Bwindi Mountain Gorilla Census 2011 – Summary of Results

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Summary of Census

- Routine censuses of the critically endangered mountain gorillas of Bwindi Impenetrable National Park have been conducted to monitor the status of the population, assess the impacts of threats, and evaluate the effectiveness of conservation strategies.
- The most recent census was conducted in 2006, resulting in an estimate of a minimum of 302 gorillas. Because this census was the first to integrate genetic analysis to reduce the possibility of double-counting gorillas, it was not possible to compare these results with previous censuses and evaluate if the population had been increasing or decreasing.
- In 2011, the population was censused using a modified 'mark-recapture' method, which involved two sweeps of the entire park, as well as incorporating genetic analysis.
- **Results reveal a minimum of 400 gorillas in Bwindi in 2011.**
- The large increase in the population estimate from 2006 to 2011 (302 to 400 gorillas) is due to a combination of improved censusing techniques leading to better detection of gorillas (eg. some groups were not detected in 2006) and actual growth of the population.
- In combination with the 2010 mountain gorilla census conducted in the Virunga Massif that found 480 gorillas, the global population of mountain gorillas is approximately 880 individuals.
- The results of this census suggest that future censusing should use a mark-recapture method with genetic analysis.

1. Introduction

To best monitor the status of endangered populations of animals, understand the impacts of the threats they face, and evaluate the effectiveness of conservation strategies, it is necessary to routinely assess their population dynamics. Mountain gorillas (*Gorilla beringei beringei*) are the best monitored ape subspecies, with routine censuses being conducted approximately every 5-10 years since the 1970's for the Virunga Massif population and since the late-1990's for the Bwindi Impenetrable National Park population. Because the habitats of both of these populations are relatively small and the terrain is difficult to traverse due to steep slopes and thick vegetation, the methods used to census mountain gorillas differs somewhat from more traditional methods used in other locations. The 'sweep' method has been used, in which several teams systematically walk throughout the forest looking for fresh signs of gorillas and estimate the number of unhabituated gorillas based upon the number of night nests found (all individuals of the habituated groups are known, providing an accurate

number for those groups). Based on the high density of reconnaissance trails covering 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 may lead to inaccuracies in the population size estimate (Gray et al., in press; Guschanski et al. 2009). First, gorillas may on occasion make more than one nest per night and that not all nests may be found at a nesting site, so the number of gorillas assigned to a group may in fact be more or less than that estimated from the nest counts. Second, the sweep method may result in 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). Given some of the limitations of the sweep method, 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 (Gray et al, in press; 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.

Additionally, since all areas of the habitat are traversed only once with the sweep method, there is the possibility that some gorilla groups or solitary males are not detected at all, resulting in an undercount of the population. A single sweep provides only a minimum estimate of the population and does not enable to put an estimate of variance around the total population size. Applying a modified 'mark-capture-recapture' method, in which the habitat is traversed more than once, so that individuals not detected during the first sweep (or 'capture') may be located in subsequent sweeps ('captures'), is a commonly applied censusing method that reduces the likelihood of an undercount and inaccurate population size estimate.

The mountain gorilla population of Bwindi Impenetrable National Park, Uganda, was censused using only the single sweep method in 1997 and 2002. In the 2006 census, the sweep method was combined with genetic analysis to reveal that there was a minimum of approximately 302 gorillas in Bwindi. Combining the sweep census with genetic analysis eliminated some sources of error (double counting gorillas) and provides a refined, more accurate estimate of the population size. However, due to the possibility of similar overcounts or undercounts in the previous Bwindi gorilla censuses, we were unable to determine if the population has been increasing or decreasing over the past decade.

The 2011 census sought to obtain an update on the status of the Bwindi mountain gorilla population as well as further refine the methods used to obtain an accurate estimate of the population size. Therefore, we used a modified mark-recapture method by combining genetic analysis with sampling obtained during two 'sweeps' of the entire park. The primary goal of this census was to obtain an estimate of the population size through the use of genetic analysis of fecal samples obtained during two sweeps (captures) of Bwindi Impenetrable National Park, Uganda.

2. Methods

2.1 Census methods & sample collection

Samples were collected in 2011 over the study area at two different time periods using the sweep method, which has been described in details in previous studies (e.g. McNeilage et al. 2001, 2006, Gray et al. 2009, in press). This study differs mainly from the previous 2006 Bwindi census (Guschanski et al. 2009) in that two sweeps were conducted at different times, henceforth referred to as Sweep 1 and Sweep 2. Sweep 1 took place between February 28 and September 2, 2011, with only between one and three teams working at any one time (see Figure 1); 746 kms of reconnaissance trails were walked. Sweep 2 was conducted from September 10 until November 3, 2011, with six teams simultaneously moving from east to west in the main portion of the park, with a total of 778 kms of reconnaissance trails walked (see Figure 2). Two teams covered the northern sector.

Figure 1. Reconnaissance trails walked during Sweep 1 of 2011 gorilla census.

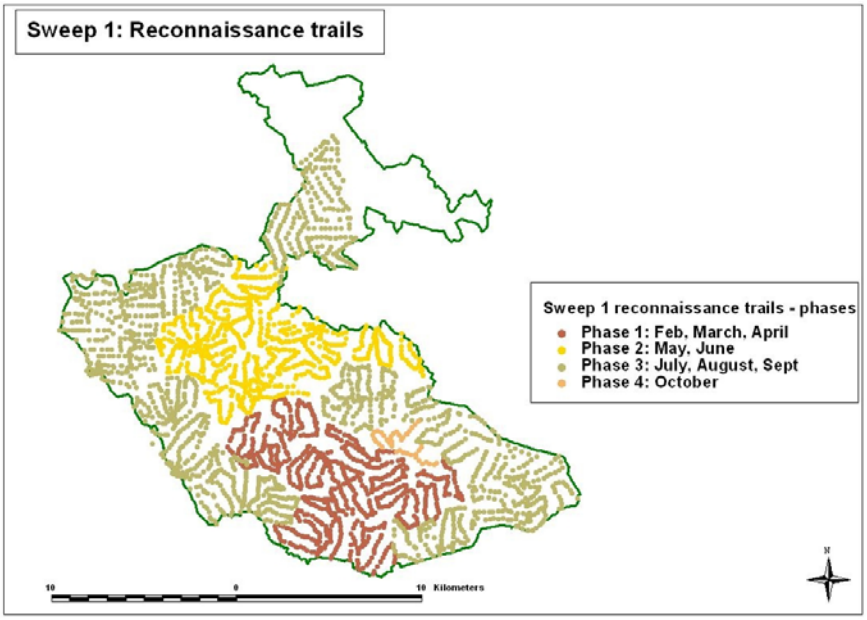
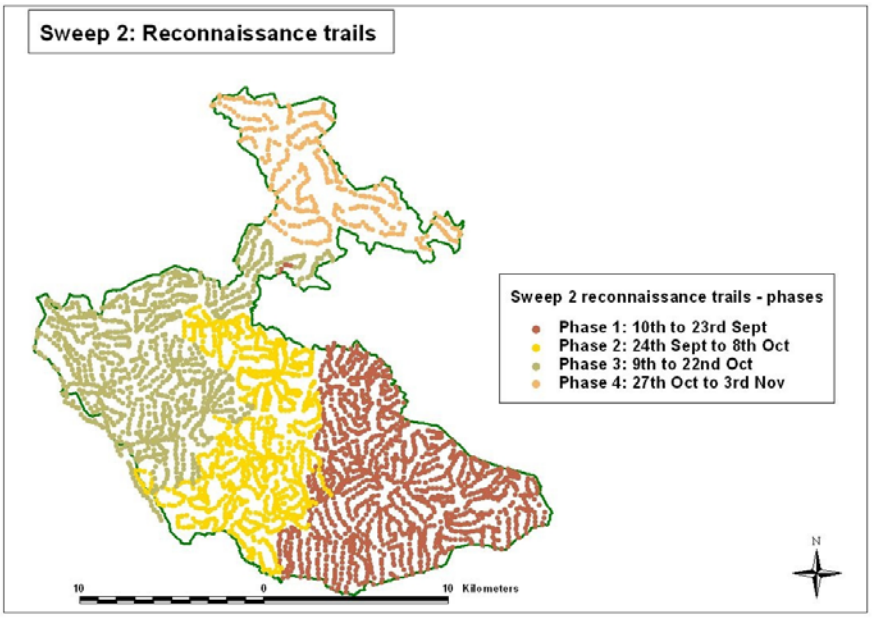


Figure 2. Reconnaissance trails walked during Sweep 2 of 2011 gorilla census.



The area of Bwindi Impenetrable National Park was divided into 33 sectors ranging in size from 4.42 to 17.38 km^2. Each sector was searched by walking an irregular network of reconnaissance routes across the area. When recent gorilla trail (less than 5-7 days old) was found, it was followed until nest sites were located. The actual direction of reconnaissance routes walked was determined largely by the terrain and the availability of existing trails. To ensure that the routes were sufficiently dense so that no area was missed that could have been large enough for a gorilla group to spend more than one week in it, the distance between adjacent trails was never greater than 500 to 700 m. Using topographic maps, along with a GPS and compass, each census team mapped as accurately as possible all paths taken. GPS readings were taken every 250 m along the trail, to ensure that it could be accurately mapped. By mapping and dating all gorilla trails and nest sites, and by marking nest sites encountered with cut sticks, it was possible to minimise the possibility that groups were missed, that none was counted twice, and to distinguish similar sized but distinct gorilla groups found close to each other. The genetic analysis was also essential to confirm the identity of groups of similar size that were found near one another.

At each nest site, nests were counted and dung size measurements, along with the presence of silver hairs, were used to establish the age-sex composition of the group. Teams aimed to find at least three nest sites for each group to confirm the composition of each group, since individual nests or dung could be missed at one nest site. Dung size classes used were as follows:

Adult male (SB): > 7.2 cm (with silver hairs)
Adult female or blackback male (MEDIUM): 5.5 – 7.2 cm
Juvenile/sub adult (JUV): < 5.5 (sleeping in own nest)
Infant (INF): generally < 4cm (sleeping in mother's nest)

Previous experience (McNeilage *et al*., 2001) has indicated that dung size alone is not a sufficiently accurate measure to distinguish between the immature age classes: infant, juvenile and sub-adult. Consequently, young individuals constructing their own nest were always considered as the combined category juveniles/subadults, and not infants, and assigned to the dung size class "JUV". Smaller dung found within the nest of an older individual was always recorded as that of an infant. In the absence of infant dung, adult female nests could not be distinguished from those of a comparable sized (blackback) male, and were therefore classified as "MEDIUM". Fecal samples were collected from all nest sites of habituated and unhabituated groups and lone silverbacks for genetic and veterinary analysis (results not presented here). While habituated groups were located in a similar manner during the sweep census, we used the known composition of these groups for the estimate of their group size (i.e. the genetic analysis was used only for the unhabituated gorillas). As done in previous censuses, groups/solitary males were assigned names based on the sector and the chronological order in which they were found, resulting in nest sites/groups having similar names for Sweep 1 and Sweep 2 (i.e. the first group found in Sector M was assigned the name M1, the second group found in Sector N was named N2, etc for each sweep). To reduce confusion of the names, following the determination of unique groups following the genetic analysis, groups were assigned numerical names and solitary males are identified by ID numbers. Fecal samples were also collected for a population-wide assessment of pathogens, specifically parasites, viruses, and bacteria, but the analysis is still underway and no results of this work are presented here.

2.1. *Survey Methods for Large Mammals and Human Disturbance*

The reconnaissance trails walked while looking for fresh gorilla trail covered a large portion of the whole of the park and therefore provided an opportunity to collect data on other large mammals as well as signs of human disturbance. Distance was measured using the tracklog function on the GPS and the total distance walked on each reconnaissance trail recorded. This distance was later corrected for the terrain using GIS (ArcGIS 9.2). However, when fresh gorilla trail was found and followed, recording of other signs stopped,

as it was not possible to record these systematically while following gorilla trail. The location of each sign or sighting was recorded as a grid reference, using a GPS.

Large mammals included were chimpanzee, elephant, bush pig, bushbuck, black-fronted duiker, yellow backed duiker, l'Hoesti monkey, blue monkey, black and white colobus, redtail monkey, baboons, and carnivores (not identified to species). For all large mammal species, all actual sightings were noted, with the species and group size at each sighting recorded. Where possible, the number of adults, juveniles and infants seen in each group were counted. For elephants, bushpig and carnivores, dung piles were recorded, with the species, and age category for all dung piles encountered ("new" being 0-3 days, "old" 4 or more days).

For human disturbance the location and age category of all signs seen were record ("recent" being less than 3 months, "old" being greater than 3 months). Such signs included snares, pitfall traps, human tracks, poachers' camps, fire damage, actual sightings of poachers, cutting of firewood, building poles and bamboo, felled trees, pitsawing, bark collection, bee hives, signs of gathering wild honey, and any other sign of people using the forest illegally.

In order to systematically compare the frequency with which signs of large mammals and human disturbance were encountered for each sector, encounter rates per kilometre of reconnaissance trail walked were calculated. Encounter rates were used rather than the actual observation value for the signs of large mammals and human disturbance as this takes into account the effort made (i.e. distance walked) by the census teams. Distance was measured using the tracklog function on the GPS and the total distance walked on each reconnaissance trail recorded. This distance was later corrected for the terrain using GIS (ArcGIS 9.2). A total of 778 km of reconnaissance trail was walked during the census.

Only preliminary analysis of signs of large mammals and human disturbance is presented in this report. Currently more in-depth analysis is underway.

2.2 Genotyping from gorilla feces

The main objectives of the genetic analysis of gorillas in different unhabituated groups were to ascertain that groups were not double-counted and to determine the number of unique individuals per group (group membership). A total of 298 and 312 fecal samples were collected from unhabituated gorillas in Bwindi Impenetrable National Park, during Sweeps 1 and 2 respectively. Although the collection of samples from multiple nesting sites for a

particular group was frequently performed in both sweeps (29 out of 45 cases), DNA was extracted from only one nesting site per group in Sweep 1 as compared from all nesting sites in Sweep 2. For both sweeps, the nesting site with the highest number of nests (field data) was fully DNA-extracted, while a minimum of three samples were also extracted from each of the other nesting sites in Sweep 2 only (two sites each for six groups, three sites each for six groups) in order to confirm that groups were consistently identified. In sum, DNA was extracted from 223 and 266 samples, respectively for Sweeps 1 and 2, using the QIAamp DNA Stool Kit (QIAGEN) with slight modifications (Nsubuga et al. 2004). Extracted samples were estimated to be 1-3 days old upon collection. DNA quality of each extract was assessed by PCR amplification of a sex-specific region of the amelogenin locus (Bradley et al. 2001).

DNA extracts which yielded PCR products at the amelogenin locus were then amplified at 12 microsatellite markers using primers tested previously in various great ape species (Arandjelovic et al. 2009): D5s1457 (Cooperative Human Linkage Center), D6s1056-D14s306 (Morin et al. 1998), and D1s550-D2s1326-D4s1627-D5s1470-D6s474-D7s817-D8s1106-D16s2624-vWf (Bradley et al. 2000). These loci were selected based upon their demonstrated efficiency to distinguish with high resolution even genotypes originating from closely related individuals, and represented a subset of loci used in the last study censusing mountain gorillas in Bwindi (Guschanski et al. 2009).

Genotypes were obtained using the two-step multiplexing approach as applied in other studies (Arandjelovic et al. 2009, Gray et al. 2012). Briefly, all microsatellite loci were initially amplified in a single reaction volume of 20 µL: 2.0 µL of 10X 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 next step, 2.5 uL of 1:100 diluted multiplex PCR product was used as template, and all reactions were independently performed in 10-µL reaction volume containing 1.0 µL of 10X reaction buffer, 0.35 µL of $MgCl_2$ (25 mM), 0.5 µL of dNTP (2.5 mM), 0.4 µL of bovine serum albumin (BSA, 20 mg/mL), 0.25 µL of each forward (FAM-, HEX-, or NED-labelled) and reverse primer (10.0 mM for each primer), and 0.04 µL of 0.5 U Super*Taq* (HT Biotechnology) premixed 2:1 with *Taq*Start Antibody (BD Biosciences). The thermocycling conditions were the same as in step 1, except that a primer-specific annealing temperature was used for each singleplex PCR

and varied from 55°C and 60°C (see Arandjelovic et al. 2009 for details). 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).

Four independent replicates of each sample were initially amplified in 96-well plates, and three negative PCR controls (H_2O) were used during the whole process to detect potential DNA contamination. 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 12 additional replicate PCRs were performed to resolve the ambiguous genotypes. Since Guschanski et al. (2009) showed that three replicate PCRs for each extract were sufficient to achieve 99% certainty that a homozygote is indeed such at a given locus, an individual was assigned as homozygote if the same allele was exclusively seen in at least three independent 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 detected in at least two positive PCRs.

2.3 Genetic data analysis

The program CERVUS 3.0.3 (Kalinowski et al. 2007) was first used to compare results from extracts with a minimum of six genotyped loci in order to identify multiple samples produced by an individual, within each sweep. Genotypes matching exactly at eight or more loci, without mismatching at any other locus, were then combined into a consensus genotype after checking for consistency in sex identification. CERVUS 3.0.3 was then launched a second time and all pairs of genotypes matching at a minimum of six loci but mismatching at up to two loci were then checked for data entry errors. These pairs were scrutinized on an individual basis, and the variables dung size, date of nesting site, group of residence and sex identification were used to assess the possibility of them originating from the same individual. As a last step, the same program allowed us to identify across both sweeps all pairs of genotypes matching at a minimum of six loci but mismatching at up to two loci. A list of unique individuals sorted by group could then be derived manually.

In addition to identity analyses, CERVUS 3.0.3 provided the following details at each microsatellite locus when considering only distinct genotypes (i.e. unique individuals): number of alleles, observed and expected heterozygosities (Nei 1978), non-exclusion probability for sib identity (PI_{sib}, Waits et al. 2001), and the Hardy-Weinberg equilibrium test (applying Bonferroni correction factor for multiple testing).

In order to ultimately infer the number of infants that were not sampled in each unhabituated group, genotypes from medium-sized dung samples genetically identified as female (i.e. potential reproductive females) were compared with those derived from small-sized dung samples (i.e. potential infants) from the same social unit. For each infant, only one mother could be assigned in the group, thus excluding all other potential mothers that did not share an allele at each locus with that infant.

3. Results

3.1 Genotyping success and individual identification

A total of 206 (92.4%) and 232 (87.2%) samples were successfully genotyped at a minimum of six loci in Sweeps 1 and 2, respectively. After the genotypes matching exactly at a minimum of eight loci were combined (no mismatch, same sex), the original 206 samples from Sweep 1 resulted in 126 individuals, while the original 232 samples from Sweep 2 yielded 134 individuals. Some individuals in the groups that were found in both Sweep 1 and 2 were not found in each sweep (eg. the final number of gorillas for those groups was based on combining results from both sweeps). Comparisons were then made among individuals identified in Sweep 1 and Sweep 2, to determine which individuals/groups were found in only one sweep or in both sweeps. Ultimately, a total of 195 unhabituated gorillas were revealed throughout the genetic analysis of samples collected during Sweeps 1 and 2.

3.2 Microsatellite marker characteristics

The 195 genotypes were overall 97.2% complete, with the majority of them (191/195, or 97.9%) confirmed at ten or more loci. The genetic markers were polymorphic with an average of 5.50 alleles per locus and a mean observed heterozygosity value of 0.664 (Table 1). The combined non-exclusion probability for sib identity (PI_{sib}) was 1.062×10^{-4} (range: 0.375 – 0.580 per locus, Table 1), thereby confirming the high resolution power of the set of markers as applied to the current population. Even if two individuals could only be compared at the eight least informative loci, the degree of discrimination remained high ($PI_{sib} = 4.005 \times 10^{-3}$). None of the loci used in this study deviated significantly from Hardy-Weinberg equilibrium ($\alpha = 0.05$).

Table 1. Summary of the genetic variation characteristics of the 12 microsatellite loci used in the study, obtained from the whole sample of 195 unique individuals (PIsib, probability of identity among siblings; HO, observed heterozygosity; HE, expected heterozygosity; HW, Hardy-Weinberg equilibrium test at $\alpha = 0.05$; NS, non significant value).

Locus	# alleles	PI_{sib}	H_O	H_E	HW
D14s306	5	0.488	0.652	0.625	NS
D16s2624	4	0.515	0.595	0.595	NS
D1s550	6	0.464	0.658	0.664	NS
D2s1326	6	0.434	0.747	0.703	NS
D4s1627	5	0.434	0.651	0.702	NS
D5s1457	7	0.442	0.731	0.684	NS
D5s1470	5	0.580	0.523	0.517	NS
D6s1056	5	0.559	0.535	0.524	NS
D6s474	5	0.424	0.718	0.719	NS
D7s817	6	0.384	0.800	0.777	NS
D8s1106	4	0.553	0.551	0.527	NS
vWf	8	0.375	0.804	0.789	NS
Overall		1.062×10^{-4}	0.664	0.652	

3.3 Group membership and group size estimate for unhabituated gorillas

The sweeps revealed the existence of 195 genetically distinct unhabituated gorillas, distributed in 26 social units (number of individuals per group: 2 – 17) and 16 solitary males (Tables 2 and 3). A total of 93 males and 102 females were identified, which is similar to the unbiased sex-ratio reported in this population in 2006 (Guschanski et al.,2009). Furthermore, the analysis of samples collected from the same group at more than one nesting site resulted in the identification of 21 individuals that were not revealed at the nesting site with the highest number of nests (field data). This observation was noted in 12 out of 26 social units.

Only 11 of the 26 social units and one of the 16 solitary males were seen in both sweeps (n = 93 gorillas, not including undetected infants). Importantly, six and nine groups (n_{total} = 35 and 52 gorillas, not including solitary males or undetected infants) were exclusively detected in Sweep 1 and Sweep 2, respectively. Nine solitary males were found exclusively in Sweep 1 and 6 solitary males were found exclusively in Sweep 2. In other words, Sweep 1 alone would have resulted in a total of 126 unhabituated gorillas (65% of the total found in the 2 sweeps combined), whereas Sweep 2 would have resulted in 134 unhabituated gorillas (69% of the total found in the 2 sweeps combined; again, not including undetected infants and other correction factors). Of particular note is the detection of a group of 17 individuals (L2) in Sweep 1, which was not detected in Sweep 2. Similarly, a group of 13 individuals (N3) was found in Sweep 2 only.

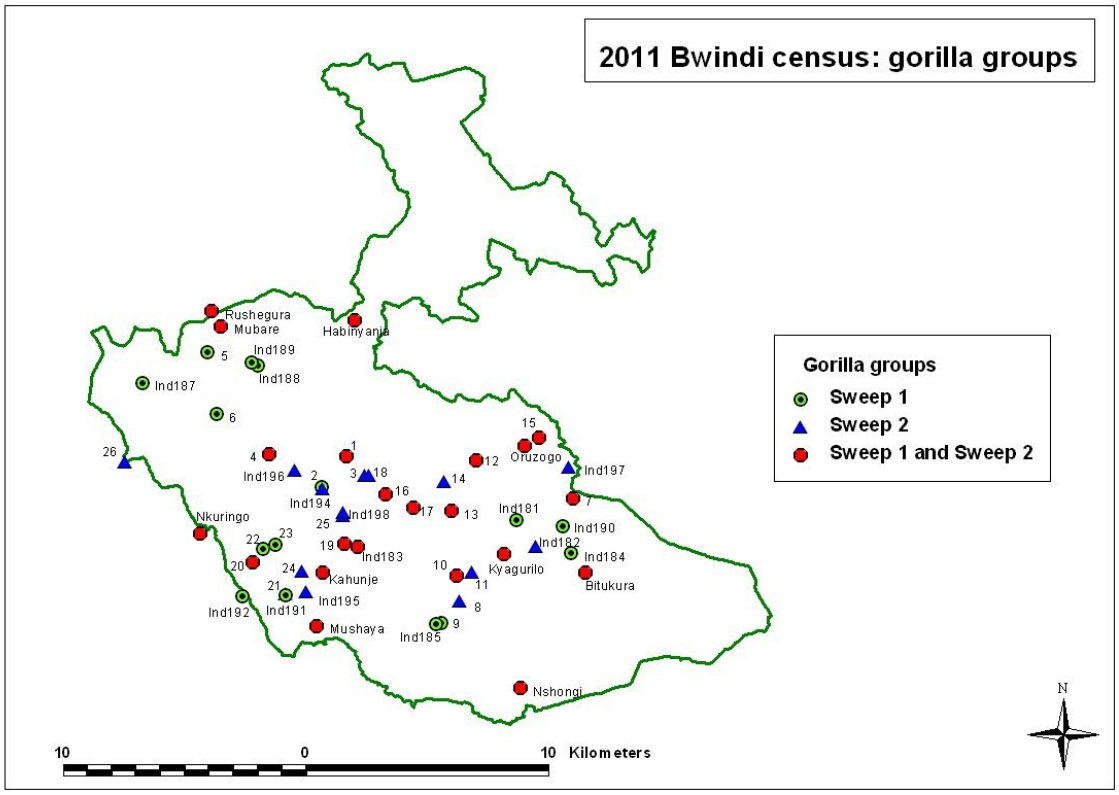
The total number of unhabituated gorillas would have been difficult to infer from nest counts alone, because there was the possibility of both overcounting and undercounting gorillas. For both Sweep 1 and Sweep 2, there were three instances in which nests which could have been attributed to different groups were found to belong to members of the same group. In Sweep 1, groups BB1, CC1 and R2 were found to be the same social unit (*n* = 13 individuals), despite the high variability in the maximal number of nests built by their members (11, 13 and 8 nests, respectively). Likewise, groups DD1 and DD2 were represented by the maximum number of 7 and 5 nests, respectively, while the genetic analysis revealed a total number of seven distinct individuals (based on Sweep 1 only). Furthermore, in Sweep 1 individuals of group M1 (2 nests) were members of groups M2 (7 nests) and M3 (4 nests). These three entities were found genetically to be the same group, which comprised a total of only 4 individuals due to the high prevalence of double-nesting in group M2 (defined as the same gorilla producing 2 nests in one night).

Table 2. Details of the social units of mountain gorillas found in Bwindi Impenetrable National Park during Sweeps 1 and 2 combined in 2011 (GR, unhabituated group; HAB, habituated group; LSB, lone silverback). The range of the number of nests (field data) is also indicated for each unhabituated group.

Social Unit	Number of gorillas	Number of males	Number of females	Field ID Sweep 1	Field ID Sweep 2	Range of number of nests
GR-1	13	8	5	BB1-CC1-R2	CC2	[5-13]
GR-2	12	4	8	N2	N1	[9-13]
GR-3	12	3	9	R1	R1A-R2	[7-12]
GR-4	9	4	5	V3	U3-V2	[7-9]
GR-5	9	1	8	U1	W3	[7-11]
GR-6	8	3	5	DD1-DD2	DD2	[5-8]
GR-7	7	4	3	I2	I2	[6-8]
GR-8	7	3	4	W1	R1B	[6-12]
GR-9	6	2	4	N3	O1	[4-6]
GR-10	5	4	1	N4	N2	{4}
GR-11	4	1	3	M1-M2-M3	M1-M3	[1-7]
GR-12	17	11	6	L2	not found	[15-19]
GR-13	5	1	4	CC2	not found	{6}
GR-14	4	1	3	GG1B	not found	{4}
GR-15	4	1	3	V1	not found	{5}
GR-16	3	2	1	GG1A	not found	{3}
GR-17	2	1	1	V4	not found	{2}
GR-18	13	5	8	not found	N3	{13}
GR-19	8	2	6	not found	W1	[6,7]
GR-20	7	5	2	not found	V5	{10}
GR-21	6	2	4	not found	CC3	{5}
GR-22	6	1	5	not found	V1	{9}
GR-23	4	3	1	not found	Y1	{5}
GR-24	3	3	0	not found	M2	{3}
GR-25	3	1	2	not found	S1	{4}
GR26	2	1	1	not found	L1	{2}
LSB	16	16	0	(10)	(7)	
Kahunje (HAB)	27					
Nshongi (HAB)	22					
Oruzogo (HAB)	20					
Rushegura (HAB)	19					
Habinyanja (HAB)	18					
Nkuringo (HAB)	17					
Kyagurilo (HAB)	16					
Bitukura (HAB)	13					
Mushaya (HAB)	11					
Mubare (HAB)	5					
Total	363	93	102	137	151	

In Sweep 2, the use of genetics helped ascertain that nest sites from two groups in close proximity were in fact the same social unit. Hence, groups M1 and M3 from Sweep 2 (4 nests each) was only one social unit (*n* = 4 individuals), as were groups U3 and V2 (8 and 9 nests respectively, *n* = 9 individuals). Interestingly, one nesting site assumed to originate from group R1 (8 nests) actually belonged to group R2 (7 nests).

Figure 3. Gorilla groups and solitary males found in Sweep 1 and Sweep 2 for a minimum estimate of gorillas in the population in 2011. Numbered groups are unhabituated, numbered individuals are solitary males, groups with names are the habituated groups.



3.4 Estimation of correction factors

Previous censuses have used a variety of correction factors to account for the undetected infants in unhabituated groups. For example, it has been previously assumed that one-third of infants are normally not found during the field census (McNeilage et al. 2001). In the current study, we used the same correction factor method as was done in Bwindi 2006 and Virunga 2010 censuses 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, Gray et al., 2012). To estimate the number of undetected infants, we first assumed that the same proportion of adult females in the unhabituated groups have infants as in the habituated groups, which was 75% of the adult females in the habituated groups (infants <3

years of age). Then, using the genetic information on whether each unhabituated gorilla was male or female in combination with the size of the dung (identified as adult female or medium), we estimated that there were 74 adult females in the unhabituated groups (38% of the 195 unhabituated gorillas). This value is within the range of the proportion of the habituated gorillas that were adult females in Virunga Massif between 1967-2008 (30-40%; Robbins et al. 2011). Assuming 75% of these females had infants, there should be 56 unhabituated infants. We confirmed the presence of 24 infants genetically, and therefore added in 32 infants to the number of unhabituated gorillas.

Another adjustment was necessary to account for the 52 cases in which a reliable genotype could not be obtained at six or more loci due to low DNA quality of the sample. For each social unit, we compared the genotypes derived from problematic samples (at confirmed loci only) to those obtained from the better-genotyped members of the group (six or more loci). In most cases it could be inferred that these samples were partial genotypes of individuals already identified with more complete genotypes. By doing so, we were able to conclude with great confidence that at least five individuals should be added to the final estimate.

In sum, the number of unhabituated gorillas was calculated by adding together the 195 individuals that were identified genetically, the five additional individuals that could not be fully genotyped, and the 32 undetected infants, for the final estimate of 232 unhabituated individuals.

3.5 Total Population Estimate – Combining Known Composition of Habituated Groups & Genetic Analysis of Unhabituated Gorillas

During the census, 10 habituated groups were being monitored on a daily basis for either research or tourism purposes, containing a total of 168 gorillas. Adding in the estimation of 232 unhabituated gorillas results in a total population size of 400 gorillas found in 36 social groups and 16 solitary males (Table 3; Figure 3).

Table 3. Summary of groups and individuals found to determine the final minimum population estimate.

	# Groups	# Gorillas
Sweep 1 Unhabituated -without undetected infants	17	126
Sweep 2 Unhabituated -without undetected infants	20	134
Unhabituated found uniquely in Sweep 1	6	44
Unhabituated found uniquely in Sweep 2	9	58
Unhabituated found in both Sweep 1 and Sweep 2	11	93
Sweep 1& 2 Unhabituated -without undetected infants	26	195
Undetected Infants – unhabituated		32
Individuals added due to incomplete genotypes		5
Habituated Gorillas	10	168
TOTAL NUMBER OF GORILLAS		**400**
% Habituated	28%	42%
% Unhabituated	72%	58%

3.6 Human Disturbance and Illegal Activities

A number of different types of human disturbance were found in Bwindi during the census (Table 4). Antelope snares, beehives, paths and tracks of people, wood cutting (of different types) were among the most commonly encountered signs. However, for any analysis of this data, signs of human paths and tracks were removed due to the concern that it was impossible to distinguish between legal (i.e. from the gorilla tourism program) and illegal paths and tracks. Signs of beehives are difficult to interpret because some of these activities are legal in the park with the multiple-use program. Signs of human disturbance, particularly of snares, found during the census were unevenly distributed across the park, with much of the disturbance concentrated in certain areas such as the northern sector, around Rushaga, Impungu, and Ndego (Figures 4a-e).

A rough comparison of snares found in the 2006 and 2011 censuses (76 snares found in ~600 km of reconnaissance trails in 2006 versus 74 snares found in ~700 km of reconnaissance trails in 2011), suggest that fewer snares were found in 2011. Additionally, fewer snares were found in Bwindi in 2011 compared to the Virunga Massif 2010 census (74 snares in 778 km of reconnaissance trails for an overall encounter rate of 0.095 snares per km walked in Bwindi compared to 218 snares found in 1141 km of reconnaissance trails for an overall encounter rate of 0.191 snare per km walked in the Virunga Massif in 2010). Nonetheless, certain areas of Bwindi should be targeted for anti-poaching efforts to further

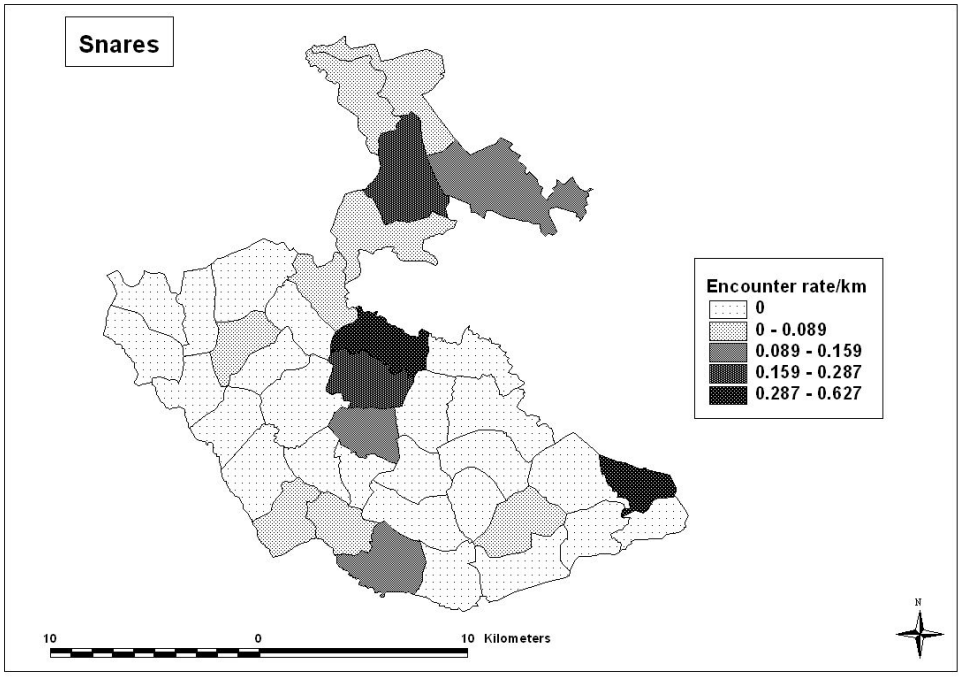
reduce the incidence of snares (Figure 4a). Further analysis of the signs of human disturbance and large mammals from the 2011 census are currently underway.

Table 4. 2011 Bwindi census – Human activity encounter rates

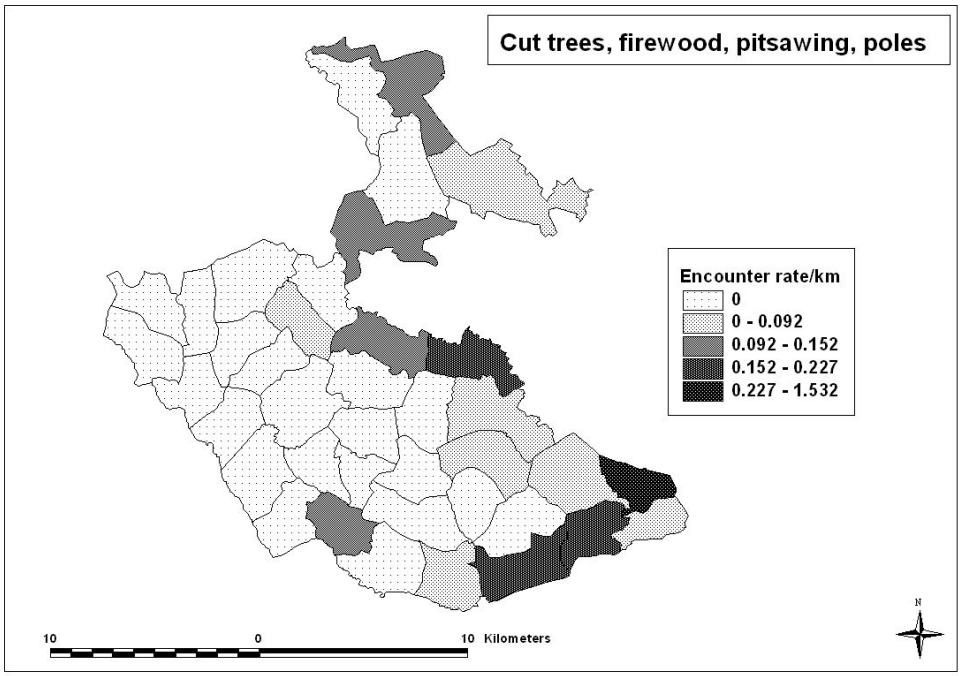
Human sign	Total number of encounters	Encounter rate	Total number of individuals/items
Bark collection	1	0.001	1
Beehives	56	0.069	123
Camp	10	0.012	10
Cut tree	16	0.020	27
Fire	2	0.002	2
firewood	9	0.011	29
Honey	6	0.007	6
Human tracks	144	0.176	211
Pitsaw	6	0.007	6
Poacher	1	0.001	1
Poles	25	0.031	65
Snare	47	0.058	74

Figure 4A-E. Encounter rates per sector of different types of human disturbance/illegal activities found in the Bwindi 2011 census (number found per km of reconnaissance trail walked). A. snares B. cut wood C. illegal camps D. beehives E. All combined.

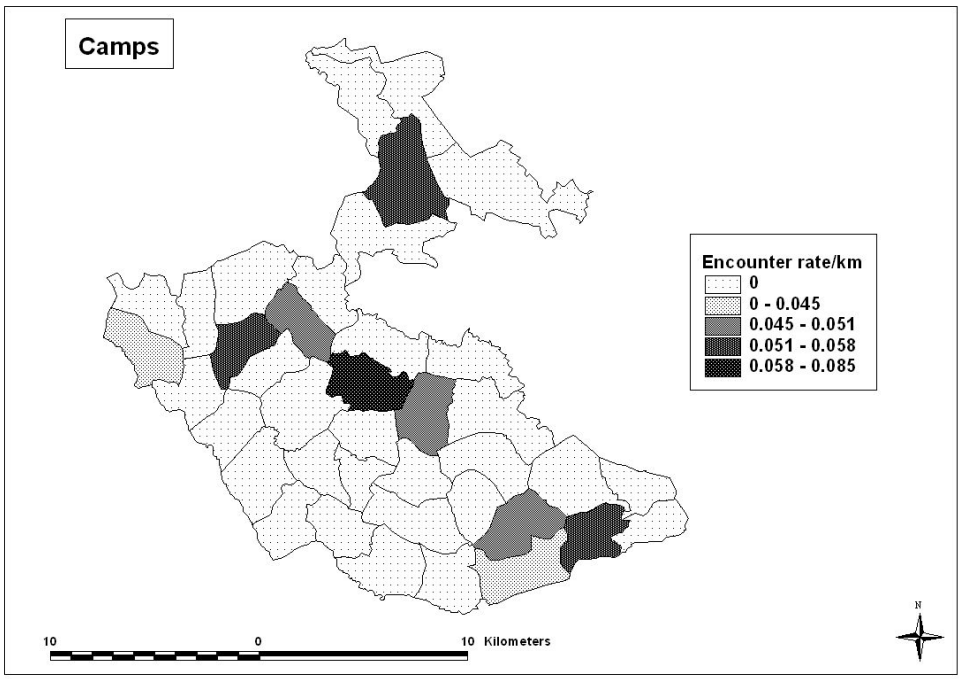
A. Snares



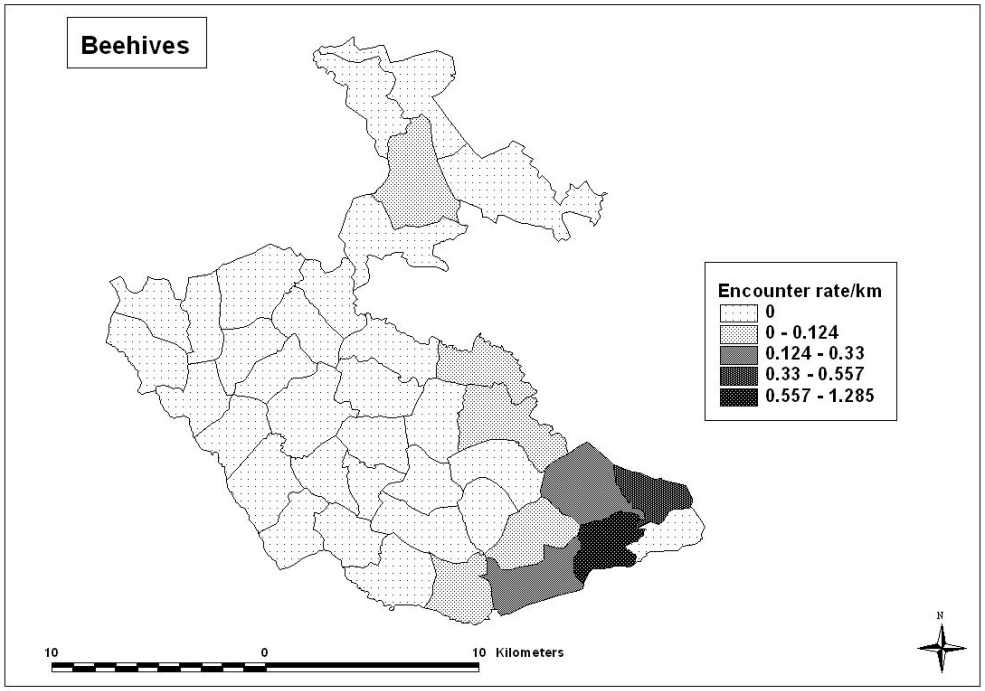
B. Cut wood.



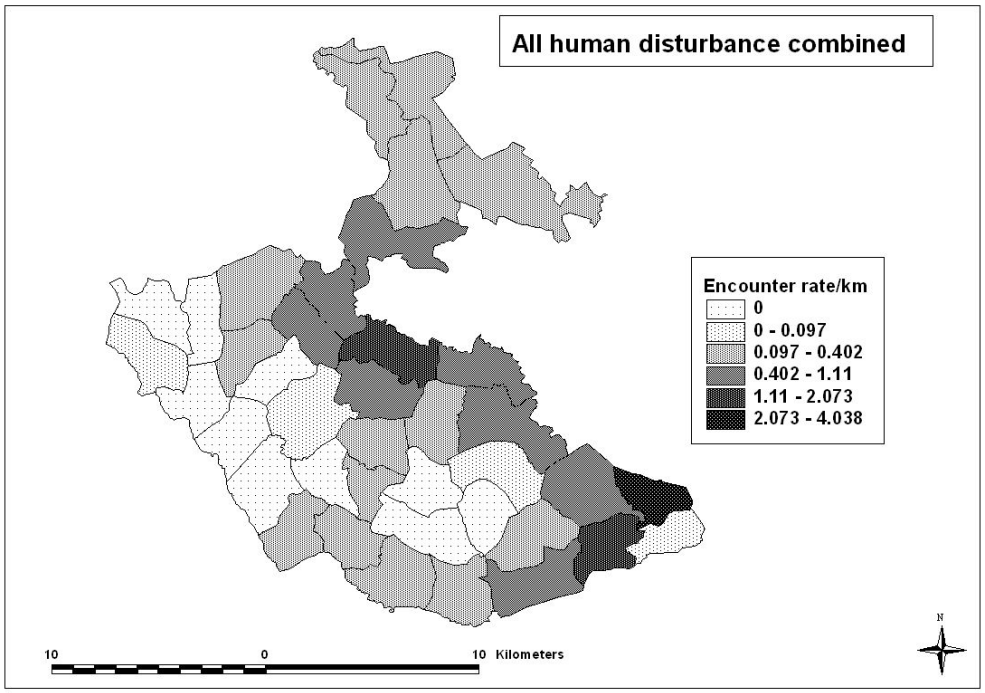
C. Illegal Camps



D. Beehives



E. All human disturbance combined.



3.7 Large Mammals

Many species of large mammals were recorded during the census (Table 5). Carnivore dung was also recorded, although this could not be attributed to a particular species.

Table 5. 2011 Bwindi census – Large mammal encounter rates. Encounter rates are based on number of encounters per kilometers of reconnaissance trails walked.

Observation	Total number of encounters	Encounter rate	Total number of individuals/items
Baboon	3	0.004	19
Bushbuck	4	0.005	4
Black fronted duiker	26	0.032	28
Blue monkey	47	0.058	193
Bush pig dung	76	0.202	165
Bush pig	5	0.006	11
Black & White Colobus	20	0.025	75
Carnivore dung	9	0.011	9
Chimp	4	0.005	18
Chimp nest	235	0.288	826
Elephant	6	0.007	51
Elephant dung	423	0.518	1555
Gorilla trail	1	0.001	1
Gorilla nest	5	0.006	27
L'hoest monkey	8	0.010	49
Redtail tail monkey	26	0.032	151
Yellow backed duiker	3	0.004	3

4. Discussion

4.1 Using genetics to monitor mountain gorilla populations

The current study reinforces the importance of using a genetic approach in estimating population size of rare and elusive species (Waits 2004, Zhan et al. 2006, Arandjelovic et al. 2010, 2011). Such an approach was applied systematically for the first time in mountain gorillas in the 2006 Bwindi census (Guschanski et al. 2009), and then again in the 2010 census in Virunga Massif (Gray et al. 2012). As suggested by both studies, the nest-count based estimate can result in either an overestimate or an underestimate of the population size, due to either double-nesting cases (individual level) and double-counting groups (group level) or conservatively assuming that two different groups found nearby one another are in fact the same group. In both sweeps, a total of 25 cases of double-nesting (out of 362 possibilities) were detected in the unhabituated groups, which is a rate of 6.91%. Because some of these cases of double-nesting were revealed when more than one nest site per group was analyzed, in sum there were 12 cases found in the 26 unhabituated groups in which the number of nests at the largest nesting site exceeded the number of distinct individuals revealed by genetic analysis. A similar value of double-nesting (7.8%) was

reported in the 2006 Bwindi census (Guschanski et al. 2009). Likewise, from both sweeps a total of seven groups that could have possibly been considered to be distinct groups in the absence of the genetic analysis were found to be the same as another group, despite the variability observed in the maximal number of nests built by their members. Without the use of genetics, it is possible that some individuals and groups would have been mistakenly included in the final population estimate, resulting in an overcount. On the other hand, without the genetic analysis the close proximity of some groups could have resulted in two distinct groups being considered the same group (eg. groups R1 and R2 in Sweep 1)

Conducting two sweeps of the park in a relatively short time period, albeit with different temporal aspects of the sampling, yet similar intensity of searching the park, was extremely beneficial and yielded unexpected results. Indeed, only 20 of the 26 unhabituated groups were located in Sweep 2, which most closely resembled previous censuses conducted in Bwindi (approximately same amount of reconnaissance trails walked in a 6 week time period). Sweep 1 found only 17 of the 26 unhabituated groups. The lower number found in Sweep 1 compared to Sweep 2 is most likely due to the fact that the 'sweep' was conducted by fewer teams over a longer time period and not as systematically from east to west as Sweep 2. Due to the natural movements of gorilla groups, it is likely that this lead to some groups being in other areas of their home range when the teams were sampling particular areas. Fifteen out of 26 unhabituated groups (57.7%) were revealed in only Sweep 1 (9 groups undetected) or Sweep 2 (6 groups undetected). Therefore, a significant number of individuals (and groups) would have been undetected if a single sweep had been conducted. This observation highlights the fact that although the genetic approach offers undeniable advantages over the traditional sweep field method, it also has some limitations in that if groups are not detected in the field, samples cannot be collected for genetic analysis. Only samples from individuals and groups that are found in the field can be genotyped and accounted for in the final estimate. It is possible that despite the intense sampling effort with the two sweeps some gorillas were still undetected, but the number of undetected individuals and groups in the field is unknown and virtually impossible to estimate. For that reason, the optimal censusing method would be to conduct more than one sweep.

In addition to providing information for the population estimate, the genetic analysis makes it possible to genetically 'track' individuals between the two time periods because such analysis was conducted in both 2006 and 2011. This will enable us to gain a better understanding of dispersal by both males and females, the formation of groups by solitary males, group fissions, and group disintegrations in the entire population. This research is currently underway and results will be presented at a later date. The focus of the genetic

analysis to date has been on the unhabituated groups because we know the group composition of the habituated groups. However, several groups that were unhabituated in 2006 were habituated in 2011, so genetic analysis on those groups is underway to enable us to make a population wide assessment of group dynamics.

4.2 Population growth of Bwindi mountain gorillas

There were a total of 36 social units and 16 solitary males identified in 2011, which is the greatest number of groups and solitary individuals ever found in Bwindi Impenetrable National Park. As for the 2006 Bwindi census, most of the unhabituated groups were found in the central region of the park, whereas the habituated groups mostly inhabit along the borders of it (Figure 3). The number of habituated groups has increased from 5 to 10 between 2006 and 2011, due to the habituation of four new groups and to the fission of one of these groups (Nshongi). Eight more groups and six more solitary males were found in 2011 as compared to 2006 (36 versus 28 groups, 16 versus 10 solitary males). The larger number of groups found is likely due to some groups being undetected in 2006 as well as some new groups being formed through either group fissions or solitary males acquiring females. Similarly, whether the detection of 16 solitary males was due to a real increase in their number since 2006 or a better detection of them through the use of two sweeps is unknown. Solitary males are difficult to find, however, so it is unclear whether those differences among censuses reflect actual changes in their prevalence.

Despite applying the sweep method twice during the same year, we can not exclude the possibility that some groups of gorillas and solitary males went undetected during the census. However, the fact that the reconnaissance trail coverage during both sweeps was good (over 700 kms walked in each) and that the 36 social groups revealed in this census represent the highest number of groups ever found in Bwindi tends to suggest that only few groups of small size might have been undetected. Similarly, the number of solitary males (n = 16) identified is the largest number ever recorded for that population. Nonetheless, the current estimate of 400 gorillas in Bwindi needs to be regarded as a minimum number of individuals inhabiting the park. This estimate is approximately 100 individuals higher than what was estimated in 2006 (Guschanski et al. 2009), when only one sweep was conducted. Because some adult gorillas were found in the 2011 census that were not detected in the 2006 census, we can conclude that the increase from 2006 to 2011 was due to both some groups being undetected in 2006 and actual growth of the population.

It is not possible to comment on changes in the population size prior to 2006 because the previous censuses used only the sweep method (one sweep) and did not use genetic analysis (Table 6). Therefore it is impossible to know if those estimates were overcounts or undercounts. Given these limitations and that the population has been undoubtedly small, numbering in only a few hundred gorillas, it would be imprudent to attempt to determine if there were changes in the population size prior to 2006.

A detailed analysis of the potential growth rate for Bwindi mountain gorillas, based on the births, deaths and dispersals of individuals in the habituated groups suggested that these groups were growing at approximately 2% annual growth rate (Robbins et al., 2009), which is lower than what has been observed in the habituated groups in the Virunga Massif (Robbins et al., 2011). One explanation for the potentially lower growth in Bwindi is that the interbirth interval is roughly 5 years compared to 4 years in the mountain gorillas of the Virunga Massif (Robbins et al., 2009). This longer interbirth interval would lead to a lower growth rate, assuming mortality is similar in the two populations. The reason behind this longer interbirth interval is unknown, but is likely due to ecological factors such as lower food availability or food of lower nutritional value (requiring the gorillas to expend more energy to meet their nutritional needs). Ongoing research on diet and habitat utilization may elucidate these differences.

Table 6. Comparison of methods used and population estimate from systematic gorilla censuses conducted in Bwindi Impenetrable National Park.

Year	Method Used	Population Estimate	Reference
1997	Single Sweep	300	McNeilage et al., 2001
2002	Single Sweep	320	McNeilage et al., 2006
2006	Single Sweep + Genetic Analysis	302	Guschanski et al., 2009
2011	Two Sweeps + Genetic Analysis	400	This report

The current estimate of 400 gorillas in Bwindi is certainly promising for this population known to be surrounded by one of the highest rural human population densities in Africa (over 300 inhabitants/km^2; Guerrera et al. 2003). Also, it shows that the creation of Bwindi as a national park approximately 20 years ago along with the implementation of effective conservation strategies has resulted in a positive impact on the mountain gorilla population dynamics. If combined with the estimate of 480 gorillas recently found in Virunga Massif

(Gray et al. in press), the total minimum number of mountain gorillas now sums up to 880 individuals, which is approximately 200 individuals more than suggested by the previous censuses conducted in these study areas in 2003 and 2006 (Gray et al 2009, Guschanski et al. 2009). This increase is due to both actual population growth and increased detection of gorillas through the refined sampling techniques and application of genetic analysis.

It should also be noted that while it was initially planned to include in the census Sarambwe Nature Reserve in the Democratic Republic of Congo, a protected area continuous with Bwindi and therefore potential habitat for mountain gorillas, it was not possible to do so due to insecurity in the Sarambwe area at the time of the census. Reports from UWA and ICCN indicate that there are gorillas utilizing Sarambwe so there is the possibility that additional mountain gorillas could be found there. Sarambwe has not be included in any of the four censuses conducted in Bwindi since the late 1990's.

4.3 Large Mammals and Human Disturbance

Ongoing analysis of the data collected on large mammals and human disturbance is underway. Nonetheless, the maps and encounter rates presented here provide an overview of the comparative rates of these variables as well as the spatial variability for each. In particular, the locations of the different types of human disturbance are useful for guiding future law enforcement activities as well as working with local communities to reduce these activities.

4.4 Guidelines for future censuses of mountain gorillas

In addition to providing an encouraging increase in mountain gorilla population size in Bwindi over the last few years, the findings of this study should also be used when designing censuses of mountain gorillas in the future. Importantly, our study showed that a significant number of individuals and groups can be detected and genetically identified through a sweep conducted by only a few teams of experienced field assistants (eg. Sweep 1 in this study), but a substantial amount of the population may go undetected with only one sweep. Indeed, a similar number of distinct unhabituated individuals were revealed in both Sweep 1 and Sweep 2, despite the time to sample the entire park being much longer and the east to west movement being less systematic in Sweep 1 due to fewer teams in the forest. Ultimately, the contribution of both sweeps was essential in providing a more accurate population estimate, because 35 and 52 individuals were exclusively detected in Sweeps 1 and 2, respectively. Conducting more sweeps would yield a more accurate population size estimate than that

obtained through the use of a single sweep or even two sweeps as done in this census because it would result in more captures (detections) and reduce the likelihood of undetected groups. This method could easily be integrated into a larger capture-mark-recapture framework (CMR, Otis et al. 1978, White et al. 1982), with individuals bearing genetic tags as temporal identifiers. Importantly, an interval of confidence around the estimate (that is, index of precision) would be calculated, allowing direct comparisons of population size estimates among future censuses. However, the increased precision due to multiple sweeps needs to be weighed against the increased effort necessary in terms of the difficult and time consuming field work, sufficient field staff, lengthy time to conduct the genetic analysis, and costs of both the field work and the genetic analysis. Additionally, if multiple sweeps are conducted, they should be done in a relatively short time frame to minimize changes in the population from one sweep to the next due to the natural processes of births, deaths, dispersals, group formations, and group fissions.

4.5 Recommendations

- Even with the increase in population size found by this census, it is important to remember that the overall population is very small, and there should be no change in its critically endangered status.

- Given that there is contiguous forest among Bwindi Impenetrable National Park in Uganda and Sarambwe Nature Reserve in the Democratic Republic of Congo, continued transboundary collaboration is important as an instrument of peace in the area (means to improve security) and for management of the transboundary forest ecosystem and the mountain gorilla population.

- Improved protection is necessary in some areas. This will require reinforcing patrols in areas where high levels of human disturbance were found. In particular, signs of human disturbance were unevenly distributed across the park, with much of the disturbance concentrated in certain areas such as the northern sector and around Rushaga, Impungu, and Ndego. Coordination with the multiple use programs should be made to reduce illegal activities in those areas and to provide a better understanding of the relationship between multiple use and illegal activities. While it is reassuring to observe that there are low levels of human disturbance in the interior of Bwindi, continued patrols should be made in this area to ensure ongoing protection. It will also require increased efforts with the local communities living to increase their understanding and awareness of

conservation issues and to work with them to find new ways of reducing pressure on forest resources.

- Since such a large proportion of the population (28% of groups; 43% of gorillas) is now habituated to human presence, and this brings inherent risks of disease transmission from humans, behavioural disturbance and potential vulnerability to poaching, this proportion should not be further increased by habituating any more groups.

- The ranger based monitoring programme is a key tool for monitoring the gorilla population and the threats to its conservation. Ongoing collaboration among UWA, MPI-EVAN, MGVP, CTPH, and IGCP to ensure that the trackers and guides can identify the individual gorillas in the habituated groups and report any changes to management is beneficial for ensuring that the demography of these groups can also be used to assess the population dynamics of the Bwindi mountain gorillas. Further analysis of long-term demographic data on the habituated groups will improve our understanding of demographic processes (birth, mortality, and dispersal rates). Such temporal analysis will compliment the detailed "snapshot" of the population that a census such as this provides.

- Further specific research studies are needed to assist management strategies and to better understand the key factors driving the abundance and distribution of the gorilla population (including ecological factors, population dynamics and human disturbance). Such research could include studies of human activities (in areas bordering the park as well as illegal activities inside the park), human/wildlife conflict, habitat utilization by the gorillas, regeneration of vegetation consumed by the gorillas, and estimating carrying capacity. Comparisons with similar studies conducted in the Virunga Massif would benefit both populations.

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