

1 Estimation of the census (Nc) and effective (Ne) population size of a wild mandrill (*Mandrillus* 2 *sphinx*) horde in the Lopé National Park, Gabon using a non-invasive genetic approach

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16 Abstract

17 Mandrills (*Mandrillus sphinx*) are enigmatic primates endemic to central Africa and are
18 threatened by habitat loss and hunting. However, effective management of this species is limited
19 by insufficient information about their numbers in the wild, since population size can impact
20 viability and genetic diversity. Here, we used for the first time a non-invasive genetic approach
21 to estimate the census and effective population size (Nc and Ne respectively) of a wild mandrill
22 horde in Lopé National Park (Gabon). We amplified a total of 232 unique genotypes using a panel
23 of 16 microsatellite loci from mandrill fecal samples collected over three years (2016-2018). Using
24 the single sample estimator in CAPWIRE, we obtained an estimate for Nc of 989 (95%CI:947-
25 1399) individuals which was close to that obtained from the multiple sample estimator
26 implemented in the program MARK [992 (95%CI:708-1453)]. These estimates approximately
27 correspond with previous visual counts obtained from the same horde. Based on a model
28 implemented in the program NeOGen, when samples were pooled across all three sampling
29 sessions, statistical power was sufficient for a robust Ne estimate. Using the three one-sample
30 estimators in the NeESTIMATORV2 program and the one in COLONY, Ne was estimated at 292
31 (95%CI:239-370) and 135 (95%CI:108-176) individuals respectively, indicating that Ne is between
32 13.6% and 29.5% of Nc. This study showed that non-invasive genetics is an effective tool for
33 providing accurate estimates of horde sizes of mandrills and other elusive primates, provided
34 enough samples and hypervariable loci are genotyped.

Keywords: *Mandrillus sphinx*, Lopé national park, non-invasive genetics, census population, effective population.

Introduction

Measures of census (N_c) and effective (N_e) population size are very important for effective management and conservation of natural populations (Charlesworth, 2009; Frankham, 2005; Gurov et al., 2017; Hedgecock et al., 2007; Mowat & Strobeck, 2000). N_c is a direct measure of the number of individuals present in a population and provides a demographic estimate of population viability. In contrast, N_e reflects the number of reproductive individuals that contribute to the next generation and is a measure of the rate at which genetic diversity is lost due to genetic drift (Frankham et al., 2002; Luikart et al., 2010). However, estimating these two population parameters in the wild remains a significant challenge, especially for rare and elusive species. Although both parameters can be estimated directly from field observation or demographic information (Bata et al., 2017; Caballero, 1994; Frankham, 1995; Gittleman, 2001; Hedwig et al., 2018; Johnson et al., 2005; Kimura & Crow, 1963; Leberg, 2005; Nunney & Elam, 1994; Ruiz-Olmo et al., 2001; Schmeller & Merilä, 2007; Wright, 1938), obtaining these data can be very logistically difficult for wild populations.

Genetic data are a common alternative (Do et al., 2014; Jones & Wang, 2010; Miller et al., 2005; Otis et al., 1978; White & Burnham, 1999) and have been applied to a wide range of wildlife species (Banks et al., 2003; Bergl & Vigilant, 2007; Frankham, 1995; Langergraber et al., 2007; Lucchini et al., 2002). Tissue samples can provide high quality genetic material, but their collection is not always feasible for at-risk species. As an alternative, genetic data can be collected from non-invasive samples, such as shed hairs or feces, without disturbing the target species. Such samples tend to be of lower quality and present numerous technical challenges (Clemento et al., 2009; Dawnay et al., 2011; Dou et al., 2016; Ernest et al., 2000; Granjon et al., 2020; Puechmaille & Petit, 2007). However, non-invasive sampling enables the collection of a higher volume of samples than may be possible if using tissue. Furthermore, a plethora of different methods have been developed to estimate N_c or N_e from genetic information using a single or multiple sample periods (Do et al., 2014; Jorde & Ryman, 2007; Miller et al., 2005; Otis et al., 1978; White & Burnham, 1999). Several studies have used one or both methods to produce credible population size values (Arandjelovic et al., 2010; Bellemain et al., 2005; England et al., 2010; Tallmon et al., 2004). Unlike methods that require multiple sampling sessions, estimating from a single sampling period is often very useful for species where sampling is costly or difficult over multiple time periods.

Nevertheless, these approaches require sufficient available data to obtain precise and accurate estimates (Miller et al., 2005; Waples, 2006; Waples & Do, 2010). Fortunately, tools such

as the NeOGen software (Blower et al., 2019) are now available that allow researchers to determine in advance the minimum number of samples and loci needed to provide a reliable estimate of N_e . Taken together, these approaches can provide crucial information and increase the essential knowledge base that informs conservation and management decisions of threatened or endangered species.

One such threatened species for which a strong knowledge base is lacking is the mandrill (*Mandrillus sphinx*). This primate species is endemic to Central Africa and is distributed across the tropical forests of Cameroon, Equatorial Guinea, Congo, and Gabon (Abernethy & Maisels, 2019; Kingdon, 1997). Mandrills are highly social and live in large groups or "hordes," which can make them particularly vulnerable to hunting pressure and habitat loss (Abernethy & Maisels, 2019). Field observations have reported that hordes may have shrunk or disappeared in some areas of the Cameroon and Equatorial Guinea forests where pressure is more intense (Abernethy & Maisels, 2019). Because of this, the mandrill is listed in Appendix I by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and categorized as "Vulnerable" on the International Union for Conservation of Nature (IUCN) Red List (Abernethy & Maisels, 2019; Oates & Butynski, 2008).

Wild mandrills are generally difficult to observe directly due to the closed habitat that they occupy (Abernethy & Maisels, 2019; Oates & Butynski, 2008), making counts of horde size difficult. Nevertheless, the first estimates of mandrill N_c were obtained using camera traps and direct observations from a focal horde at the Station d'Etudes des Gorilles et Chimpanzés (SEGC) in the Lopé National Parc (LNP), Gabon (Abernethy et al., 2002; Rogers et al., 1996). This horde frequents the savanna-forest mosaic in the northern portion of the park during the breeding season (June to September, with a peak in reproductive effort in July-August), enabling direct counts. The size of the horde was first estimated to be over 600 individuals (Rogers et al., 1996), and a second count reported a range of 340-845 individuals, with an average of 620 (Abernethy et al., 2002). More recent unpublished observations have suggested as many as 1,250 mandrills in the horde (Lehmann D., 2019, personal communication). In contrast, observational estimates of N_c from another horde in Moukalaba-Doudou National Park in Gabon are comparatively smaller (169-442 individuals (Hongo, 2014). Although these intensive field studies have provided valuable information on the likely range in the horde sizes, it is difficult to replicate these kinds of studies in other parts of the mandrill range without taking a non-invasive genetic approach.

Therefore, the objective of this study was to use a panel of 16 microsatellite loci to genotype fecal samples obtained from successive annual sampling (2016-2018) of the SEGC mandrill horde to: (1) estimate the census size (N_c) of the SEGC horde using several mark-recapture genetic estimators and compare these estimates with those previously obtained in the field, (2) validate the minimum number of samples and loci needed to obtain accurate estimates of N_e , and (3) derive estimates of N_e using a range of available genetic estimators. This research

will also allow us to evaluate the feasibility of non-invasive genetics to monitor the population size of wild mandrills at other sites across their range.

Materials and methods

Study site and sample collection

Samples were collected in the northern part (0012S, 1136E) of the LNP, adjacent to the SEGC field station in Gabon. Although the LNP covers an area of approximately 5,000 km² of lowland tropical rainforest, the northern part of the SEGC is dominated by a mosaic vegetation cover of grassy savannahs and fragments of natural forest (White, 1994; White & Abernethy, 1997). The Ogooué River borders the park at its northern-most extent and provides a natural barrier for many animals (Abernethy et al., 2002). The site is characterized by two dry seasons: the little dry season from December to February and the long dry season that extends from mid-June to mid-September. Temperatures at the site vary little with a mean monthly minimum of 20 ± 23.8 °C in the dry season and 26 ± 33.8 °C in the wet season (1984 ± 98) (Abernethy et al., 2002).

We sampled mandrill feces ($n=927$) from the SEGC horde over three successive years (2016-2018) during the long summer dry season (July and August). This period corresponds to the mandrill breeding season, when mature males and females are present in the horde (Abernethy et al., 2002). We collected only fresh (< 3 hours) fecal samples to maximize DNA quality for downstream molecular analyses (Regazzi, 2007). Mandrill fecal samples are similar to that of other large primates, in that they are generally solid and physically preserve well. We placed fecal samples in a 50 mL Falcon tube half-filled with silica gel beads, as previous work has shown that this storage medium is the best for preserving nuclear DNA in central African forest antelope (Soto-Calderón et al., 2009). We stored the samples in a freezer at -20°C prior to DNA extraction. In an unrelated study, a small number of females and males of the horde were fitted with radio collars, allowing us to locate the focal horde and collect fecal samples more easily. Aliquots of blood ($n = 14$) and hair ($n = 9$) samples were also collected from a subset of these individuals.

DNA extraction and amplification of microsatellites

We extracted DNA from fecal samples collected 1 to 2 months after collection using the QIAamp Fast DNA Stool Mini Kit (Qiagen, CA). DNA from blood and hair was extracted using the DNeasy Blood & Tissue kit (Qiagen, CA). All extractions included blanks to control for DNA contamination. We performed all extractions in a dedicated fecal DNA extraction room, which was kept separate from all other sources of DNA to minimize the risk of contamination.

We selected a panel of 16 microsatellite loci previously isolated from mandrills (Benoit et al., 2014) and amplified them in four multiplex reaction mixes (M1-4), each containing four loci

(Supplementary Table 1). Forward primers were labelled with fluorophore dyes (labeled 6-FAM, HEX, or NED) to discriminate individual loci within each multiplex. We performed polymerase chain reaction (PCR) amplification of each multiplex in a total volume of 10 μ l. PCRs contained 0.1 μ l of each primer (reverse and forward) at 0.2 μ M final concentration, 0.5 μ l of 20mg/ml BSA (Bovine Serum Albumin), 5 μ l of 2X multiplex PCR kit (Qiagen, CA), 1.7 μ l of RNase-free water, and 2 μ l of DNA extract. We performed PCR amplification using a touch-down protocol, with duration of cycling steps following the PCR kit's manufacturer instructions. The cycle began with an initial denaturation step for 15 minutes at 95°C to allow activation of the hot start Taq polymerase. For M1 and M3, we then followed this step with 10 cycles of 30 seconds at 95°C for denaturation, 90 s of annealing at 60°C (with a 1°C decrease after each cycle), and a 60-s extension step at 72°C. We then performed 30 additional cycles using the following conditions: 94°C for 30 s, 50°C for 90 s, and 72°C for 60 s, followed by a final extension step of 60°C for 30 min. PCR conditions for M2 and M4 were the same as for M1 and M3, except that the initial annealing temperature during the first 10 cycles of the protocol started at 63°C, and decreased to 53°C over the course of the reaction. PCR products were then analyzed on an ABI3130xl sequencer at either the Department of Biological Sciences, University of New Orleans, USA, or the Georgia Genomics and Bioinformatics Core (Georgia, USA).

Microsatellite genotyping

We determined raw allele sizes for each microsatellite locus using the GENEIOUS R 6.1.8 program (Kearse et al., 2012), and binned alleles using the TANDEM program (Matschiner & Salzburger, 2009). Because of the generally low amounts of DNA in fecal samples and the high risk of genotyping errors, we quantified rates of allelic dropout in a pilot study to determine the number of replicates needed to reduce the probability of obtaining a false homozygote to less than 0.05 (Bellemain et al., 2005; Flagstad et al., 2004; Paetkau, 2003). Calculation of error rates from this preliminary analysis revealed that three replicates were sufficient to minimize the risk of genotyping false homozygotes (Supplementary Table 2). In this pilot study, we also calculated the probability of identity (PID), or the probability of individuals having the same genotype by chance. In the absence of information on the kinship structure or level of genetic diversity in the focal horde, we used the PIDsibs estimator because it provides conservative estimates of PID based on the possibility that individuals in the population may be related (Evetts & Weir, 1998; Waits et al., 2001). We estimated the per-locus values of PIDsibs using the GIMLET version 1.3.3 program (Valière, 2002). To determine the minimum number of loci needed to differentiate individuals, we ranked loci from highest to lowest PIDsibs and calculated cumulative scores across ordered loci until the PIDsibs value fell to < 0.01 (Supplementary Table 3). Our estimates of PID indicated that a minimum of six least informative loci are needed in order to reliably differentiate individuals. Therefore, assuming that data for some loci may be lost due to conflicts between PCR

replicates, only samples that amplified for at least 9 loci in the first replicate were genotyped for the remaining two. From these three replicates, we constructed multi-locus consensus genotypes using GIMLET (Valière, 2002). Based on error rates calculated in the pilot study, we called genotypes as heterozygous when two alleles appeared in at least two independent replicates, whereas homozygotes were only accepted if the same allele appeared alone in all three replicates (Bonin et al., 2004). Samples that did not have consensus genotypes for at least seven loci, which is one more than the minimum required as per our PID estimation, were discarded from downstream analyses.

We performed tests of deviation from Hardy-Weinberg equilibrium (HWE) and linkage equilibrium (LE) using the program ARLEQUIN version 3.5 (Excoffier & Lischer, 2015) and corrected for multiple hypothesis testing using the Holm-Bonferroni method (Gaetano, 2018; Holm, 1979). We also evaluated consensus genotypes for the presence of three common genotyping errors: non-amplification of specific alleles (null alleles), small allele bias, and errors due to stutter using the program MICROCHECKER version 2.2.3 (Van Oosterhout et al., 2004).

We identified duplicate genotypes in the dataset using a custom Python script that counted matching loci in all possible pairwise combinations of multi-locus genotypes. We considered two samples to belong to the same individual when they shared six or more matching genotypes with no more than two mismatching alleles (Paetkau, 2003). Because genotypes from noninvasive samples tend to have missing data, we also considered any multi-locus pairs with fewer than six matching loci as duplicates if their shared loci had a cumulative PIDsibs < 0.01 (Waits et al., 2001). For downstream analyses requiring unique genotypes, the least informative genotype of the duplicated pair was removed from the dataset. In cases where missing data resulted in pairs of genotypes with fewer than six loci that amplified in both genotypes, it was impossible to determine whether the two originated from the same individual. In these ambiguous cases, the least informative genotype of the pair was also removed from the dataset.

Genetic estimation of N_c using single and multiple sampling periods

We estimated the N_c of the focal mandrill horde of the SEGC using several genetic models based on single and multiple sampling periods. All these N_c estimators assume that each multi-locus genotype can be "captured" one or more times during the same or different sampling periods and that capture heterogeneity may exist. Here, duplicate samples represent recaptures. These estimators also assume a closed population (Miller et al., 2005; White & Burnham, 1999).

We estimated N_c from each individual sampling period (2016, 2017, and 2018) by applying two estimators from the CAPWIRE package (Miller et al., 2005) implemented in the program R (R Development Core Team, 2017). The two estimators are: the equal capture model (ECM), which assumes no capture heterogeneity in the dataset, and the two-rate innate model (TIRM), which accounts for heterogeneity in capture probabilities. Both estimators calculate N_c

on a maximum likelihood basis from a single sampling session, utilizing multiple captures of genotypes from that session (Miller et al., 2005). To examine the effect of sample size, we also pooled the samples from the three periods into a single dataset to estimate N_c , since the successive sampling periods were only one year apart and are likely to reflect the same cohort.

We also compared estimates of N_c using the multi-sample estimators implemented in the program MARK version 9.0 (White & Burnham, 1999). The program estimates N_c using several closed population models that each incorporate different capture probabilities: the M_0 model, where capture probabilities are assumed to be constant; M_t , where capture probabilities vary with time; M_b , where there is a behavioral response to capture; and M_{h2} , where capture probabilities vary by individual animal. MARK also allows combinations of these factors (M_{th} , M_{tb} , M_{tbh}). For analyses carried out in the program, we first aggregated individual multi-locus genotypes observed during the three sampling periods and compiled a "capture" and "recapture" history using the GenCapture version 1.4.9 program (McKelvey & Schwartz, 2005; Schwartz et al., 2006). To choose the best model for our data, we compared each model's AICc (Akaike information criterion corrected for small sample size) and respective weighting values (w).

Estimation of the minimum number of samples and loci needed to estimate N_e

We used the program NeOGen (genetic N_e for Overlapping Generations) Ver. 1.3.0.6.a1 (Blower et al., 2019) to estimate the minimum number of samples and loci needed to provide an accurate and precise estimate of N_e . NeOGen estimates the number of samples and loci required to provide a reliable N_e estimate using species-specific demographic and genetic parameters (Blower et al., 2019) and the degree of linkage disequilibrium based on the LDNe algorithm (Waples & Do, 2010). The model is applicable to iteroparous species with overlapping generations, as is the case for mandrills. Demographic and genetic data on wild populations of mandrills are unfortunately scarce. We therefore gathered available data on reproductive age and male mortality rates from captive populations of mandrills at the Centre International de Recherches Médicales de Franceville (CIRMF), Gabon (Setchell et al., 2005) and from expert opinion (Abernethy K. and Lehmann D., personal communication) (Table 1). As data on female mortality for mandrills was lacking, we used demographic data available from baboon populations (Bronikowski et al., 2016). We evaluated the power of N_e estimation using 13 or 10 loci and a maximum sample size of 400 genotypes, with confidence intervals for N_e assessed at every 100 genotypes. This simulated sample size is greater than the actual sample size in the present study, allowing us to determine the minimum number of samples needed to obtain an accurate estimate of N_e . Ten loci represent the average number of loci that were amplified in all samples, and 13 is the maximum number of loci used. Since the exact size of the mandrill horde is not known, we ran NeOGen using N_c values of 620, 845, and 1250. These values are drawn from Abernethy et al. (2002) and from D. Lehmann (personal communication, 2019).

Genetic estimation of effective population size (Ne) using single and combined sample period

We used the unique genotypes to provide estimates of effective population size (Ne). We first estimated Ne using the samples from each individual sampling period using available one-sample estimators available in the program NeESTIMATOR Version 2.01 (Do et al., 2014), namely: the linkage disequilibrium method between loci (LDNe), the excess heterozygosity method (HeNe), and the molecular coancestry method. We also applied the sibship structure approach using the "Maximum Likelihood" model implemented in the program COLONY Version 2.0.6.4 (Jones & Wang, 2010). As a comparison, we also estimated Ne using genotypes pooled across all three-year sampling periods. In all methods, we used an exclusion criterion for rare alleles (Pcrit) equal to 0.02 (alleles with frequency < Pcrit are excluded) (Do et al., 2014). Finally, Ne estimates were combined across years using an unweighted harmonic mean, as suggested by other researchers (Waples & Do, 2010). To incorporate all estimates into the analyses, infinite estimates were converted to a value of 99999 (Do et al., 2014).

Results

Microsatellite genotyping

From 927 samples collected in the field, a total of 329 samples or 35.5% (with 91, 103 and 135 samples respectively for each individual year from 2016-2018) amplified successfully with a minimum of seven out of 16 microsatellite loci. From each individual year period from 2016-2018, a total of 83, 93 and 98 individual genotypes were identified respectively after removal of within-year duplicates. After removal of between-year recaptures, we identified a total of 232 unique genotypes across all three years combined. All loci were consistently amplified with a success rate of at least 45%, except for the MaCh312 locus, which only amplified in 10% of samples (Supplementary Table 1). We detected evidence of null alleles in only two loci: MaCh868 and MaCh834. Both loci also showed evidence of significant deviation from HWE proportions after Holm-Bonferroni correction and were removed from all subsequent analyses. We also removed the MaCh312 locus due to insufficient data. In the individual year data, all loci appeared to be independent of each other. All remaining loci (n=13) were highly polymorphic (Table 2), with an average allele number of 8.38 ± 1.74 and an overall mean observed and expected heterozygosity of 0.76 ± 0.08 and 0.77 ± 0.10 , respectively.

Estimates of Nc from genetic methods based on single and multiple sampling periods

Estimates of Nc obtained for each individual year (2016, 2017, and 2018) and for combined data from across all three time periods using CAPWIRE are shown in Table 3. Overall, the TIRM model provided larger estimates, while the ECM model provided smaller point

estimates with narrower 95% confidence intervals. The N_c estimates for TIRM from the combined 3-year data were larger and the confidence intervals narrower than those obtained using the individual period data, except for 2018, which had even smaller confidence intervals. The ECM estimates were similar to each other, with the exception of the one produced with the 2018 data, which was smaller. In addition, use of the likelihood ratio test (LRT) indicated that TIRM was a better fit to the data compared to ECM in the analyses of the 2018 data and when the data were combined. However, ECM was a better fit only for data from the individual periods of 2016 and 2017.

Comparison of the different models implemented in MARK shows that both the Mo and Mh2 models fit the data well based on the Delta AICc values (Table 4), implying that there may be heterogeneity in the detection probabilities. Nevertheless, the null model (Mo) and the heterogeneity model (Mh2) in MARK produced similar estimates and associated confidence intervals (Table 4).

The minimum number of samples needed to estimate effective population size (N_e)

The results of our simulation of the power of N_e estimation indicate that, if the census population size is 620, a minimum of 200 samples is required when 10 or 13 loci are used for estimation (Figure S1). When a population size of 845 is used, for 10 or 13 loci, a minimum of 300 or 200 samples are sufficient respectively to obtain an accurate N_e estimate (Figure 1). The results of the analysis using $N_c=1,250$ showed that for 10 loci, 400 samples are required, while for 13 loci, 300 samples are sufficient to provide an accurate estimate of N_e (Figure S1). These observations show that fluctuations in the population size parameter can affect NeOGen results. Furthermore, they suggest that the strength of the N_e estimates determined here may be improved with additional samples or loci when the population size is larger than 620.

Estimates of N_e from genetic methods based on single and combined sampling periods

Estimates of effective population size (N_e) varied considerably between methods (Table 5). Overall, the estimates produced by the individual period samples were generally smaller than those provided by the three-year samples combined. Finite population N_e estimates based on individual period data ranged from 58.71 to 234.14 individuals for all methods. Results based on excess heterozygosity (HeNe) and the molecular coancestry model were inconsistent or yielded infinite estimates. In contrast, estimates from the linkage disequilibrium (LDNe) and sibship (COLONY) models appeared more consistent across sample periods, although the LDNe estimates using data from the 2017 individual period were comparatively large. Combining data from across all three sampling periods yielded larger estimates of N_e for both the LDNe and sibship models. In contrast, the HeNe method still yielded infinite estimates whereas the molecular coancestry

model produced unrealistically low estimates. Given the most robust estimates of N_e from our models, N_e appears to range between 13.6% and 29.5% of N_c (Table 6).

Discussion

Census size estimates (N_c) of the mandrill population

We used a non-invasive genetic approach to provide measures of population size based on single and multiple sampling strategies. We found that both methods can be effective, given a sufficient sample size. Estimates from the TIRM model implemented in CAPWIRE were improved when genotypes from the three sampling sessions were pooled. Those estimates, along with those from the program MARK were most similar to previous estimates determined by direct field observations (Abernethy et al., 2002; Lehmann D., person. Communication, 2019). In accordance with past studies, our estimates revealed a larger group size than many other highly social primates from other regions, such as focal groups of northern yellow baboon (Wallis, 2020), the southern Chacma baboon (Sithaldeen & Rylands, 2020; Stone et al., 2012) and macaques (Boonratana et al., 2020; Chetry et al., 2003). The only other primate with a larger estimated group size is from gelada monkeys (*Theropithecus gelada*; $N_c \geq 1500$ individuals; Beehner et al., 2007; Kifle et al., 2013).

The low N_c values obtained in our study from the single sample period data using ECM and TIRM in CAPWIRE (Miller et al., 2005) appear to underestimate the population size. In addition, the wide confidence intervals of these values show low precision around the point estimate. These results can be explained by the small number of samples used. Indeed, consistent with the results of other studies, an insufficient number of samples can produce unreliable estimates when using these one-sample models (Miller et al., 2005). In contrast, using a greater number of samples improves the population estimates and reduces the width of the confidence intervals (Miller et al., 2005).

When we used a larger sample size by combining data from all three years, the ECM produced a point estimate that appeared very similar to previous estimates from the same model based on single-year samples. In contrast, the TIRM estimate was much larger and had reasonably small confidence intervals. Other researchers have obtained similar results (Miller et al., 2005). It has been shown that, despite using a sufficient number of samples, ECM tended to produce lower and less credible estimates when there was heterogeneity in the probability of sample capture (Miller et al., 2005), which may also be the case in our study. These results have also been observed in other simulation and empirical studies, for example in population estimates for gorillas (Arandjelovic et al., 2010; Dou et al., 2016) and bats (Puechmaille & Petit, 2007). In these studies, the authors used a sufficient number of samples and found that CAPWIRE performed better using TIRM rather than ECM when capture heterogeneity was suspected in the data. Thus, our results suggest that the insufficient number of samples obtained from individual

years in our study leads to less precise estimates with wider confidence intervals and low point values of N_c , as previously demonstrated (Miller et al., 2005). In contrast, the use of a larger data set and the TIRM model that accounts for heterogeneity in capture probabilities between samples appears to produce a more robust estimate.

Interestingly, using either the null model (Mo, suggesting a constant capture probability) or the heterogeneity model (Mh2) in MARK (White & Burnham, 1999) gave results that were comparable to those given by TIRM when applied to a large number of samples from all three sampling years. The comparison of the MARK and TIRM results thus shows that using the combined samples from the three sampling periods produced relatively robust N_c estimates of mandrills. These results also support the suggestion by other researchers that accounting for heterogeneity in capture probability can produce good results of N_c (Bellemain et al., 2005; Dou et al., 2016; White & Burnham, 1999).

Our genetic estimates of 989 (95%CI:947-1399) and 992 (95%CI:708-1453) mandrills obtained with the TIRM and MARK estimators respectively are substantially larger than the initial maximum field estimates of up to 700 (Rogers et al., 1996) or 845 individuals (Abernethy et al., 2002) using observational data of the same horde. Recent unpublished observations suggest as many as 1,250 mandrills in the SEGC horde (Lehmann D. 2019, personal communication), and although this number is included within our confidence intervals, our point estimates show a somewhat smaller value.

Previous studies have compared genetic and standard field methods to estimate N_c in other species such as mountain gorillas (Guschanski et al., 2009), otters (Arrendal et al., 2007; Hájková et al., 2009) and giant pandas (Zhan et al., 2006). In these studies, the authors found that genetic estimators most often provide reliable results, whereas standard field methods tend to overestimate or underestimate true population sizes. The usefulness of standard traditional methods for estimating population size, such as cameras or direct counts, may indeed be limited when individuals form a large horde and live in closed forest habitats (Bata et al., 2017; Buckland, 1980; Christman, 2004; Frankham, 1995; Leberg, 2005). Studies carried out on mandrill populations have shown that this species is difficult to observe in nature due to their dense forest habitat and reclusive behavior (Abernethy et al., 2002; Hoshino et al., 1984; Jouventin, 1975; Rogers et al., 1996).

As mentioned above, there are some discrepancies between our genetic estimates and the historical estimates from Rogers et al. (1996) and Abernethy et al. (2002). It is possible that past researchers did not observe the entire horde, as we have noted that the horde often divides into smaller sub-hordes to better occupy different habitats in search of new resources (Lehmann D., 2019, personal communication). Predation by panthers may also lead to subdivision of the horde but is expected to be of short duration, while subdivision due to foraging may extend for about one to two months before the larger horde rebuilds (Lehmann D., 2019, personal communication). However, mandrill counts by Abernethy et al. (2002) occurred over a 39-month

period, from June 1996-August 1999, and therefore should have captured the majority of individuals within the SEGC horde. The difference in our estimates is more likely to be explained by growth of the focal horde. LNP contains favorable habitat, with minimal hunting pressure and seasonally stable resources. Given that more than 20 years have elapsed between the past studies and the present one, horde growth would be unsurprising. The recent unpublished counts by D. Lehmann (2019) also point to an increase in horde size since the late 1990s.

The apparent growth of the mandrill horde reflects the conservation efforts of the park's wildlife brigade and ecoguard patrols, as well as the park's recognition in 2007 as a World Heritage Site (<https://papaco.org/gabon/>). However, similar protection may not be provided in other areas of the mandrill's range and monitoring the population size of other hordes may prove essential in management. It remains to be seen whether direct counts are as accurate as genetic methods in other habitat types where mandrills are more difficult to observe. In this case, genetic methods appear to offer a reliable alternative.

Genetic estimates of N_e in mandrills

In this study, we provided for the first time N_e estimates for the SEGC focal horde of mandrills using a range of genetic methods (Do et al., 2014; Jones & Wang, 2010). We compared the estimates based on individual sample period samples (2016-2018) and data combined from all three periods. Our results indicated that both strategies can provide good estimates but only if sufficient sample sizes are obtained. Comparison of N_e estimates allowed us to estimate N_e of the SEGC horde to be between 135 (95%CI: 108-176) and 292 (95%CI: 239-370) individuals, using the two best performing estimators in this study: sibship structure and linkage disequilibrium (LD N_e) respectively. Nevertheless, these estimates should be interpreted with caution, since our NeOGen analyses showed that our dataset may lack sufficient power if the census size is large.

Estimates produced using the individual period samples and those based on the combined dataset varied between methods. Excess heterozygosity (He N_e) and molecular coancestry methods gave unreliable results. However, the linkage disequilibrium (LD N_e) and sibship methods gave close finite estimates using both single-period data and combined sample periods, although the value obtained from the COLONY sibship was much lower when single-period samples were used.

These differences in N_e estimates obtained from different sampling designs (single-period versus combined samples) are consistent with previous studies that have reported variable estimates of N_e depending on the method used (Do et al., 2014; Wang, 2009; Waples & Do, 2010). These results reflect the limitations of the approaches used to estimate N_e , with genetic estimators generally losing performance with small numbers of samples and loci (Do et al., 2014; England et al., 2006, 2010; Luikart et al., 2010; Richards & Leberg, 1996; Tallmon et al., 2004;

Wang, 2009; Waples, 1989; Waples & Do, 2010). The results provided by the HeNe and molecular coancestry methods are not surprising, as in most cases of simulation studies, these methods have often produced poor results due to biases caused by sample number (Do et al., 2014; Luikart et al., 2010). The downward bias observed using the sibship method could be due to the increase in related individuals or the sensitivity of the method to sample size, as previously demonstrated by other researchers (Wang, 2009; Waples, 1989; Waples & Do, 2010). Indeed, the sibship method is based on the principle that the estimate of N_e increases when the number of non-related individuals increases (Jones & Wang, 2010). Thus, the results produced by the sibship model suggest that the estimates obtained using the combined samples from the three sampling years appear to be the best.

Nevertheless, the results provided by NeOGen (Blower et al., 2019) revealed that more than 300 samples may be required to obtain an accurate N_e for a large population using 10 loci, which is the average number in our dataset. Somewhat fewer samples would have been required for 13 loci. From these results, it appears that our N_e estimates would be improved by the use of either additional samples or additional loci, if the census size is as large as is suggested from our analyses. In addition, other studies have shown that using a large number of loci with high allelic richness and high P_{crit} values (i.e., $P_{crit} > 1/2N$ with N the number of samples) can minimize bias, and thus improve estimates of these variables (Do et al., 2014; Waples, 2006; Waples & Do, 2010), which was likely the case for the LDNe and sibship methods.

Previous studies have indicated that levels of N_e that are less than 50 can be detrimental to a population, since small effective sizes can reduce adaptive capacity and cause severe inbreeding risk (Frankham et al., 2014; Madsen et al., 1999; Westemeier et al., 1998). Therefore, an N_e of 135 or 292 individuals in the SEGC mandrill horde is likely sustainable given the large size of this mandrill horde and its apparent growth over 22 years of study (1999-2018).

Here, we noted that N_e values appear to be between 13.6% and 29.4%, of N_c , which is higher than many other wildlife studies (Frankham, 1995; Harpending & Cowan, 1986; Kinnaird & O'Brien, 1991; Palstra & Ruzzante, 2008). Our analyses did not identify the exact factors that might influence N_e in this population, but factors such as the large numbers of individuals (N_c) and connectivity between hordes may be key. Although studies of gene flow between mandrill hordes have not yet been done, observational studies of the SEGC horde have reported that male mandrills leave the natal horde to be solitary before they reach 6 years of age. When these individuals reach adulthood (>9 years), they return to the horde during the breeding season (Abernethy et al., 2002). It is not yet known whether these mature males return to their natal horde or emigrate to other populations. However, these field observations of Abernethy et al., (2002) may suggest that mandrills may disperse into neighboring hordes and thus avoid inbreeding. In addition, other observations reveal that mandrills appear to move between habitat fragments by crossing the intervening savanna (Abernethy & Maisels, 2019) and thus may exchange genes with other hordes to maintain a viable population.

The lack of demographic data on wild mandrills limits the extent to which we can understand the dynamics of this population. This study is limited by the number of loci and genotypes available, which could affect the reliability of N_c and N_e estimates. However, mark-release-recapture estimates of N_c and single sample estimates of N_e from a larger pooled set of samples yielded meaningful results. Thus, our research shows that non-invasive sampling is a viable strategy to estimate horde size in mandrills and is the first study to provide a genetic estimate of this species in the wild.

Conclusion

This study shows that population assessment of wild mandrills using a non-invasive sampling approach is feasible and likely to be effective in providing important data that would otherwise be difficult to obtain. While standard field methods are often limited when it is difficult to observe mandrills in the wild, the non-invasive genetic approach may become one of the most efficient and cost-effective ways to study the species in areas where populations are suspected to be declining. This study also shows the importance of combining a range of genetic estimators, because not all estimators perform equally well. However, a sufficient number of samples is required to obtain an accurate estimate, so it may be necessary to sample in multiple sessions. We recommend the use of non-invasive genetics as an effective tool to study wild mandrill, provided sufficient samples and loci are available. Studies on the reproductive system, assessment of bottlenecks, gene flow between populations, and population viability are needed to better understand the genetic status, management, and long-term conservation of mandrills.

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References

- Abernethy, K. A., & Maisels, F. (2019). *Mandrillus sphinx*. *The IUCN Red List of Threatened Species 2019: E.T12754A17952325*. <https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T12754A17952325.en>.
- Abernethy, K. A., White, L. J. T., & Wickings, E. J. (2002). Hordes of mandrills (*Mandrillus sphinx*): Extreme group size and seasonal male presence. *Journal of Zoology*, 258(1), 131–137. <https://doi.org/10.1017/s0952836902001267>
- Arandjelovic, M., Head, J., Kuehl, H., Boesch, C., Robbins, M. M., Maisels, F., & Vigilant, L. (2010). Effective non-invasive genetic monitoring of multiple wild western gorilla groups. *Biological Conservation*, 143(7), 1780–1791.
- Arrendal, J., Vila, C., & Björklund, M. (2007). Reliability of noninvasive genetic census of otters compared to field censuses. *Conservation Genetics*, 8(5), 1097–1107.
- Banks, S. C., Hoyle, S. D., Horsup, A., Sunnucks, P., & Taylor, A. C. (2003). Demographic monitoring of an entire species (the northern hairy-nosed wombat, *Lasiorninus krefftii*) by genetic analysis of non-invasively collected material. *Animal Conservation Forum*, 6(2), 101–107.
- Bata, M. N., Easton, J., Fankem, O., Wachter, T., Bruce, T., Elisé, T., Taguieteu, P. A., & Olson, D. (2017). Extending the Northeastern Distribution of Mandrills (*Mandrillus sphinx*) into the Dja Faunal Reserve, Cameroon. *African Primates*, 12, 65–67.
- Beehner, J. C., Berhanu, G., Bergman, T. J., & McCann, C. (2007). Population estimate for geladas (*Theropithecus gelada*) living in and around the Simien Mountains National Park, Ethiopia. *SINET: Ethiopian Journal of Science*, 30(2), 149–154.
- Bellemain, E. V. A., Swenson, J. E., Tallmon, D., Brunberg, S., & Taberlet, P. (2005). Estimating population size of elusive animals with DNA from hunter-collected feces: Four methods for brown bears. *Conservation Biology*, 19(1), 150–161.
- Benoit, L., Mboumba, S., Willaume, E., Kappeler, P. M., & Charpentier, M. J. E. (2014). Using next-generation sequencing methods to isolate and characterize 24 simple sequence repeat loci in mandrills (*Mandrillus sphinx*). *Conservation Genetics Resources*, 6(4), 903–905. <https://doi.org/10.1007/s12686-014-0237-1>
- Bergl, R. A., & Vigilant, L. (2007). Genetic analysis reveals population structure and recent migration within the highly fragmented range of the Cross River gorilla (*Gorilla gorilla diehli*). *Molecular Ecology*, 16(3), 501–516.

- Blower, D. C., Riginos, Cynthia., & Ovenden, J. R. (2019). neogen: A tool to predict genetic effective population size (N_e) for species with generational overlap and to assist empirical N_e study design. *Molecular Ecology Resources*, 19(1), 260–271.
<https://doi.org/10.1111/1755-0998.12941>
- Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F., Brochmann, C., & Taberlet, P. (2004). How to track and assess genotyping errors in population genetics studies. *Molecular Ecology*, 13(11), 3261–3273.
- Boonratana, R., Chalise, M., Chetry, D., Htun, S., & Timmins, R. J. (2020). *Macaca assamensis ssp. Assamensis*. *The IUCN Red List of Threatened Species 2020: E.T39766A17985704*.
<https://dx.doi.org/10.2305/IUCN.UK.20202.RLTS.T39766A17985704.en>
- Bronikowski, A. M., Cords, M., Alberts, S. C., Altmann, J., Brockman, D. K., Fedigan, L. M., Pusey, A., Stoinski, T., Strier, K. B., & Morris, W. F. (2016). Female and male life tables for seven wild primate species. *Scientific Data*, 3(1), 1–8.
- Buckland, G. (1980). *Fox Talbot and the invention of photography*. David R Godine Pub.
- Caballero, A. (1994). Developments in the prediction of effective population size. *Heredity*, 73(6), 657–679.
- Charlesworth, B. (2009). Effective population size and patterns of molecular evolution and variation. *Nature Reviews Genetics*, 10(3), 195–205.
- Chetry, D., Medhi, R., & Bhattacharjee, P. (2003). Anti-predator behaviour of stump-tail macaques in Gibbon Wildlife Sanctuary, Assam, India. *Asian Primates*, 8(4), 20–22.
- Christman, M. C. (2004). Sequential sampling for rare and geographically clustered populations. *Sampling Rare or Elusive Species*. Island Press, Washington, DC, 134–145.
- Clemente, A. J., Anderson, E. C., Boughton, D., Girman, D., & Garza, J. C. (2009). Population genetic structure and ancestry of *Oncorhynchus mykiss* populations above and below dams in south-central California. *Conservation Genetics*, 10(5), 1321.
- Dawnay, N., Dawnay, L., Hughes, R. N., Cove, R., & Taylor, M. I. (2011). Substantial genetic structure among stocked and native populations of the European grayling (*Thymallus thymallus*, Salmonidae) in the United Kingdom. *Conservation Genetics*, 12(3), 731–744.
- Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Molecular Ecology Resources*, 14(1), 209–214.
- Dou, H., Yang, H., Feng, L., Mou, P., Wang, T., & Ge, J. (2016). Estimating the population size and genetic diversity of Amur tigers in Northeast China. *PloS One*, 11(4), e0154254.
- England, P. R., Cornuet, J.-M., Berthier, P., Tallmon, D. A., & Luikart, G. (2006). Estimating effective population size from linkage disequilibrium: Severe bias in small samples. *Conservation Genetics*, 7(2), 303.

- England, P. R., Luikart, G., & Waples, R. S. (2010). Early detection of population fragmentation using linkage disequilibrium estimation of effective population size. *Conservation Genetics*, 11(6), 2425–2430.
- Ernest, H. B., Penedo, M. C. T., May, B. P., Syvanen, M., & Boyce, W. M. (2000). Molecular tracking of mountain lions in the Yosemite Valley region in California: Genetic analysis using microsatellites and faecal DNA. *Molecular Ecology*, 9(4), 433–441.
- Evetts, I., & Weir, B. (1998). *Interpreting DNA evidence: Statistical genetics for forensic scientists*.
- Excoffier, L., & Lischer, H. (2015). Arlequin (Version 3.5). *Swiss Institute of Bioinformatics*.
- Flagstad, Ø., Hedmark, E., Landa, A., Brøseth, H., Persson, J., Andersen, R., Segerström, P., & Ellegren, H. (2004). Colonization History and Noninvasive Monitoring of a Reestablished Wolverine Population. *Conservation Biology*, 18(3), 676–688.
<https://doi.org/10.1111/j.1523-1739.2004.00328.x-i1>
- Frankham, R. (1995). Conservation Genetics. *Annual Review of Genetics*, 29(1), 305–327.
<https://doi.org/10.1146/annurev.ge.29.120195.001513>
- Frankham, R. (2005). Genetics and extinction. *Biological Conservation*, 126(2), 131–140.
- Frankham, R., Ballou, S. E. J. D., Briscoe, D. A., & Ballou, J. D. (2002). *Introduction to conservation genetics*. Cambridge university press.
- Frankham, R., Bradshaw, C. J., & Brook, B. W. (2014). Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation*, 170, 56–63.
- Gaetano, J. (2018). *Holm-Bonferroni sequential correction: An Excel calculator (1.3) [Microsoft Excel workbook]*. https://www.researchgate.net/publication/322568540_Holm-Bonferroni_sequential_correction_An_Excel_calculator_13
- Gittleman, J. L. (2001). *Carnivore conservation*.
- Granjon, A.-C., Robbins, M. M., Arinaitwe, J., Cranfield, M. R., Eckardt, W., Mburanumwe, I., Musana, A., Robbins, A. M., Roy, J., Sollmann, R., Vigilant, L., & Hickey, J. R. (2020). Estimating abundance and growth rates in a wild mountain gorilla population. *Animal Conservation*, 23(4), 455–465. <https://doi.org/10.1111/acv.12559>
- Gurov, T., Atanassov, E., Karaivanova, A., Serbezov, R., & Spassov, N. (2017). Statistical Estimation of Brown Bears (*Ursus arctos* L.) Population in the Rhodope Mountains. *Procedia Computer Science*, 108, 2028–2037.
<https://doi.org/10.1016/j.procs.2017.05.272>
- Guschanski, K., Vigilant, L., McNeillage, A., Gray, M., Kagoda, E., & Robbins, M. M. (2009). Counting elusive animals: Comparing field and genetic census of the entire mountain gorilla population of Bwindi Impenetrable National Park, Uganda. *Biological Conservation*, 142(2), 290–300.

- Hájková, P., Zemanová, B., Roche, K., & Hájek, B. (2009). An evaluation of field and noninvasive genetic methods for estimating Eurasian otter population size. *Conservation Genetics*, 10(6), 1667–1681. <https://doi.org/10.1007/s10592-008-9745-4>
- Harpending, H., & Cowan, S. (1986). Primate population structure: Evaluation of models. *American Journal of Physical Anthropology*, 70(1), 63–68.
- Hedgecock, D., Launey, S., Pudovkin, A. I., Naciri, Y., Lapègue, S., & Bonhomme, F. (2007). Small effective number of parents (Nb) inferred for a naturally spawned cohort of juvenile European flat oysters *Ostrea edulis*. *Marine Biology*, 150(6), 1173–1182. <https://doi.org/10.1007/s00227-006-0441-y>
- Hedwig, D., Kienast, I., Bonnet, M., Curran, B. K., Courage, A., Boesch, C., Kühl, H. S., & King, T. (2018). A camera trap assessment of the forest mammal community within the transitional savannah-forest mosaic of the Batéké Plateau National Park, Gabon. *African Journal of Ecology*, 56(4), 777–790. <https://doi.org/10.1111/aje.12497>
- Holm, S. (1979). A simple sequential rejective method procedure. 6, 65–70.
- Hongo, S. (2014). New evidence from observations of progressions of mandrills (*Mandrillus sphinx*): A multilevel or non-nested society? *Primates*, 55(4), 473–481.
- Hoshino, J., Mori, A., Kudo, H., & Kawai, M. (1984). Preliminary report on the grouping of mandrills (*Mandrillus sphinx*) in Cameroon. *Primates*, 25(3), 295–307.
- Johnson, A. E., Knott, C. D., Pamungkas, B., Pasaribu, M., & Marshall, A. J. (2005). A survey of the orangutan (*Pongo pygmaeus wurmbii*) population in and around Gunung Palung National Park, West Kalimantan, Indonesia based on nest counts. *Biological Conservation*, 121(4), 495–507. <https://doi.org/10.1016/j.biocon.2004.06.002>
- Jones, O. R., & Wang, J. (2010). COLONY: A program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, 10(3), 551–555.
- Jorde, P. E., & Ryman, N. (2007). Unbiased Estimator for Genetic Drift and Effective Population Size. *Genetics*, 177(2), 927–935. <https://doi.org/10.1534/genetics.107.075481>
- Jouventin, P. (1975). Observations sur la socio-écologie du mandrill. *La Terre et La Vie*.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12), 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kifle, Z., Belay, G., & Bekele, A. (2013). Population size, group composition and behavioral ecology of geladas (*Theropithecus gelada*) and human-gelada conflict in Wonchit Valley, Ethiopia. *Pak J Biol Sci*, 16, 1248–1259.
- Kimura, M., & Crow, J. F. (1963). The measurement of effective population number. *Evolution*, 279–288.
- Kingdon, J. (1997). *The Kingdon ®eld guide to African mammal*.

- Kinnaird, M. F., & O'BRIEN, T. G. (1991). Viable populations for an endangered forest primate, the Tana River crested mangabey (*Cercocebus galeritus galeritus*). *Conservation Biology*, 5(2), 203–213.
- Langergraber, K. E., Mitani, J. C., & Vigilant, L. (2007). The limited impact of kinship on cooperation in wild chimpanzees. *Proceedings of the National Academy of Sciences*, 104(19), 7786–7790.
- Leberg, P. (2005). Genetic Approaches for Estimating the Effective Size of Populations. *The Journal of Wildlife Management*, 69(4), 1385–1399. [https://doi.org/10.2193/0022-541X\(2005\)69\[1385:GAFETE\]2.0.CO;2](https://doi.org/10.2193/0022-541X(2005)69[1385:GAFETE]2.0.CO;2)
- Lucchini, V., Fabbri, E., Marucco, F., Ricci, S., Boitani, L., & Randi, E. (2002). Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps. *Molecular Ecology*, 11(5), 857–868.
- Luikart, G., Ryman, N., Tallmon, D. A., Schwartz, M. K., & Allendorf, F. W. (2010). Estimation of census and effective population sizes: The increasing usefulness of DNA-based approaches. *Conservation Genetics*, 11(2), 355–373.
- Madsen, T., Shine, R., Olsson, M., & Wittzell, H. (1999). Restoration of an inbred adder population. *Nature*, 402(6757), 34–35.
- Matschiner, M., & Salzburger, W. (2009). TANDEM: Integrating automated allele binning into genetics and genomics workflows. *Bioinformatics*, 25(15), 1982–1983. <https://doi.org/10.1093/bioinformatics/btp303>
- McKelvey, K. S., & Schwartz, M. K. (2005). Dropout: A program to identify problem loci and samples for noninvasive genetic samples in a capture-mark-recapture framework. *Molecular Ecology Notes*, 5(3), 716–718.
- Miller, C. R., Waits, L. P., & Joyce, P. (2005). A new method for estimating the size of small populations from genetic mark–recapture data. *Molecular Ecology*, 14(7), 1991–2005.
- Mowat, G., & Strobeck, C. (2000). Estimating population size of grizzly bears using hair capture, DNA profiling, and mark-recapture analysis. *The Journal of Wildlife Management*, 183–193.
- Nunney, L., & Elam, D. R. (1994). Estimating the effective population size of conserved populations. *Conservation Biology*, 8(1), 175–184.
- Oates, J. F., & Butynski, T. M. (2008). *Mandrillus sphinx*. *IUCN Red List of Threatened Species*. Version.
- Otis, D. L., Burnham, K. P., White, G. C., & Anderson, D. R. (1978). Statistical inference from capture data on closed animal populations. *Wildlife Monographs*, 62, 3–135.
- Paetkau, D. (2003). An empirical exploration of data quality in DNA-based population inventories. *Molecular Ecology*, 12(6), 1375–1387. <https://doi.org/10.1046/j.1365-294X.2003.01820.x>

- Palstra, F. P., & Ruzzante, D. E. (2008). Genetic estimates of contemporary effective population size: What can they tell us about the importance of genetic stochasticity for wild population persistence? *Molecular Ecology*, 17(15), 3428–3447. <https://doi.org/10.1111/j.1365-294X.2008.03842.x>
- Puechmaille, S. J., & Petit, E. J. (2007). Empirical evaluation of non-invasive capture–mark–recapture estimation of population size based on a single sampling session. *Journal of Applied Ecology*, 44(4), 843–852.
- Regazzi, R. (Ed.). (2007). *Molecular mechanisms of exocytosis*. Landes Bioscience/Eurekah.com ; Springer Science+Business Media.
- Richards, C., & Leberg, P. L. (1996). Temporal changes in allele frequencies and a population’s history of severe bottlenecks. *Conservation Biology*, 10(3), 832–839.
- Rogers, M. E., Abernethy, K. A., Fontaine, B., Wickings, E. J., White, L. J., & Tutin, C. E. (1996). Ten days in the life of a mandrill horde in the Lope Reserve, Gabon. *American Journal of Primatology*, 40(4), 297–313.
- Ruiz-Olmo, J., Saavedra, D., & Jiménez, J. (2001). Testing the surveys and visual and track censuses of Eurasian otters (*Lutra lutra*). *Journal of Zoology*, 253(3), 359–369.
- Schmeller, D. S., & Merilä, J. (2007). Demographic and Genetic Estimates of Effective Population and Breeding Size in the Amphibian *Rana temporaria*. *Conservation Biology*, 21(1), 142–151. <https://doi.org/10.1111/j.1523-1739.2006.00554.x>
- Schwartz, M. K., Cushman, S. A., McKelvey, K. S., Hayden, J., & Engkjer, C. (2006). Detecting genotyping errors and describing American black bear movement in northern Idaho. *Ursus*, 17(2), 138–148.
- Setchell, J. M., Charpentier, M., & Wickings, E. J. (2005). Sexual selection and reproductive careers in mandrills (*Mandrillus sphinx*). *Behavioral Ecology and Sociobiology*, 58(5), 474–485.
- Sithaldeen, R., & Rylands, A. B. (2020). *Papio ursinus ssp. Ursinus*. *The IUCN Red List of Threatened Species 2020:e.T136856A17986139*. <https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T136856A17986139.en>
- Soto-Calderón, I. D., Ntie, S., Mickala, P., Maisels, F., Wickings, E. J., & Anthony, N. M. (2009). Effects of storage type and time on DNA amplification success in tropical ungulate faeces. *Molecular Ecology Resources*, 9(2), 471–479. <https://doi.org/10.1111/j.1755-0998.2008.02462.x>
- Stone, O. M. L., Laffan, S. W., Curnoe, D., Rushworth, I., & Herries, A. I. R. (2012). Distribution and population estimate for the chacma baboon (*Papio ursinus*) in KwaZulu-Natal, South Africa. *Primates*, 53(4), 337–344. <https://doi.org/10.1007/s10329-012-0303-9>
- Taberlet, P., Waits, L. P., & Luikart, G. (1999). Noninvasive genetic sampling: Look before you leap. *Trends in Ecology & Evolution*, 14(8), 323–327. [https://doi.org/10.1016/S0169-5347\(99\)01637-7](https://doi.org/10.1016/S0169-5347(99)01637-7)

- Tallmon, D. A., Luikart, G., & Beaumont, M. A. (2004). Comparative evaluation of a new effective population size estimator based on approximate Bayesian computation. *Genetics*, 167(2), 977–988.
- Valière, N. (2002). gimlet: A computer program for analysing genetic individual identification data. *Molecular Ecology Notes*, 2(3), 377–379. <https://doi.org/10.1046/j.1471-8286.2002.00228.x-i2>
- Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4(3), 535–538.
- Waits, L. P., Luikart, G., & Taberlet, P. (2001). Estimating the probability of identity among genotypes in natural populations: Cautions and guidelines. *Molecular Ecology*, 10(1), 249–256. <https://doi.org/10.1046/j.1365-294X.2001.01185.x>
- Wallis, J. (2020). *Papio cynocephalus ssp. Ibeanus*. *The IUCN Red List of Threatened Species 2020: E.T136862A92251072*. <https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T136862A92251072.en>
- Wang, J. (2009). A new method for estimating effective population sizes from a single sample of multilocus genotypes. *Molecular Ecology*, 18(10), 2148–2164.
- Waples, R. S. (1989). A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics*, 121(2), 379–391.
- Waples, R. S. (2006). A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conservation Genetics*, 7(2), 167.
- Waples, R. S., & Do, C. H. I. (2010). Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: A largely untapped resource for applied conservation and evolution. *Evolutionary Applications*, 3(3), 244–262.
- Westemeier, R. L., Brawn, J. D., Simpson, S. A., Esker, T. L., Jansen, R. W., Walk, J. W., Kershner, E. L., Bouzat, J. L., & Paige, K. N. (1998). Tracking the long-term decline and recovery of an isolated population. *Science*, 282(5394), 1695–1698.
- White, Gary C., & Burnham, K. P. (1999). Program MARK: Survival estimation from populations of marked animals. *Bird Study*, 46(sup1), S120–S139. <https://doi.org/10.1080/00063659909477239>
- White, L., JT. (1994). The effects of commercial mechanised selective logging on a transect in lowland rainforest in the Lopé Reserve, Gabon. *Journal of Tropical Ecology*, 10(3), 313–322.
- White, Lee., & Abernethy, Kate. (1997). *A guide to the vegetation of the Lopé Reserve*. Wildlife Conservation Society.
- Wright, S. (1938). Size of population and breeding structure in relation to evolution. *Science*, 87, 430–431.

Zhan, X. J., Li, M., Zhang, Z. J., Goossens, B., Chen, Y. P., Wang, H. J., Bruford, M. W., & Wei, F. W. (2006). *Molecular censusing doubles giant panda population estimate in a key nature reserve.*

Statements & Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the conception and design of the study. Material preparation, data collection and analysis were carried out by Amour Guibinga Mickala, Anna Weber, Stephan Ntie, Prakhar Gahlot, Nicola Anthony, David Lehmann, Katherine Abernethy and Patrick Mickala. The first draft of the manuscript was written by Amour Guibinga Mickala and all authors commented on earlier drafts of the manuscript. All authors read and approved the final manuscript.

Data Availability

Upon acceptance, all data will be made available through an online data repository (DataDryad.org).

Figures legend

Figure 1: Graph showing the results of NeoGen software simulations to estimate the number of samples and loci needed to obtain an accurate N_e , assuming a census size of 845. The power of the N_e estimate is evaluated at every 100 genotypes, with a maximum of 400, using 10 loci (a), and 13 loci (b). The x-axis shows the combination of the number of samples and loci. The y-axis shows the corresponding estimate of N_e (blue circles) with 95% confidence intervals (Cis). All estimates of N_e are represented by two values in parentheses. The first value indicates the relevant estimate, and the second indicates the number of times the estimate was incalculable (i.e., negative, or close to infinity) in all replicates. Incalculable CIs are indicated by a red arrow and CIs with adequate power are in blue with a flat base. The precision of N_e for each combination is evaluated by the width of the Cis. The precision of the point estimates of N_e can be judged by their similarity to the shaded dashed "precision guideline," which is equal to the N_e estimated from all loci and all individuals in the same age cohorts as sampled for the sample/locus combinations.

795 Tables

796 **Table 1:** Mandrill (*Mandrillus sphinx*) input parameters used in the NeOGen software

Parameters	Values
Maximum age	22
Maximum mating age	20
Minimum mating age	4
Offspring per litter distribution	Absolute
Litter size	1
Population size	845
Mortality rates, Females Age 0-1:	22.6 ± 2.26
Subadult, Age 1-4:	8.6 ± 0.86
Adults, Age 4-20	8.6 ± 0.86
Males Age 0-1:	17.4 ± 1.74
Subadult, Age 1-10:	8.43 ± 0.843
Adults, Age 10-14:	70 ± 7.00
Alleles per locus distribution	Binomial
Mean allele number	8.38 ± 1.70
Number of population replicates	20
Maximum samples	400
Maximum loci	13
LDNe Pcrit	0.02
Number of Ne replicates	50

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Table 2: Summary statistics for the 16 microsatellite loci

Locus	Multiplex	Gen	Na	Hobs	Hexp	pHWE	%
MaCh868 ^{a, b}	1	140	9	0.776	0.543	0	61
MaCh726	1	204	9	0.803	0.892	0.393	88
MaCh303	1	199	8	0.782	0.804	0.264	86
MaCh834 ^{a, b}	1	160	7	0.662	0.494	0	69
MaCh866	2	192	6	0.705	0.75	0.291	83
MaCh070	2	118	12	0.87	0.78	0.067	51
MaCh184	2	133	10	0.832	0.767	0.1	58
MaCh372	2	146	9	0.815	0.842	0.446	63
MaCh419	3	173	6	0.696	0.786	0.477	75
MaCh129	3	173	10	0.572	0.665	0.879	74
MaCh409	3	190	9	0.812	0.826	0.636	82
MaCh141	3	103	7	0.777	0.913	0.09	45
MaCh581	4	181	8	0.844	0.74	0.061	78
MaCh007	4	217	7	0.77	0.839	0.372	94
MaCh312 ^a	4	24	8	0.83	0.542	0.009	10
MaCh262	4	213	8	0.782	0.812	0.386	92

Gen=number of genotypes, Na=number of alleles, Hobs=observed heterozygosity, Hexp=expected heterozygosity, pHWE= probability of deviation from Hardy-Weinberg equilibrium, and %=percentage of genotypes for which a consensus could be reached; a: Represents microsatellite loci that have been removed from the data set, b: Significant pHWE after sequential Holm-Bonferroni correction.

Table 3: Estimates of Nc from individual sample period data (2016 to 2018) and data from all three periods combined into a single sample design, using two single sample based genetic estimators.

Single sample				Combined data
Sampling period	2016	2017	2018	Allyears
Sample size	91	103	135	329
ECM (CAPWIRE)	573 (340-1392)	507 (335-1054)	204 (177-246)	548 (491-607)
TIRM (CAPWIRE)	616 (390-1230)	603 (472-1407)	420 (370-692)	989 (947-1399)

ECM - maximum likelihood, equal capture model (constant capture probability model), TIRM - maximum likelihood, two innate rate model (heterogeneity detection probabilities model), n=sample size.

Table 4: Population size estimate [N_c (95%IC)], and AICc scores corrected for sample size using the program MARK. AICc, delta AICc and Akaike weights (W) are ranked in relation to the best supported model.

Model	Description	AICc	Delta AICc	AICc W	N_c (95%IC)
Mo	constant detection probability	-1552.0822	0	0.52401	979 (712-1399)
Mh2	heterogeneity in detection probabilities	-1550.1126	1.9696	0.19573	992 (708- 1453)
Mth2	heterogeneity and temporal variation in detection probability	-1549.4679	2.6143	0.14179	990 (707- 1450)
Mt	temporal variation in detection probability	-1549.4205	2.6617	0.13847	977 (711- 1396)

Table 5: Estimates of N_e from individual sample period data (2016 to 2018) and data from all three periods combined into a single sample design, using four single sample based genetic estimators.

Single sample					
Period	n	LDNe	HeNe	Coancestry	Sibship
2016	83	154.1 (95.5-356)	∞ (18.4- ∞)	2175 (2.2-10918)	56 (38-84)
2017	93	801.5 (232.3- ∞)	∞ (17.1- ∞)	∞	56 (38-81)
2018	98	197 (124.3-428.7)	∞ (24.3- ∞)	30.3 (9.1-64)	65 (48-94)
Hmean (unweighted)		234.14	∞	89.62	58.71
Combined data					
Period	n	LDNe	HeNe	Coancestry	Sibship
All years	232	292 (239-370)	∞	26 (10-50)	135 (108-176)

Harmonic mean (unweighted), Harmonic mean of N_e estimates from each estimator, LDNe, N_e estimate with the one-sample method of linkage disequilibrium; HeNe, heterozygote excess method; Coancestry, molecular coancestry method; Sibship method based on COLONY; n=sample size.

Table 6: Ratio of population size estimates

	N_e	N_c	Ratio
minimum N_e/N_c ratio	135 (Sibship method)	992 (MARK)	13.6%
maximum N_e/N_c ratio	292 (LDNe)	989 (TIRM)	29.5%

Fig 1a

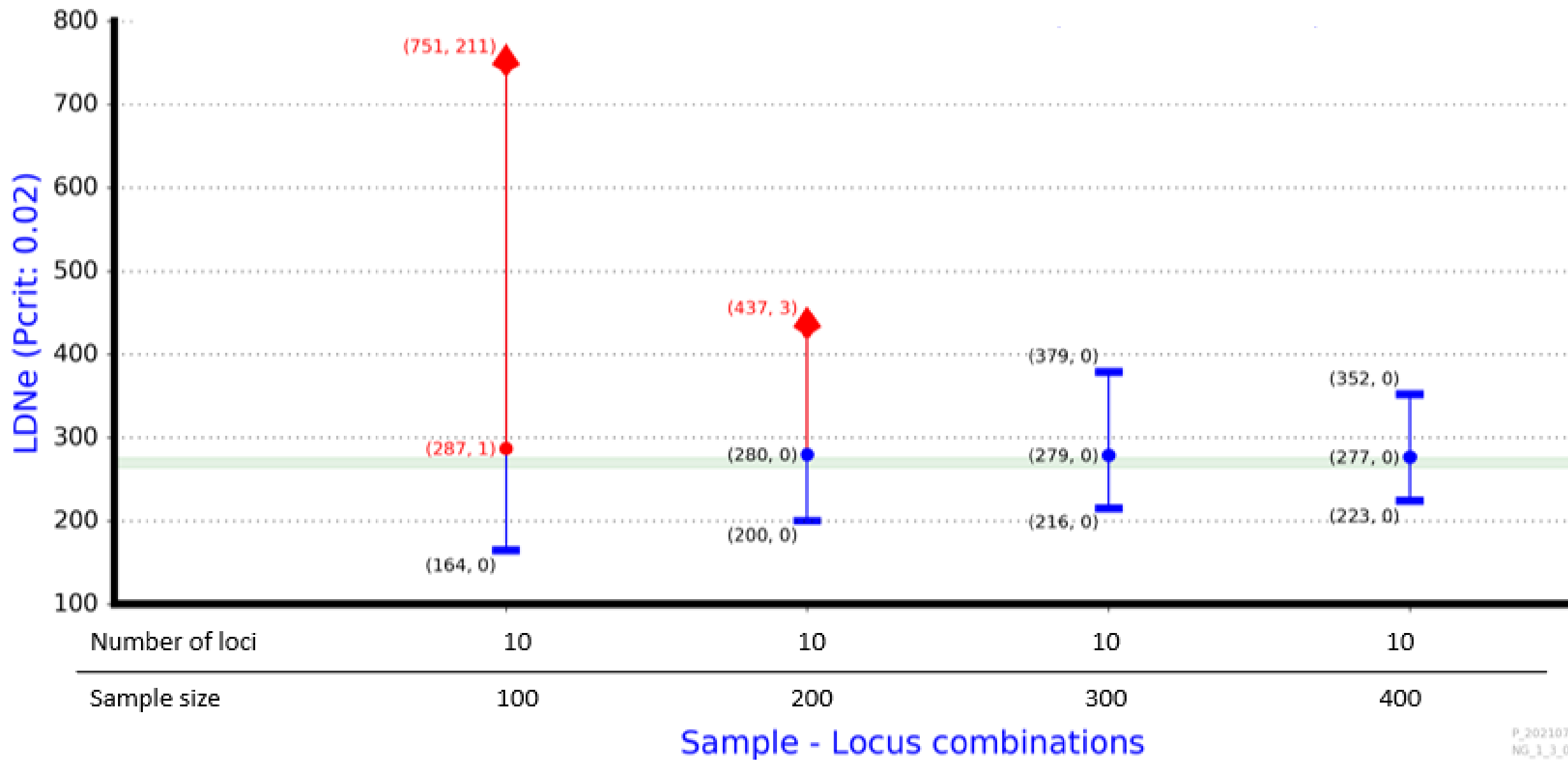
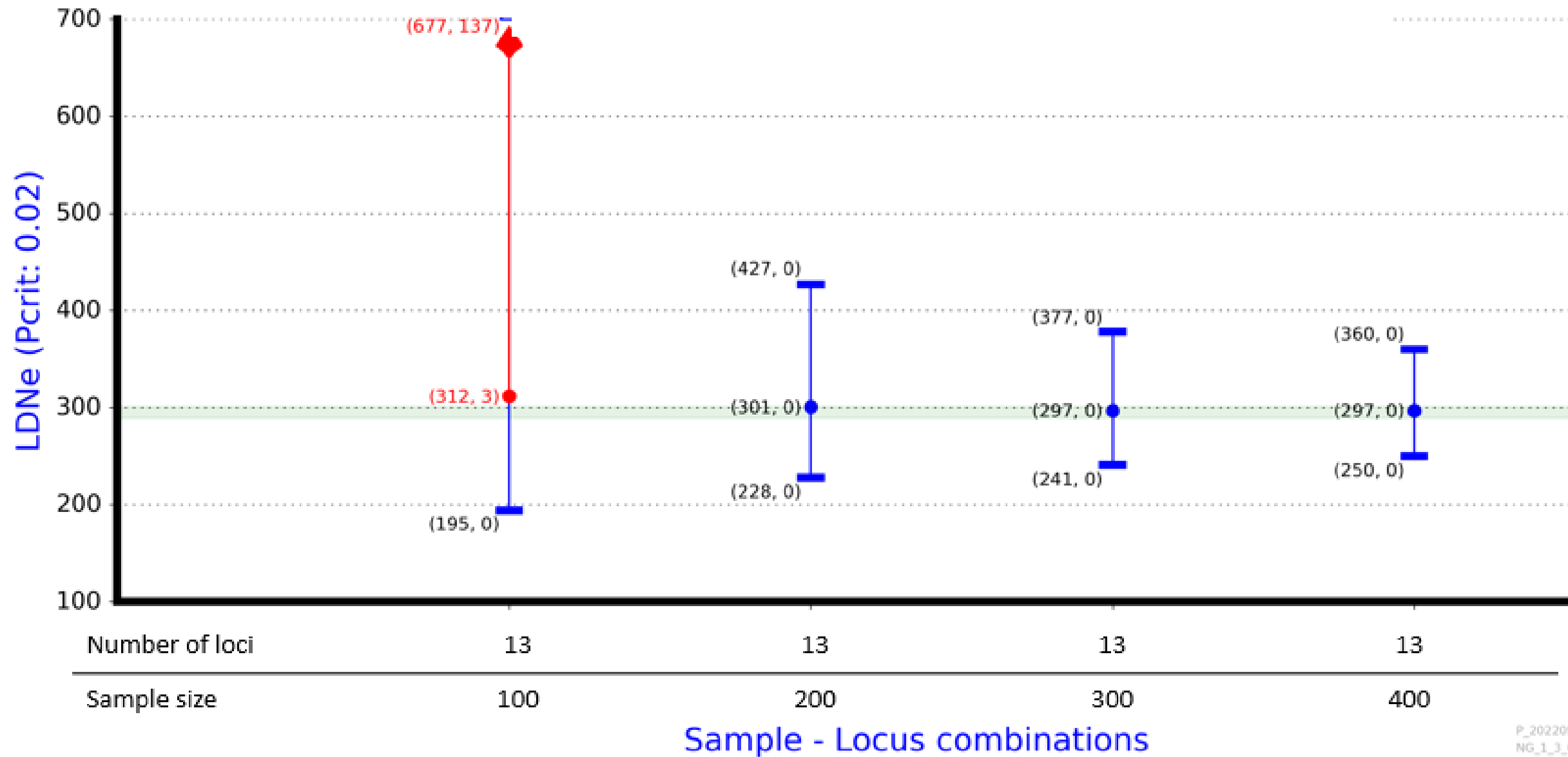


Fig 1b



1 **Conservation Genetics**

2 **Supplementary material:**

3 **Estimation of the census (Nc) and effective (Ne) population size of a wild mandrill (*Mandrillus sphinx*) horde in the Lopé National**
4 **Park, Gabon using a non-invasive genetic approach**

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19 **Supplementary Table 1**

20 Assembled microsatellite multiplexes, the identity of each locus (Locus ID), multiplex number, type of repeat motif, fluorophore label,
21 allele range and the corresponding accession number of each locus.

ID# locus	Multiplex	Repeat motif	Code Fluorophore	Color fluorophore	Allele size	Genbank accession No
MaCh0868	1	TCTA	NED	Yellow	80 - 120	KJ881174
MaCh0726	1	TCCA	6-FAM	Blue	140 - 190	KJ881193
MaCh0303	1	TCCA	HEX	Green	220 - 240	KJ881183
MaCh0834	1	GTT	6-FAM	Blue	230 - 250	KJ881172
MaCh0866	2	TAGA	6-FAM	Blue	140 - 180	KJ881173
MaCh0070	2	TATC	NED	Yellow	180 - 220	KJ881178
MaCh0184	2	AC	HEX	Green	210 - 240	KJ881181
MaCh0372	2	CA	6-FAM	Blue	240 - 280	KJ881185
MaCh0419	3	ATGG	HEX	Green	120 - 150	KJ881187
MaCh0129	3	CAT	6-FAM	Blue	160 - 180	KJ881179
MaCh0409	3	CTAT	NED	Yellow	170 - 210	KJ881186
MaCh0141	3	CATC	6-FAM	Blue	220 - 260	KJ881180
MaCh0581	4	CCAT	6-FAM	Blue	150 - 190	KJ881188
MaCh0007	4	TCTA	HEX	Green	180 - 220	KJ881176
MaCh0312	4	AC	NED	Yellow	220 - 250	KJ881184
MaCh0262	4	TGG	6-FAM	Blue	230 - 260	KJ881182

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26 **Detailed results of error rate calculations from the pilot study**

27 We genotyped a randomly selected subset of samples (n=19) six times to quantify error rates and determine the number of
28 replicates needed to reduce the probability of obtaining a false homozygote to less than 0.05. We obtained locus-specific estimates
29 of allelic dropout (ADO) and false alleles (FA) using the program GIMLET version 1.3.3 (Valiere, 2002). We called the consensus
30 genotypes using a modification of the strict threshold method (Taberlet & Fumagalli, 1996) in which a genotype was considered
31 heterozygous if two alleles appeared at least twice in six independent replicates, and homozygous if one allele was typed in at least
32 five of the six replicates. If neither of these cases applied, we treated the genotypes as missing data. The ADO rate was determined
33 for each sample at each locus by calculating the proportion of replicates in which ADO occurred (Table 2a). An average rate was
34 calculated for each locus, representing the probability of ADO occurring in a single replicate. To find the number of replicates
35 needed to reduce this number to less than 0.05, we chose the locus with the highest probability of loss and multiplied this number
36 by itself once for each replicate (Table 2b). The number of replicates needed to reduce this number below 0.05 represents the
37 number of replicates needed to produce a genotype with a sufficiently low probability of obtaining a false homozygote.

38 **Supplementary Table 2**

Table 2a. The ADO rate for each of the 16 loci. * Indicates the locus with the highest loss frequency.

MaCh868	0.142	MaCh866	0.225	MaCh419	0.107	MaCh581	0.252
MaCh726	0.113	MaCh070	0.158	MaCh129	0.194	MaCh007	0.143
MaCh303*	0.315*	MaCh184	0.171	MaCh409	0.228	MaCh312	0
MaCh834	0.063	MaCh372	0.171	MaCh141	0	MaCh262	0.222

Table 2b. The probability of loss of the MaCh303 locus in each of the three replicates. The probability of loss occurring in all three replicas is reliably negligible.

	1 st replicate	2 nd replicate	3 rd replicate
Probability of ADO	0.315	$0.315^2 = 0.099$	$0.315^3 = 0.031$

41 After applying this strategy, we found that a reliable genotype can be determined after three replicates of the locus with the highest
42 ADO rate, MaCh303 (ADO rate = 0.315).

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44 **Determining the Microsatellite Panel Power to Differentiate Individuals**

45 In addition to determining the number of replicates needed for reliable genotyping, it is also necessary to determine the panel
46 strength of the 16 loci in terms of differentiation of individuals, as it is a fundamental assumption of this study that each individual will
47 have a unique genotype. From the same 19 samples as with the ADO test, we calculated a par-locus probability of identity (PID) (Table
48 3a) using the PIDsibs estimator intended for populations with very low diversity (Evetts & Weir, 1998, Taberlet & Luikart, 1999). The
49 true value is probably lower. Starting with the locus with the most reliable PID and in descending order, the PID values were multiplied
50 together until the cumulative value was <0.01 (Table 3b), thus showing the number of loci needed to differentiate individuals with
51 confidence, as the probability of two individuals having the same genotype at all of these loci would be negligibly reliable.

52 **Supplementary Table 3**

Table 3a- The PID of each locus, or the probability that two individuals would share a genotype at these loci by chance.							
MaCh868	0.384	MaCh866	0.404	MaCh419	0.426	MaCh581	0.349
MaCh726	0.341	MaCh070	0.327	MaCh129	0.507	MaCh007	0.409
MaCh303	0.351	MaCh184	0.339	MaCh409	0.352	MaCh312	0.436
MaCh834	0.438	MaCh372	0.375	MaCh141	0.387	MaCh262	0.379

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Table 3b- The loci with the highest five PIDs still produce cumulative PID values that are acceptable.						
	MaCh129	MaCh834	MaCh312	MaCh419	MaCh007	MaCh866
Locus PID	0.507	0.436	0.438	0.426	0.409	0.404

Cumulative PID	0.507	0.221	0.097	0.041	0.017	0.007
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54 This test shows that even if PCR amplification is only successful at the six least informative microsatellite loci (MaCh129, MaCh834,
55 MaCh312, MaCh419, MaCh007, and MaCh866) the microsatellite panel is still robust enough to differentiate individuals.

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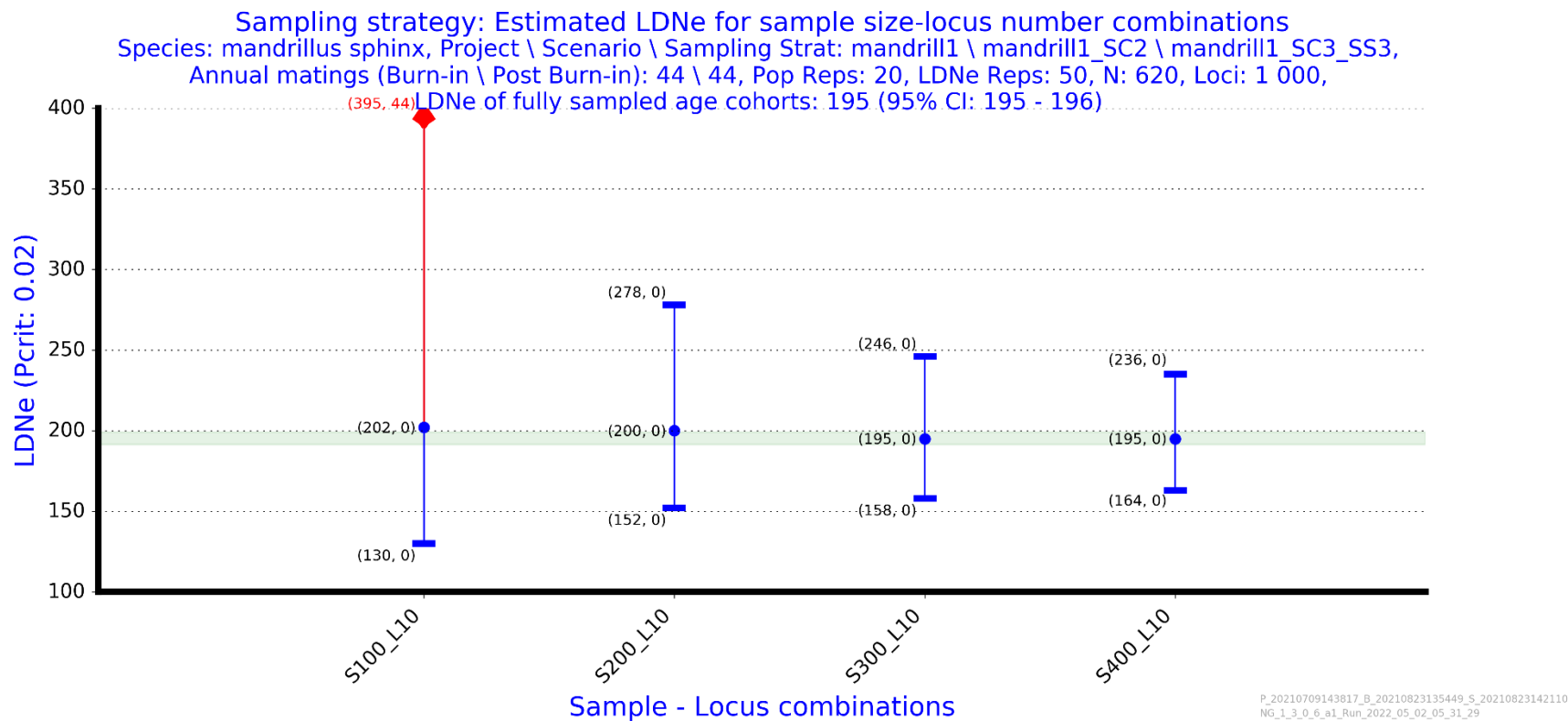
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72 **Supplementary Figure 1**

73 **Figure 1a**



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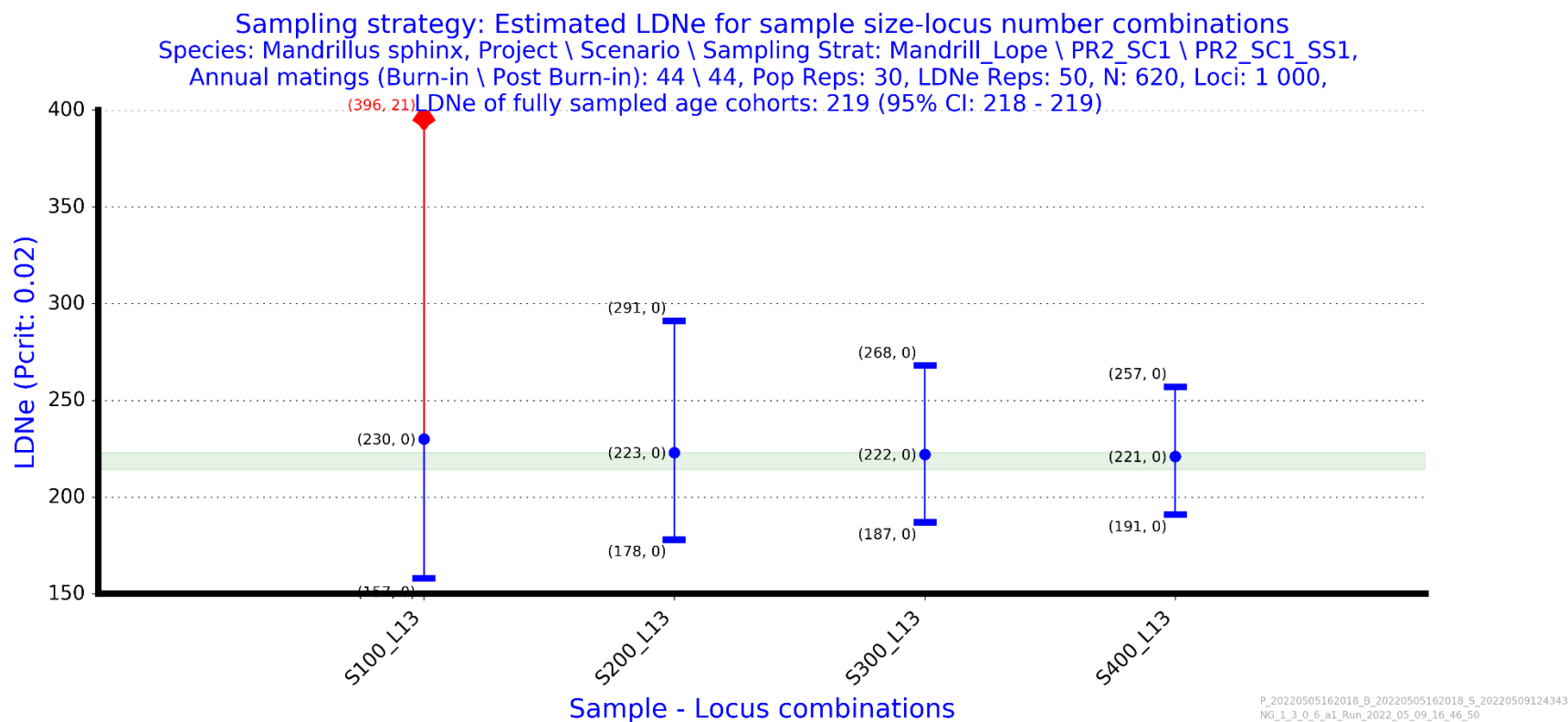
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79 **Figure 1b**



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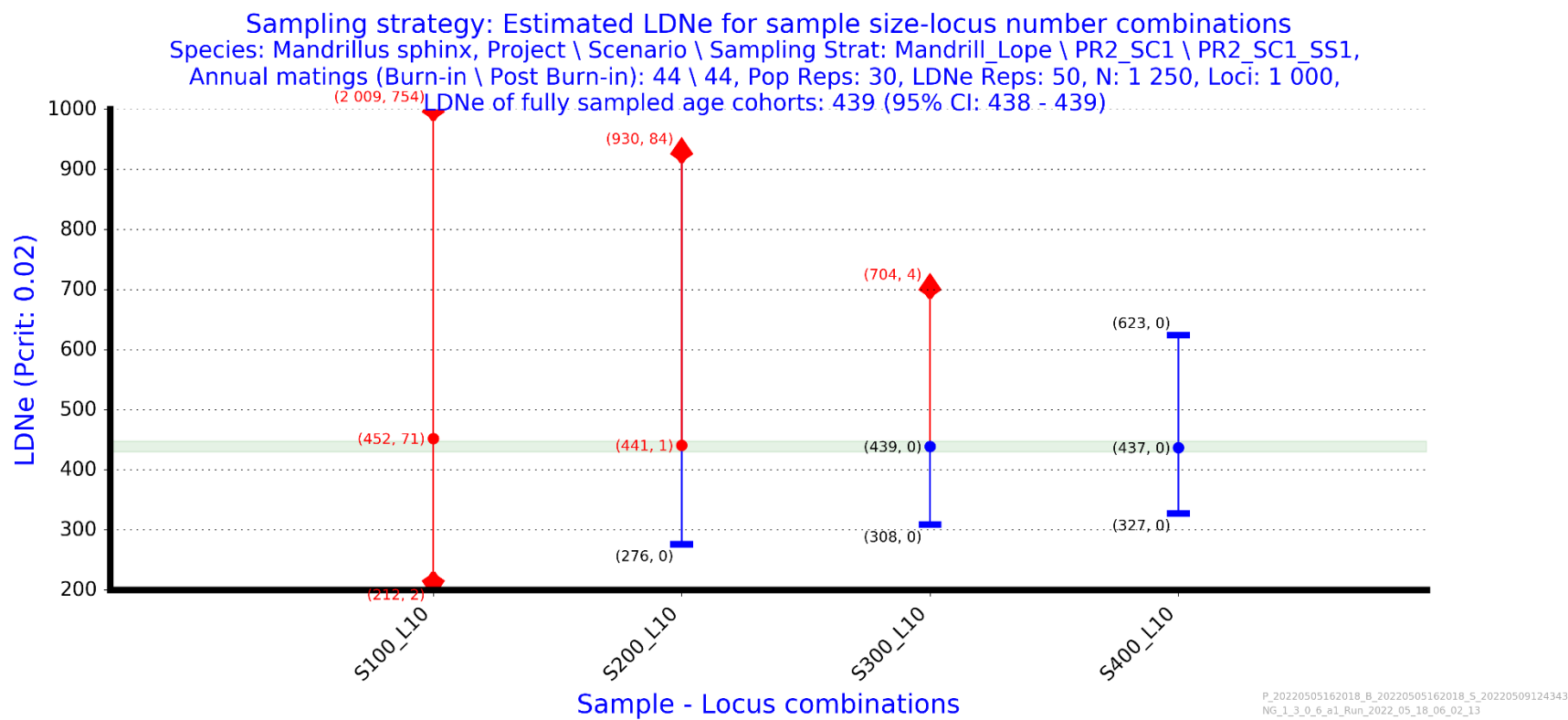
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86 **Figure 1c**



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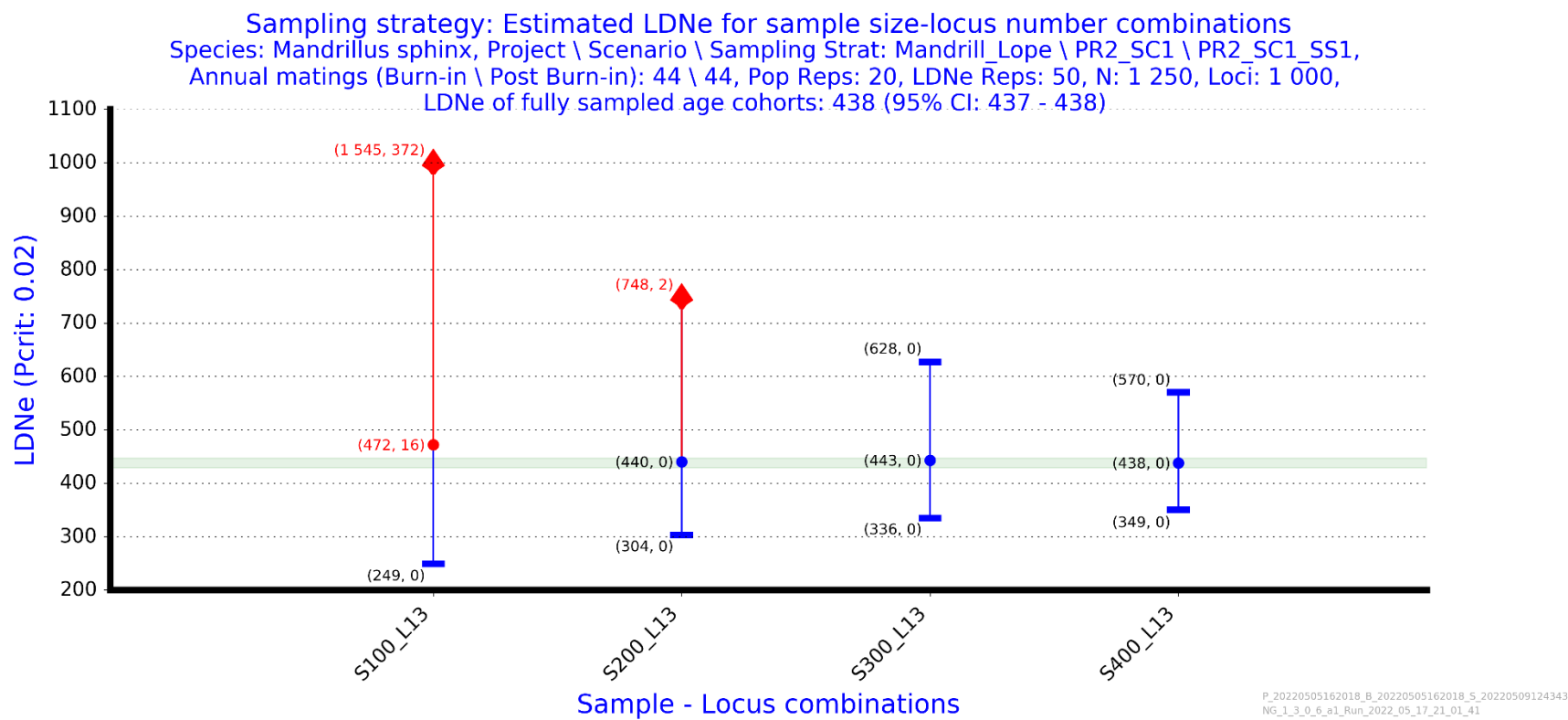
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93 **Figure 1d**



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