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Challenges in the use of genetic mark-recapture to estimate the population size of Bwindi mountain gorillas (*Gorilla beringei beringei*)

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ABSTRACT

Monitoring the population dynamics of endangered species is a critical component of conservation management strategies, but attaining accurate and precise estimates of population sizes using cost and time effective methods can be challenging. Routine censuses of the two populations of critically endangered mountain gorillas (*Gorilla beringei beringei*) have been conducted over the last decades to monitor populations and evaluate the effectiveness of conservation strategies. A census in 2006 of the mountain gorillas in Bwindi Impenetrable National Park, Uganda, showed the value of genetic analysis of fecal samples collected at nest sites by revealing discrepancies between the numbers of nests and uniquely identified gorillas. In this study, we censused the Bwindi gorilla population using a 'mark-recapture' method which involved genetic analysis of fecal samples collected in 2011 during two 'sweep' surveys of the entire park. We found that a notable proportion of gorillas were missed in either of the two sweeps (minimum 35% and 31%, respectively). Based on the number of genotyped gorillas and correction factors, we estimated the population to contain a minimum of 400 individuals. Using the mark-recapture approach, we infer possibly as many as 430 gorillas (95% confidence interval: 398–487). As the 2010 census of the Virunga Massif population found 480 gorillas, the total number of mountain gorillas worldwide is at least 880 individuals. Simulations using different mark-recapture models suggest that a future census of Bwindi mountain gorillas would benefit by increasing the number of sweeps in order to achieve accurate and precise results. Finally, based on our results, we recommend a sequential approach incorporating a pilot study and simulations for optimizing time and resources in large mammal genetic census studies.

1. Introduction

To 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 (Nichols and Williams, 2006). Many approaches have been used to make population assessments of rare, elusive species including line transects (Kühl et al., 2008; Marques et al., 2001; Morgan et al., 2006), audio recordings (Wrege et al., 2010), and camera traps (Head et al., 2013; Rowcliffe et al., 2008). Additionally, genetic tracking and mark-recapture methods are being used with increasing frequency to estimate population sizes in a variety of animals (e.g. grizzly bear (*Ursus arctos*): Boulanger et al., 2002; imperial eagle (*Aquila heliaca*): Katzner et al., 2011; river otter (*Lontra canadensis*): Mowry et al., 2011; giant panda (*Ailuropoda melanoreuca*): Zhan et al., 2006), including several populations of great apes (Arandjelovic et al., 2010,2011; Gray et al., 2013; Guschanski et al., 2009; Moore and Vigilant, 2014).

Mountain gorillas (*Gorilla beringei beringei*) are arguably the best monitored ape subspecies, with routine censuses conducted approximately every 5–10 years since the 1970s for the Virunga Massif population (Gray et al., 2013) and since the late 1990s for the population in Bwindi Impenetrable National Park, Uganda

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(Guschanski et al., 2009). Because the habitats of both of these populations are relatively small (Virunga: 450 km^2, Bwindi: 330 km^2) and the terrain is difficult to traverse due to steep slopes and thick vegetation, the method used to census mountain gorillas differs from transect-based methods used elsewhere for other great apes (e.g. Kühl et al., 2008; Murai et al., 2013; Nakashima et al., 2013). As the gorilla groups move through the forest, they leave easily detectable trails and construct groups of individual nests every evening. Therefore, researchers have devised a 'sweep' method in which several teams walk systematically through the forest looking for fresh signs of gorillas and subsequently estimate the number of unhabituated gorillas based upon the number of night nests found, as detailed in (McNeilage et al., 2001,2006). Briefly, the number of nests found at a given nesting location represents the number of individuals assumed to be part of the detected group, and two identically-sized groups found in close proximity to each other are considered to be the same group if the estimated dates of the nesting sites are similar. To identify recent nesting locations, field members aim to localize gorilla trails less than five days old. Using this approach, the simultaneous presence of numerous teams over the limited habitat is expected to result in nearly 100% detection of gorilla groups although such a counting approach does not permit gauging the magnitude of the potential error associated with the population size estimate.

However, the sweep method incorporates several assumptions that may lead to inaccuracies in the population size estimate (Gray et al., 2013; Guschanski et al., 2009). First, gorillas may on occasion make more than one nest per night and not all nests may be found at a nesting site, resulting in potential over and underestimation of the number of gorillas represented by a nesting site. Second, the sweep method may result in the counting of a particular group more than once (for example, if its nest sites are found in different locations with differing numbers of nests) or the misattribution of two or more unique groups as the same group (for example, if their nest sites are found in the same area and have similar numbers of nests). Both forms of error were detected in a census of Bwindi mountain gorillas conducted in 2006 which employed genetic analysis of fecal samples collected in parallel with the nest counts (Guschanski et al., 2009). Overall, including genetic analysis greatly reduces the problems of possibly under- or overcounting gorillas using only physical evidence. However, since all areas of the habitat are traversed only once with a single sweep method, there is the possibility that some gorillas are not detected physically or genetically at all, resulting in an undercount of the population. Application of a genetic 'mark-recapture' method, in which the habitat is traversed more than once, so that individuals not detected during the first sweep (or initial 'capture' session) may be located in subsequent sweeps (potential 'recapture' session for already tagged individuals), is an increasingly common censusing approach that reduces the likelihood of a downwardly biased population size estimate (e.g. Arrendal et al., 2007; Harris et al., 2010; Marucco et al., 2012; Mowry et al., 2011). In addition to increasing the chance of detecting all individuals in the population, multiple sampling sessions increase the frequency with which individuals are sampled more than once ('recaptured') and such data are essential for accurate model-based estimation of population size and associated uncertainty values (Otis et al., 1978).

The mountain gorilla population of Bwindi Impenetrable National Park, Uganda, was censused using the single sweep method in 1997 and 2002, yielding point estimates of 300 and 320 gorillas (McNeilage et al., 2001,2006). In the 2006 Bwindi census, use of genetic analysis reduced the number of gorillas inferred solely from the sweep method by some 10%, to a minimum of approximately 300 gorillas (Guschanski et al., 2009). However, due to the possibility of similar overcounts or undercounts in the previous Bwindi gorilla censuses, it is not clear if the population has been increasing or decreasing over the past decade. In 2006, five groups comprising 76 individuals were habituated to human observation, so the census focus was upon ascertaining the number of individuals in the remaining ~75% of the population. Similarly, although a total of 10 groups (including the five groups newly habituated between 2006 and 2011) containing 168 individuals were habituated in 2011, a census was required to estimate the number of unhabituated gorillas in order to obtain a total population size estimate.

The census conducted in 2011 thus aimed to assess the number of unhabituated Bwindi mountain gorillas and evaluate the impact of methodological changes to the census procedure. We used a mark-recapture method by combining genetic analysis with samples obtained during two 'sweeps' of the entire park. The use of two surveys closely-spaced in time is important, as it allows evaluation of the assumption that the sweep method encounters all gorillas in addition to minimizing the violation of the assumption of demographic closure of the population. By using two sweeps characterized by a similar total distance traversed by field members and a similar number of samples collected (see Material and methods section), we predicted that nearly all groups and individuals detected in one sweep would also be found in the other sweep, thus yielding a very high individual recapture rate which would then lead to a narrow confidence interval around the population size estimate. The primary goals of this census were to (a) obtain a minimum estimate of the total population size by counting the number of unhabituated gorillas genetically detected during two sweeps and adding the known number of habituated gorillas; (b) apply mark-recapture models to obtain an error estimate around the estimate of the number of unhabituated gorillas; (c) compare the results of this study with the previous 2006 genetic census in order to get an insight into the population growth of Bwindi mountain gorillas, (d) produce recommendations for future improvements in mountain gorilla census methodology, and (e) describe an approach towards the efficient implementation of large mammal genetic census studies.

2. Material and methods

2.1. Census methods and sample collection

The sweep method was conducted as in previous mountain gorilla studies (e.g. Gray et al., 2009,2013; Guschanski et al., 2009; McNeilage et al., 2001,2006). As in the previous Bwindi surveys (Guschanski et al., 2009; McNeilage et al., 2001,2006), 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 a team walking an irregular network of reconnaissance routes across the area. Each team contained individuals experienced in tracking gorillas and in estimating the ages of trails signs and nests. When a 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. The distance between adjacent trails was never greater than 500 to 700 m to minimize the possibility that an area was missed that could have been large enough for a gorilla group to spend more than one week in it. Using topographic maps, along with a GPS and compass, each census team mapped as accurately as possible all paths walked, and mapped and dated all gorilla trails and nesting sites encountered. GPS readings were taken every 250 m. Sweep 1 took place between February 28 and September 2, 2011, with between one and three teams (four or five individuals per team) working at any one time. A total of 746 km of reconnaissance trails were walked (Fig. 1).

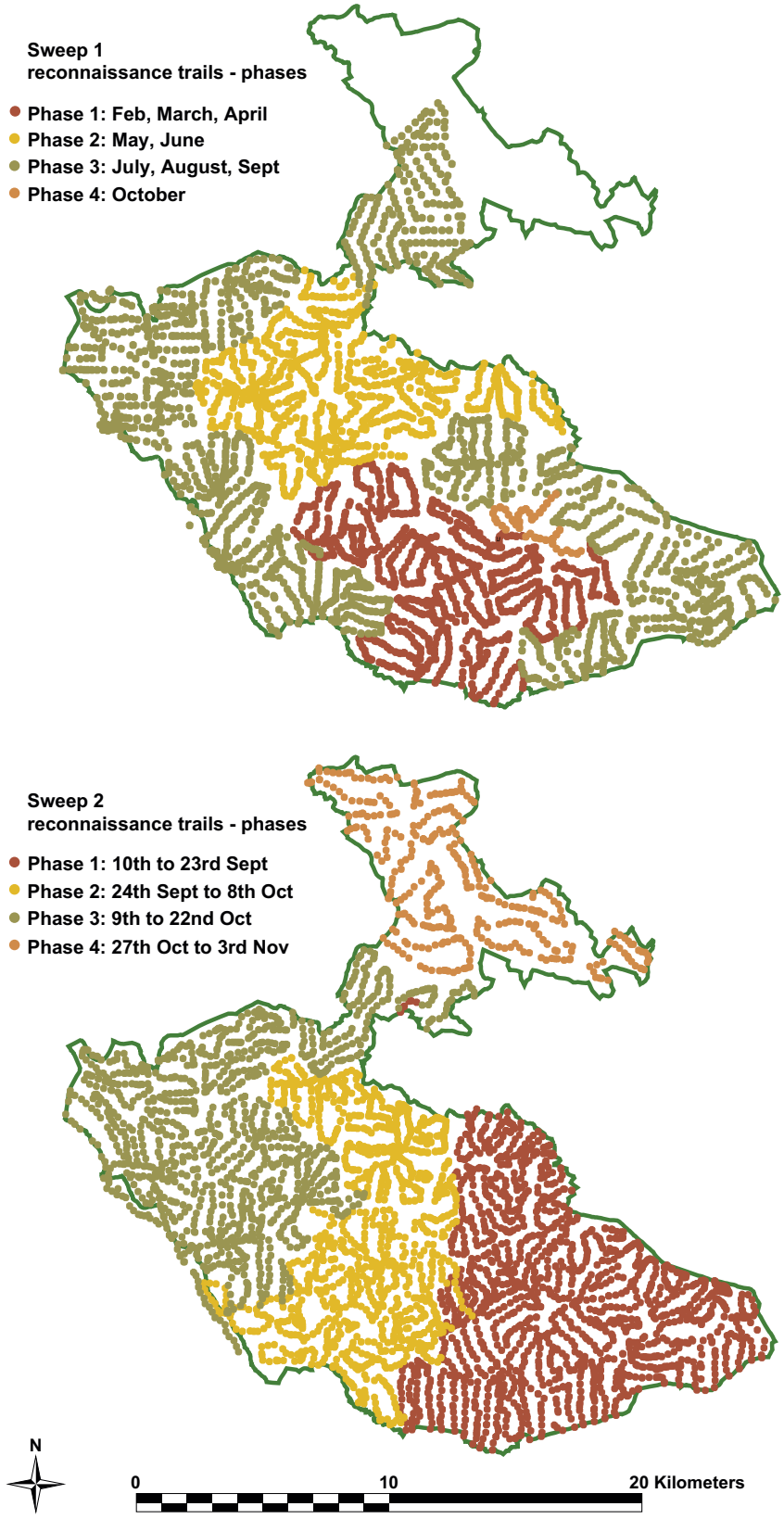

Fig. 1. Reconnaissance trails walked during Sweep 1 and Sweep 2 of the 2011 Bwindi mountain gorilla census. Approximate survey dates are given. The duration of Sweep 1 was longer than of Sweep 2, but fewer teams were involved in Sweep 1 and the total number of km surveyed and samples collected was similar between sweeps.

Sweep 2 was conducted from September 10 until November 3, 2011, with six teams (four or five individuals per team) simultaneously moving from east to west in the main portion of the park. They walked a total of 778 kms of reconnaissance trails (Fig. 1). Coverage of the northern sector was light in accordance with the unsuitable habitat and reported lack of gorillas in much of this area (Guschanski et al., 2009; McNeilage et al., 2001,2006).

At each nesting site, the number of nests was counted and dung size measurements, along with the presence of silver hairs, were used to estimate the age–sex composition of the group. By following apparent gorilla trails, teams aimed to find at least three nesting sites for each putative group, since individual nests or dung could be missed at any nesting site. Dung size alone is not a sufficiently accurate measure to distinguish between the immature age classes of infant, juvenile and sub-adult (McNeilage et al., 2001). Consequently, young individuals with their own nests 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". Nests containing larger dung and silver hairs were considered to be from silverbacks (fully mature males). Fecal samples for genetic analysis were collected from all encountered nesting sites of habituated and unhabituated groups and lone silverbacks. Although habituated groups were located in a similar manner during the census, we used the known composition of these groups for estimation of their group sizes. As in previous censuses, unhabituated groups as well as solitary males were assigned names based on the sector and the chronological order in which they were found, resulting in nesting 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, and so on, for each sweep). To reduce confusion, after the determination of unique groups following the genetic analysis, groups were assigned numerical names and solitary males were identified by ID numbers.

2.2. Genotyping from gorilla feces

A total of 298 and 312 fecal samples were collected during Sweeps 1 and 2, respectively. Collection of samples from multiple nesting sites for a particular group was frequently performed in both sweeps (29 of 45 cases). Samples were collected and stored using the two-step method consisting of a short period of storage in ethanol followed by desiccation using silica (Nsubuga et al., 2004). For both sweeps, all of the samples from the nesting site with the highest number of nests (based on field data) were extracted, while a minimum of three samples estimated by size to originate from mature individuals 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). This additional step was employed to confirm that groups were consistently identified. In addition, if the nest site with the highest number of samples for a particular putative group from Sweep 1 failed to produce usable DNAs, we analyzed samples from a second nest site. In sum, DNA was extracted from 223 (75%) and 266 (85%) samples from Sweeps 1 and 2, respectively, using the QIAamp DNA Stool Kit (QIAGEN) with slight modifications (Nsubuga et al., 2004). Extracted samples were estimated by experienced field researchers 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 loci analyzed 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 of distinguishing with high resolution even genotypes originating from closely related individuals, and represented a subset of the 16 loci used in the last genetic census of mountain gorillas in Bwindi (Guschanski et al., 2009).

Genotypes were obtained using the two-step multiplexing approach as recently described (Arandjelovic et al., 2009; Gray et al., 2013). Briefly, all microsatellite loci were initially amplified in a single reaction 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 next step, 2.5 μL 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 10× 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-labeled) 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 DNA-extracted sample were initially amplified in 96-well plates for both the multiplex and the singleplex PCR steps (Arandjelovic et al., 2009), and three negative PCR controls (H_2O) were used throughout the entire 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 ambiguous genotypes. Since an earlier study of the same population using the same sample collection and preservation methods showed that three replicate PCRs for each extract were sufficient to achieve 99% certainty that a homozygote is indeed such at a given locus (Guschanski et al., 2009), we assigned an individual as homozygote if the same allele was exclusively seen in at least three independent PCRs. For sex identification, an individual was assigned as female if the approximately 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 approximately 110-bp band was also detected in at least two positive PCRs.

2.3. Genetic data analysis

We used the program CERVUS 3.0.3 (Kalinowski et al., 2007) to compare results from extracts with a minimum of eight 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 first 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, and we then manually examined these matches to see if they indeed represented the same individual. If allelic dropout was observed by comparison of genotypes from different samples from what was evidently the same individual, the missing allele was incorporated into the final genotype. A list of unique individuals sorted by group was then derived manually by comparing directly the individuals found in Sweep 1 with those found in Sweep 2. Individuals were either gathered as part of the same group or considered lone silverbacks if their samples were genetically revealed to be a male and not found in spatial proximity to other samples.

In addition to identity analyses, CERVUS 3.0.3 provided the following details at each microsatellite locus when applied to the dataset of unique individual genotypes: 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 ($\alpha = 0.05$).

Because the dung of small infants may be absent or difficult to find, in order to ultimately infer the number of infants that were not sampled in each unhabituated group, we compared genotypes from medium-sized dung samples genetically identified as female (i.e. potential reproductive females) to those derived from small-sized dung samples (i.e. potential infants) from the same social unit. As in the previous 2006 genetic census in Bwindi (Guschanski et al., 2009), we assumed that 75% of adult females had an infant in the group at the time of the census. For each group we compared the genotypes from samples of adult size which were genetically determined to represent females with genotypes from infant size dung, thus providing an indication of the relative numbers of samples and expected infants in the group We found only one genotypically compatible mother genotype for each infant genotype.

2.4. Estimate of number of habituated gorillas from field information

The ten habituated groups are checked daily and all births, deaths, and transfers are recorded. The group compositions on October 1, 2011 during the second sweep were used for including the number of habituated gorillas in the total population size estimate. In this study, we do not report an estimate of the number of unhabituated gorillas solely from field data, but briefly note that such previous estimates were based upon the largest number of nests observed from one to several nesting sites for a purported group, and that groups were distinguished based upon apparent numbers of members and locations (Guschanski et al., 2009; McNeilage et al., 2001,2006).

2.5. Mark-recapture unhabituated population size estimation using genetic data

We used our genetic record of captures and recaptures to estimate the number of unhabituated gorillas by applying four capture-mark-recapture (CMR) models designed for closed populations in the software MARK (White and Burnham, 1999), using the *Full Closed Captures with Heterogeneity pi, p, and c* data type. This category of models includes the desired term of abundance (N) in the likelihood expression as part of one of the parameters to be estimated (Otis et al., 1978). In addition to the null model M_o {N, $p(.) = c(.)$}, three other models were applied to the dataset, each accounting for a different potential source of variation in the encounter probability: behavioral effect M_b {N, $p(.)$, $c(.)$}, time effect M_t {N, $p(t) = c(t)$}, and heterogeneity effect M_h with 2 mixtures {N, $p(a) = c(a)$, $p(b) = c(b)$, Π} (Pledger, 2000). All models were created manually using the Parameter Index Matrices (PIMs) windows in the software MARK. An averaged abundance estimate (with 95% confidence interval calculated manually) was subsequently derived using the model averaging procedure (Burnham and Anderson, 2002) implemented in the software. Although we did try initially to apply to our dataset more general models accounting for a combination of the above factors (b, t, h), we do not present the results here because these models were very poorly supported based on the corrected Akaike Information Criterion (AICc), likely a consequence of the relatively small sample size and low number of sampling occasions (i.e. sweeps).

2.6. Simulations as guidelines for future improvement of census methodology

We also used MARK to conduct two rounds of simulations to determine how many sampling occasions would be necessary in a future census. We determined the minimum number of sampling occasions according to the following criteria (1) we required that the simulations accurately produce an estimate of 400 gorillas, or an estimate close to that number (2) we required that the most realistic model among those defined in Section 2.5, that is the 2-mixture heterogeneity model, be the best supported of the four models employed and (3) finally, we required a 95% confidence interval around the estimate that would allow a 2% increase in the annual growth rate to be detected with statistical confidence after five years between censuses conducted under the specified conditions. Because heterogeneity in capture probability among individuals is a predominant feature of virtually all CMR studies (Otis et al., 1978; Pledger, 2000), we emphasize that the sampling methodology in future censuses should be defined in a way that the 2-mixture heterogeneity model is the basis of an accurate and precise estimate of the population size. We note that in both rounds of simulations we conservatively included a large number of gorillas (400) as compared to the number of unhabituated gorillas (230–319) estimated in this study, but do not expect this to affect the inferences obtained.

For the first round of simulations, we increased the number of sampling occasions stepwise from two to six and for each performed 1000 simulations using the 2-mixture heterogeneity model as the true model and then applied the same four CMR models as described above (Section 2.5). For these simulations, fixed parameters of the true model were based on parameters estimated from the 2011 Bwindi genetic census dataset (this study). Since it is *a priori* reasonable to assume that the capture probability of an individual might differ according to group size, we divided the dataset into two categories (mixtures), namely (a) groups of 5 or more individuals (encompassing ca. 75% of the unhabituated gorillas), and (b) groups of 4 individuals or less, including lone silverbacks (encompassing ca. 25% of the unhabituated gorillas). For each category, we estimated the capture probability to be at most 0.69 and 0.59, respectively. For simulation purposes, we mimicked a worse-case scenario by entering the following parameters: probability that an individual belongs to mixture $A = 0.70$, probability of capture of an individual in mixture $A = 0.65$, probability of capture of an individual in mixture $B = 0.40$, and population size of 400 individuals. The optimal number of sampling occasions was determined by looking at the accuracy and the 95% CI of the abundance estimate obtained under the true 2-mixture heterogeneity model (M_h) as well as the percentage of the first 100 simulations for which this model received the best support based on AICc value.

For the second round of simulations, we mimicked a scenario in which the capture probability for each of the two mixtures is increased substantially while keeping constant the other

parameters: probability that an individual belongs to mixture $A = 0.70$, probability of capture of an individual in mixture $A = 0.95$, probability of capture of an individual in mixture $B = 0.70$, and population size of 400 individuals. As for the first round of simulations, we performed 1000 simulations using the 2-mixture heterogeneity model as the true model and then applied the same four CMR models as described previously. By doing these simulations, we were interested in investigating the impact of increased sampling effort on the number of sweeps needed to reach high accuracy and precision of the abundance estimate under model M_h. We expected this number to be smaller than for the first round of simulations since the simulations were here performed with higher capture probabilities. For that reason, we tested only two and three sampling occasions. Ultimately, we aim to compare the recommended number of sweeps obtained in the first and second rounds of simulations.

3. Results

3.1. Genotyping success and genetic count of unhabituated individuals

The two sweeps covered 746 and 778 km, respectively and produced 298 and 312 samples, revealing similar sampling intensity for each of the two sweeps despite the differing number of teams employed. 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 same-sex genotypes matching exactly at a minimum of eight loci were combined, the 206 analyzed samples from Sweep 1 reduced to 126 individuals, while the 232 analyzed samples from Sweep 2 yielded 134 individuals. We compared genotypes of individuals identified in Sweep 1 and Sweep 2, to determine which individuals or groups were found in only one or both sweeps. 61 individuals were found only in Sweep 1, 69 individuals were found only in Sweep 2, and 65 individuals were found in both sweeps. Thus, a total of 195 different unhabituated gorillas were detected through the genetic analysis of samples collected during Sweeps 1 and 2. Six, nine, and 11 groups were detected in Sweep 1, 2 and both sweeps, respectively (Fig. 2).

3.2. Microsatellite marker characteristics

The genotypes from 195 gorillas were on average 97.2% complete, with the majority of them (191/195, or 97.9%) confirmed at ten or more loci. There were 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 ($PI_{sib} = 4.005 \times 10^{-3}$), suggesting that we are unlikely to have missed individuals due to insufficient resolution in our genotyping. Only one pair of genotypes mismatched at fewer than three loci while matching at six loci, thus suggesting that the incidence of overcounting individuals because of errors causing genotypes from different samples from the same individual to look different is extremely low. None of the loci used in this study deviated significantly from Hardy–Weinberg equilibrium ($\alpha = 0.05$).

3.3. Group membership and group size estimates for unhabituated gorillas

The genetic analysis identified 195 unhabituated gorillas, including members of 26 social units (number of individuals per group: 2–17) and 16 solitary silverback males (Table 2). A total of 93 males and 102 females were identified. The genetic analysis of samples collected from the same putative group at more than one nesting site, an analysis we undertook either when the first nest site produced a limited number of usable DNAs or the nest sites varied substantially in number, resulted in the identification of 21 individuals that were not detected at the nesting site with the highest number of nests. This occurred in 12 out of 26 social units, and represents the number of individuals that might have been erroneously missed if only nest count data were used. It is important to note that analysis of multiple nest sites of a putative field-identified group did not identify cases in which nest sites attributed to one particular putative group actually derived from different groups. This suggests that even though we limited our analysis to 75% and 85% of the samples collected in Sweep 1 and 2, respectively, analysis of the remaining samples would only have identified a limited number of individuals from already identified groups. As expected based on previous studies (Guschanski et al., 2009), the number of nests detected at a nest site did not always correspond to the number of genotypes detected and a total of 25 cases of double-nesting (out of 362 possibilities) were detected in the unhabituated groups, which is a rate of 6.9%.

There were six instances (three in each sweep) in which nest sites that might have been attributed to different groups were found via genetic analysis to belong to members of the same group (Table 2). The number of unhabituated gorillas inferred solely from counts of these specific nest sites might have been as high as 82 individuals, but genetics revealed the presence of 37 individuals, a reduction of some 55%. If only field data were employed, the potential overcount of 45 gorillas would have been partly compensated by the likely undercount of 21 individuals mentioned above, but at an obvious cost in terms of understanding true group numbers and composition.

Only 11 of the 26 social units and one of the 16 solitary males were detected in samples from both sweeps ($n = 65$ gorillas, Table 3; Fig. 2). Specifically, six and nine groups ($n_{total} = 35$ and 52 gorillas, not including solitary males or undetected infants) were detected only in Sweep 1 and Sweep 2, respectively. Nine solitary males were found exclusively in Sweep 1 and six solitary males were found exclusively in Sweep 2. Genetic analysis from Sweep 1 alone would have resulted in the detection of 126 unhabituated gorillas (65% of the total found in the 2 sweeps combined), whereas samples from Sweep 2 alone would have resulted in the detection of 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 large 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.

3.4. Adjusted minimum genetic count of the number of unhabituated gorillas

We know from genetic analysis of nest sites from habituated groups of known composition that we are less likely to sample the feces of infants, defined as offspring under the age of three who do not typically make their own nests. Hence, we needed to correct our estimate of the number of unhabituated gorillas by accounting for missed infants. As we did previously (Gray et al., 2013; Guschanski et al., 2009), we first assumed that the same proportion of adult females in the unhabituated groups have infants as do females in the habituated groups (75%). Using the genetic information on whether each unhabituated gorilla was male or female in combination with the dung size classification (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 consistent with the estimated proportion of adult females in the

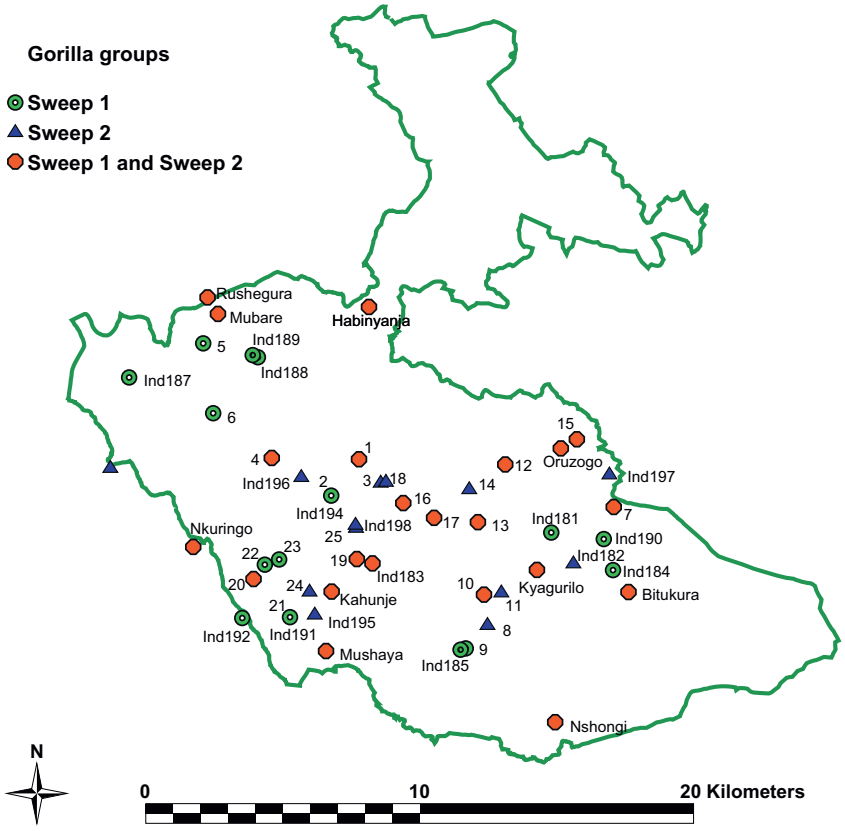

Fig. 2. Locations of gorilla groups and solitary males found in Sweep 1 and Sweep 2. Numbers designate unhabituated groups, symbols with 'Ind' represent solitary males, and named groups are habituated.

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 (PI_{sib}, probability of identity among siblings; H_O, observed heterozygosity; H_E, 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	

habituated groups in the Virunga Massif between 1967–2008 (30–40%; Robbins et al., 2011). Assuming that 75% of these females had infants, there should be 56 unhabituated infants. We confirmed the presence of 24 infants genetically, and therefore added 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 problematic samples had yielded partial genotypes of individuals already identified with more complete genotypes from other samples. By doing so, we were able to conclude that a minimum of five individuals should be added to the final genetic count of unhabituated gorillas. In sum, the minimum number of unhabituated gorillas was estimated 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 a final minimum estimate of 232 unhabituated individuals.

3.5. Mark-recapture population estimate of number of unhabituated gorillas

The mark-recapture population estimate was based upon the frequency with which we detected each of the 195 genetically identified individuals in Sweeps 1 and 2. The null model (M_o) was the most supported model for our dataset based on the AICc for model selection. Its AICc weight (0.444) was more than twice that of the second and third most supported models (M_t: 0.206, M_b: 0.189, respectively), and nearly three times higher than the AICc weight for the heterogeneity model (M_h: 0.161). However, since ΔAICc between any two models was ⩽2.0312 (results not shown), it was necessary to use the model averaging procedure while estimating the abundance parameter (N). By doing so, we obtained an averaged estimate (95% CI) of 262 unhabituated gorillas (230–319).

3.6. Total population size estimates based on both minimum genetic count and mark-recapture approaches

At the time of the census, 10 habituated groups containing a total of 168 gorillas were being monitored on a daily basis for either

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	

Table 3
Summary of data used to arrive at 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	61
Unhabituated found uniquely in Sweep 2	9	69
Unhabituated found in both Sweep 1 and Sweep 2	11	65
Sweeps 1 & 2 Unhabituated-without undetected infants	26	195
Undetected Infants – unhabituated		32
Individuals added due to incomplete genotypes		5
Total number of unhabituated gorillas		
Genetic count-based		232
Mark-recapture estimate		262 (230–319)
Known number of habituated gorillas	10	168
Total number of gorillas		
Genetic count-based	36	400
Mark-recapture estimate		430 (398–487)

research or tourism purposes. Adding these to the estimated 232 unhabituated gorillas derived from genotype counts and consideration of missed individuals (see above) results in a total minimum estimated population size of 400 gorillas found in 36 social groups and 16 solitary males (Fig. 2; Table 3). When the 168 habituated gorillas are added to the mark-recapture population size estimate of 262 gorillas (95% CI: 230–319), the total estimated population in Bwindi Impenetrable National Park is 430 individuals (95% CI: 398–487). Thus, the first minimum population size estimate of 400, which incorporates the count of unhabituated gorillas detected genetically, can be compared to a second population size estimate of 430 that incorporates a model-based mark-recapture estimate of the number of unhabituated gorillas based upon recapture frequencies.

3.7. Simulation of sampling effort needed for informative population size estimates

As a guide to future censusing of mountain gorillas aimed at providing a measure of population growth rate since the 2011 census, we used information from this genetic census, including the observed detection probabilities, to evaluate the impact of the number of sweeps (sampling occasions) on the accuracy and the precision (95% CI) of the unhabituated population size estimate obtained under the 2-mixture heterogeneity model, as well as on the support received by this model when compared to other, less realistic models. When the parameters used in the simulations are based on values derived from our study, the estimate based on the 2-mixture heterogeneity model (true model used for all simulations) reached high accuracy when four sweeps are simulated (Table 4). However, at this stage, the model M_h which allows for differing sampling probabilities, received the best support among all four applied models in only 38% of the simulations as compared to 50% for the null model, M_o. It is only when the number of sweeps is increased by one (i.e. five sampling occasions) that the model M_h receives the best support for more than 50% (namely 63%) of the simulations. Likewise, the precision of the estimate under model M_h increases from a width equivalent to 12.5% of the population size estimate with four sampling sessions to a narrower confidence interval corresponding to 7.75% or less of the population size estimate when using five sampling sessions. If one assumes population growth at 2% per year, the population is expected to increase from 400 to 440 gorillas in five years. Thus, if two censuses using this methodology (five sweeps with the assumed detection probabilities) are held at an interval of five years, it would just be possible to infer a growth rate of 2% with statistical confidence, but lower rates of growth would not be detectable.

For the second round of simulations in which capture probabilities of the two mixtures were increased from 65% and 40% to 95% and 70%, respectively, we found that the abundance estimate derived from model M_h was rather accurate (398 rather than 400 individuals estimated) when three sweeps are simulated (Table 5). For three sweeps, the model M_h receives the best support among all four applied models in 69% of the simulations as compared to 19% for the null model (M_o), the latter being the preferred model when only two sweeps are performed. Three sweeps also resulted in a rather precise estimate with a confidence interval corresponding to only 2% of the population size estimate. Thus, it can be

Table 4
Results from the 1000 simulations performed with the software MARK using the 2-mixture heterogeneity model M_h as the true model and a population size of 400 individuals, for each of the four models applied under a number of sampling occasions varying from two to six. For each model are displayed the average and the 95% confidence interval (into brackets) of the estimated population size (N). The column "Percentage of simulations" shows the percentage of the first 100 simulations for which the model received the best support among all four models based on the AICc value. M_o is the null model, M_b is the behavioral effect model, M_t is the temporal effect model, and M_h is the 2-mixture heterogeneity model.

# Sampling occasions	Model used for estimation	N	Percentage of simulations (%)
2	M_o	383 [357–411]	82
	M_b	383 [347–432]	9
	M_t	383 [356–411]	9
	M_h	383 [357–411]	0
3	M_o	388 [373–402]	65
	M_b	389 [370–408]	10
	M_t	388 [373–402]	14
	M_h	392 [375–409]	11
4	M_o	391 [381–400]	50
	M_b	392 [380–403]	9
	M_t	391 [381–400]	3
	M_h	400 [384–434]	38
5	M_o	394 [386–400]	25
	M_b	394 [387–401]	6
	M_t	393 [386–400]	6
	M_h	400 [389–420]	63
6	M_o	395 [389–400]	10
	M_b	396 [390–401]	4
	M_t	395 [389–400]	1
	M_h	400 [392–413]	85

Table 5
Results from the 1000 simulations performed with the software MARK using the 2-mixture heterogeneity model M_h as the true model and a population size of 400 individuals, for each of the four models applied under a number of 2 and 3 sampling occasions. For each model are displayed the average and the 95% confidence interval (into brackets) of the estimated population size (N). The column "Percentage of simulations" shows the percentage of the first 100 simulations for which the model received the best support among all four models based on the AICc value. M_o is the null model, M_b is the behavioral effect model, M_t is the temporal effect model, and M_h is the 2-mixture heterogeneity model.

# Sampling occasions	Model used for estimation	N	Percentage of simulations (%)
2	M_o	393 [385–399]	85
	M_b	393 [385–400]	8
	M_t	393 [385–399]	7
	M_h	393 [385–399]	0
3	M_o	397 [393–400]	19
	M_b	397 [393–401]	7
	M_t	397 [393–400]	5
	M_h	398 [394–402]	69

presumed that two censuses, each incorporating three sweeps with high detection probabilities and separated by five years would allow for sensitive estimate of population growth rates as low as 0.5%.

4. Discussion

4.1. Genetic versus nest-count based estimation of mountain gorilla population size

Our study shows that the fundamental assumption of the genetic sweep method for censusing mountain gorillas in Bwindi, namely that all or nearly all groups are encountered during a single survey employing intense coordinated effort in the field, is not met. By comparing the results obtained from analysis of genetic samples collected during two separate sweeps, we showed that more than half (58%, 15 of 26) of the social groups were found in only one of the sweeps, an observation which stands in sharp contrast with our expectation. This meant that in each of the two sweeps we missed approximately one-third of the identified unhabituated gorilla groups, and this proportion gets even higher when undetected individuals are considered. Although the two sweeps were of different durations, lasting twenty-seven and seven weeks, respectively, a similar total distance was traversed by field members and similar numbers of samples were collected in each sweep, indicating that the two sweeps were of equivalent intensities. Not only were the number of samples collected in each sweep similar, but the number of groups and individuals detected in each sweep were statistically indistinguishable. The first sweep detected 17 of the 26 unhabituated groups and the second sweep found 20 such groups (Fisher's exact test, two-tailed, $p = 0.5414$), while the total number of unhabituated gorillas found in each sweep was similar at 126 and 134, respectively, suggesting that at these time scales the duration of the sweep may not be a critical factor. Overall, conducting two genetic sweeps prevented an undercount of the total population size.

As in previous work (Guschanski et al., 2009), we investigated whether a nest-count based estimate can result in either an overestimate or an underestimate of the population size, due to either double-nesting cases (overcount on the individual level) and double-counting groups (overcount on the group level) or by assuming that two different groups found nearby one another are in fact the same group (undercount on the group level). Our estimate of a 6.9% rate at which individual gorillas construct more than one nest is similar to the value (7.8%) reported in the 2006 Bwindi census (Guschanski et al., 2009). As in that earlier study, there were also multiple instances of a particular group being represented by different numbers of nests at different sites, and these different sites may have erroneously been attributed to different putative groups in the absence of the genetic analysis. Although it is possible that different groups with similar numbers of members might produce nearby nest sites that could be mistakenly be attributed to a single putative group, we detected no instances of such 'missing groups' in our analyses of multiple nest sites from 12 of the 26 groups. This suggests that although for practical reasons we limited our analysis to 75% and 85% of the samples from Sweep 1 and 2, respectively, analysis of the remaining samples would not be expected to reveal additional groups. In sum, the use of genetic analysis in the 2011 Bwindi census improved the ability to conduct a simple count of the gorillas.

4.2. Population growth of Bwindi mountain gorillas

Application of mark-recapture models of population size to our data on the capture history of the 195 genetically identified gorillas resulted in an estimate of 262 unhabituated gorillas with a 95% confidence interval of 230–319, which with the addition of the 168 habituated gorillas resulted in a population estimate of 430 gorillas (95% CI: 398–487). Despite the uncertainty around the estimate and methodological differences among the studies we can make some comparisons to population size estimates from previous censuses. Our results identified a total of 36 social units and 16 solitary males in 2011, which is the largest number of groups and solitary individuals ever described in Bwindi Impenetrable National Park. The number of habituated groups increased from five to ten 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. Lastly, it should be noted that as in all previous censuses of Bwindi, no gorillas were detected in the easternmost portion of

the park (Fig. 2). The suitable habitat in this region is indicative of the potential for further growth of the gorilla population.

Both the direct, genetic count-based estimate of a minimum of 400 gorillas as well as the mark-recapture estimate of 430 gorillas present in 2011 represent substantial increases (>30%) over the approximately 300 gorillas detected in 2006 using only one sweep and genetic analysis (Guschanski et al., 2009). Because this increase is far in excess of the 2% annual growth rate estimated from demographic data from the habituated groups (Robbins et al., 2009), we suggest that the increase from 2006 to 2011 was due to some groups not being detected in 2006 and to a smaller extent to actual growth of the population. It should also be noted that Sarambwe Nature Reserve in the Democratic Republic of Congo, contiguous to Bwindi, has due to insecurity in the area not been surveyed in any of the four censuses conducted thus far, leading to the possibility that the few groups which may sometimes use this reserve may have been detected in some but not all of the censuses.

It is not possible to comment on changes in the population size prior to 2006 because the previous censuses in 1997 and 2002 used only the sweep method (one sweep) without the support of genetic analysis (McNeilage et al., 2001,2006). Therefore, it is impossible to know if those estimates were overcounts or undercounts and to determine if there were actual changes in the population size prior to 2006. Although habituation facilitates counting of the gorillas, since such a large proportion of the population (28% of groups; 42% of gorillas) is now habituated to human presence, and this brings inherent risks of disease transmission from humans (Woodford et al., 2002; Spelman et al., 2013) and potentially increased vulnerability to poaching, we suggest that the proportion of habituated gorillas should not be further increased by habituation of additional groups.

Despite applying the sweep method twice in the same year, we cannot exclude the possibility that some gorilla groups and solitary males went undetected during the census. However, the facts that the reconnaissance trail coverage during both sweeps was extensive (over 700 km walked in each sweep) and that the 36 social groups revealed in this census represent the highest number of groups ever found in Bwindi may suggest that few groups might have been undetected. Nonetheless, the current estimate of 400 gorillas in Bwindi should be regarded as a minimum number of individuals inhabiting the park. This estimate is encouraging for a 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). If combined with the estimate of 480 gorillas recently found in Virunga Massif (Gray et al., 2013), the total minimum number of mountain gorillas now exceeds 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. Nonetheless, as vulnerable populations numbering only in the hundreds, both should continue to be considered critically endangered.

4.3. Guidelines for future censuses of mountain gorillas

The current study reinforces the importance of using a genetic approach, rather than relying exclusively upon indirect signs, in estimating population sizes of rare and elusive species (Arandjelovic et al., 2010,2010; Waits, 2004; Zhan et al., 2006). However, it is also apparent that the use of two sampling surveys with the observed moderate capture probabilities downwardly biased the minimum population size estimate as a substantial proportion of groups and individuals were missed in either sweep. Furthermore, performing only two sweeps in a CMR AICc-based model selection framework will always result in favoring the intrinsically unrealistic null model (M_o). Heterogeneity in capture probability among individuals exists in virtually any wildlife CMR study, including ours, and should be taken into account despite the difficulty of adequately modeling this parameter (White and Burnham, 1999). In our case, we observe an inconsistent effect of group size upon capture probability, with only one of 16 lone silverbacks sampled more than once, while the largest detected group of 17 individuals was only found in one of the sweeps. Nonetheless, we expect that a more powerful dataset would demonstrate capture heterogeneity associated with group size or other variables.

Our first round of simulations, which assumed a similar capture probability per sweep as observed in our study, showed that four sweep surveys would be sufficient in future censuses to achieve an accurate population size estimate under the 2-mixture heterogeneity model. However, performing four sweeps does not allow this model to receive reasonable support in the model averaging procedure, nor would it allow for the detection of a 2% annual growth rate or higher and for those reasons we would argue in favor of five sweeps, assuming similar capture probabilities as observed in the present study and an interval of five years between censuses.

Our second round of simulations revealed that, when capture probabilities of both mixtures are substantially increased to 0.95 and 0.70 while all other parameters are held constant, three sweeps would be sufficient to yield an accurate population size estimate, higher support for the 2-mixture heterogeneity model relative to the other models, and sufficient precision to estimate even low (0.5% per year) population growth rates, assuming an interval of five years between censuses. In the field the overall capture probability could potentially be improved by reducing the distance between reconnaissance trails and ensuring that teams are particularly careful to search along boundaries among sectors. Censuses could be conducted less frequently than every five years, allowing for more resources to be devoted to additional sweeps.

Given the financial and logistical challenges of conducting multiple intensive sweeps, we should also consider alternative ways to estimate the population growth and demography of the population. Assuming that a sufficient amount of genotypes obtained from a number of consecutive censuses could be compared in the future, we note that more advanced demographic models might potentially be investigated to better understand the gorilla population dynamics in Bwindi, such as Pollock's robust design model (Kendall et al., 1995). Since many of these advanced models were initially developed for open populations, they could potentially be used to estimate other demographically relevant parameters such as birth and mortality rates in addition to population size. Birth and mortality rates may be estimated from the habituated groups (Robbins et al., 2009) but may not reflect the situation for the entire population (Gray et al., 2013; Robbins et al., 2011). Another option may be to use an alternative approach to censusing, such as camera trapping (Head et al., 2013; Kühl and Burghardt, 2013; Loos et al., 2011).

Although it would not provide a precise mark-recapture population size estimate, a future census using only two sweeps would still allow for a minimum population size estimate. Genotypes obtained could be compared with those obtained in 2006 and 2011, allowing 'molecular tracking' of individuals. For example, we found that 92 individuals (of 257) genotyped as part of the 2006 Bwindi genetic census were once again sampled and genotyped in this study (data not shown), where the low proportion refound is likely due to the fact that we did not type the groups that became habituated in the interval between the censuses. This census provided genotypes from the majority (195 of an estimated

262) of the currently unhabituated gorillas. Additional sampling in the future will enhance the ability to 'follow' unhabituated individuals, including comparing group membership and inferring group dynamics and genetic relationships between individuals, thus complementing and providing context for a population size estimate.

4.4. Recommendations for use of a sequential survey approach in large mammal genetic census studies

The combination of genetics and mark-recapture methods is commonly used to estimate population sizes in several large mammal species (e.g. Arandjelovic et al., 2011; Boulanger et al., 2002; Zhan et al., 2006). Given the numerous challenges in such demographic studies, we argue that researchers would benefit from adopting a sequential approach to determining the optimal sampling scheme for obtaining accurate and precise abundance estimates. This would include an initial study (pilot study) aimed at assessing difficulties related to implementation in the field of the initial sampling scheme and estimating the approximate genotyping success and error rates. This pilot study may thus yield a first approximation of the individual capture probability in the target population. A subsequent, larger-scale study may then be conducted using an appropriately revised sampling methodology and design, which should lead to higher capture probabilities. Finally, as we did in this study, the genetic record of captures and recaptures could be further utilized to simulate the number of capture sessions that is required to reach the desired level of accuracy and precision around the population size estimate. To that end, we advocate the testing of a large number of realistic CMR models in the model selection step, such as those implemented in the program MARK (White and Burnham, 1999). To our knowledge, few authors (but see Lampa et al., 2013) have performed simulations in their study to determine the optimal sampling scheme to adopt in regards to their expectations. This step should improve the prospect of obtaining reliable results while saving a considerable amount of time and resources.

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References

Arandjelovic, M., Guschanski, K., Schubert, G., Harris, T.R., Thalmann, O., Siedel, H., Vigilant, L., 2009. Two-step multiplex polymerase chain reaction improves the speed and accuracy of genotyping using DNA from noninvasive and museum samples. Mol. Ecol. Resour. 9, 28–36. http://dx.doi.org/10.1111/j.1755-0998.2008.02387.x.

Arandjelovic, M., Head, J., Kühl, H., Boesch, C., Robbins, M.M., Maisels, F., Vigilant, L., 2010. Effective non-invasive genetic monitoring of multiple wild western gorilla groups. Biol. Conserv. 143, 1780–1791. http://dx.doi.org/10.1016/j.biocon.2010.04.030.

Arandjelovic, Mimi, Head, Josephine, Rabanal, L.I., Schubert, Grit, Mettke, E., Boesch, Christophe, Robbins, Martha M., Vigilant, Linda, 2011. Non-invasive genetic monitoring of wild central chimpanzees. PLoS ONE 6, e14761. http://dx.doi.org/10.1371/journal.pone.0014761.

Arrendal, J., Vilà, C., Björklund, M., 2007. Reliability of noninvasive genetic census of otters compared to field censuses. Conserv. Genet. 8, 1097–1107. http://dx.doi.org/10.1007/s10592-006-9266-y.

Boulanger, J., White, G.C., McLellan, B.N., Woods, J., Proctor, M., Himmer, S., 2002. A meta-analysis of grizzly bear DNA mark-recapture projects in British Columbia, Canada. Ursus 13, 137–152.

Bradley, B.J., Boesch, Christophe, Vigilant, Linda, 2000. Identification and redesign of human microsatellite markers for genotyping wild chimpanzee (Pan troglodytes verus) and gorilla (Gorilla gorilla gorilla) DNA from faeces. Conserv. Genet. 1, 289–292.

Bradley, B.J., Chambers, K.E., Vigilant, Linda, 2001. Accurate DNA-based sex identification of apes using non-invasive samples. Conserv. Genet. 2, 179–181.

Burnham, K., Anderson, D., 2002. Model selection and multimodel inference – A practical information-theoretic approach, 2nd ed. Springer, New York, USA.

Gray, Maryke, McNeilage, A., Fawcett, K., Robbins, Martha M., Ssebide, B., Mbula, D., Uwingeli, P., 2009. Censusing the mountain gorillas in the Virunga Volcanoes: complete sweep method versus monitoring. Afr. J. Ecol. 48, 588–599. http://dx.doi.org/10.1111/j.1365-2028.2009.01142.x.

Gray, Maryke, Roy, J., Vigilant, Linda, Fawcett, K., Basabose, A., Cranfield, M., Uwingeli, P., Mburanumwe, I., Kagoda, E., Robbins, Martha M., 2013. Genetic census reveals increased but uneven growth of a critically endangered mountain gorilla population. Biol. Conserv. 158, 230–238. http://dx.doi.org/10.1016/j.biocon.2012.09.018.

Guerrera, W., Sleeman, J.M., Jasper, S.B., Lonny, B., Ichinose, T.Y., Reif, J.S., 2003. Medical survey of the local human population to determine possible health risks to the mountain gorillas of Bwindi Impenetrable Forest National Park, Uganda. Int. J. Primatol. 24, 197–207.

Guschanski, Katerina, Vigilant, Linda, McNeilage, A., Gray, Maryke, Kagoda, E., Robbins, Martha M., 2009. Counting elusive animals: comparing field and genetic census of the entire mountain gorilla population of Bwindi Impenetrable National Park, Uganda. Biol. Conserv. 142, 290–300. http://dx.doi.org/10.1016/j.biocon.2008.10.024.

Harris, R.B., Winnie, J., Amish, S.J., Beja-Pereira, A., Godinho, R., Costa, V., Luikart, Gordon, 2010. Argali abundance in the Afghan Pamir using capture–recapture modeling from fecal DNA. J. Wildlife Manage. 74, 668–677. http://dx.doi.org/10.2193/2009-292.

Head, J.S., Boesch, C., Robbins, M.M., Rabanal, L.I., Makaga, L., Kühl, H.S., 2013. Effective sociodemographic population assessment of elusive species in ecology and conservation management. Ecol. Evolut. 3, 2903–2916. http://dx.doi.org/10.1002/ece3.670.

Kalinowski, S.T., Taper, M.L., Marshall, T.C., 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16, 1099–1106. http://dx.doi.org/10.1111/j.1365-294X.2007.03089.x.

Katzner, T.E., Ivy, J.A.R., Bragin, E.A., Milner-Gulland, E.J., DeWoody, J.A., 2011. Conservation implications of inaccurate estimation of cryptic population size. Anim. Conserv. 14, 328–332. http://dx.doi.org/10.1111/j.1469-1795.2011.00444.x.

Kendall, W.L., Pollock, K.H., Brownie, C., Kendall, W.L., 1995. A likelihood-based approach to capture-recapture estimation of demographic parameters under the robust design. Biometrics 51, 293–308.

Kühl, H.S., Burghardt, T., 2013. Animal biometrics: quantifying and detecting phenotypic appearance. Trends Ecol. Evol. 28, 432–441. http://dx.doi.org/10.1016/j.tree.2013.02.013.

Kühl, H.S., Maisels, F., Ancrenaz, M., Williamson, E.A., 2008. Best practice guidelines for surveys and monitoring of great ape populations. IUCN SSC Primate Specialist Group (PSG), Gland, Switzerland.

Lampa, S., Henle, K., Klenke, R., Hoehn, M., Gruber, B., 2013. How to overcome genotyping errors in non-invasive genetic mark-recapture population size estimation – a review of available methods illustrated by a case study. J. Wildl. Manag. 77, 1490–1511. http://dx.doi.org/10.1002/jwmg.604.

Loos, A., Pfitzer, M., Aporius, L., 2011. Identification of great apes using face recognition. In: 19th European Signal Processing Conference (EUSIPCO). pp. 922–926.

Marques, F.F.C., Buckland, S.T., Goffin, D., Dixon, C.E., Borchers, D.L., Mayle, B.A., Peace, A.J., 2001. Estimating deer abundance from line transect surveys of dung: sika deer in southern Scotland. J. Appl. Ecol. 38, 349–363. http://dx.doi.org/10.1046/j.1365-2664.2001.00584.x.

Marucco, F., Vucetich, L.M., Peterson, R.O., Adams, J.R., Vucetich, J.A., 2012. Evaluating the efficacy of non-invasive genetic methods and estimating wolf survival during a ten-year period. Conserv. Genet. 13, 1611–1622. http://dx.doi.org/10.1007/s10592-012-0412-4.

McNeilage, A., Plumptre, A.J., Brock-Doyle, A., Vedder, A., 2001. Gorilla Census 1997, Bwindi Impenetrable National Park, Uganda. Oryx 35, pp. 39–47. doi:10.1046/j.1365-3008.2001.00154.x.

McNeilage, A., Robbins, Martha M., Gray, Maryke, Olupot, W., Babaasa, D., Bitariho, R., Kasangaki, A., Rainer, H., Asuma, S., Mugiri, G., Baker, J., 2006. Census of the mountain gorilla *Gorilla beringei beringei* population in Bwindi Impenetrable National Park, Uganda. Oryx 40, 419–427. http://dx.doi.org/10.1017/S0030605306001311.

Moore, D., Vigilant, L., 2014. A population estimate of chimpanzees (*Pan troglodytes schweinfurthii*) in the Ugalla region using standard and spatially explicit genetic capture-recapture methods. Am. J. Primatol. 76, 335–346. http://dx.doi.org/10.1002/ajp.22237.

Morgan, D., Sanz, C., Onononga, J.R., Strindberg, S., 2006. Ape abundance and habitat use in the Goualougo Triangle, Republic of Congo. Int. J. Primatol. 27, 147–179. http://dx.doi.org/10.1007/s10764-005-9013-0.

Morin, P.A., Mahboubi, P., Wedel, S., Rogers, J., 1998. Rapid screening and comparison of human microsatellite markers in baboons: allele size is conserved, but allele number is not. Genomics 53, 12–20. http://dx.doi.org/10.1006/geno.1998.5460.

Mowry, R.A., Gompper, M.E., Beringer, J., Eggert, L.S., 2011. River otter population size estimation using noninvasive latrine surveys. J. Wildl. Manag. 75, 1625–1636. http://dx.doi.org/10.1002/jwmg.193.

Murai, M., Ruffler, H., Berlemont, A., Campbell, G., Esono, F., Agbor, A., Mbomio, D., Ebana, A., Nze, A., Kühl, H.S., 2013. Priority areas for large mammal conservation in Equatorial Guinea. PLoS ONE 8, e75024. http://dx.doi.org/10.1371/journal.pone.0075024.

Nakashima, Y., Iwata, Y., Ando, C., Nze Nkoguee, C., Inoue, E., Akomo, E.-F.O., Nguema, P.M., Bineni, T.D., Banak, L.N., Takenoshita, Y., Ngomanda, A., Yamagiwa, J., 2013. Assessment of landscape-scale distribution of sympatric great apes in African rainforests: concurrent use of nest and camera-trap surveys. Am. J. Primatol. 75, 1220–1230. http://dx.doi.org/10.1002/ajp.22185.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.

Nichols, J.D., Williams, B.K., 2006. Monitoring for conservation. Trends Ecol. Evol. 21, 668–673.

Nsubuga, A.M., Robbins, M.M., Roeder, A.D., Morin, P.A., Boesch, C., Vigilant, L., 2004. Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. Mol. Ecol. 13, 2089–2094. http://dx.doi.org/10.1111/j.1365-294X.2004.02207.x.

Otis, D.L., Burnham, K.P., White, G.C., Anderson, D.R., 1978. Statistical inference from capture data on closed animal populations. Wildlife Monographs, 3–135.

Pledger, S., 2000. Unified maximum likelihood estimates for closed capture-recapture models using mixtures. Biometrics 56, 434–442.

Robbins, Martha M., Gray, Maryke, Kagoda, E., Robbins, A.M., 2009. Population dynamics of the Bwindi mountain gorillas. Biol. Conserv. 142, 2886–2895. http://dx.doi.org/10.1016/j.biocon.2009.07.010.

Robbins, Martha M., Gray, Markye, Fawcett, K.A., Nutter, F.B., Uwingeli, P., Mburanumwe, I., Kagoda, E., Basabose, A., Stoinski, T.S., Cranfield, M.R., Byamukama, J., Spelman, L.H., Robbins, A.M., 2011. Extreme conservation leads to recovery of the Virunga mountain gorillas. PLoS ONE 6, e19788. http://dx.doi.org/10.1371/journal.pone.0019788.

Rowcliffe, J.M., Field, J., Turvey, S.T., Carbone, C., 2008. Estimating animal density using camera traps without the need for individual recognition. J. Appl. Ecol. 45, 1228–1236. http://dx.doi.org/10.1111/j.1365-2664.2008.0.

Spelman, L.H., Gilardi, K.V.K., Lukasik-Braum, M., Kinani, J.-F., Nyirakaragire, E., Lowenstine, L.J., Cranfield, M.H., 2013. Respiratory disease in mountain gorillas (Gorilla beringei beringe) in Rwanda, 1990–2010: outbreaks, clinical course, and medical management. J. Zoo Wildlife Med. 44, 1027–1035.

Waits, L., 2004. Using noninvasive genetic sampling to detect and estimate abundance of rare wildlife species. In: Thompson, W. (Ed.), Sampling Rare or Elusive Species. Island Press, Washington DC, USA, pp. 211–228.

Waits, L.P., Luikart, G., Taberlet, P., 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Mol. Ecol. 10, 249–256.

White, G.C., Burnham, K.P., 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46, S120–S139. http://dx.doi.org/10.1080/00063659909477239.

Woodford, M.H., Butyinski, T.M., Karesh, W.B., 2002. Habituating the great apes: the disease risks. Oryx 36, 153–160. http://dx.doi.org/10.1017/S0030605302000224.

Wrege, P.H., Rowland, E.D., Thompson, B.G., Batruch, N., 2010. Use of acoustic tools to reveal otherwise cryptic responses of forest elephants to oil exploration. Conserv. Biol. 24, 1578–1585. http://dx.doi.org/10.1111/j.1523-1739.2010.01559.x.

Zhan, X., Li, M., Zhang, Z., Goossens, B., Chen, Y., Wang, H., Bruford, M.W., Wei, F., 2006. Molecular censusing doubles giant panda population estimate in a key nature reserve. Curr. Biol. 16, R451–2. http://dx.doi.org/10.1016/j.cub.2006.05.042.