRC (A1) who died during the census so he was not included in the final calculation of the population size.

Among all pair wise comparisons of the unique genotypes, we observed only one pair of genotypes mismatching at a single locus. Since the sex identification was different and a confirmed allele at one incomplete locus for one individual was not shared by the other individual whose genotype was complete at this locus, we concluded that this pair of genotypes likely originated from two individuals. For the six cases where a pair of genotypes differs at two loci, information about sex and residence group supported the inferences that these genotypes indeed came from different individuals. Furthermore, all individuals involved in such comparisons were characterized by a low individual-level value of non-exclusion probability for sib identity (PI_{sib}: 6.471×10^{-3} or less).

3.2. Microsatellite marker characteristics

None of the loci used in this study deviated significantly from Hardy–Weinberg equilibrium. Thus, all loci were used in subsequent analyses. Overall, the genetic markers were polymorphic with an average of 5.09 alleles per locus and an average observed heterozygosity value of 0.598 (Table 1). The combined non-exclusion probability for sib identity (PI_{sib}) was 4.988×10^{-4} (range:

Table 1
Summary of the characteristics of the 11 microsatellite markers used in the study, obtained from the whole sample of 107 individuals (HO, observed heterozygosity; HE, expected heterozygosity; PIsib, non-exclusion probability for sib identity).

Locus	# Alleles	H_O	H_E	PI_{sib}
D14s306	4	0.594	0.611	0.504
D16s2624	4	0.617	0.649	0.473
D1s550	5	0.624	0.649	0.475
D2s1326	5	0.618	0.615	0.496
D4s1627	6	0.610	0.644	0.472
D5s1470	6	0.632	0.678	0.455
D6s1056	5	0.529	0.532	0.552
D6s474	4	0.548	0.588	0.511
D7s817	6	0.642	0.605	0.501
D8s1106	7	0.614	0.572	0.528
vWF	4	0.552	0.543	0.555
Overall	5.09	0.598	0.608	4.988×10^{-4}

0.455–0.555 per locus, Table 1), thereby confirming a statistically significant ability to discriminate among individuals in the study area. Even if two samples could only be compared at the eight least informative loci, the degree of discrimination was also high, as suggested by the combined non-exclusion probability for sib identity (PI_{sib}) of 4.917×10^{-3}.

3.3. Determination of group membership of unhabituated gorillas using genetic analysis

The genetic census of the unhabituated groups revealed the existence of 106 genetically distinct individuals, consisting of 57 males and 49 females (Table 2). Of the 106 gorillas, 98 were distributed into 11 unhabituated gorilla groups (average number of individuals per group: 8.91; range: 2–17, Table 2). Eight males were found alone, and are henceforth referred to as lone silverbacks.

The total number of unhabituated gorillas would have been difficult to infer from nest counts alone, because there was the possibility of both over counting and undercounting gorillas. There were two instances in which nests attributed to different groups were found to belong to members of the same group, and the genetic analysis prevented an over count. Groups O1 and O3 were each found to have a maximum of five nests, but we found that samples from O1 and O3 yielded a total of only five different genotypes which were found in both O1 and O3, and hence label this unit O1–O3 (Table 2). This result is consistent with the close proximity of the nest sites for these two groups. Likewise, groups B1 and B4 were represented by a maximal number of 2 and 8 nests respectively. We found that the two samples in B1 came from a single individual, and that this individual was also found in B4, resulting in the identification of nine different genotypes, and hence label this unit B1–B4 (Table 2). Samples Gb7 and Gb8 were the same solitary male. Hence, genetic analysis prevented a possible over count of seven individuals. The number of nests may also exceed the number of gorillas in the group if an individual constructs more than one nest during the night. There were a total of four cases in which the number of nests at the largest nesting site exceeded the number of genotypes produced from the samples (A2, D1, Ja2, Ja3), and in just one case (Ka1) did the use of samples collected from the same group at different nesting sites result in the identification of one more individual than was evident from the nest count at the most numerous nesting site.

An underestimation of the number of gorillas can occur if two sets of nests from different groups are mistakenly attributed to the same group. We ascertained that the many unhabituated groups all found in relatively close proximity to one another (which in many cases had similar number of nests) to the west and north of Mt Bisoke were in fact all unique groups (Groups D2, Ga1, Ga2, Ja3, Ka1, Kb2). In the absence of the genetic analysis, it is possible that some of these unique groups would have been considered to be the same group, resulting in an undercount.

Table 2
Details of the unhabituated social units of the mountain gorillas inhabiting the Virunga Massif (GR, group; LSB, lone silverback). Asterisks indicate groups which turned out to be the same group by genetic analysis and were hence combined.

Field ID	Social unit	# Nests (largest nesting site)	# Individuals (genetic census)	# Males	# Females
Kb2	GR	16	16	7	9
O1–O3*	GR	5 and 5	5	1	4
A2	GR	18	17	9	8
B1–B4*	GR	2 and 8	9	4	5
D1	GR	3	2	2	0
D2	GR	12	12	5	7
Ga1	GR	7	7	6	1
Ga2	GR	11	11	5	6
Ja2	GR	11	10	6	4
Ja3	GR	3	2	1	1
Ka1	GR	6	7	3	4
F1	LSB	2	1	1	
O4	LSB	1	1	1	
Ga3	LSB	1	1	1	
Gb5	LSB	1	1	1	
Gb6	LSB	1	1	1	
Gb7	LSB	1	1	1	
Jb1	LSB	1	1	1	
Jb2	LSB	1	1	1	
Total		116	106	57	49

3.4. Population size and growth rate

Other adjustments were necessary to account for problematic samples. Due to low DNA quality of the samples collected in sector V (group V1), it was impossible to genotype them reliably. However, since three samples were collected for each of the three nesting sites for this group, it is highly likely that this group is composed of three distinct individuals. The same issue applied to three other samples, each found independently in the study area and located far from the nearest sample, thus corresponding most likely to three lone silverbacks.

Census approaches based on nest counts or genetic analyses of fecal samples may not detect individuals, such as infants, who do not build nests and leave no dung or only small amounts of it. Therefore, previous censuses have used a variety of correction factors to account for these undetected infants. For example, it has been previously assumed that one-third of infants are normally missed during the field census (McNeilage et al., 2001). In this census we used the same correction factor method as was done in the Bwindi 2006 census because the genetic results enable us to have more accurate information on the sex of gorillas than from the nest site data alone (Guschanski et al., 2009). To estimate the number of undetected infants, first we assumed that the same proportion of adult females in the unhabituated groups have infants as in the habituated groups. 75% of the adult females in the habituated groups had infants (<3 years of age). Next, using the information on whether each unhabituated gorilla was male or female (from genetic analysis) in combination with the size of the dung (identified as adult female or medium), we estimated that there were 31 adult females in the unhabituated groups. There were an additional five gorillas that genotyped as female, but the field data provided ambiguous results on the dung size (typically it was UNK or smashed). We have considered these to be adult females. In sum, this results in 36 of the 112 unhabituated gorillas being classified as adult female (32%). This value is within the range of the proportion of the habituated gorillas that were adult females between 1967 and 2008 (30–40%; Robbins et al., 2011). Assuming 75% of these females had infants, there should be 27 unhabituated infants. We confirmed the presence of 11 infants genetically, and therefore added in 16 infants to the number of unhabituated gorillas.

In sum, the number of unhabituated gorillas was calculated by adding together the 106 individuals that were identified genetically, the six additional samples that could not be genotyped, and the 16 undetected infants, for the final estimate of unhabituated gorillas of 128 individuals. In addition, 24 habituated groups and three habituated solitary males were being monitored on a daily basis for either research or tourism purposes at the time of the census, containing a total of 352 gorillas. Adding to the estimated 128 unhabituated gorillas results in a total population size of 480 gorillas consisting of in 36 social groups and 14 solitary males (Table 3).

The estimated population size of 480 gorillas represents a 26.3% increase since the previous census in 2003 that estimated a popu-

Table 3
Group compositions from the 2010 census: habituated and unhabituated gorillas.

Group	Group or LSB	Habituated or not	Research or tourism	Country found	Largest nesting site (Unhabituated only)	Number
Munyaga	Group	Habituated	Tourism	DRC		6
Kabririzi	Group	Habituated	Tourism	DRC		36
Humba	Group	Habituated	Tourism	DRC		12
Rugendo	Group	Habituated	Tourism	DRC		5
Lulengo	Group	Habituated	Tourism	DRC		6
Mapuwa	Group	Habituated	Tourism	DRC		16
Susa	Group	Habituated	Tourism	Rwanda		28
Karisimbi	Group	Habituated	Tourism	Rwanda		15
Amahoro	Group	Habituated	Tourism	Rwanda		16
Umubano	Group	Habituated	Tourism	Rwanda		12
Kwitonda	Group	Habituated	Tourism	Rwanda		19
Sabyinyo	Group	Habituated	Tourism	Rwanda		10
Group13	Group	Habituated	Tourism	Rwanda		22
Hirwa	Group	Habituated	Tourism	Rwanda		12
Pablo	Group	Habituated	Research	Rwanda		47
Ugenda	Group	Habituated	Research	Rwanda		13
Urugamba	Group	Habituated	Research	Rwanda		6
Ntambara	Group	Habituated	Research	Rwanda		10
Titus	Group	Habituated	Research	Rwanda		7
Kuryama	Group	Habituated	Research	Rwanda		15
Bwenge	Group	Habituated	Research	Rwanda		11
Isabakura	Group	Habituated	Research	Rwanda		10
Inshuti	Group	Habituated	Research	Rwanda		6
Nyakagezi	Group	Habituated	Tourism	Uganda		9
A2	Group	Unhabituated		DRC	18	17
B4/1	Group	Unhabituated		DRC	2 and 8	9
D1	Group	Unhabituated		DRC	3	2
D2	Group	Unhabituated		DRC	12	12
Ga1	Group	Unhabituated		DRC	7	7
Ga2	Group	Unhabituated		DRC	11	11
Ja2	Group	Unhabituated		DRC	11	10
Ja3	Group	Unhabituated		DRC	3	2
Ka1	Group	Unhabituated		DRC	6	7
Kb2	Group	Unhabituated		DRC	16	16
O1/3	Group	Unhabituated		DRC	5 and 5	5
V1	Group	Unhabituated		Rwanda	3	3
Mukunda	LSB	Habituated		DRC		1
Ruzirabwoba	LSB	Habituated		DRC		1
Karateka	LSB	Habituated		DRC		1
F1	LSB	Unhabituated		Rwanda	1	1
Ga3	LSB	Unhabituated		DRC	1	1
Gb5	LSB	Unhabituated		Rwanda	1	1
Gb6	LSB	Unhabituated		Rwanda	1	1
Gb7/8	LSB	Unhabituated		Rwanda	1 and 1	1
Jb1	LSB	Unhabituated		Rwanda	1	1
Jb2	LSB	Unhabituated		Rwanda	1	1
Ka2	LSB	Unhabituated		DRC	1	1
M1	LSB	Unhabituated		DRC	1	1
O4	LSB	Unhabituated		DRC	1	1
T2	LSB	Unhabituated		Uganda	1	1
						464
Summary				# Groups		# Gorillas
		Habituated		24		349
		Unhabituated – without undetected infants		12		101
		Undetected infants – unhabituated				16
		Habituated LSB's				3
		Unhabituated LSB's				11
		Total number				480
		% Habituated				73%
		% Unhabituated				27%

lation size of 380 gorillas. This translates into a 3.7% annual growth rate, which is higher than the projected growth rate calculated from Leslie matrix models using birth rates and age-specific survivorship values in the habituated groups from 1967 to ~2008 (3.1%; Robbins et al., 2011). On a longer time scale, the population has grown at a 2% annual growth rate since the 1989 census (320 gorillas). Between 2003 and 2010, the habituated groups have thus grown at an annual rate of 4.7%, whereas the unhabituated groups have experienced only 0.9% annual growth rate during the same time. Furthermore, extending these estimates to when the population monitoring began, since 1972 the habituated gorillas have had a 4.2% annual growth rate, whereas the unhabituated gorillas have

Table 4
Population parameters for Virunga Mountain Gorilla Population from 1971 to 2010. See text for explanation of estimated population size. Numbers in parentheses for mean group size is the standard deviation. na = data not available for calculating this variable.

Census year	Total gorillas counted	Estimated population size	# Of social groups	Mean group size	Median group size	# Of solitary males	% Multimale groups	% Immature	% of Social groups with >20 individuals
1971–73[c]	261	274	31	7.9 (na)	na	15	42	39.8	Na
1976–78[d]	252	268	28	8.8 (4.4)	7	6	39	35.8	3.5
1981[e]	242	254	28	8.5 (na)	na	5	40	39.7	Na
1986[f]	279	293	29	9.2 (5.5)	8	11	14	48.1	7
1989[g]	309	324	32	9.2 (7.1)	7	6	28	45.5	9
2000[i]	359	359–395	32	10.9 (9.7)	8	10	53[a]	44.7[a]	15.6
2003[j]	360	380	32	11.4 (11.2)	7.5	11	36*[†]	41.0[b]	15.6
2010[h]	464	480	36	12.5 (9.1)	10.5	14	61[a]	45.2[a]	11.1

[a] For 2000 and 2010, % multimale groups and% immature are calculated from the 17 habituated groups only.
[b] These numbers do not include the four groups found only by RBM, for which only the number of nests was observed.
[c] Harcourt and Groom (1972), Groom (1973).
[d] Weber and Vedder (1983).
[e] Aveling and Harcourt (1984).
[f] Vedder and Aveling (1986).
[g] Sholley (1991).
[h] This study.
[i] Kalpers et al. (2003).
[j] Gray et al. (2009).

had a negative growth rate (−0.3% annually; calculations adjust for groups that were habituated throughout the four decades; see Robbins et al., 2011 for methods).

3.5. Population structure and spatial distribution

There were 36 social groups found in 2010, which is 12.5% more than the 32 groups found in 2003, and is the greatest number of groups found in the Virunga Massif since routine censuses began. The number of habituated groups increased from 16 to 24, due to four groups forming through group fissions in habituated groups, three habituated solitary males forming new groups, and the habituation of one new group. The number of unhabituated groups found was the same as in 2003 (12 groups). Fourteen solitary males were found in 2010, which is more than in any census except 1971–1973 (Table 4). Solitary males are difficult to find, however, so it is unclear whether those differences among censuses reflect actual changes in their prevalence.

The mean and median group sizes were 12.5 ± 9.1 SD and 10.5 respectively, which are also the highest values that have been observed since routine censuses began. Only 11% of the social groups contained more than 20 individuals, however, which is fewer than in 2000 and 2003 (15.6% in both censuses). The habituated groups were significantly larger than the unhabituated (*t*-test: mean = 14.5 versus 8.4, $t = 2.42$, df = 35, $p = 0.021$). Among the habituated groups, 45.2% of the gorillas were immature, and 61% of those groups contained more than one adult male.

Although there are clear international boundaries among the three countries, assessments of the spatial distribution of gorillas are somewhat qualitative because the regions of the Virunga Massif do not have well-defined internal boundaries that could limit the movements of the gorillas. As in previous censuses, the majority of the gorillas were found in the central region, spanning northeast of Mikeno and Karisimbi, around Bisoke, and into the saddle area between Bisoke and Sabyinyo (Fig. 1). Fewer groups were found in the southern region, as has been the case for the past several decades. For example, only one group was found around Mikeno in 2010 (Group B1/4), so there has been little change since 2003. Historically, the region to the east of Mt. Sabyinyo has contained few gorillas and has shown little change over time. In the northwestern region, the habituated groups in the DRC had only one less gorilla in 2010 than in 2003 (84 versus 85), even though 10 gorillas were killed there in 2007.

4. Discussion

4.1. Population size and growth rate

The Virunga mountain gorilla population has increased by 26.3% from 2003 to 2010, which equals an annual growth rate of 3.7% (Fig. 2). The population size has nearly doubled in the past three decades. The sustained growth suggests that the mountain gorilla population had been well below the carrying capacity of the Virunga Massif. It also suggests that sustained conservation efforts on a transboundary scale can be beneficial in areas experiencing civil strife. Even with the recent increases, however, the population still contains only 480 individuals and mountain gorillas remain critically endangered.

Between 2003 and 2010, habituated groups grew at a higher rate than unhabituated groups (4.7% versus 0.9% annually, Fig. 2). The 0.9% growth rate of the unhabituated groups seems better than the annual decline of 0.7% that they experienced from 1972 to 2003, but it is unclear how much of the apparent difference was due to improved conditions versus demographic stochasticity, differences in census methodology, and measurement errors. However, the growth rate of habituated groups was also higher than

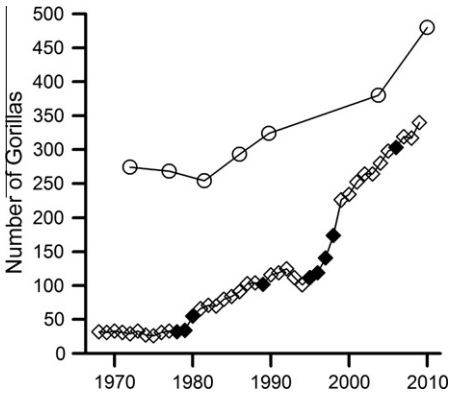

Fig. 2. Change over time in the population size and growth rate. Size of the total population is represented in the line with circles, each point representing a census conducted throughout the Massif. The line with diamonds represents the number of habituated gorillas since 1967 (groups habituated for both research and tourism). Filled diamonds indicate years when additional groups were habituated. Figure modified from Robbins et al. (2011).

unhabituated groups in all five of the previous intervals between consecutive censuses, which is likely due to the benefits of daily surveillance and the ability to perform veterinary interventions if necessary (Robbins et al., 2011). Despite these recurring differences in growth rates, it would be imprudent to habituate additional groups due to the inherent risks of disease transmission from humans, behavioral disturbance and potential vulnerability to poaching. Instead, park management should consider other ways to improve the growth rate of the unhabituated gorillas such as preventative measures to protect them from disease, snares and poaching.

4.2. Population structure and spatial distribution

The number of social groups was higher than all previous censuses, as was the mean and median group size (Table 4). Agent based modeling has been used to predict that as the population increases in size, we should expect to see an increase in the number of groups with less of an increase in the average group size (Robbins and Robbins, 2004); this is what was observed in this census. Among habituated groups, immature gorillas accounted for more than 40% of the population, which has been considered an indication of a healthy population (Weber and Vedder, 1983). The majority of habituated groups were multimale, which is expected to reduce the risk of population loss via infanticide when the dominant male dies (Watts, 1989).

As observed in previous censuses, the gorillas were not evenly distributed across the Virunga Massif. The highest concentration occurs in the central region, where most of the gorillas are habituated and the vegetation quality is relatively high (McNeilage, 1995; Robbins et al., 2011). The lower concentration of gorillas in the southern region may be due to past human disturbances. Much of the habitat around Mikeno appears to be suitable for gorillas, so there appears to be room for growth. In the eastern region, the area to the south of Sabyinyo and Mgahinga appears to be good gorilla habitat, whereas the areas around Muhabura include some grassland and other habitat that may be unable to sustain many gorillas. Population growth in the northwestern region was stalled by the killing of 10 habituated gorillas in 2007. Thus the spatial distribution of gorillas could be influenced by a variety of factors including ecological conditions and levels of human disturbance (past and present), as well as the natural movement of groups whose home ranges span more than one region.

4.3. Benefits of genetic analysis

The genetic analyses improved the precision of the population estimate by identifying several instances in which gorillas otherwise would have been undercounted or double-counted. Genetic analyses indicated a net overestimate of 10% when the population estimate was based solely on nest counts during the 2006 census of mountain gorillas at Bwindi (Guschanski et al., 2009), but we cannot extrapolate from one census to another given that both under and over counts are possible (see Results). Such errors should offset each other to some degree. Because a relatively low proportion (approximately 25%) of the total population is unhabituated and the entire Virunga Massif was intensively covered during the sweep censuses, it is unlikely that in previous censuses enough gorillas went undetected or were counted twice to have a significant impact on the estimates in growth rate among the unhabituated gorillas or the population as a whole. The growth rate would remain 4.7% for the habituated groups because their exact counts are known from daily observations. From that perspective, the data seems sufficiently precise to show a higher growth rate for habituated groups than unhabituated groups, and the estimate of overall growth rate is insensitive to census errors because more than 70% of the population is habituated.

Despite the benefits of genetic analyses, it remains difficult to fully assess the precision of the unhabituated population estimates because the nest counts do not provide information about how many groups may have been missed entirely. Such information may begin to emerge if genetic analyses of future censuses find adult gorillas who were not previously detected. Use of genetic analysis in a mark-recapture sampling approach (e.g. Zhan et al., 2006; Arandjelovic et al., 2010), incorporating repeated rounds of nest sampling efforts following a systematic search design would also improve the accuracy and precision of population estimates but would be challenging to implement in the Virunga Massif and Bwindi Impenetrable National Park given the effort necessary to survey the area. If park rangers collected fecal samples from unhabituated gorillas on a systematic basis, it may be possible to incorporate a genetic mark-recapture approach into routine patrols of the entire region. This would also potentially yield data on group movement and individual dispersal events.

4.4. Implications for conservation

While monitoring changes in population dynamics are typically time consuming, logistically challenging, and expensive to implement, it is crucial that conservationists have up to date information on a routine basis if they want to best understand the effectiveness of different management strategies and utilize them in the most cost-effective manner possible. Rapidly developing methods such as genetic analysis and remote-sensor camera traps are providing innovative ways of obtaining more precise and accurate estimates of population size (Kühl et al., 2008; O'Connell et al., 2011). In many cases, particularly for small populations, the benefits of a more precise and accurate estimate outweigh the additional cost and time that genetic analysis requires, and it allows to the monitoring of known individuals over time if applied repeatedly. The feasibility of applying these methods to other endangered species will depend on the availability of funds and human resources. Applying 'extreme' conservation strategies such as daily monitoring and/or veterinary care for habituated individuals to other endangered species is not always possible due to logistical or financial limitations, but this study provides further evidence of their enhanced effectiveness combined with traditional, population-wide approaches (e.g. park wide ranger patrols, community conservation activities; see Robbins et al., 2011). Such conventional approaches may vary in their effectiveness in time and space depending on a variety of factors that may be difficult to control, such as responses to ecological conditions, levels of human disturbance, and political instability. A balance of conventional and extreme measures is important for cost-effective, habitat-wide protection in some cases. Combined, these issues serve as a stern reminder of the complexities involved in conserving endangered species.

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