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DOI: https://doi.org/10.1111/csp2.12898

Received: 4 February 2022 | Revised: 29 November 2022 | Accepted: 23 January 2023

DOI: 10.1111/csp2.12898

CONTRIBUTED PAPER

Conservation Science and Practice
A journal of the Society for Conservation Biology
Open Access

WILEY

Genetics as a novel tool in mining impact assessment and biomonitoring of critically endangered western chimpanzees in the Nimba Mountains, Guinea

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Funding information
Alfred Benzon Foundation; Gates Cambridge Trust; Graduate Teaching PhD Scholarship, University of Kent; Homerton College, University of Cambridge; Ministry of Education, Culture, Sports, Science and Technology, Grant/Award Number: 120020096; Mohamed bin Zayed Species Conservation Fund; Newnham College, University of Cambridge; Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: PCEFP3_186967; Société des Mines de Fer de Guineé S.A. (Conakry, Guinea); Stichting Lucie Burgers, The Netherlands

Abstract

Western chimpanzees (*Pan troglodytes verus*) are Critically Endangered and Guinea is a key stronghold for this subspecies. However, Guinea is also rich in minerals with some of the highest-grade iron-ore deposits in the world. Specifically, the Nimba Mountains, home to western chimpanzees, is one of the sites under consideration for mining activities. To assess the impact of mining activities in the area, we used non-invasive genetic sampling to estimate chimpanzee population size, sex ratio, community composition, and range boundaries on the western flank of the massif. The level of genetic diversity and affinity between communities was estimated and recommendations for future genetic censusing provided. Between 2003 and 2018, we collected 999 fecal samples of which 663 were analyzed using a panel of 26 microsatellites. We identified a minimum of 136 chimpanzees in four communities, with evidence of migratory events, a high level of shared ancestry and genetic diversity. We assessed sampling intensities and capture rates for each community. Saturation was reached in two communities with sampling between 3.2 and 4.3 times the estimated number of chimpanzees. Our findings highlight the utility of genetic censusing for temporal monitoring of ape abundance, as well as capturing

migratory events and gauging genetic diversity and population viability over time. We recommend genetic sampling, combined with camera trapping, for use in future Environmental and Social Impact Assessments, as these methods can yield robust baselines for implementing the mitigation hierarchy, future biomonitoring and conservation management.

KEYWORDS

conservation management, environmental impact assessment, genetic censusing, *Pan troglodytes verus*, UNESCO world heritage site

1 | INTRODUCTION

Habitat loss and fragmentation, disease transmission and illegal hunting activities are increasingly threatening chimpanzees (*Pan troglodytes*) and other non-human primates across their range (Humle et al., 2016; Estrada et al., 2017). Due to these threats and the predicted future decline in chimpanzee populations across Africa, this great ape species is listed as Endangered on the IUCN Red List (Humle et al., 2016). The drivers of habitat destruction include logging, subsistence and industrial agriculture, and energy and extractive industry projects, such as hydroelectric dams (Arcus Foundation, 2014, 2015). In addition, infrastructures associated with large scale developments, such as roads and rail lines, tend to increase direct and indirect impacts on ape habitats (Arcus Foundation, 2018). In some chimpanzee range countries, industrial mining is also often associated with unregulated artisanal mining that develops in parallel, thus further exacerbating the impact on the local environment (World Bank, 2009). The mining sector in Africa is rapidly developing, attracting billions of dollars in foreign investment, and national governments perceive this sector as critical to rapid economic growth and development (Edwards et al., 2014). Even though mining represents a substantial opportunity for socioeconomic development, extractive industries also pose a significant challenge when it comes to balancing economic growth and the protection and preservation of biodiversity and ecosystem services (Arcus Foundation, 2014; Kühl et al., 2017).

Guinea is a key stronghold for the Critically Endangered western chimpanzee subspecies (*Pan troglodytes verus*) (Humle et al., 2016; IUCN SSC Primate Specialist Group, 2020). However, Guinea is also a country rich in mineral resources, including bauxite, gold and iron, and mining sites often overlap with chimpanzee habitat (Kormos et al., 2014). The two highest-grade iron ore deposits globally occur in the Simandou and Nimba Mountain ranges of Guinea. Additionally, mining concessions for bauxite dominate Guinea's western coast in localities where chimpanzees occur (Kormos et al., 2014). Even though avoidance is the first and foremost element of the mitigation hierarchy (Phalan et al., 2018), many extractive industries are currently developing projects in chimpanzee habitat. Mining companies are required to meet legal and, in some cases, financial standard requirements in order to be granted exploitation permission and/or financial backing from the banking sector (Evans et al., 2021; Kormos et al., 2014). Indeed, to be eligible for funding from the International Finance Corporation (IFC), mining companies have to conform to Performance Standard 6 with regards to Biodiversity Conservation and Sustainable Management of Living Natural Resources (IFC, 2019). Performance Standard 6 states that, in areas with great apes present (i.e., critical habitat) activities are only permitted if "*the project does not lead to a net reduction in the global and/or national/regional population of any Critically Endangered or Endangered species over a reasonable period of time*". It is therefore crucial to generate rigorous ape population abundance estimates and to understand population structure and spatial distribution, in order to effectively assess the potential impact of a project, as well as the avoidance and mitigation options. At the same time, it is essential to establish solid baselines in terms of population size, structure and genetic diversity for future monitoring and adaptive management if project activities should proceed in an area with great ape presence.

Most wild ape populations are not habituated to humans, and thus cannot be counted directly. The survey methods traditionally used to estimate the distribution and abundance of unhabituated wild ape populations include reconnaissance walks, or "recces", and nest count surveys (i.e., standing crop and marked-nest counts) along line transects (Kühl et al., 2008). Weaned individuals of all great ape species build nests to sleep in at night, and sometimes to rest in during the day (Goodall, 1968). These nests remain visible in the forest for a period of time (i.e., weeks or months). Nest count surveys rely on the number of nests encountered to calculate ape abundance. However, these methods have

TABLE 1 Comparison of survey methods for non-human great apes in terms of time investment, financial cost, and output type and quality (i.e., precision level, depth of information)

Survey method	Time investment	Financial cost	Output type and quality
Reconnaissance walks ("recces")	*Low* - One survey	*Low* - Survey effort	*Very low* - Presence/absence - Relative habitat utilization
Standing crop nest counts	*Low/Medium* - Single survey - Nest decay rate	*Low* - Survey effort	*Low/Medium* - Population abundance
Marked nest counts	*Medium* - Repeated surveys	*Medium* - Survey effort	*Low/Medium* - Population abundance
Motion-triggered cameras (MTC)	*High* - Camera monitoring - Video analyses	*Medium/High* - Equipment cost - Monitoring cost	*Medium/High* - Population abundance - Sex ratio - Group/community membership - Age classes - Ranging - Health (disease, injuries)
Genetic census	*Medium/High* - Sample collection - Laboratory analyses	*High* - Sampling effort - Analyses cost	*High* - Population abundance - Sex ratio - Group/community membership - Kinship - Ranging - Gene flow (dispersal patterns) - Genetic diversity/health

considerable limitations (Table 1), including the lack of precision in estimating population size and structure, i.e., the number and composition of communities or groups present (Plumptre, 2000).

Chimpanzee social structure and population dynamics are complex. Chimpanzees live in fission-fusion societies, which means that they travel in parties (or subgroups) of varying size and composition (Goodall, 1968; Nishida, 1968). Chimpanzee communities often defend partially overlapping home ranges and lethal aggression between communities is common (Boesch & Boesch-Achermann, 2000; Wilson et al., 2014; Wrangham et al., 2006). Female chimpanzees typically disperse when they reach sexual maturity, whereas males usually stay in their natal community (Pusey & Packer, 1987; Thompson, 2013). The level of gene flow depends on communities being able to exchange females, i.e., the ability of these females to disperse within the landscape. The number of communities present will influence the likelihood of inter-community aggression resulting from any potential displacement of individuals and shifts in home ranges. Hence, information on the number of chimpanzee communities present, and their respective home ranges, is crucial in order to assess the potential impact of habitat disturbances. Traditional survey methods, like recces and nest count surveys, do not provide the necessary information to establish chimpanzee community ranges and membership. Moreover, recces and nest surveys do not generate information on population or community-level demographics, such as sex ratios and age-class composition, or their genetic health and viability.

In recent years, motion-triggered cameras (or camera traps) have contributed significantly to obtaining additional information on the number of elusive apes inhabiting a site, along with information on age and sex of the individuals present (McCarthy et al., 2018; Garriga et al., 2019; van Leeuwen et al., 2020; Table 1). Camera trapping can generate invaluable spatial and temporal data on the number of apes present, while also providing insight into their behavior and physical health, such as potential disabilities resulting from snaring or disease (e.g., leprosy, Hockings et al., 2021). The repeated observations of identified individuals on camera traps can help determine community size and home ranges (McCarthy et al., 2018). However, the quality of imagery and videos, as well as the angle of capture can in some cases severely constrain reliable identification of individuals. Automated detection of individual apes from camera traps has been a challenge despite major advances in artificial intelligence, and manual identification of individual apes is time-consuming and requires a rigorous inter-recorder

protocol (Green et al., 2020; Schofield et al., 2019). Moreover, camera traps typically underestimate the number of chimpanzees in a party due to some individuals passing the camera out of frame or actively avoiding cameras (Després-Einspenner et al., 2017; McCarthy et al., 2018).

Genetic censusing solves many of the problems outlined above. As a result, the characterization of individual DNA profiles based on non-invasively collected fecal or hair samples is increasingly being used to estimate African great ape populations (mountain gorillas, Uganda: Guschanski et al., 2009; western lowland gorillas, Gabon: Arandjelovic et al., 2010; chimpanzees, Uganda: McCarthy et al., 2015). Genetic estimates of ape densities and abundance are more accurate and precise than the estimates obtained using other more traditional survey methods (Arandjelovic et al., 2010; Chancellor et al., 2012; Roy et al., 2014; McCarthy et al., 2015; Granjon et al., 2017; Table 1). Moreover, genetic surveys can provide additional information on kinship, ranging patterns, gene flow, and genetic diversity (e.g., Arandjelovic et al., 2010, 2011, 2014; Table 1).

A number of methods exist to assess population size based on genetics sampling, but care has to be taken to ensure that all methodological assumptions are met. A single genetic census can yield a number of distinct genotypes, which can generate a minimum count of individuals present. Genetic capture-recapture population size estimators can help to account for the number of individuals that went undetected (e.g., Arandjelovic et al., 2011; McCarthy et al., 2015), but the methodology relies on a number of assumptions that cannot always be met (e.g., no death and/or migration events, i.e., a closed population), as well as a sufficient number of individual genetic capture-recapture events. Moreover, the generated dataset should include a complete genotyping record for all loci across all individuals, yet, this is rarely feasible for non-invasive sampling. For cases with partial datasets (i.e., including missing data), the number of matching loci used in determining the number of unique individuals can greatly influence the resulting population size estimates. For instance, if 50% missing data is allowed, there is no power to detect recaptures if none of the loci overlap between compared pairs of individuals. Therefore, a stricter threshold for matching *and* overlapping loci is essential to obtain an accurate census size estimate for patchy data sets, than required for ideal data sets with complete genotypes. In addition, saturation curves, as the relationship between sampling intensity and the identified number of individuals, can be informative to assess the number of samples needed to reach saturation, where increased sampling does not result in identification of additional individuals (e.g., forest elephants: Eggert et al., 2003). Nevertheless, saturation curves are rarely reported and it is therefore often unclear whether the target population has been sufficiently sampled or not.

This study presents findings on the use of extensive non-invasive sampling and genetic analyses to estimate chimpanzee abundance and population structure in the Nimba Mountains in Guinea, West Africa. The Nimba Mountains form a natural boundary between Guinea, Côte d'Ivoire and Liberia. A large portion of the mountains, spanning Guinea and Côte d'Ivoire, forms the Mount Nimba Strict Nature Reserve, a UNESCO World Heritage Site in Danger (World Heritage Committee, 2019). Covering 18,500 ha, this internationally protected area was established in recognition of the extraordinary biodiversity it harbors, including the western chimpanzee. It has been on the list of World Heritage in Danger since 1992 due to the threat of iron-ore mining activities on its outstanding universal value. Here, we used non-invasive genetic sampling to estimate chimpanzee population size and composition (i.e., the number and spatial distribution of communities) on the western flank of the Nimba Mountains. The specific goals of our study were to: (1) Provide an accurate minimum chimpanzee population estimate based on genotyping of fecal samples; (2) Assess the number of communities and their ranges based on genetic analyses; (3) Assess the number of chimpanzees in each community, as well as the sex ratio; (4) Assess the level of genetic diversity and affinity between communities to elucidate levels of gene flow between them; (5) Evaluate genetic sampling strategies in terms of saturation curves, and finally, (6) Provide practical recommendations for future genetic censusing endeavors for Environmental and Social Impact Assessments (ESIA) and biomonitoring and for the future conservation of the Nimba chimpanzee population.

2 | METHODS

2.1 | Samples and genotyping

The chimpanzee distribution area was surveyed and fresh fecal samples were collected when encountered on chimpanzee trails or below nests. A total of 999 chimpanzee fecal samples were collected during 2003–2018. Of these, 707 samples were collected at the Seringbara study site, south of Gouoton (Mt. Leclerc), between 2003–March 2014. Another 292 samples were collected in the areas north of Gouoton in and around the mining enclave (i.e., mining permit): 214 samples were collected between November 2012 – March 2014 and 78 samples in 2017–2018.

For community-specific analyses, samples were grouped into communities according to prior knowledge

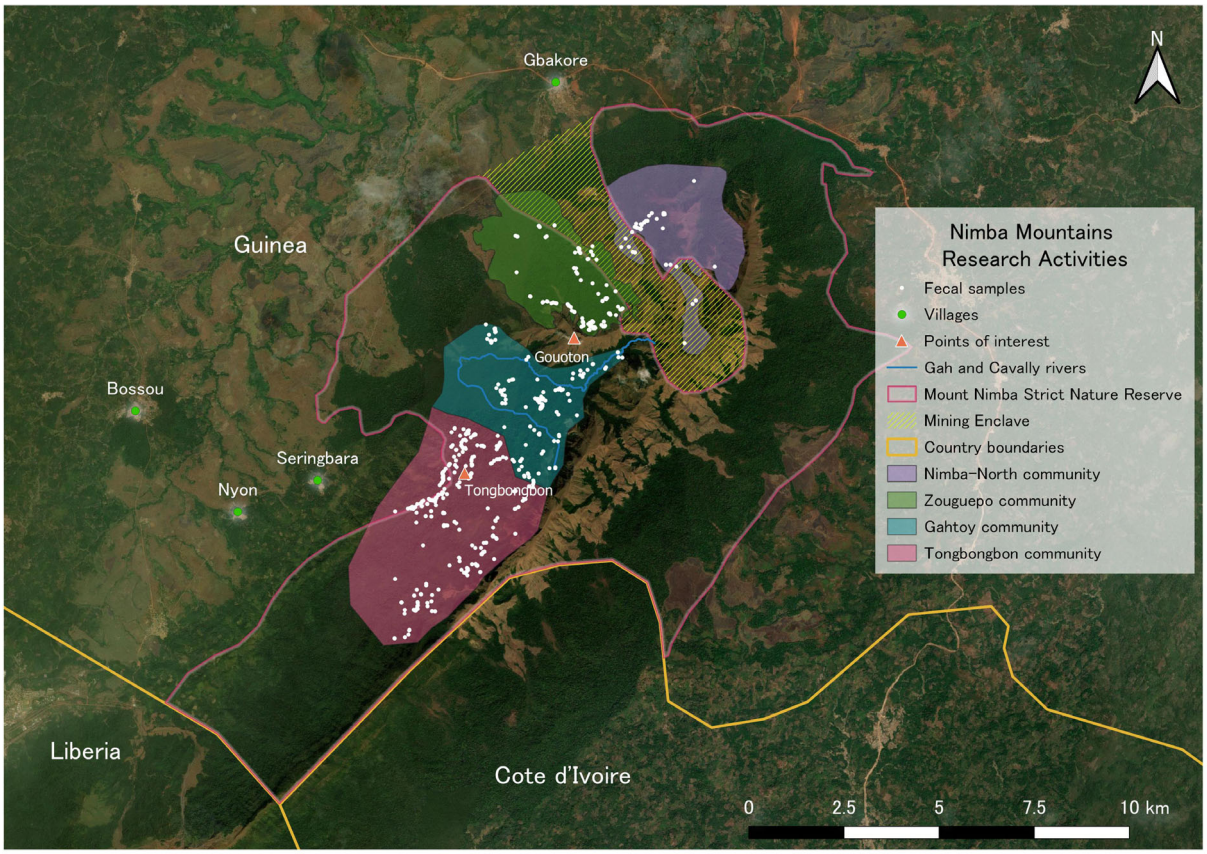

FIGURE 1 Location of amplified fecal samples with GPS coordinates (N = 663) collected within the Nimba Mountains.

from camera trapping, direct observations, and topography. In the Nimba Mountains, we defined four communities; Nimba-North (situated within the north and northeast areas of the mining enclave and just north of the mining enclave), Zouguepo (located in the Zouguepo forest just north of Gouoton), Gahtoy (spanning the Gah and Cavalley river valleys on the southern side of Gouoton), and Tongbongbon (south of the Gahtoy community and named after a hilltop in that area, Tongbongbon; Figure 1). For the two little-studied communities in the North (Zouguepo, Nimba-North), the topography (a mountain ridge separating the two northern from the two southern communities) and the mining enclave (intersecting the two northern communities) likely impede movement between the communities. For the two communities in the South (Tongbongbon, Gahtoy), we had pre-existing knowledge based on long-term camera trap data, direct observations, and mtDNA data (Koops, 2011; Koops et al., 2012). Based on the GPS locations of repeated observations of the same individuals (camera traps, direct sightings), as well as the recordings of community unique haplotypes (mtDNA data, Koops et al., 2012), we were able to assign samples to one of the two southern communities based on sampling location.

The fecal samples were stored in RNAlater or on silica gel beads (Sigma-Aldrich) and kept at room temperature whilst in the field and subsequently at −20°C or 4°C, respectively. DNA was extracted using QIAamp DNA Stool Kit (Qiagen) following the manufacturer's protocol, except for an initial saline water wash step for the RNAlater preserved fecal samples. The endogenous DNA content was quantified using forward and reverse primers (Morin et al., 2001) and probe Fam-TGCCCTGCGTGACCAGATCC-BHQ1. Each PCR was carried out in a 20 ul reaction volume containing 1 ul DNA, 10 ul Master mix and 0.3 ul reference dye from the Brilliant III Ultra-Fast SYBR® Green QRT-PCR (Agilent Technologies), 300 nM each primer, 200 nM probe and 3.1 ul H20. Human genomic DNA (Bioline) was used to create the standard curve. Negative controls were included in each set of amplification to check for contamination. Amplification was performed on AriaMX (Agilent Technologies) and included 95°C for 3 min, followed by 40 cycles of 95°C for 5 s and annealing at 59°C for 10 min. Amplification analysis was performed, and endogenous DNA quantities assessed using the AriaMX software. A total of 746 endogenous chimpanzee samples were identified and analyzed for genetic variation at

25 autosomal loci and one Y-chromosome locus following the protocol of Hvilsom et al. (2013). The tetra and tri repeat loci were selected based on the following criteria: (1) distributed throughout all chromosomes; (2) no more than two loci on any chromosome; (3) with two loci on a chromosome, the location should be on the p and q arm and should be located far from each other to maximize the impact of recombination and minimize linkage disequilibrium; and (4) the informativeness (Rosenberg et al., 2003) should be as high as possible. The loci were originally selected for a human panel and subsequently applied to large chimpanzee datasets (Becquet et al. 2007; Hvilsom et al., 2013), including invasive and non-invasive samples. The robustness of the panel of highly polymorphic loci (between 9 and31 alleles at each locus) has been tested and used to e.g., discriminate between relatives, self-self and to assign paternities. As fecal samples collected in the field can vary in terms of endogenous DNA, we performed genotyping in quadruplicates on a subset of the samples, as a first test and quality control of the samples and performance of the loci. The samples tested in quadruplicates resulted in identical genotypes. The rest of the samples which contained chimpanzee DNA were assessed in duplicates and included if each of the sets of duplicates resulted in identical genotypes. In case of a discrepancy in one allele, the sample was re-analyzed to ensure a match with one of the duplicates, or discarded if not. Additional details on the microsatellite loci can be found in Supplementary Table S1 in Hvilsom et al. (2013).

For temporal monitoring purposes, hyper variable microsatellites were chosen instead of single nucleotide polymorphisms (SNPs), since microsatellite variation arises more frequently than novel SNP variation. Also, while the methodology to sequence SNPs from fecal samples was in its infancy at the time of the start of this study, the used panel of microsatellites had been carefully selected and thoroughly validated in more than 400 chimpanzees from the population managed under the European Association of Zoos and Aquaria. Moreover, the panel was successfully used and validated in the original publication (Becquet et al., 2007) and later applied in Hvilsom et al. (2013). As the present study includes data with a considerably greater amount of missing data, we calculated the polymorphic information content and probability of identity for all included loci using CERVUS (Marshall et al., 1998).

Data were analyzed and fragment lengths scored with Genemapper version 3.7 (Applied Biosystems). A subset of all the samples were amplified in quadruplicates, on separate dates, to assess the amplification performance and consistency. The remaining samples were genotyped in duplicates, amplified on separate dates, and any given locus was retained for further analysis if the genotyping matched (within 2 bp discrepancy) between duplicates. Genotypic inconsistencies due to variability in genotype calling, chemistry null alleles and large allele dropout, were tested for in Microchecker version 2.2.3. Genotypes retained for analysis are available in the Supplementary Table A1 and online https://doi.org/10.5061/dryad.p8cz8w9rg.

2.2 | Ethics statement

This research was non-invasive, complied with the laws of Guinea, and was approved by the Direction General de la Recherche Scientifique et l'innovation Technologique (DGERSIT). Moreover, this research adhered to guidelines as set forth by the Division of Biological Anthropology, Department of Archaeology, University of Cambridge (UK), and was approved by the Research Ethics Committee of the School of Anthropology and Conservation, University of Kent (UK).

2.3 | Genotypic diversity and census size

Common statistics were calculated and tested for genotypic linkage disequilibrium using the Markov chain method implemented in Arlequin version 3.5.2.2 (Escoffier & Lischer, 2010) with 10,000 dememorizations, 1000 batches and 10,000 iterations per batch, using Fisher's method for combining independent test results (Manly, 1985). The statistical significance of these tests was determined by adjusting the probability values for multiple comparisons using sequential Bonferroni correction (Rice, 1989). Allele frequencies, gene diversity (H_e) and deviation from Hardy–Weinberg proportions (Weir & Cockerham, 1984) was estimated using Arlequin 3.5.2.2 (Escoffier & Lischer, 2010). Deviation from random mating was calculated for each locus and overall. Samples with low quantity or quality of extracted DNA, were excluded from amplification, resulting in 746 amplified samples. Of these, 83 had failed location coordinates, leading to 663 samples retained for analysis (Figure 1).

When, as in the present study, samples are collected over a prolonged time period, assumptions (e.g., closed populations, deaths and births) used in traditional genetic capture-recapture population size estimators are violated leading to inflated census size. Preliminary analysis with often favored software like CERVUS (Marshall et al., 1998), confirmed these concerns, producing a vastly inflated census estimate of N = 287 which would correspond to a density far beyond what has been previously reported for a part of this chimpanzee population

(Koops, 2011). Furthermore, when inspecting the retained sample set by eye, a substantial number of pairwise sample comparisons were clearly miscalled as non-identical. The relatively incomplete nature of our data also highlighted a sensitivity to, for example, missing genotypes and inconsistent repeat patterns, when using publicly available software (see also Appendix A for this rationale). Therefore, with an in-house statistical program, we identified resampled individuals, *within* each predefined community by pairwise comparisons of samples with overlapping genotypes in a minimum of 16 genotyped loci while allowing for minor genotyping errors ($+-2$ base pairs per locus). Any pair of samples with identical genotypes in more than 12 of the compared loci were labeled as resampled individuals (i.e., "recaptures"). This method is therefore different from a standard capture-recapture model, where census size is extrapolated from a recapture rate. Here, we assume that we, in the 999 collected samples, have sampled all individuals in the area at least once (confirmation of this assumption is later explored through our sampling intensity analyses). The census size is therefore instead estimated by excluding all duplicate samples, referred to here as "recaptures." One sample from each identified resampled pair was kept for downstream analyses, unless specified otherwise. Lower and upper estimates of census population sizes were estimated by an ad-hoc bootstrap method of randomized shuffling and resampling of the data set ten times (see also section on sampling intensity below). The total census size was finally adjusted by running the above procedure *across* communities to correct for any individuals that might have occurred in more than one community over the course of the sampling time span (see also section on observed migration events). Sex was determined by the presence-absence of genotyping scores for the Y-chromosome specific locus. The male-to-female ratio was calculated both within and across communities. Summary statistics were calculated on the census population using the R package *adegenet* version 1.4-2 (Jombart, 2008).

2.4 | Sampling intensity

To evaluate sampling intensities in each of the four preassigned communities, we used the above-mentioned bootstrap method of randomly down-sampling our full dataset (i.e., before removing resampled individuals) in bins of increasing size at increments of 20. The full dataset was randomly shuffled before each down-sampling and iterated five times for each sample bin size, allowing an evaluation of the sampling intensity saturation, that is, when additional samples do not lead to newly identified individuals and hence, the sampling curve flattens asymptotically. This could, for the present study, reveal potentially under-sampled communities, recognizable by a non-flattening sampling curve and in general provide empirical evidence for future monitoring of the species or community, allowing a baseline for the sampling intensity required to sample all individuals at least once. We further calculated the ratio between the total number of genotyped samples and the total number of identified individuals in each community to assess an approximate capture rate, informing how many samples would be needed in future monitoring efforts to capture each individual in each of the four communities.

2.5 | Genetic population structure

To characterize the genetic population structure and connectivity between the sampled chimpanzee communities in the Nimba Mountains, we applied a range of complementary statistical analyses. While communities defined by field observation and camera traps are not necessarily the same as genetically random mating populations, the structural analyses were intended to explore how these entities line up and test how the a priori defined communities resemble any structure that could be inferred from genetics. Underlying structural trends in our data (without any community priors) were explored through principal component analysis (PCA) and spatial principal component analysis (SPCA) using the R package *adegenet* version 1.4-2 (Jombart, 2008).

Population structure and ancestry sharing between communities were further explored in a maximum likelihood framework as implemented in STRUCTURE v. 2.3.4 (Pritchard et al., 2000). We applied the admixture model to allow individuals to have ancestry from multiple communities and assumed correlated allele frequencies. We ran two parallel parameter setups where (1) was blinded for prior geographical sampling locations, and (2) with a priori community labels based on exact GPS coordinate recordings of samples as location priors. For both the blinded and the location prior setup, we explored a wide range of K values ($K = 2–8$) with a burn-in of 100,000 MCMC iterations and 1000,000 follow-on MCMC iterations. For each K value, we ran 20 individual repetitions to check for convergence of the obtained likelihoods between runs. Likelihood scores were evaluated using an ad hoc procedure as recommended by Evanno et al. (2005). The R package *construct* v. 1.0.4 (Bradburd, 2019) was used to visualize ancestries as pie-charts spatially distributed according to sampling location. Genetic differentiation between communities was calculated with Jost´s unbiased estimator of

differentiation D_{est} (Jost, 2008) as the arithmetic mean across loci using the R package *graph4lg* v. 1.2.0 (Savary et al., 2021).

Genetic dissimilarities and potential barriers to migration between communities were further explored using Estimated Effective Migration Surface (EEMS) (Petkova et al., 2016). This method provides a visual and intuitive representation of the spatial differentiation between individual samples within communities and possible differences in migration rates between the inferred communities. In 10 parallel MCMC chains, each with a different seed, we ran the analysis for 1000,000 iterations, with a burn-in of 500,000, and 9999 thin iterations. A polygon of the outer bounds of our spatial analysis field, covering the northern region of the Nimba Mountains, was drawn with the online software http://apps.headwallphotonics.com/ and converted to longitude and latitude degrees with http://www.zonums.com/online/coords/cotrans.php?module=13. The R-package *reemsplot2* (Petkova et al., 2016) was used to plot the migration and genetic dissimilarities as well as convergence of the 10 MCMC chains and finally, exploration of isolation by distance were included to either reject or confirm if the pre-defined communities were indeed reflected in the data or should be considered one panmictic population.

2.6 | Observed migration events

From the identified recaptures (see above) within and across communities, we mapped the observed movement patterns by connecting repeated sample locations of the same individuals over time. This allowed us to visualize the outlines of community boundaries and migration events across communities.

3 | RESULTS

3.1 | Genetic diversity and census size

Of the 999 collected samples, 748 samples contained enough endogenous DNA and were amplified at, at least one locus. Of these, 663 had reliable GPS information and were retained for the downstream analyses (Figure 1; see distribution of genotyped loci at different stages of the filtering steps in Appendix A Figure A2). Allelic dropouts, and therefore deviations from Hardy–Weinberg, were observed. The duplicate genotyping was consistent. All microsatellite loci in the set of amplified samples (N = 746) were highly polymorphic, except GATA125D11N, which was therefore excluded from further analyses. Despite an excess of homozygotes indicating allele dropouts or Wahlund effect due to substructure, the gene diversity was high (H_e total mean 0.77) and comparable with levels previously published (Hvilsom et al., 2013). The polymorphic information content was also high (PIC = 0.79) across all loci and the probabilities of not identifying two identical samples were low (Table A1) despite a considerable high amount of missing data compared to previous studies.

TABLE 2 Number of samples and sex ratio within and across communities

Community	$N_{filtered}$	$\tilde{N}_{identified}$	$\tilde{N}_{adjusted}$	M/F
Nimba-North	55	24 (23–24)	22	1.20
Zouguepo	132	29 (27–31)	25	0.92
Gahtoy	152	50 (50–51)	47	1.47
Tongbongbon	324	46 (44–48)	42	1.00
Total	663	149 (144–154)	136	1.16

Abbreviations: M/F, the ratio between males and females; $N_{filtered}$, number of samples after filtering out samples with >50% missing genotypes; $\tilde{N}_{identified}$, number of samples identified after removing duplicates *within* each community with upper and lower estimates from resampling the dataset ten times; $\tilde{N}_{adjusted}$, number of samples after removing duplicates *between* communities.

From the census estimation by identity exclusion, we identified a total of 149 individuals (144–154, upper and lower estimates respectively) when summarized over all four communities (Table 2). When adjusting for individuals that appeared in more than one community over the sampling period, we obtain a final census estimate of 136 unique chimpanzees in the sampling region. Sex ratios were slightly male-biased in all communities, with the exception of the Zouguepo community (Table 2). A selection of summary statistics calculated from the census population are reported in Table A2, showing high observed gene diversity across the four communities but also elevated inbreeding coefficients (overall Fis = 0.29).

Genetic differentiation followed a south-northern gradient of divergence with the highest observed differentiation between the geographically most distant communities. Pair-wise, we observed the lowest D_{est} value between the neighboring communities Gahtoy and Tongbongbon (Table 3).

3.2 | Sampling intensity

By subsampling our data in each of the four communities and iterating our census size estimates, we were able to assess whether our sampling intensity had saturated. Two of the most intensively sampled communities, Tongbongbon and Zouguepo, show a clear saturation as the sampling curves flatten asymptotically prior to reaching

TABLE 3 Pairwise genetic differentiation (Jost's Dest[a])

Community[a]	Nimba-north	Zouguepo	Gahtoy	Tongbongbon
Nimba-North		0.140	0.144	0.182
Zouguepo			0.103	0.105
Gahtoy				0.024
Tongbongbon				

[a]Calculated as the arithmetic mean across all loci.

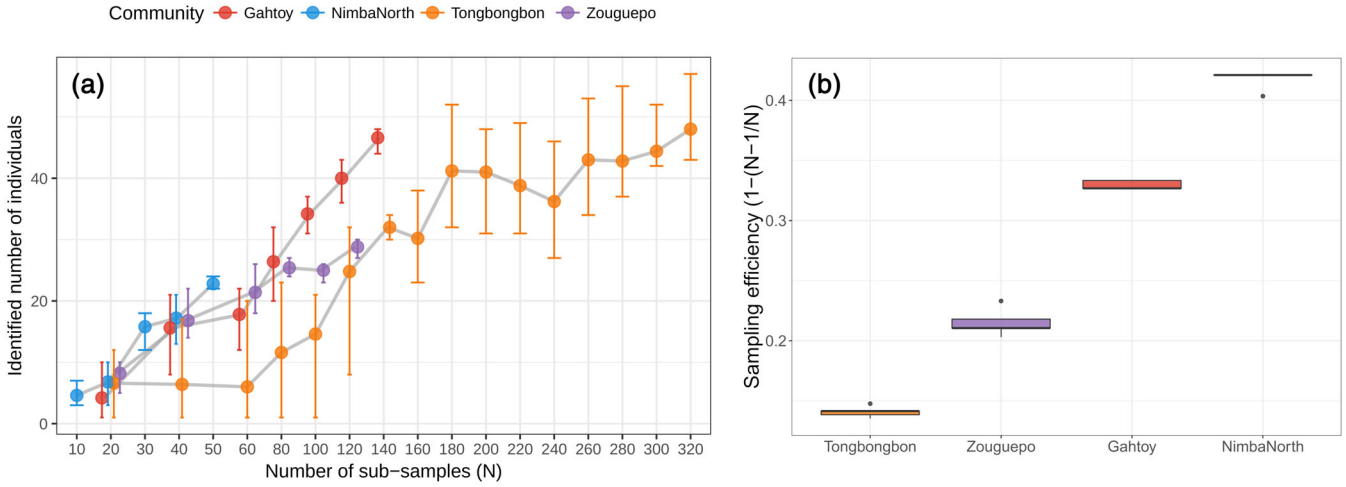

FIGURE 2 Sampling efficiency. (a) Sampling curves across communities. With asymptotically flattening curves, Tongbongbon and Zouguepo exhibit a saturation after 180 and 80 samples, respectively. Non-flattening curves in Gahtoy and Nimba-North, suggests under sampling in these communities. (b) The approximate discovery rate as a ratio between the number of samples (N) and identified individuals (Ñ) for each community.

the total number of samples (Figure 2a). This is further reflected in the approximate discovery rates (Figure 2b), with Tongbongbon, followed by Zouguepo, having the lowest ratios between the number of samples (N) and identified individuals (Ñ). In contrast, the Nimba-North and Gahtoy communities did not seem to reach saturation, which would suggest under-sampling of these communities. The relatively high approximate discovery rates additionally point towards an under-sampling in the Nimba-North and Gahtoy communities, as every two new samples capture approximately one newly identified individual in Nimba-North (avg. rate 0.42) and slightly less in Gahtoy (avg. rate 0.33) (Figure 2b).

3.3 | Genetic population structure

Initial population structure analyses *without* location priors, revealed little evidence of population stratification resembling that of the a priori defined communities. The principal components analysis only showed hints of a differentiation between the two northern communities (i.e., Nimba-North, Zouguepo) and the two southern communities (i.e., Gahtoy, Tongbongbon), with clusters of samples from north and south being separated along the first principal component (Figure A1). However, the large majority of samples cluster together, indistinguishable by any of the most explanatory principal components (Figure A3). Signals of population structure were also relatively weak in the location blinded STRUCTURE analyses. Only the northernmost community (i.e., Nimba-North) showed evidence of distinct genetic clustering (Figure A4).

Adding location priors from exact GPS recordings, largely improved our ability to infer patterns of population structure that approximate the assumed communities. At K = 4, the two northern communities (i.e., Nimba-North, Zouguepo) are clearly distinguishable with some evidence of shared ancestries (Figure 3a). The two southern communities, Gahtoy and Tongbongbon, appear less differentiated with ancestry components shared across the range, only with some clustering of a fourth component mid-range (Figure 3a). At K = 4, the substructural clustering in Gahtoy appears to share ancestry with Nimba-North. At K = 5, this substructure is assigned to a separate cluster (Figure 3b). Following the ad-hoc method of Evanno et al. (2005) to evaluate the "best" of the eight explored K values (2–8) for both

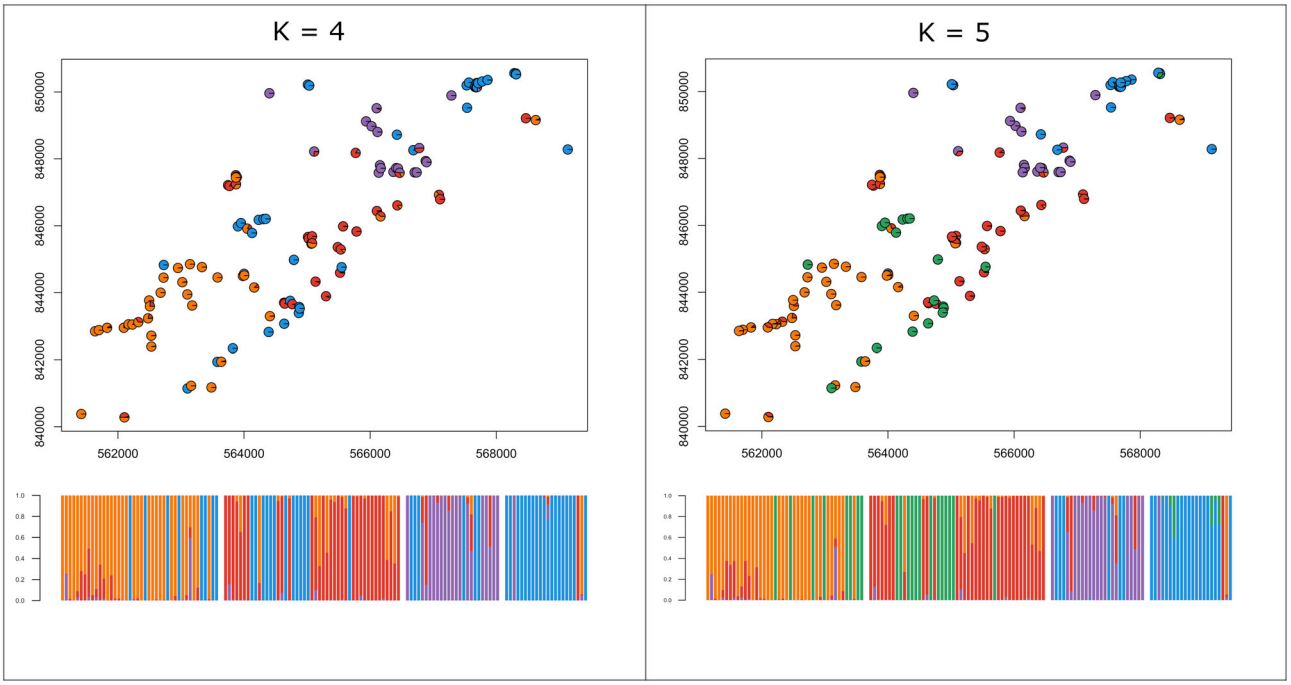

FIGURE 3 Population genetic structure. (Left) Pie charts (top) of shared ancestries between the four communities inferred from the structure analysis with locality priors (K = 4) sorted according to GPS sample location and bar plot (bottom) of shared ancestries sorted according to community and geographic location (south-to-north gradient). (Right) Pie charts (top) and bar plot (bottom) ordered as on the left but showing structure results for K = 5.

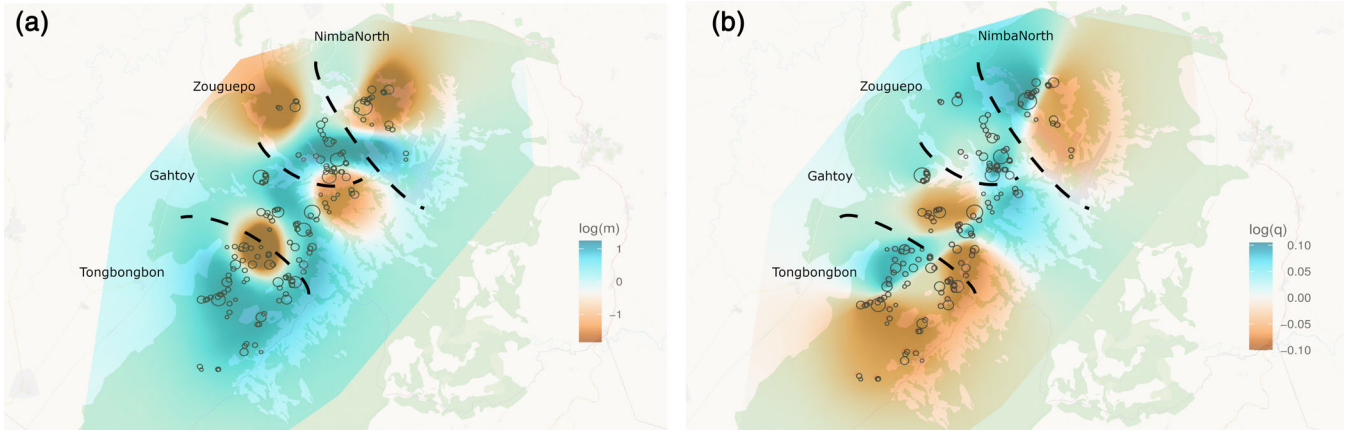

FIGURE 4 Effective migration and diversity surfaces show areas with effective migration and diversity rates higher or lower than the overall average rate across all samples, where blue shades indicate higher rates than the average and brown shades indicate lower rates than the average. Dotted lines indicate assumed boundaries between communities following prior knowledge from field observations and topology (mountain ridges are here shaded in light colors surrounded by green forest cover). (a) Effective migration rate surfaces, *m*, characterizing genetic dissimilarities between distinct demes. (b) Effective diversity dissimilarities rates, (*q*), between distinct individuals within the same deme.

blinded and location prior estimates, actually indicates a discrepancy between a priori defined communities and genetically inferred population, with most support, by the data, for five ancestral genetic clusters, where the fifth cluster is located within the Gahtoy community (Figure A6).

The inferred population structure was further corroborated by the EEMS analyses that identified migration and diversity dissimilarity rate surfaces in the Nimba range (Figure 4). Our results show a migration edge between the northernmost community, Nimba-North and the south, corresponding geographically to the mountain ridge and the

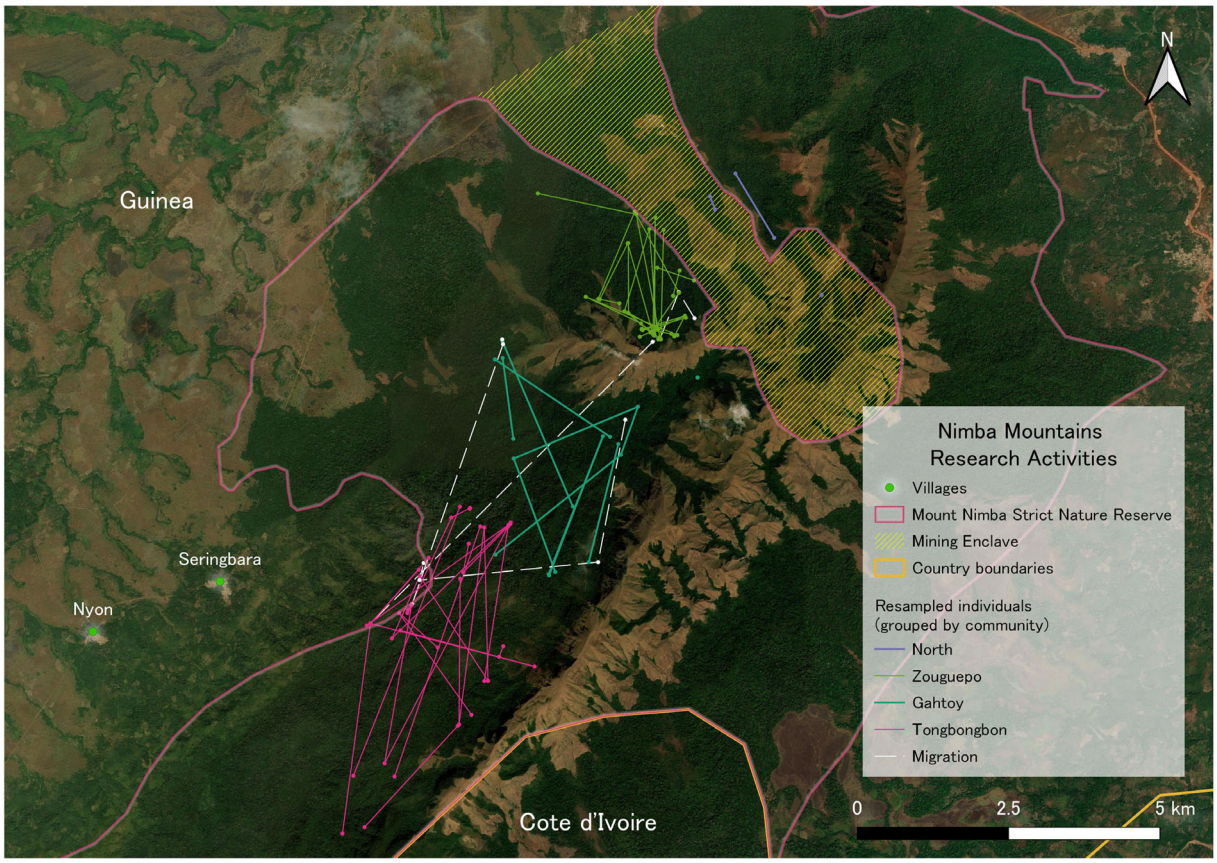

FIGURE 5 Map of resampled individuals within each of the four communities. Lines connect samples that have been identified as the same individual. Possible migration events of individual chimpanzees between communities are represented by white dashed lines.

Nimba mining enclave, possibly acting as a partial barrier to migration (Figure 4a). Migration edges are further observed between two distant sites within the Zouguepo community and towards the Gahtoy community along the mountain ridge (i.e., Gouoton). Surprisingly, we also identify a reduced migration rate between the Gahtoy and Tongbongbon communities, where no apparent geographical barrier exists. These results are further corroborated by the sPCA analyses that showed little connectivity between these areas (Figure A7 and A8). The effective dissimilarity rates further showed a reduced dissimilarity (i.e., high genetic similarity) in the eastern range of the Nimba-North community, within the Gahtoy community in areas overlapping with the inferred sub-structure, and the southern region of Tongbongbon (Figure 4b). Lastly, the EEMS analyses did not show any evidence of isolation by distance when measured as the observed dissimilarities as a function of circular distances between demes (Figure A9).

3.4 | Observed migration events

From the identified recaptures, we mapped the observed movement patterns within and across community boundaries (Figure 5). We recorded numerous recaptures within each of the four separate communities, as well as three potential migration events (i.e., two females and one male) across community ranges (i.e., white lines, Figure 5).

4 | DISCUSSION

Our genetic censusing approach allowed us to successfully and extensively sample the largely unhabituated chimpanzees inhabiting the steep and rugged terrain of the Nimba Mountains. The number of discrete communities spanning the Guinean portion of the massif had previously been difficult to ascertain with confidence due to the challenging topography and the generally low levels of habituation of the chimpanzees. Moreover, a previous population size estimate was based on nest count methodology, generating wide confidence intervals and hence a relatively high degree of uncertainty in abundance estimates (Koops, 2011). Our findings confirmed a genetically viable and sizable chimpanzee population living on the sampled western flank of the Nimba Mountains in Guinea. We confirmed the presence of at least 136 individuals living in four communities (Table 2). This

number is, however, an underestimate of the actual population size, since immature individuals, particularly infants and juveniles, are not reliably included in fecal sampling. Moreover, the sampling curves suggested that the Nimba-North and Gahtoy communities were under sampled (Figure 2). Hence, the actual number of chimpanzees (i.e., adults and immatures) living on the western flank of the Nimba Mountains range will exceed the minimum estimate of 136 chimpanzees. Although sampling saturation was not reached in the Gahtoy community, it is likely close to the actual population size. Compared to Nimba-North, the discovery rate is lower in Gahtoy, suggesting a sampling efficiency nearing saturation.

The genetic data revealed a distinct population structure and ancestry sharing between the four sampled communities. Of these four communities sampled, the two communities in the North (i.e., Nimba-North and Zouguepo) were the most genetically distinct, albeit with some observable ancestry sharing (Figure 3). Genetic differentiation was highest between communities separated by physical barriers like mountain ridges and the mining enclave (Table 3). The two most southern communities (i.e., Gahtoy and Tongbongbon) showed a less defined population structure, with some evidence of sub-structuring in both communities (Figure 3). However, the actual observable movement patterns inferred from the genetic resampling of the same individuals (i.e., recaptures) confirmed the existence of distinct home ranges of the two communities in the south (Figure 5). Low levels of genetic differentiation (Table 2) and clear evidence of a high degree of ancestry sharing (Figure 3) also further indicate that any effective barriers to gene flow are only minimal in this south-western region of the massif.

Our data revealed three cases of likely chimpanzee migration events: One from the Tongbongbon community to the Zouguepo community, one between the Tongbongbon and Gahtoy communities, and another from the Gahtoy to the Tongbongbon community (Figure 5). Specifically, in 2010, a female chimpanzee was sampled in the Tongbongbon community and then, in 2013, multiple times in the Zouguepo community (Figure 5). Similarly, another female was first sampled in the Gahtoy community in 2008 and a year later in the Tongbongbon community (Figure 5). More surprisingly, a male chimpanzee was observed first in the Gahtoy community in 2009, and subsequently in the Tongbongbon community in 2010. This finding could reflect an incursion of a male patrol into neighboring territory. It could also potentially concern an immature male migrating with his mother, or an actual migration event of an adult male (see also Ishizuka et al., 2020). Ensuring continued future connectivity between these communities is key in securing the genetic viability of the Nimba chimpanzee population.

Based on our genetics results, the male/female sex ratio observed in this region of the Nimba Mountains is roughly 1.2, and thus slightly male-biased. However, since some subadult individuals may have also been sampled, this ratio may not accurately reflect adult sex ratio which is typically reported in the literature. Previous findings for the mean adult sex ratio of parties for the Gahtoy and Tongbongbon communities combined based on camera trap data (2011–2014) was 0.9, compared to our results of 1.0 for Tongbongbon and 1.5 for Gahtoy (van Leeuwen et al., 2020). The Nimba sex ratio is considerably more male-biased compared to other forested West African sites, such as nearby Bossou in Guinea (M/F = 0.5–0.13; Sugiyama, 2004) and the Taï forest in Côte d'Ivoire (M/F = 0.77–0.23; Boesch & Wittig, 2019). The sex ratio at Nimba approaches that of the heavily male-biased savannah site of Fongoli in Senegal (i.e., M/F = 1.7–1.4, Pruetz et al., 2017). At Fongoli, the skewed sex ratio may be due to opportunistic hunting by people of female chimpanzees and their offspring as they represent easier targets and capture of young potentially to fuel the pet trade (Pruetz et al., 2017). However, on the Guinean side of the Nimba massif, direct hunting of chimpanzees has never been witnessed, most likely since chimpanzees are the totem animal of the Manon people, one of the dominant ethnic groups in the locality (Kortlandt, 1986). The reason for the relatively skewed sex ratio in Nimba could be a male-biased sex ratio at birth, as seen at Taï (Boesch & Wittig, 2019). Future research using both genetic sampling and camera traps may shed more light on adult sex ratios compared to sex ratios at birth in Nimba.

Our findings show that non-invasive genetic sampling can provide valuable data on chimpanzee population size and structure both for baseline studies in environmental impact assessments and for long-term population monitoring. By evaluating the sampling intensity in each community, we also obtained important insights into whether sampling effort had reached saturation, or not. Towards saturation, the sampling curve for the identified individuals in a given community flattens (Figure 2a) and the approximate capture rate (Figure 2b) decreases. When comparing the four sampled communities in Nimba, we showed that the required sampling intensity varies substantially between communities, even on this relatively small geographical scale. In Zouguepo (N = 25 individuals), saturation was approached after 80 samples, while about 180 samples were required in Tongbongbon (N = 42 individuals). Community size, as well as home range size, may influence the level at which saturation is reached in a given community. It is therefore crucial to establish a robust baseline for the required sampling intensity in each given community if future assessments

are to provide reliable estimates of population trends (i.e., increase or decrease), which is essential for effective species abundance estimates and monitoring. In addition, temporal sampling can provide useful information about potential changes in genetic diversity and health of the sampled communities over time.

For a complete picture in terms of species monitoring, as well as an initial assessment of population size and structure for ESIA purposes, we propose a combination of genetic censusing and camera trapping. Although genetic sampling provides crucial insights into a variety of chimpanzee community characteristics (e.g., genetic diversity, effective and census population size, sex ratio), it does not generate information on the number of immatures in a community (including their sex ratio), and thus reproductive and growth rate, nor does it inform us about the presence of snare injuries and other observable health issues. Hence, a two-pronged approach of genetic data and camera trapping provides the most comprehensive view of a chimpanzee community's demographics, health and viability.

Our results confirm that the Nimba chimpanzee population on the Guinean side of the massif represents a viable and genetically healthy population comprising at least four communities. Considering the prevalence of chimpanzees in the Ivorian and Liberian portions of the massif (Matsuzawa et al., 2011; Norman et al., 2015), our results confirm the importance of the Mount Nimba Strict Nature Reserve as a key priority site for the conservation of the western subspecies of chimpanzees. Mining development in the region will pose a significant threat to this population if infrastructure development and extraction activities affect chimpanzee movement, such as dispersal between the southern and northern communities, and if any habitat loss or disturbance reduces access to areas of high suitability for food or nesting, especially predominant in the higher altitudes (Fitzgerald et al., 2018). Such impacts, unless avoided entirely or mitigated successfully, could reduce the genetic viability of the population, and potentially induce overlap between community home ranges. Such range shifts can readily expose chimpanzees to inter-community lethal aggression and enhance intra- and inter-community competition for food, thereby increasing individual stress levels and reducing immune system resistance and reproductive rates (Arcus Foundation, 2014). Other threats to chimpanzees across the Mount Nimba Strict Nature Reserve include hunting, via snaring and use of guns, and habitat loss or disturbance most often associated with slash and burn agriculture at the edges of the reserve boundaries, and bush fires linked to livestock farming in the lowlands (Matsuzawa et al., 2011). The presence of people in areas frequented by chimpanzees also presents a risk of disease transmission, that is, zoonoses, which can result in significant chimpanzee mortality (Humle, 2011; Köndgen et al., 2008). These threats are likely to continue to affect the Nimba chimpanzee population until an effective transnational management plan of the reserve is in place and accepted and implemented by all stakeholders. This plan must aim to balance human development with conservation goals through effective law enforcement, increased access to education, enhanced community environmental awareness, land use planning, "one health" initiatives, alternative livelihoods programs, and strategies for managing the likely influx of humans into the region seeking economic opportunities.

In sum, of the 999 fecal samples collected, 66.3% (N = 663) were retained for analysis. Although these samples most likely did not capture infants or juveniles, our study identified a minimum of 136 individual chimpanzees distributed across four communities, exhibiting dispersal behavior with evidence of migratory events, a high level of shared ancestry and genetic diversity with a mean H_e of 0.81 and a slightly male sex-biased ratio. Sampling curves for the four defined communities revealed that the most heavily sampled communities (i.e., Tongbongbon, Zouguepo) reached sampling saturation while the other two did not, suggesting that the number of individuals in these communities are underestimated. Our findings suggest that there is no single rule of thumb for how many samples are required for estimating population or community abundance using genetics, even on such a small spatial scale. Our data show that saturation was reached with sampling ranging between 3.2 and 4.3 times the estimated number of chimpanzees. In future studies employing non-invasive genetic sampling, we highly recommend that saturation curves be reported to help validate abundance estimates and genetic health parameters.

Finally, this study highlights the significant utility and value of genetic censusing using fecal samples for chimpanzees not only for temporal monitoring of chimpanzee abundance, but also its ability to capture migratory events and gauge genetic diversity and population viability over time. Our study affirms that non-invasive genetic sampling is an extremely powerful tool that can inform us about the abundance, the structure and genetic health of a chimpanzee population. With emerging improvements in fecal sample extraction techniques, the use of SNP data could be a feasible direction in future projects, adding more power to population diagnostic inferences (e.g., Fontsere et al., 2021) As such, genetic sampling (in combination with camera trap monitoring), should be used in ESIA assessments to yield robust baselines for implementing the mitigation hierarchy, future monitoring and adaptive management and conservation plans.

ACKNOWLEDGMENTS

We thank the Direction General de la Recherche Scientifique et l'innovation Technologique (DGERSIT) and the Institut de Recherche Environnementale de Bossou (IREB) in Guinea for research authorization; research assistants, K. Doré, F. Doré, F. Zogbila, N. Doré, D. Zogbila, Y. Zogbila, C. Samy, H. Camara, N. Gbouomy, K. van Leeuwen, M. O'Reilly, P. Le Sommer, J. Caraway, W. Edwards, D. Montanari, N. James, I. Vélez del Burgo Guinea, D. Hassler, M. McCann, S. Canington, G. Mamy, C.V. Traoré, L. Camara, C. Bamamou, B. Doré, M. Keita, S. Bamba, T. Fanghamou, B. Zogbila, G. Zogbila, J. Doré, for help in the field. We thank T. N. Gade, N. V. Jochumsen and A. L. Jørgensen for assisting with the lab work. Sample collection was supported by grants from the Gates Cambridge Trust (Cambridge, UK), Lucie Burgers Foundation for Comparative Behavior Research (Netherlands), Homerton College and Newnham College (Cambridge, UK), and a SNSF Eccellenza Professorial Fellowship (no. PCEFP3_186967) to KK, Mohamed Bin Zayed Species Conservation Fund and Graduate Teaching PhD Scholarship, University of Kent, UK, to LD, and MEXT (#12002009, #16002001, #20002001, #24000001, #16H06283) grants to T. Matsuzawa. PF was supported by a postdoctoral fellowship grant from Alfred Benzon Foundation. Genetic analyses and sample collection (Nimba-North) were supported by the Société des Mines de Fer de Guineé S.A. (Conakry, Guinea). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

DATA AVAILABILITY STATEMENT

The raw data are available upon reasonable request to the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Koops, K., Humle, T., Frandsen, P., Fitzgerald, M., D'Auvergne, L., Jackson, H. A., Børsting, C., Siegismund, H. R., Soumah, A. G., & Hvilsom, C. (2023). Genetics as a novel tool in mining impact assessment and biomonitoring of critically endangered western chimpanzees in the Nimba Mountains, Guinea. *Conservation Science and Practice*, e12898. https://doi.org/10.1111/csp2.12898