

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.

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

37

38 **Introduction**

39 Measures of census (N_c) and effective (N_e) population size are very important for
40 effective management and conservation of natural populations (Charlesworth, 2009; Frankham,
41 2005; Gurov et al., 2017; Hedgecock et al., 2007; Mowat & Strobeck, 2000). N_c is a direct measure
42 of the number of individuals present in a population and provides a demographic estimate of
43 population viability. In contrast, N_e reflects the number of reproductive individuals that
44 contribute to the next generation and is a measure of the rate at which genetic diversity is lost
45 due to genetic drift (Frankham et al., 2002; Luikart et al., 2010). However, estimating these two
46 population parameters in the wild remains a significant challenge, especially for rare and elusive
47 species. Although both parameters can be estimated directly from field observation or
48 demographic information (Bata et al., 2017; Caballero, 1994; Frankham, 1995; Gittleman, 2001;
49 Hedwig et al., 2018; Johnson et al., 2005; Kimura & Crow, 1963; Leberg, 2005; Nunney & Elam,
50 1994; Ruiz-Olmo et al., 2001; Schmeller & Merilä, 2007; Wright, 1938), obtaining these data can
51 be very logistically difficult for wild populations.

52 Genetic data are a common alternative (Do et al., 2014; Jones & Wang, 2010; Miller et
53 al., 2005; Otis et al., 1978; White & Burnham, 1999) and have been applied to a wide range of
54 wildlife species (Banks et al., 2003; Bergl & Vigilant, 2007; Frankham, 1995; Langergraber et al.,
55 2007; Lucchini et al., 2002). Tissue samples can provide high quality genetic material, but their
56 collection is not always feasible for at-risk species. As an alternative, genetic data can be collected
57 from non-invasive samples, such as shed hairs or feces, without disturbing the target species.
58 Such samples tend to be of lower quality and present numerous technical challenges (Clemento
59 et al., 2009; Dawnay et al., 2011; Dou et al., 2016; Ernest et al., 2000; Granjon et al., 2020;
60 Puechmaille & Petit, 2007). However, non-invasive sampling enables the collection of a higher
61 volume of samples than may be possible if using tissue. Furthermore, a plethora of different
62 methods have been developed to estimate N_c or N_e from genetic information using a single or
63 multiple sample periods (Do et al., 2014; Jorde & Ryman, 2007; Miller et al., 2005; Otis et al.,
64 1978; White & Burnham, 1999). Several studies have used one or both methods to produce
65 credible population size values (Arandjelovic et al., 2010; Bellemain et al., 2005; England et al.,
66 2010; Tallmon et al., 2004). Unlike methods that require multiple sampling sessions, estimating
67 from a single sampling period is often very useful for species where sampling is costly or difficult
68 over multiple time periods.

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

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

76 One such threatened species for which a strong knowledge base is lacking is the mandrill
77 (*Mandrillus sphinx*). This primate species is endemic to Central Africa and is distributed across
78 the tropical forests of Cameroon, Equatorial Guinea, Congo, and Gabon (Abernethy & Maisels,
79 2019; Kingdon, 1997). Mandrills are highly social and live in large groups or "hordes," which can
80 make them particularly vulnerable to hunting pressure and habitat loss (Abernethy & Maisels,
81 2019). Field observations have reported that hordes may have shrunk or disappeared in some
82 areas of the Cameroon and Equatorial Guinea forests where pressure is more intense (Abernethy
83 & Maisels, 2019). Because of this, the mandrill is listed in Appendix I by the Convention on
84 International Trade in Endangered Species of Wild Fauna and Flora (CITES) and categorized as
85 "Vulnerable" on the International Union for Conservation of Nature (IUCN) Red List (Abernethy
86 & Maisels, 2019; Oates & Butynski, 2008).

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

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

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

110 **Materials and methods**

111 Study site and sample collection

112 Samples were collected in the northern part (0012S, 1136E) of the LNP, adjacent to the
113 SEGC field station in Gabon. Although the LNP covers an area of approximately 5,000 km² of
114 lowland tropical rainforest, the northern part of the SEGC is dominated by a mosaic vegetation
115 cover of grassy savannahs and fragments of natural forest (White, 1994; White & Abernethy,
116 1997). The Ogooué River borders the park at its northern-most extent and provides a natural
117 barrier for many animals (Abernethy et al., 2002). The site is characterized by two dry seasons:
118 the little dry season from December to February and the long dry season that extends from mid-
119 June to mid-September. Temperatures at the site vary little with a mean monthly minimum of 20
120 ± 23.8 °C in the dry season and 26 ± 33.8 °C in the wet season (1984 ± 98) (Abernethy et al., 2002).

121 We sampled mandrill feces (n=927) from the SEGC horde over three successive years
122 (2016-2018) during the long summer dry season (July and August). This period corresponds to
123 the mandrill breeding season, when mature males and females are present in the horde
124 (Abernethy et al., 2002). We collected only fresh (< 3 hours) fecal samples to maximize DNA
125 quality for downstream molecular analyses (Regazzi, 2007). Mandrill fecal samples are similar to
126 that of other large primates, in that they are generally solid and physically preserve well. We
127 placed fecal samples in a 50 mL Falcon tube half-filled with silica gel beads, as previous work has
128 shown that this storage medium is the best for preserving nuclear DNA in central African forest
129 antelope (Soto-Calderón et al., 2009). We stored the samples in a freezer at -20°C prior to DNA
130 extraction. In an unrelated study, a small number of females and males of the horde were fitted
131 with radio collars, allowing us to locate the focal horde and collect fecal samples more easily.
132 Aliquots of blood (n = 14) and hair (n = 9) samples were also collected from a subset of these
133 individuals.

134 DNA extraction and amplification of microsatellites

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

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

142 (Supplementary Table 1). Forward primers were labelled with fluorophore dyes (labeled 6-FAM,
143 HEX, or NED) to discriminate individual loci within each multiplex. We performed polymerase
144 chain reaction (PCR) amplification of each multiplex in a total volume of 10 μ l. PCRs contained
145 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
146 (Bovine Serum Albumin), 5 μ l of 2X multiplex PCR kit (Qiagen, CA), 1.7 μ l of RNase-free water,
147 and 2 μ l of DNA extract. We performed PCR amplification using a touch-down protocol, with
148 duration of cycling steps following the PCR kit's manufacturer instructions. The cycle began with
149 an initial denaturation step for 15 minutes at 95°C to allow activation of the hot start Taq
150 polymerase. For M1 and M3, we then followed this step with 10 cycles of 30 seconds at 95°C for
151 denaturation, 90 s of annealing at 60°C (with a 1°C decrease after each cycle), and a 60-s extension
152 step at 72°C. We then performed 30 additional cycles using the following conditions: 94°C for 30
153 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
154 conditions for M2 and M4 were the same as for M1 and M3, except that the initial annealing
155 temperature during the first 10 cycles of the protocol started at 63°C, and decreased to 53°C over
156 the course of the reaction. PCR products were then analyzed on an ABI3130xl sequencer at either
157 the Department of Biological Sciences, University of New Orleans, USA, or the Georgia Genomics
158 and Bioinformatics Core (Georgia, USA).

159 Microsatellite genotyping

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

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

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

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

203 Genetic estimation of N_c using single and multiple sampling periods

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

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

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

218 We also compared estimates of N_c using the multi-sample estimators implemented in the
219 program MARK version 9.0 (White & Burnham, 1999). The program estimates N_c using several
220 closed population models that each incorporate different capture probabilities: the M_0 model,
221 where capture probabilities are assumed to be constant; M_t , where capture probabilities vary
222 with time; M_b , where there is a behavioral response to capture; and M_{h2} , where capture
223 probabilities vary by individual animal. MARK also allows combinations of these factors (M_{th} ,
224 M_{tb} , M_{tbh}). For analyses carried out in the program, we first aggregated individual multi-locus
225 genotypes observed during the three sampling periods and compiled a "capture" and "recapture"
226 history using the GenCapture version 1.4.9 program (McKelvey & Schwartz, 2005; Schwartz et al.,
227 2006). To choose the best model for our data, we compared each model's AICc (Akaike
228 information criterion corrected for small sample size) and respective weighting values (w).

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

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

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

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

263 Results

264 Microsatellite genotyping

265 From 927 samples collected in the field, a total of 329 samples or 35.5% (with 91, 103 and
266 135 samples respectively for each individual year from 2016-2018) amplified successfully with a
267 minimum of seven out of 16 microsatellite loci. From each individual year period from 2016-2018,
268 a total of 83, 93 and 98 individual genotypes were identified respectively after removal of within-
269 year duplicates. After removal of between-year recaptures, we identified a total of 232 unique
270 genotypes across all three years combined. All loci were consistently amplified with a success
271 rate of at least 45%, except for the MaCh312 locus, which only amplified in 10% of samples
272 (Supplementary Table 1). We detected evidence of null alleles in only two loci: MaCh868 and
273 MaCh834. Both loci also showed evidence of significant deviation from HWE proportions after
274 Holm-Bonferroni correction and were removed from all subsequent analyses. We also removed
275 the MaCh312 locus due to insufficient data. In the individual year data, all loci appeared to be
276 independent of each other. All remaining loci (n=13) were highly polymorphic (Table 2), with an
277 average allele number of 8.38 ± 1.74 and an overall mean observed and expected heterozygosity
278 of 0.76 ± 0.08 and 0.77 ± 0.10 , respectively.

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

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

283 estimates with narrower 95% confidence intervals. The N_c estimates for TIRM from the combined
284 3-year data were larger and the confidence intervals narrower than those obtained using the
285 individual period data, except for 2018, which had even smaller confidence intervals. The ECM
286 estimates were similar to each other, with the exception of the one produced with the 2018 data,
287 which was smaller. In addition, use of the likelihood ratio test (LRT) indicated that TIRM was a
288 better fit to the data compared to ECM in the analyses of the 2018 data and when the data were
289 combined. However, ECM was a better fit only for data from the individual periods of 2016 and
290 2017.

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

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

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

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

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

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

319 Discussion

320 Census size estimates (N_c) of the mandrill population

321 We used a non-invasive genetic approach to provide measures of population size based
322 on single and multiple sampling strategies. We found that both methods can be effective, given
323 a sufficient sample size. Estimates from the TIRM model implemented in CAPWIRE were
324 improved when genotypes from the three sampling sessions were pooled. Those estimates, along
325 with those from the program MARK were most similar to previous estimates determined by
326 direct field observations (Abernethy et al., 2002; Lehmann D., person. Communication, 2019). In
327 accordance with past studies, our estimates revealed a larger group size than many other highly
328 social primates from other regions, such as focal groups of northern yellow baboon (Wallis,
329 2020), the southern Chacma baboon (Sithaldeen & Rylands, 2020; Stone et al., 2012) and
330 macaques (Boonratana et al., 2020; Chetry et al., 2003). The only other primate with a larger
331 estimated group size is from gelada monkeys (*Theropithecus gelada*; $N_c \geq 1500$ individuals;
332 Beehner et al., 2007; Kifle et al., 2013).

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

341 When we used a larger sample size by combining data from all three years, the ECM
342 produced a point estimate that appeared very similar to previous estimates from the same model
343 based on single-year samples. In contrast, the TIRM estimate was much larger and had
344 reasonably small confidence intervals. Other researchers have obtained similar results (Miller et
345 al., 2005). It has been shown that, despite using a sufficient number of samples, ECM tended to
346 produce lower and less credible estimates when there was heterogeneity in the probability of
347 sample capture (Miller et al., 2005), which may also be the case in our study. These results have
348 also been observed in other simulation and empirical studies, for example in population
349 estimates for gorillas (Arandjelovic et al., 2010; Dou et al., 2016) and bats (Puechmaille & Petit,
350 2007). In these studies, the authors used a sufficient number of samples and found that CAPWIRE
351 performed better using TIRM rather than ECM when capture heterogeneity was suspected in the
352 data. Thus, our results suggest that the insufficient number of samples obtained from individual

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

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

365 Our genetic estimates of 989 (95%CI:947-1399) and 992 (95%CI:708-1453) mandrills
366 obtained with the TIRM and MARK estimators respectively are substantially larger than the initial
367 maximum field estimates of up to 700 (Rogers et al., 1996) or 845 individuals (Abernethy et al.,
368 2002) using observational data of the same horde. Recent unpublished observations suggest as
369 many as 1,250 mandrills in the SEGC horde (Lehmann D. 2019, personal communication), and
370 although this number is included within our confidence intervals, our point estimates show a
371 somewhat smaller value.

372 Previous studies have compared genetic and standard field methods to estimate N_c in
373 other species such as mountain gorillas (Guschanski et al., 2009), otters (Arrendal et al., 2007;
374 Hájková et al., 2009) and giant pandas (Zhan et al., 2006). In these studies, the authors found that
375 genetic estimators most often provide reliable results, whereas standard field methods tend to
376 overestimate or underestimate true population sizes. The usefulness of standard traditional
377 methods for estimating population size, such as cameras or direct counts, may indeed be limited
378 when individuals form a large horde and live in closed forest habitats (Bata et al., 2017; Buckland,
379 1980; Christman, 2004; Frankham, 1995; Leberg, 2005). Studies carried out on mandrill
380 populations have shown that this species is difficult to observe in nature due to their dense forest
381 habitat and reclusive behavior (Abernethy et al., 2002; Hoshino et al., 1984; Jouventin, 1975;
382 Rogers et al., 1996).

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

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

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

404 405 Genetic estimates of N_e in mandrills

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

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

421 These differences in N_e estimates obtained from different sampling designs (single-period
422 versus combined samples) are consistent with previous studies that have reported variable
423 estimates of N_e depending on the method used (Do et al., 2014; Wang, 2009; Waples & Do,
424 2010). These results reflect the limitations of the approaches used to estimate N_e , with genetic
425 estimators generally losing performance with small numbers of samples and loci (Do et al., 2014;
426 England et al., 2006, 2010; Luikart et al., 2010; Richards & Leberg, 1996; Tallmon et al., 2004;

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

437 Nevertheless, the results provided by NeOGen (Blower et al., 2019) revealed that more
438 than 300 samples may be required to obtain an accurate N_e for a large population using 10 loci,
439 which is the average number in our dataset. Somewhat fewer samples would have been required
440 for 13 loci. From these results, it appears that our N_e estimates would be improved by the use
441 of either additional samples or additional loci, if the census size is as large as is suggested from
442 our analyses. In addition, other studies have shown that using a large number of loci with high
443 allelic richness and high P_{crit} values (i.e., $P_{crit} > 1/2N$ with N the number of samples) can minimize
444 bias, and thus improve estimates of these variables (Do et al., 2014; Waples, 2006; Waples & Do,
445 2010), which was likely the case for the LD N_e and sibship methods.

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

451 Here, we noted that N_e values appear to be between 13.6% and 29.4%, of N_c , which is
452 higher than many other wildlife studies (Frankham, 1995; Harpending & Cowan, 1986; Kinnaird
453 & O'Brien, 1991; Palstra & Ruzzante, 2008). Our analyses did not identify the exact factors that
454 might influence N_e in this population, but factors such as the large numbers of individuals (N_c)
455 and connectivity between hordes may be key. Although studies of gene flow between mandrill
456 hordes have not yet been done, observational studies of the SEGC horde have reported that male
457 mandrills leave the natal horde to be solitary before they reach 6 years of age. When these
458 individuals reach adulthood (>9 years), they return to the horde during the breeding season
459 (Abernethy et al., 2002). It is not yet known whether these mature males return to their natal
460 horde or emigrate to other populations. However, these field observations of Abernethy et al.,
461 (2002) may suggest that mandrills may disperse into neighboring hordes and thus avoid
462 inbreeding. In addition, other observations reveal that mandrills appear to move between habitat
463 fragments by crossing the intervening savanna (Abernethy & Maisels, 2019) and thus may
464 exchange genes with other hordes to maintain a viable population.

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

472 **Conclusion**

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

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503 **References**

- 504 Abernethy, K. A., & Maisels, F. (2019). *Mandrillus sphinx*. *The IUCN Red List of Threatened*
505 *Species 2019: E.T12754A17952325*. [https://dx.doi.org/10.2305/IUCN.UK.2019-](https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T12754A17952325.en)
506 [3.RLTS.T12754A17952325.en](https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T12754A17952325.en).
- 507 Abernethy, K. A., White, L. J. T., & Wickings, E. J. (2002). Hordes of mandrills (*Mandrillus sphinx*
508): Extreme group size and seasonal male presence. *Journal of Zoology*, *258*(1), 131–137.
509 <https://doi.org/10.1017/s0952836902001267>
- 510 Arandjelovic, M., Head, J., Kuehl, H., Boesch, C., Robbins, M. M., Maisels, F., & Vigilant, L.
511 (2010). Effective non-invasive genetic monitoring of multiple wild western gorilla
512 groups. *Biological Conservation*, *143*(7), 1780–1791.
- 513 Arrendal, J., Vila, C., & Björklund, M. (2007). Reliability of noninvasive genetic census of otters
514 compared to field censuses. *Conservation Genetics*, *8*(5), 1097–1107.
- 515 Banks, S. C., Hoyle, S. D., Horsup, A., Sunnucks, P., & Taylor, A. C. (2003). Demographic
516 monitoring of an entire species (the northern hairy-nosed wombat, *Lasiorninus krefftii*)
517 by genetic analysis of non-invasively collected material. *Animal Conservation Forum*,
518 *6*(2), 101–107.
- 519 Bata, M. N., Easton¹, J., Fankem¹, O., Wachter, T., Bruce, T., Eliséé¹, T.,
520 Taguieteu¹, P. A., & Olson, D. (2017). Extending the Northeastern Distribution of
521 Mandrills (*Mandrillus sphinx*) into the Dja Faunal Reserve, Cameroon. *African Primates*,
522 *12*, 65–67.
- 523 Beehner, J. C., Berhanu, G., Bergman, T. J., & McCann, C. (2007). Population estimate for
524 geladas (*Theropithecus gelada*) living in and around the Simien Mountains National
525 Park, Ethiopia. *SINET: Ethiopian Journal of Science*, *30*(2), 149–154.
- 526 Bellemain, E. V. A., Swenson, J. E., Tallmon, D., Brunberg, S., & Taberlet, P. (2005). Estimating
527 population size of elusive animals with DNA from hunter-collected feces: Four methods
528 for brown bears. *Conservation Biology*, *19*(1), 150–161.
- 529 Benoit, L., Mboumba, S., Willaume, E., Kappeler, P. M., & Charpentier, M. J. E. (2014). Using
530 next-generation sequencing methods to isolate and characterize 24 simple sequence
531 repeat loci in mandrills (*Mandrillus sphinx*). *Conservation Genetics Resources*, *6*(4), 903–
532 905. <https://doi.org/10.1007/s12686-014-0237-1>
- 533 Bergl, R. A., & Vigilant, L. (2007). Genetic analysis reveals population structure and recent
534 migration within the highly fragmented range of the Cross River gorilla (*Gorilla gorilla*
535 *diehli*). *Molecular Ecology*, *16*(3), 501–516.

- 536 Blower, D. C., Riginos, Cynthia., & Ovenden, J. R. (2019). neogen: A tool to predict genetic
537 effective population size (N_e) for species with generational overlap and to assist
538 empirical N_e study design. *Molecular Ecology Resources*, 19(1), 260–271.
539 <https://doi.org/10.1111/1755-0998.12941>
- 540 Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F., Brochmann, C., & Taberlet, P.
541 (2004). How to track and assess genotyping errors in population genetics studies.
542 *Molecular Ecology*, 13(11), 3261–3273.
- 543 Boonratana, R., Chalise, M., Chetry, D., Htun, S., & Timmins, R. J. (2020). *Macaca assamensis*
544 *spp. Assamensis*. *The IUCN Red List of Threatened Species 2020: E.T39766A17985704*.
545 <https://dx.doi.org/10.2305/IUCN.UK.20202.RLTS.T39766A17985704.en>
- 546 Bronikowski, A. M., Cords, M., Alberts, S. C., Altmann, J., Brockman, D. K., Fedigan, L. M., Pusey,
547 A., Stoinski, T., Strier, K. B., & Morris, W. F. (2016). Female and male life tables for seven
548 wild primate species. *Scientific Data*, 3(1), 1–8.
- 549 Buckland, G. (1980). *Fox Talbot and the invention of photography*. David R Godine Pub.
- 550 Caballero, A. (1994). Developments in the prediction of effective population size. *Heredity*,
551 73(6), 657–679.
- 552 Charlesworth, B. (2009). Effective population size and patterns of molecular evolution and
553 variation. *Nature Reviews Genetics*, 10(3), 195–205.
- 554 Chetry, D., Medhi, R., & Bhattacharjee, P. (2003). Anti-predator behaviour of stump-tail
555 macaques in Gibbon Wildlife Sanctuary, Assam, India. *Asian Primates*, 8(4), 20–22.
- 556 Christman, M. C. (2004). Sequential sampling for rare and geographically clustered populations.
557 *Sampling Rare or Elusive Species*. Island Press, Washington, DC, 134–145.
- 558 Clemente, A. J., Anderson, E. C., Boughton, D., Girman, D., & Garza, J. C. (2009). Population
559 genetic structure and ancestry of *Oncorhynchus mykiss* populations above and below
560 dams in south-central California. *Conservation Genetics*, 10(5), 1321.
- 561 Dawnay, N., Dawnay, L., Hughes, R. N., Cove, R., & Taylor, M. I. (2011). Substantial genetic
562 structure among stocked and native populations of the European grayling (*Thymallus*
563 *thymallus*, Salmonidae) in the United Kingdom. *Conservation Genetics*, 12(3), 731–744.
- 564 Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NeEstimator
565 v2: Re-implementation of software for the estimation of contemporary effective
566 population size (N_e) from genetic data. *Molecular Ecology Resources*, 14(1), 209–214.
- 567 Dou, H., Yang, H., Feng, L., Mou, P., Wang, T., & Ge, J. (2016). Estimating the population size and
568 genetic diversity of Amur tigers in Northeast China. *PloS One*, 11(4), e0154254.
- 569 England, P. R., Cornuet, J.-M., Berthier, P., Tallmon, D. A., & Luikart, G. (2006). Estimating
570 effective population size from linkage disequilibrium: Severe bias in small samples.
571 *Conservation Genetics*, 7(2), 303.

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

- 608 Hájková, P., Zemanová, B., Roche, K., & Hájek, B. (2009). An evaluation of field and noninvasive
609 genetic methods for estimating Eurasian otter population size. *Conservation Genetics*,
610 10(6), 1667–1681. <https://doi.org/10.1007/s10592-008-9745-4>
- 611 Harpending, H., & Cowan, S. (1986). Primate population structure: Evaluation of models.
612 *American Journal of Physical Anthropology*, 70(1), 63–68.
- 613 Hedgecock, D., Launey, S., Pudovkin, A. I., Naciri, Y., Lapègue, S., & Bonhomme, F. (2007). Small
614 effective number of parents (N_b) inferred for a naturally spawned cohort of juvenile
615 European flat oysters *Ostrea edulis*. *Marine Biology*, 150(6), 1173–1182.
616 <https://doi.org/10.1007/s00227-006-0441-y>
- 617 Hedwig, D., Kienast, I., Bonnet, M., Curran, B. K., Courage, A., Boesch, C., Kühl, H. S., & King, T.
618 (2018). A camera trap assessment of the forest mammal community within the
619 transitional savannah-forest mosaic of the Batéké Plateau National Park, Gabon. *African*
620 *Journal of Ecology*, 56(4), 777–790. <https://doi.org/10.1111/aje.12497>
- 621 Holm, S. (1979). *A simple sequential rejective method procedure*. 6, 65–70.
- 622 Hongo, S. (2014). New evidence from observations of progressions of mandrills (*Mandrillus*
623 *sphinx*): A multilevel or non-nested society? *Primates*, 55(4), 473–481.
- 624 Hoshino, J., Mori, A., Kudo, H., & Kawai, M. (1984). Preliminary report on the grouping of
625 mandrills (*Mandrillus sphinx*) in Cameroon. *Primates*, 25(3), 295–307.
- 626 Johnson, A. E., Knott, C. D., Pamungkas, B., Pasaribu, M., & Marshall, A. J. (2005). A survey of
627 the orangutan (*Pongo pygmaeus wurmbii*) population in and around Gunung Palung
628 National Park, West Kalimantan, Indonesia based on nest counts. *Biological*
629 *Conservation*, 121(4), 495–507. <https://doi.org/10.1016/j.biocon.2004.06.002>
- 630 Jones, O. R., & Wang, J. (2010). COLONY: A program for parentage and sibship inference from
631 multilocus genotype data. *Molecular Ecology Resources*, 10(3), 551–555.
- 632 Jorde, P. E., & Ryman, N. (2007). Unbiased Estimator for Genetic Drift and Effective Population
633 Size. *Genetics*, 177(2), 927–935. <https://doi.org/10.1534/genetics.107.075481>
- 634 Jouventin, P. (1975). Observations sur la socio-écologie du mandrill. *La Terre et La Vie*.
- 635 Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper,
636 A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A.
637 (2012). Geneious Basic: An integrated and extendable desktop software platform for the
638 organization and analysis of sequence data. *Bioinformatics*, 28(12), 1647–1649.
639 <https://doi.org/10.1093/bioinformatics/bts199>
- 640 Kifle, Z., Belay, G., & Bekele, A. (2013). Population size, group composition and behavioral
641 ecology of geladas (*Theropithecus gelada*) and human-gelada conflict in Wonchit Valley,
642 Ethiopia. *Pak J Biol Sci*, 16, 1248–1259.
- 643 Kimura, M., & Crow, J. F. (1963). The measurement of effective population number. *Evolution*,
644 279–288.
- 645 Kingdon, J. (1997). *The Kingdon®eld guide to African mammal*.

- 646 Kinnaird, M. F., & O'BRIEN, T. G. (1991). Viable populations for an endangered forest primate,
647 the Tana River crested mangabey (*Cercocebus galeritus galeritus*). *Conservation Biology*,
648 5(2), 203–213.
- 649 Langergraber, K. E., Mitani, J. C., & Vigilant, L. (2007). The limited impact of kinship on
650 cooperation in wild chimpanzees. *Proceedings of the National Academy of Sciences*,
651 104(19), 7786–7790.
- 652 Leberg, P. (2005). Genetic Approaches for Estimating the Effective Size of Populations. *The*
653 *Journal of Wildlife Management*, 69(4), 1385–1399. [https://doi.org/10.2193/0022-](https://doi.org/10.2193/0022-541X(2005)69[1385:GAFETE]2.0.CO;2)
654 [541X\(2005\)69\[1385:GAFETE\]2.0.CO;2](https://doi.org/10.2193/0022-541X(2005)69[1385:GAFETE]2.0.CO;2)
- 655 Lucchini, V., Fabbri, E., Marucco, F., Ricci, S., Boitani, L., & Randi, E. (2002). Noninvasive
656 molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps.
657 *Molecular Ecology*, 11(5), 857–868.
- 658 Luikart, G., Ryman, N., Tallmon, D. A., Schwartz, M. K., & Allendorf, F. W. (2010). Estimation of
659 census and effective population sizes: The increasing usefulness of DNA-based
660 approaches. *Conservation Genetics*, 11(2), 355–373.
- 661 Madsen, T., Shine, R., Olsson, M., & Wittzell, H. (1999). Restoration of an inbred adder
662 population. *Nature*, 402(6757), 34–35.
- 663 Matschiner, M., & Salzburger, W. (2009). TANDEM: Integrating automated allele binning into
664 genetics and genomics workflows. *Bioinformatics*, 25(15), 1982–1983.
665 <https://doi.org/10.1093/bioinformatics/btp303>
- 666 McKelvey, K. S., & Schwartz, M. K. (2005). Dropout: A program to identify problem loci and
667 samples for noninvasive genetic samples in a capture-mark-recapture framework.
668 *Molecular Ecology Notes*, 5(3), 716–718.
- 669 Miller, C. R., Waits, L. P., & Joyce, P. (2005). A new method for estimating the size of small
670 populations from genetic mark–recapture data. *Molecular Ecology*, 14(7), 1991–2005.
- 671 Mowat, G., & Strobeck, C. (2000). Estimating population size of grizzly bears using hair capture,
672 DNA profiling, and mark-recapture analysis. *The Journal of Wildlife Management*, 183–
673 193.
- 674 Nunney, L., & Elam, D. R. (1994). Estimating the effective population size of conserved
675 populations. *Conservation Biology*, 8(1), 175–184.
- 676 Oates, J. F., & Butynski, T. M. (2008). *Mandrillus sphinx*. *IUCN Red List of Threatened Species*.
677 *Version*.
- 678 Otis, D. L., Burnham, K. P., White, G. C., & Anderson, D. R. (1978). Statistical inference from
679 capture data on closed animal populations. *Wildlife Monographs*, 62, 3–135.
- 680 Paetkau, D. (2003). An empirical exploration of data quality in DNA-based population
681 inventories. *Molecular Ecology*, 12(6), 1375–1387. [https://doi.org/10.1046/j.1365-](https://doi.org/10.1046/j.1365-294X.2003.01820.x)
682 [294X.2003.01820.x](https://doi.org/10.1046/j.1365-294X.2003.01820.x)

- 683 Palstra, F. P., & Ruzzante, D. E. (2008). Genetic estimates of contemporary effective population
684 size: What can they tell us about the importance of genetic stochasticity for wild
685 population persistence? *Molecular Ecology*, *17*(15), 3428–3447.
686 <https://doi.org/10.1111/j.1365-294X.2008.03842.x>
- 687 Puechmaille, S. J., & Petit, E. J. (2007). Empirical evaluation of non-invasive capture–mark–
688 recapture estimation of population size based on a single sampling session. *Journal of*
689 *Applied Ecology*, *44*(4), 843–852.
- 690 Regazzi, R. (Ed.). (2007). *Molecular mechanisms of exocytosis*. Landes Bioscience/Eurekah.com ;
691 Springer Science+Business Media.
- 692 Richards, C., & Leberg, P. L. (1996). Temporal changes in allele frequencies and a population’s
693 history of severe bottlenecks. *Conservation Biology*, *10*(3), 832–839.
- 694 Rogers, M. E., Abernethy, K. A., Fontaine, B., Wickings, E. J., White, L. J., & Tutin, C. E. (1996).
695 Ten days in the life of a mandrill horde in the Lope Reserve, Gabon. *American Journal of*
696 *Primatology*, *40*(4), 297–313.
- 697 Ruiz-Olmo, J., Saavedra, D., & Jiménez, J. (2001). Testing the surveys and visual and track
698 censuses of Eurasian otters (*Lutra lutra*). *Journal of Zoology*, *253*(3), 359–369.
- 699 Schmeller, D. S., & Merilä, J. (2007). Demographic and Genetic Estimates of Effective Population
700 and Breeding Size in the Amphibian *Rana temporaria*. *Conservation Biology*, *21*(1), 142–
701 151. <https://doi.org/10.1111/j.1523-1739.2006.00554.x>
- 702 Schwartz, M. K., Cushman, S. A., McKelvey, K. S., Hayden, J., & Engkjer, C. (2006). Detecting
703 genotyping errors and describing American black bear movement in northern Idaho.
704 *Ursus*, *17*(2), 138–148.
- 705 Setchell, J. M., Charpentier, M., & Wickings, E. J. (2005). Sexual selection and reproductive
706 careers in mandrills (*Mandrillus sphinx*). *Behavioral Ecology and Sociobiology*, *58*(5),
707 474–485.
- 708 Sithaldeen, R., & Rylands, A. B. (2020). *Papio ursinus ssp. Ursinus*. *The IUCN Red List of*
709 *Threatened Species 2020:e.T136856A17986139*.
710 <https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T136856A17986139.en>
- 711 Soto-Calderón, I. D., Ntie, S., Mickala, P., Maisels, F., Wickings, E. J., & Anthony, N. M. (2009).
712 Effects of storage type and time on DNA amplification success in tropical ungulate
713 faeces. *Molecular Ecology Resources*, *9*(2), 471–479. [https://doi.org/10.1111/j.1755-](https://doi.org/10.1111/j.1755-0998.2008.02462.x)
714 [0998.2008.02462.x](https://doi.org/10.1111/j.1755-0998.2008.02462.x)
- 715 Stone, O. M. L., Laffan, S. W., Curnoe, D., Rushworth, I., & Herries, A. I. R. (2012). Distribution
716 and population estimate for the chacma baboon (*Papio ursinus*) in KwaZulu-Natal, South
717 Africa. *Primates*, *53*(4), 337–344. <https://doi.org/10.1007/s10329-012-0303-9>
- 718 Taberlet, P., Waits, L. P., & Luikart, G. (1999). Noninvasive genetic sampling: Look before you
719 leap. *Trends in Ecology & Evolution*, *14*(8), 323–327. [https://doi.org/10.1016/S0169-](https://doi.org/10.1016/S0169-5347(99)01637-7)
720 [5347\(99\)01637-7](https://doi.org/10.1016/S0169-5347(99)01637-7)

- 721 Tallmon, D. A., Luikart, G., & Beaumont, M. A. (2004). Comparative evaluation of a new
722 effective population size estimator based on approximate Bayesian computation.
723 *Genetics*, 167(2), 977–988.
- 724 Valière, N. (2002). gimlet: A computer program for analysing genetic individual identification
725 data. *Molecular Ecology Notes*, 2(3), 377–379. [https://doi.org/10.1046/j.1471-](https://doi.org/10.1046/j.1471-8286.2002.00228.x-i2)
726 8286.2002.00228.x-i2
- 727 Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). MICRO-CHECKER:
728 Software for identifying and correcting genotyping errors in microsatellite data.
729 *Molecular Ecology Notes*, 4(3), 535–538.
- 730 Waits, L. P., Luikart, G., & Taberlet, P. (2001). Estimating the probability of identity among
731 genotypes in natural populations: Cautions and guidelines. *Molecular Ecology*, 10(1),
732 249–256. <https://doi.org/10.1046/j.1365-294X.2001.01185.x>
- 733 Wallis, J. (2020). *Papio cynocephalus ssp. Ibeanus*. *The IUCN Red List of Threatened Species*
734 2020: E.T136862A92251072. [https://dx.doi.org/10.2305/IUCN.UK.2020-](https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T136862A92251072.en)
735 2.RLTS.T136862A92251072.en
- 736 Wang, J. (2009). A new method for estimating effective population sizes from a single sample of
737 multilocus genotypes. *Molecular Ecology*, 18(10), 2148–2164.
- 738 Waples, R. S. (1989). A generalized approach for estimating effective population size from
739 temporal changes in allele frequency. *Genetics*, 121(2), 379–391.
- 740 Waples, R. S. (2006). A bias correction for estimates of effective population size based on
741 linkage disequilibrium at unlinked gene loci. *Conservation Genetics*, 7(2), 167.
- 742 Waples, R. S., & Do, C. H. I. (2010). Linkage disequilibrium estimates of contemporary Ne using
743 highly variable genetic markers: A largely untapped resource for applied conservation
744 and evolution. *Evolutionary Applications*, 3(3), 244–262.
- 745 Westemeier, R. L., Brawn, J. D., Simpson, S. A., Esker, T. L., Jansen, R. W., Walk, J. W., Kershner,
746 E. L., Bouzat, J. L., & Paige, K. N. (1998). Tracking the long-term decline and recovery of
747 an isolated population. *Science*, 282(5394), 1695–1698.
- 748 White, Gary C., & Burnham, K. P. (1999). Program MARK: Survival estimation from populations
749 of marked animals. *Bird Study*, 46(sup1), S120–S139.
750 <https://doi.org/10.1080/00063659909477239>
- 751 White, L., JT. (1994). The effects of commercial mechanised selective logging on a transect in
752 lowland rainforest in the Lopé Reserve, Gabon. *Journal of Tropical Ecology*, 10(3), 313–
753 322.
- 754 White, Lee., & Abernethy, Kate. (1997). *A guide to the vegetation of the Lopé Reserve*. Wildlife
755 Conservation Society.
- 756 Wright, S. (1938). Size of population and breeding structure in relation to evolution. *Science*, 87,
757 430–431.

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

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762 **Statements & Declarations**

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767 **Competing Interests**

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

769 **Author Contributions**

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

775 **Data Availability**

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

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781 **Figures legend**

782 **Figure 1:** Graph showing the results of NeoGen software simulations to estimate the number of
783 samples and loci needed to obtain an accurate N_e , assuming a census size of 845. The power of
784 the N_e estimate is evaluated at every 100 genotypes, with a maximum of 400, using 10 loci (a),
785 and 13 loci (b). The x-axis shows the combination of the number of samples and loci. The y-axis
786 shows the corresponding estimate of N_e (blue circles) with 95% confidence intervals (Cis). All
787 estimates of N_e are represented by two values in parentheses. The first value indicates the
788 relevant estimate, and the second indicates the number of times the estimate was incalculable
789 (i.e., negative, or close to infinity) in all replicates. Incalculable CIs are indicated by a red arrow
790 and CIs with adequate power are in blue with a flat base. The precision of N_e for each
791 combination is evaluated by the width of the CIs. The precision of the point estimates of N_e can
792 be judged by their similarity to the shaded dashed "precision guideline," which is equal to the N_e
793 estimated from all loci and all individuals in the same age cohorts as sampled for the
794 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

797

798 **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

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

803 **Table 3:** Estimates of Nc from individual sample period data (2016 to 2018) and data from all
 804 three periods combined into a single sample design, using two single sample based genetic
 805 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)

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

808

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

Model	Description	AICc	Delta AICc	AICc W	Nc (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)

812 **Table 5:** Estimates of Ne from individual sample period data (2016 to 2018) and data from all
 813 three periods combined into a single sample design, using four single sample based genetic
 814 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)

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

818 **Table 6:** Ratio of population size estimates

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

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Fig 1a

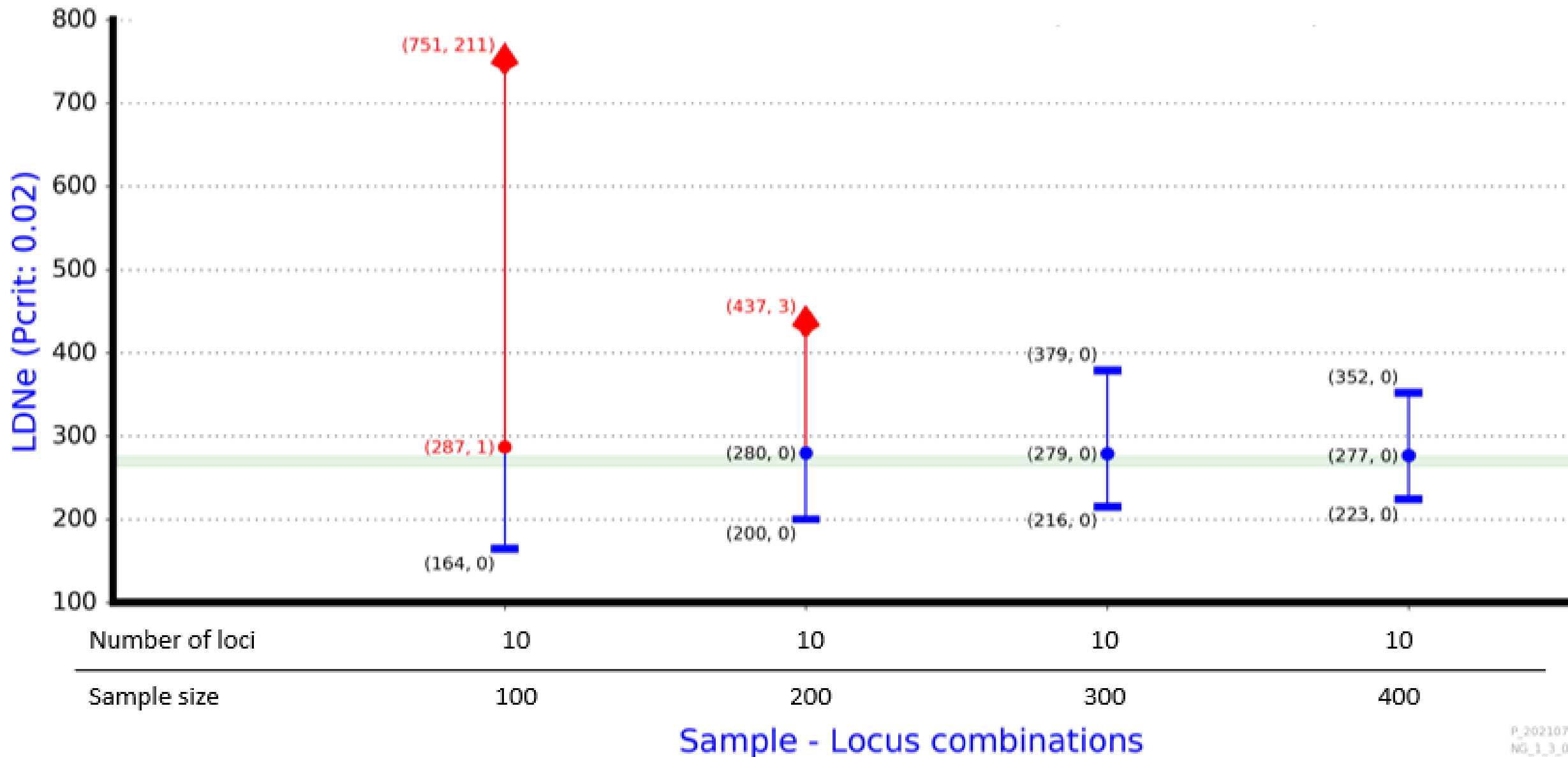
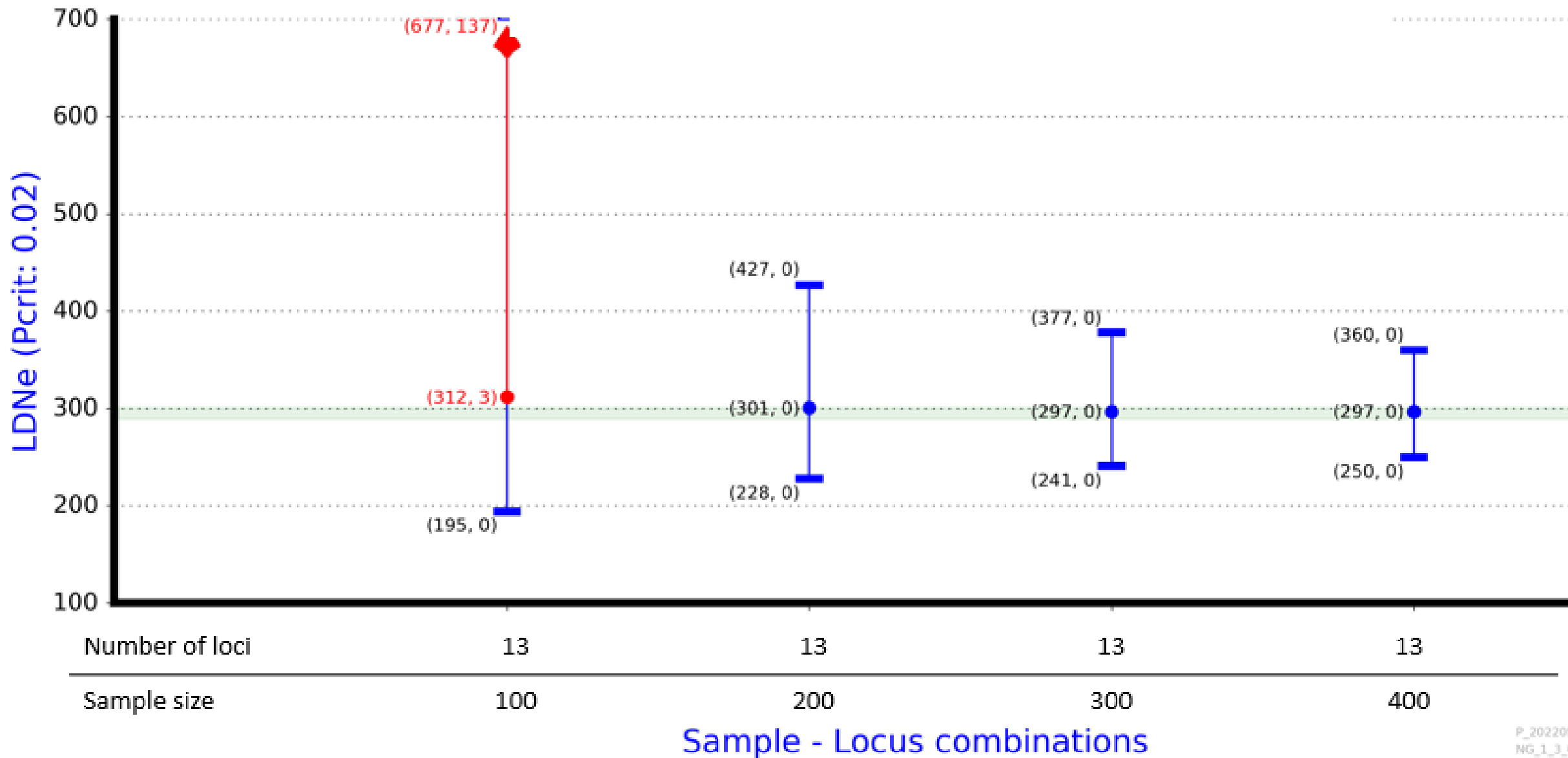


Fig 1b



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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7

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18

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

39

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	$0.315^2 = 0.099$	$0.315^3 = 0.031$

40

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).

43

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

53

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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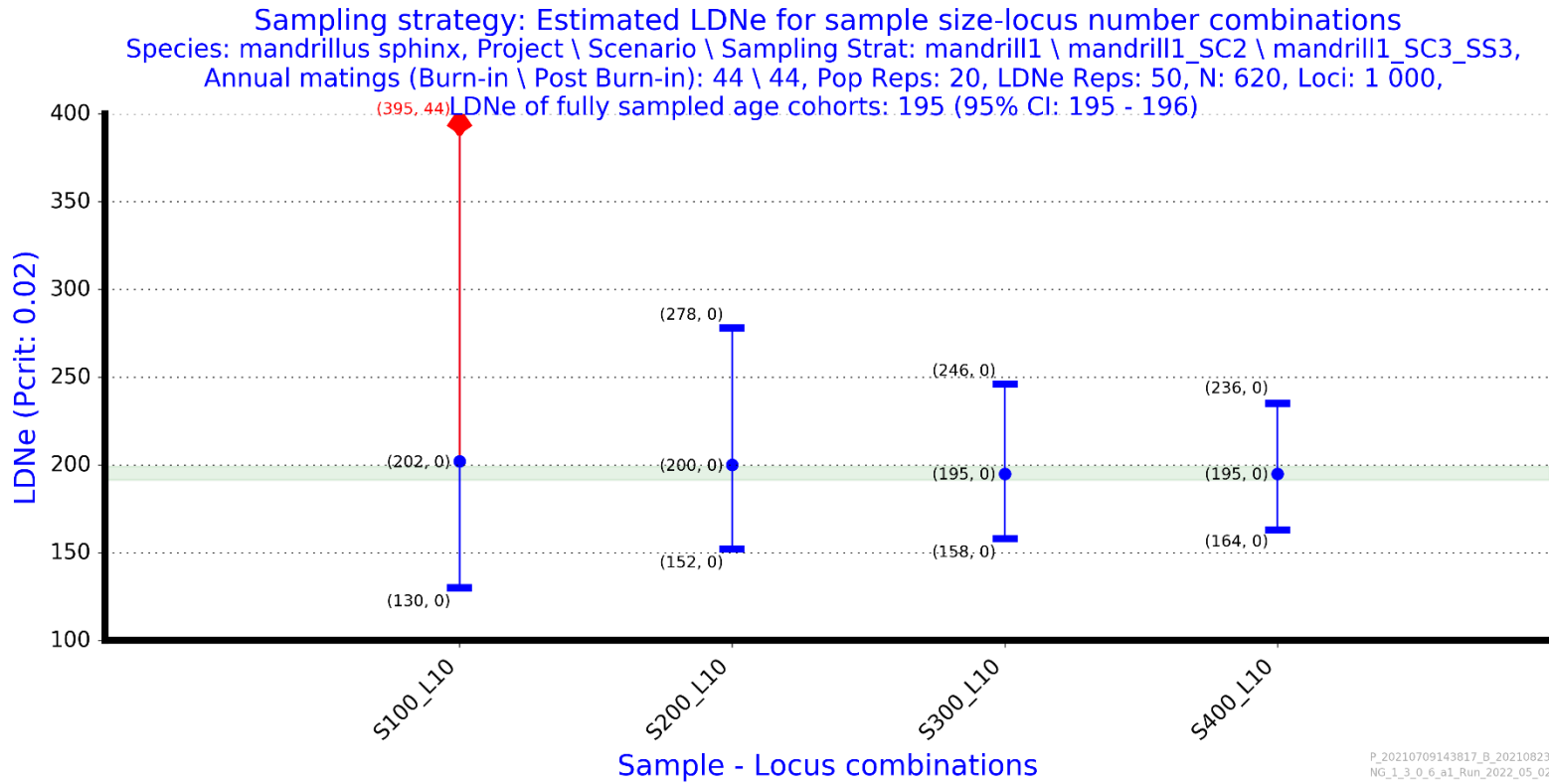
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72 **Supplementary Figure 1**

73 **Figure 1a**



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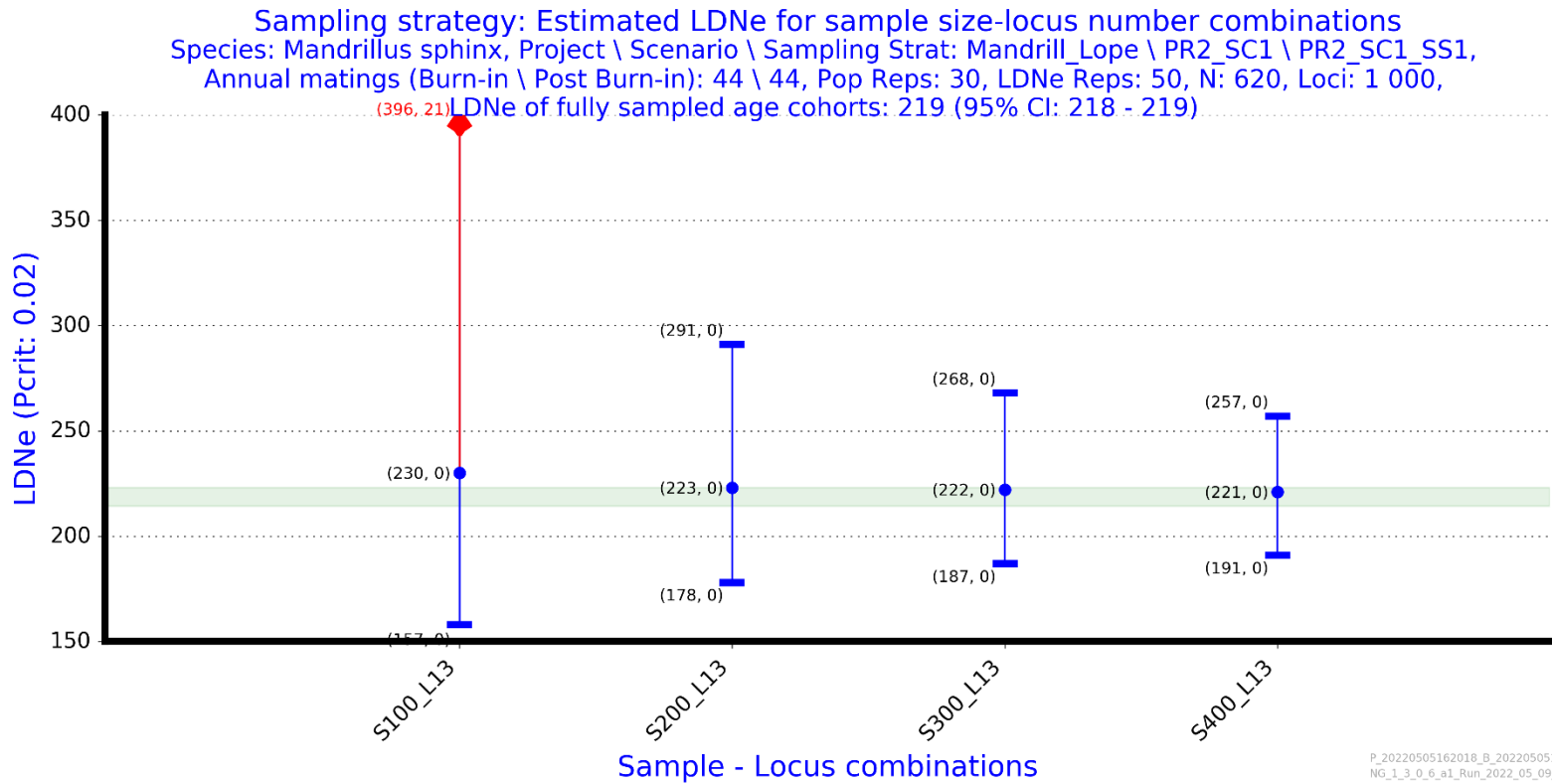
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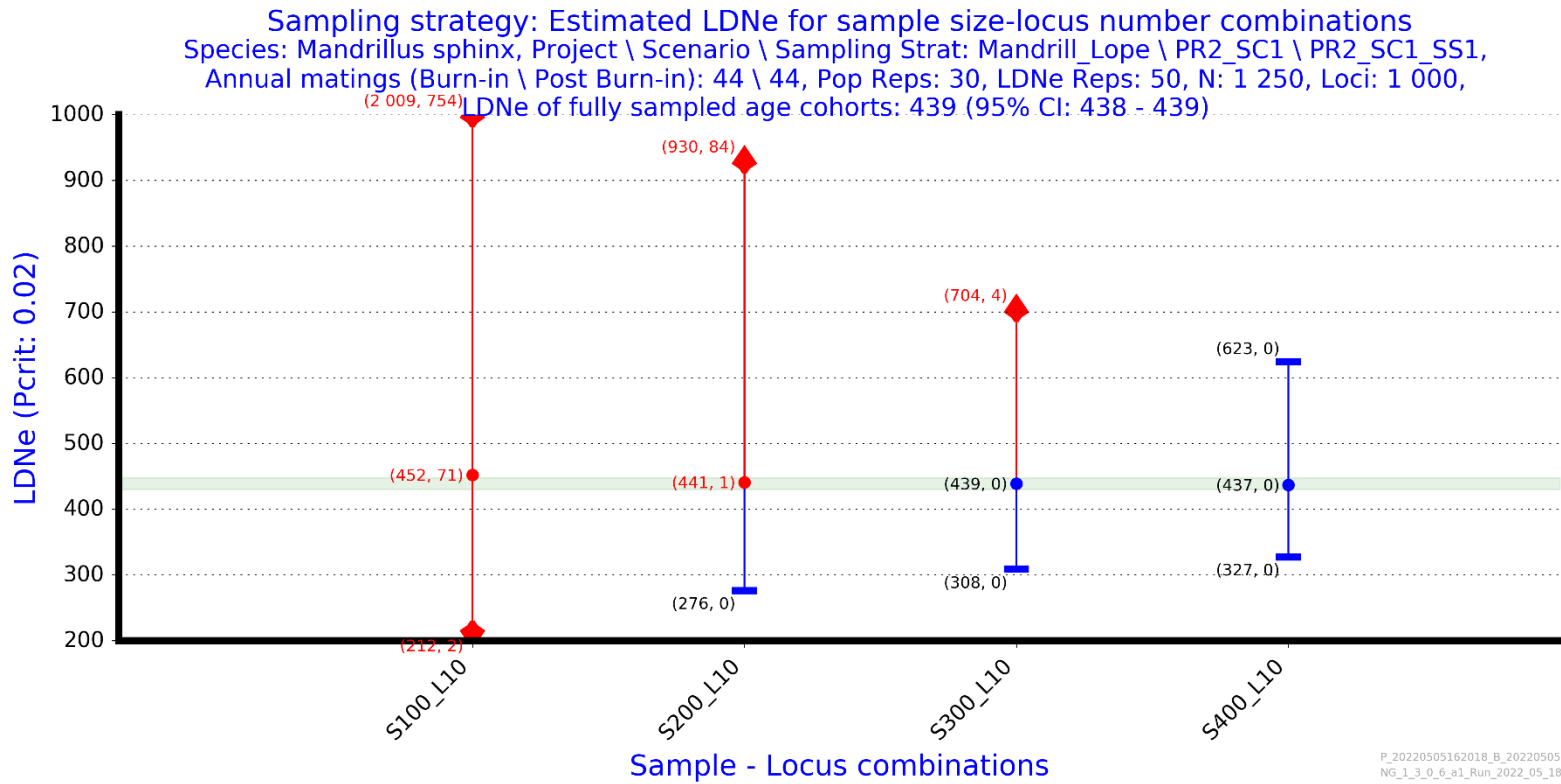
79 **Figure 1b**



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86 **Figure 1c**



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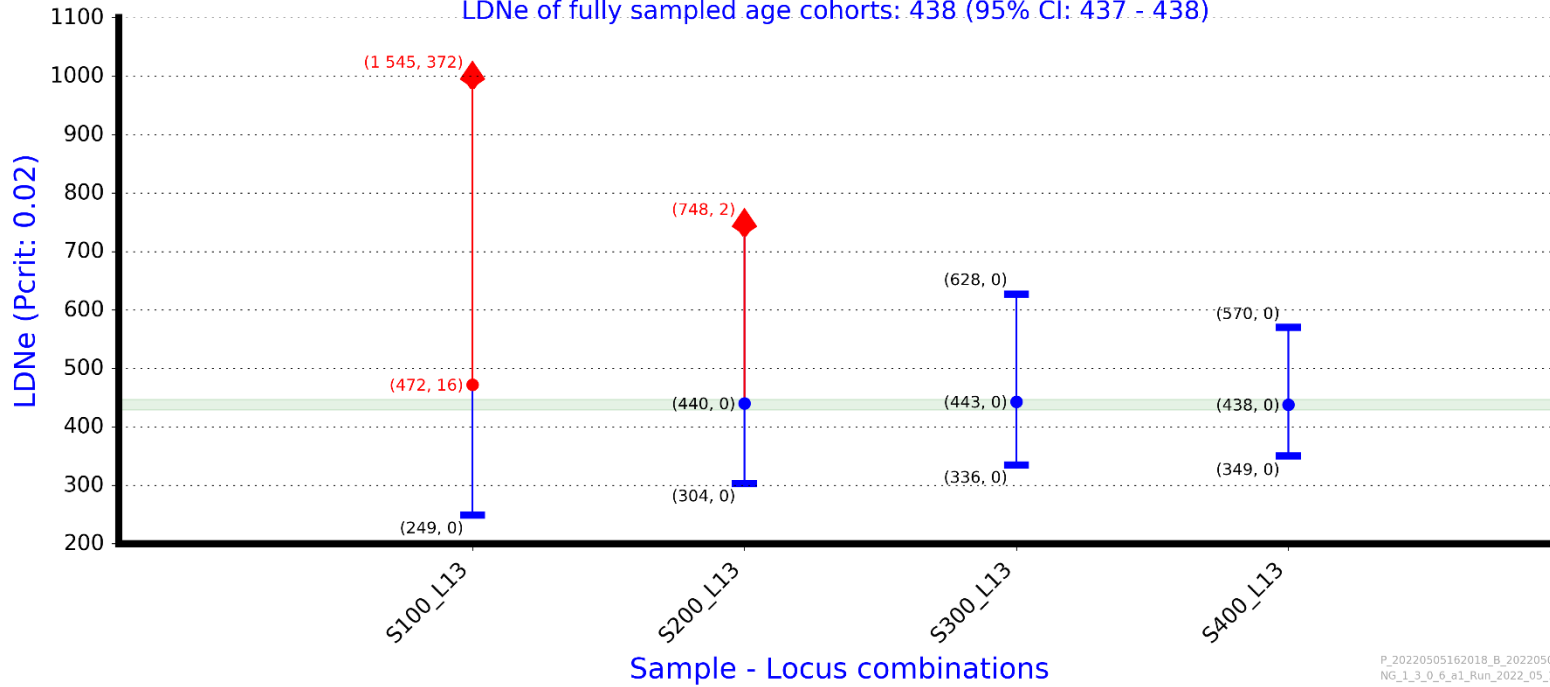
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Sampling strategy: Estimated LDNe for sample size-locus number combinations
 Species: *Mandrillus sphinx*, Project \ Scenario \ Sampling Strat: Mandrill_Lope \ PR2_SC1 \ PR2_SC1_SS1,
 Annual matings (Burn-in \ Post Burn-in): 44 \ 44, Pop Reps: 20, LDNe Reps: 50, N: 1 250, Loci: 1 000,
 LDNe of fully sampled age cohorts: 438 (95% CI: 437 - 438)



P_20220505162018_B_20220505162018_S_20220509124343
 NG_1_3_0_6_a1_Run_2022_05_17_21_01_41