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The importance of well protected forests for the conservation genetics of West African colobine monkeys

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Abstract

In tropical forests, anthropogenic activities are major drivers of the destruction and degradation of natural habitats, causing severe biodiversity loss. African colobine

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monkeys (Colobinae) are mainly folivore and strictly arboreal primates that require large forests to subsist, being among the most vulnerable of all nonhuman primates. The Western red colobus *Piliocolobus badius* and the King colobus *Colobus polykomos* inhabit highly fragmented West African forests, including the Cantanhez Forests National Park (CFNP) in Guinea-Bissau. Both species are also found in the largest and best-preserved West African forest—the Taï National Park (TNP) in Ivory Coast. Colobine monkeys are hunted for bushmeat in both protected areas, but these exhibit contrasting levels of forest fragmentation, thus offering an excellent opportunity to investigate the importance of well-preserved forests for the maintenance of evolutionary potential in these arboreal primates. We estimated genetic diversity, population structure, and demographic history by using microsatellite loci and mitochondrial DNA. We then compared the genetic patterns of the colobines from TNP with the ones previously obtained for CFNP and found contrasting genetic patterns. Contrary to the colobines from CFNP that showed very low genetic diversity and a strong population decline, the populations in TNP still maintain high levels of genetic diversity and we found no clear signal of population decrease in Western red colobus and a limited decrease in King colobus. These results suggest larger and historically more stable populations in TNP compared to CFNP. We cannot exclude the possibility that the demographic effects resulting from the recent increase of bushmeat hunting are not yet detectable in TNP using genetic data. Nevertheless, the fact that the TNP colobus populations are highly genetically diverse and maintain large effective population sizes suggests that well-preserved forests are crucial for the maintenance of populations, species, and probably for the evolutionary potential in colobines.

KEYWORDS
arboreal primates, demographic history, evolutionary potential, habitat fragmentation, West Africa

1 | INTRODUCTION

Tropical forests harbor over 60% of all biodiversity despite covering only 7% of the earth's surface (Dirzo & Raven, 2003; Hoekstra & Wiedmann, 2014; Laurance, 1999; Struhsaker et al., 2005). In recent decades, tropical regions have been experiencing the drastic impacts of human population growth. Unsustainable human activities such as intensive agriculture and commercial hunting for bushmeat consumption (Haddad et al., 2015; Malhi et al., 2014) are leading to major declines in wildlife populations (Laurance et al., 2012; Laurance & Peres, 2006; Tranquilli et al., 2014). In particular, habitat loss and fragmentation reduce the availability of habitat and suitable areas for biodiversity to thrive, and frequently lead to resource shortages, greater accessibility of wildlife to hunters, population declines, and demographic isolation, all of which might result in loss of genetic diversity (Frankham et al., 2002; Haddad et al., 2015; Hoffmann & Sgrò, 2011; Minhós et al., 2016; Radespiel & Bruford, 2014). Consequently, populations may experience inbreeding depression

and loss of evolutionary potential, thus reducing the likelihood of a response when faced with sudden environmental changes, such as those driven by humans (Frankham et al., 2002; Radespiel & Bruford, 2014). Various nonhuman primates, from lemurs to colobines to great apes, show reduced genetic diversity in populations inhabiting degraded habitats (Bergl et al., 2008; Craul et al., 2009; Minhós et al., 2016; Sharma et al., 2012; Ruiz-Lopez et al., 2016). All these studies, across different primate taxa, stress the importance of preserving large areas of pristine habitat for the long-term survival of populations.

Adding to the effect of habitat loss and fragmentation, overhunting is a major direct threat to wildlife throughout tropical environments (Tranquilli et al., 2014). Specifically, the Afrotropical region is considered one of those with the highest hunting pressure: estimates show that one to five million tons of biomass are hunted for bushmeat annually (Laurance et al., 2006). Food dependency on bushmeat has resulted in hunting exceeding habitat destruction as the main source of primate population loss in the Central and

Western regions of Africa (Linder & Oates, 2011; Rovero et al., 2012). Hunting is associated with traditional subsistence food consumption in tropical countries owing to the lack of other protein sources (Cronin et al., 2015). However, the consumption of wildlife as snacks and delicacies outside rural areas is also increasing, leading to the overexploitation of natural populations, and bushmeat markets becoming an important economic activity (Covey & McGraw, 2014; Cronin et al., 2015; Harrison et al., 2016; Kun-Rodrigues et al., 2014; Linder & Oates, 2011; Minhós et al., 2013b; Refisch & Koné, 2005a; Rovero et al., 2012). By itself, hunting can have devastating consequences for local fauna, but when combined with deforestation, the pressure is exacerbated synergistically (Benchimol & Peres, 2013; Núñez-Regueiro et al., 2015).

Nonhuman primates are particularly vulnerable to the effects of habitat loss and fragmentation, and hunting (Estrada et al., 2017). Forest dwelling primates that require a large matrix of forest to disperse and achieve daily dietary requirements are often the most affected in human-dominated landscapes (Liu et al., 2015; Sharma et al., 2012). African colobine monkeys are particularly vulnerable, as they are forest dwelling primates with highly specialized folivore diets, low dispersal ability across fragmented forests, and whose populations are known to experience declines in disturbed habitats (Minhós et al., 2016; Minhós et al., 2013a; Oates, 1994; Onderdonk & Chapman, 2000). African colobines (genera *Colobus*, *Piliocolobus*, and *Procolobus*) are among the most threatened primates globally (IUCN, 2020). Of the three groups, *Colobus* sp. (black-and-white colobus) have the most continuous distribution throughout equatorial Africa with five recognized species, while *Piliocolobus* sp. (red colobus) are distributed in a more fragmented manner, with 17 recognized species (IUCN, 2020). West African colobines (Western black-and-white colobus or King colobus *Colobus polykomos* and Western red colobus *Piliocolobus badius*) inhabit the Upper Guinean Forests hotspot, occupying small and isolated forest fragments surrounded by a matrix of human-modified habitat in one of the most severely anthropogenically fragmented regions on the planet (McGraw et al., 2020b; Minhós et al., 2020; Tranquilli et al., 2014). As a result, local and taxon-wide extinctions are ongoing in this region, for example, Miss Waldron's red colobus, which has not been observed since 2002, with probably only a few individuals remaining (Oates et al., 2020).

Here we investigate the importance of well-protected forests for the maintenance of the genetic diversity and sustainable populations of two threatened colobus monkeys—the King colobus, *C. polykomos* (classified as Vulnerable, Gonedelé et al., 2020) and the Western red colobus, *P. badius* (classified as Endangered, McGraw et al., 2020b). These two species are distributed from Gambia-Senegal to Ivory Coast, mostly in small and isolated populations. They also inhabit the largest and best protected forest in West Africa—the Taï National Park, Ivory Coast (TNP, Figure 1; Gonedelé et al., 2020; McGraw et al., 2020a). We conducted the first genetic study of both primates inhabiting this large and continuous forest, in which we examined the genetic diversity and structure, and inferred the demographic history of the two species. We then compared the genetic patterns of TNP colobines with those of colobus populations inhabiting the smaller and highly fragmented forest patches at the Cantanhez Forests National Park, Guinea-Bissau (CFNP; Figure 1). Previous studies found CFNP colobus monkeys to exist in small and decreasing populations (<300 reproductive individuals), exhibiting extremely low levels of genetic diversity (Minhós et al., 2013a, 2016). Colobus populations living in both TNP and CFNP are hunted for bushmeat (Minhós et al., 2013b; Refisch & Koné, 2005a), and it is possible that the difference in the size and connectivity of the two forests may result in different hunting pressures. It is known that populations inhabiting fragmented forests are more accessible to hunters and subjected to intensive hunting (Robinson, 1996; Turner & Corlett, 1996). The severe forest fragmentation in CFNP may contribute to an increased hunting pressure in this protected area compared to TNP, which would still be a direct consequence of the difference in forest size and preservation between the two parks. Additionally, TNP is home to a long-term research targeting several primate species, including the colobine monkeys. These primates have been studied and followed by researchers, on a more or less continuous basis, for the past 40 years. This is now known to have lessened the hunting pressure toward these primates in areas with a strong presence of researchers and tourists (Campbell et al., 2011; Hoppe-Dominik et al., 2011). Although primate researchers are also present at CFNP, their continuous permanence is more recent (past 15 years), and it is likely that their effect on decreasing hunting pressure is not so evident as it is at TNP. Thus, the levels of hunting between the two protected areas may differ not only due to the differences in the level of forest fragmentation but also due to differences in the duration of researchers' presence in each area. Our comparative study therefore offers a suitable setting to assess the importance of forest preservation for the maintenance of the evolutionary potential of the arboreal colobus monkeys. We hypothesized that the populations of colobine monkeys from TNP are genetically more diverse than their CFNP conspecifics, as a result of a large and well-preserved forest in the former protected area, leading to more stable demographic histories.

1.1 | Study areas

Taï National Park (TNP) (Figure 1), is located in the Southwest of Ivory Coast (5°15′ to 6°7′N; 7°25′ to 7°54′W). It is the largest well-preserved block of tropical forest in West Africa, covering an area of 5,364 km² (Brou et al., 2004; Chatelain et al., 2010). It is managed by the Office Ivorien des Parcs et Réserves, was classified as a UNESCO World Heritage Site in 1982 and is one of the 25 global biodiversity hotspots as part of the Upper Guinean Tropical Forests of West Africa (Myers et al., 2000). The TNP harbors several human communities that depend on natural resources for subsistence (Budelman & Zander, 1990; de Rouw, 1993).

In 2005, funding allowed the implementation of an effective monitoring program (N'Goran et al., 2013) and, in 2014, an ecotourism plan ("Nature and Culture") seeking to value the natural and cultural heritage was created with the local community of Taï village. This safeguards TNP while simultaneously generating an
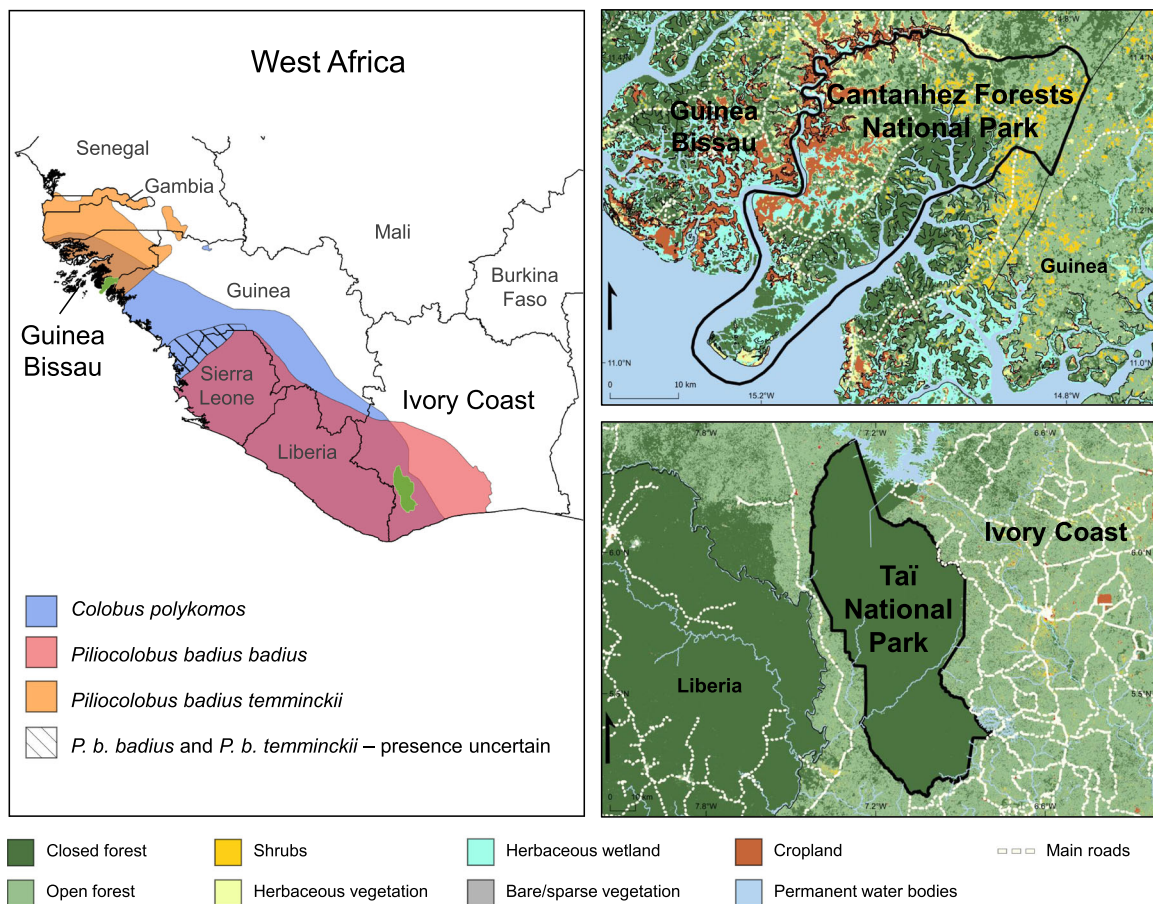
FIGURE 1 Map showing the distribution of the taxa on the study area (IUCN, 2020) and the location of the protected areas in West Africa (left panel). Detail of Cantanhez Forests National Park (CFNP), Guinea-Bissau (top right panel), and of Taï National Park, Ivory Coast (bottom right panel). The details of land cover in each park (right panels) are based on satellite imagery, which explains the differences between the two images. A similar figure showing the main villages in CFNP can be seen in the Supporting Information: Figure S8 (the same information was not available for TNP). *Source of layers*: IUCN; Copernicus Global Land Service; UN OCHA; OpenStreetMap. Map done using QGIS v3.16.

economic contribution to the communities inhabiting the park. TNP is not entirely threat-free and hunting, above all activities, poses a daily risk to primate populations (Covey & McGraw, 2014; Hoppe-Dominik et al., 2011; N'Goran et al., 2013; Refisch & Koné, 2005a,b), notwithstanding its prohibition by law in the whole country. Approximately 250,000 kg of Cercopithecidae bushmeat are sold annually in the markets of the villages adjacent to TNP (Refisch & Koné, 2005b). Among the 12 different primate species inhabiting TNP, the Bay colobus *P. badius badius* (one of the two Western red colobus subspecies in this study) and the King colobus *C. polykomos* are amongst the most hunted (Refisch & Koné, 2005a).

Cantanhez Forests National Park (CFNP), in Guinea-Bissau, is located in the southwest area of the Tombali administrative region, in the south of the country, and represents the northern border of the Guinean subhumid forests (Figure 1). The park is managed by IBAP—Instituto da Biodiversidade e das Áreas Protegidas ("Institute for Biodiversity and Protected Areas") and covers an agroforest mosaic area of 1,057 km^2 (11° 02″N; 15° 19″W). Contrary to TNP, the forest in CFNP is highly fragmented. There are over 20,000 people distributed among over 100 communities, living in-between forest

blocks and converting the forest into human settlements and plantations (Hockings & Sousa, 2013; IBAP, 2014). CFNP harbors six primate species, including the Temminck's red colobus *P. badius temminckii* (the second subspecies of the Werstern red colobus featuring in this study) and the King colobus (Gonedelé et al., 2020; Minhós et al., 2020). Although CFNP is identified as a key area for the protection of the King colobus and the Temminck's red colobus (Gonedelé et al., 2020; Minhós et al., 2020), genetic analyses revealed that both colobines underwent a recent severe demographic bottleneck consistent with the intensification of anthropogenic activities, such as forest degradation and hunting (Minhós et al., 2016).

2 | METHODS

2.1 | Sample information, DNA extraction, and amplification

We analyzed 31 DNA samples of Western red colobus and 9 DNA samples of King colobus from TNP. Tissue samples were collected

from carcasses or darted specimens found in several surveys between 2004 and 2010 (approximate central point of the surveyed area: 5°50.34′N to 7°19.26′W; for further details please see Calvignac-Spencer et al., 2013; Schubert et al., 2015). In CFNP, colobines' fecal samples were collected between 2009 and 2010. The genetic analyses were conducted using 72 Western red colobus from six social groups and 52 King colobus from eight social groups (see details in Minhós et al., 2016).

We ensured DNA preservation by storing the samples in liquid nitrogen while in the field and at −80°C once in the laboratory until DNA extraction (see detailed information in Leendertz et al., 2010). DNA was extracted from tissue using the DNeasy® Blood and Tissue kit (Qiagen®). Total DNA concentrations of all extracts were measured with a Nanodrop device (Thermo Fisher Scientific). For details on the CFNP fecal sample storage and DNA extraction protocol, please see Minhós et al. (2013a).

All TNP samples were genotyped for 15 human-derived microsatellites, multiplexed in three panels containing five loci each (of which 10 and 11 loci constituted the final data set for Western red colobus and King colobus respectively, see below). For detailed protocols and features of the microsatellite loci see Minhós et al. (2013a). Microsatellite loci were amplified by polymerase chain reaction (PCR). Cycling conditions followed the protocol optimized by Minhós et al. (2013a) with adjustment steps required for the use of tissue samples instead of feces, including the removal of BSA (bovine serum albumin) from the PCR mixture and reduction of the number of cycles from 40 to 25. PCR products were analyzed using an ABI 3130XL Automatic Sequencer at the Genomics Unit at Instituto Gulbenkian de Ciência, Portugal. Alleles were scored using GENEMAP-PER® Software version 4.1 (Applied Biosystems).

2.2 | Quality control of genetic data

Due to the limitations of genotyping microsatellite loci from fecal DNA, as was the case for the CFNP data set, we used the simulation software GEMINI v.1.4.1. (Valiere et al., 2002) to estimate the number of PCR repeats per loci and the number of times an allele needed to be scored to produce genotypes with 95% confidence (see further details in Minhós et al., 2013a). Since the TNP data set was produced from blood-derived DNA, loci were genotyped up to three times. Two independent observers scored the genotypes and the genotypes were considered as heterozygous after each allele was observed in at least two independent PCR reactions. Wright's F_{IS}, quantifying departures from the Hardy–Weinberg equilibrium (HWE), and linkage disequilibrium (LD) between pairs of loci were calculated using GENEPOP (Raymond & Rousset, 1995; Rousset, 2008). The loci were tested for null alleles, large allele dropout and scoring errors, using MICRO-CHECKER (Van Oosterhout et al., 2004). The probability of identity among siblings (PI_{sibs}) by locus, accounting for the presence of related individuals in the sample (Waits et al., 2001), was estimated using GENALEX 6.5 (Peakall & Smouse, 2006, 2012). The quality index, a genotype reliability evaluation method which scores consensus

genotypes with a value between zero and one depending on the consistency among replicates, was calculated following Miquel et al. (2006).

2.3 | Genetic diversity and population structure

Genetic diversity was estimated for each species as the number of alleles per locus (n_A), effective number of alleles (n_E), the observed (H_O) and unbiased expected heterozygosity (H_E, Nei, 1978) using GENALEX 6.5 (Peakall & Smouse, 2006, 2012). The uneven allelic richness and the coefficient of inbreeding (F_{IS}) following Weir and Cockerham (1984) were estimated in FSTAT (Goudet, 2001). Departures from the HWE and LD between pairs of loci were calculated using GENEPOP (Raymond & Rousset, 1995; Rousset, 2008).

Population genetic structure within TNP was assessed using STRUCTURE 2.3.4 (Pritchard et al., 2000). Due to the small sample size for King colobus ($n = 8$), this analysis was only run for the Western red colobus. We varied the number of genetic clusters (K) between one and five and for each K value we performed five independent runs with an initial 100,000 burn-in period followed by 1,000,000 MCMC iterations. We ran the program under the admixture model with correlated allele frequencies among populations, which assumes that each individual has contributions from one or more genetically distinct sources. We visually checked the convergence of the obtained likelihood of K for each run. We applied the Evanno et al. (2005) summary statistic ΔK which finds the uppermost level of structure in a given data set through comparing the rate of change in the estimated likelihood between sequential K values, as implemented in STRUCTURE HARVESTER (Earl & vonHoldt, 2012) and estimated the posterior probability of K (Pritchard et al., 2000) as to confirm the most likely number of K that better explains the distribution of genetic diversity among the sampled individuals.

We also assessed patterns of genetic variation and structure within the TNP large forest through principal components analysis (PCA) in R (R Core Team, 2021) using the packages adegenet (Jombart, 2008; Jombart & Ahmed, 2011), ade4 (Bougeard & Dray, 2018; Chessel et al., 2004; Dray & Dufour, 2007; Dray et al., 2007; Thioulouse et al., 2018), ggplot2 (Wickham, 2016), and cowplot (Wilke et al., 2021). We used the dudi.pca function, replacing missing values by the mean frequency, centering by the mean, and representing the two first axes. This analysis was performed for both taxa.

2.4 | Demographic history

To detect past population size changes in Western red colobus and King colobus at TNP, we used a likelihood-based Bayesian method as implemented in MSVAR 1.3 (Storz & Beaumont, 2002). This method estimates the posterior probability distribution of N_0, N_1, T, and μ, that is, current and past population sizes, time (in years) and per locus mutation rate, respectively, under a simple

model of exponential population size change (allowing increase or decrease). For each data set (King colobus and Western red colobus), we performed four independent runs with different starting points and different sets of priors and hyperpriors to test the influence of the prior on the posterior distributions (see Supporting Information: Table S1). We varied prior distributions such that they would assume (a priori) either constant (same prior for N_0 and N_1), decreasing (prior values larger for N_1) or increasing (prior values larger for N_0) population demographic models. For the stationary population we fixed N_0 (present) and N_1 (past) population sizes to 10,000 (mean $\log(N) = 4$) and prior T to 100,000 years (mean $\log(T) = 5$). Lognormal prior distributions were chosen for the scenarios of population size change. For the population decrease we set the mean N_1 to 100,000 (mean log $(N_1) = 5$), N_0 to 1,000 (mean $\log(N_0) = 3$) and prior T to 100,000 years ($\log(T) = 5$). For the two population expansions we fixed N_0 to 100,000 (mean $\log(N_0) = 5$) and we varied mean N_1 between 10,000 and 1,000 (mean $\log(N_1) = 4$ and 3, respectively) corresponding to relatively moderate and strong expansions, respectively, and we set the prior mean T to 10,000 years ($\log(T) = 4$ scale). We set a mean mutation rate $\log(u)$ to -3.5, supporting mutation rates of 10^{-4} to 10^{-3} as widely assumed in demographic analyses (Storz & Beaumont, 2002). We fixed the standard variation to 1 (in $\log_{10}$ scale) for all parameters. Each run was performed with 300,000 thinning update steps and 30,000 thinning intervals, that is, 9×10^9 steps. We removed the first 10% of each independent simulation to avoid influence in parameter estimation by starting conditions (burn-in). The Brooks, Gelman, and Rubin convergence diagnostic test (Brooks & Gelman, 1998; Gelman & Rubin, 1992) was used to check the convergence of the chains in all runs (close to one for both species) in addition to a visual check of the MCMC chains.

Since we were interested in understanding if recent anthropogenic events were responsible for possible signals of population decline (or changes in connectivity, but see Section 4), we estimated the probability that the data (posterior T) favored recent over more ancient demographic events. To do that, we calculated the Bayes factor (BF) to detect regions that confer high support for a given T interval. We divided time in windows of 50 years length corresponding to a total of 2,000-time intervals, from 0 up to 100,000 years before present. For each time interval we tested the hypothesis that a demographic change occurred within that time interval (for instance, $100 < T \leq 150$) relative to the hypothesis of it having occurred in any other time interval (i.e., outside that interval; $T \leq 100$ & $T > 150$). The weight of evidence of a demographic change within versus outside the defined time window is given by the BF, which calculates the ratio of the posterior densities over the ratio of the prior densities. In other words, this ratio shows how much the posterior support (likelihood) for a given interval has improved in comparison to the prior support for that same time interval. We considered BF values >3 as positive evidence for demographic change at a given time interval and values >7 as significant (Storz & Beaumont, 2002).

2.5 | Mitochondrial DNA (mtDNA)

We sequenced a fragment of the hypervariable domain of the mitochondrial control region for 31 Western red colobus and 9 King colobus from TNP. We used the primers L15449 (5′-CCRC CAATACCCAAAACTGG-3′) and H15973 (5′-AGGAGAGTAGCACT CTTGTGC-3′), and the concentration of the PCR reagents described in Minhós et al. (2013a). The PCR conditions were: initial denaturation for 15 min at 95°C; 35 cycles of denaturation for 30 s at 94°C, annealing for 90 s at 63°C, and extension for 90 s at 72°C; and a final extension for 15 min at 72°C. PCR products were sequenced in both directions using the Sanger method. One Western red colobus sample and one King colobus sample were discarded after several failed attempts to produce good quality sequences. For all other samples, the forward and the reverse sequences were aligned to reach a consensus sequence for each sample. All consensus sequences were then aligned and trimmed to the length of the shortest sequence (463 bp for Western red colobus and 444 bp for King colobus). The final data set of mtDNA sequences consists of 28 Western red colobus and 7 King colobus individuals.

We calculated haplotype and nucleotide diversity, constructed haplotype networks, and performed a PCA in R (R Core Team, 2021) using the packages pegas (Paradis, 2010), ape (Paradis & Schliep, 2019), adegenet (Jombart, 2008; Jombart & Ahmed, 2011) and ade4 (Bougeard & Dray, 2018; Chessel et al., 2004; Dray & Dufour, 2007; Dray et al., 2007; Thioulouse et al., 2018). We also estimated haplotype richness (Hurlbert, 1971) as a way to control for differences in sample sizes between the two protected areas, using the software Contrib v1.4 (Petit et al., 1998) and setting the rarefaction size to seven (number of the smallest data set in this study).

3 | RESULTS

3.1 | Genotyping and quality control

Three markers were excluded from the Western red colobus data set (D1s548, D12s372, D10s1432) and two markers were removed from the King colobus data set (D12s372, D10s1432) due to peaks with unexpected shapes or falling outside marker bins. Further, one marker in each species was removed due to the lack of amplification in all samples except one (D10s611 for both Western red colobus and King colobus). One marker was removed in each species due to significant deviations from the HWE (D2s442 and D10s676 in Western red colobus and King colobus, respectively). We removed one King colobus sample and two Western red colobus samples which showed no positive amplification for any of the 15 microsatellites.

The MICRO-CHECKER analysis did not reveal the presence of null alleles, large allele dropout, or scoring errors for the Western red colobus. It highlighted an excess of homozygotes at the loci D2s1326

and D11s2002 for the King colobus, which might be due to the low sample size of this database.

The PI$_{sibs}$ was estimated as 1.5×10^{-5} for Western red colobus and 2.1×10^{-5} for King colobus, thus supporting that different genotypes in the data set correspond to different individuals. After all procedures, the mean number of successfully genotyped loci per sample was 9.93 (7–10 microsatellite loci) and 10.38 (7–11 microsatellite loci) for Western red colobus and King colobus, respectively. The percentage of missing data was 1.7% for Western red colobus and 5.7% for King colobus. The final data set for TNP contained 29 Western red colobus and 8 King colobus, with quality indices of 0.84 and 0.82, respectively. The same detailed information is available for the CFNP databases in Minhós et al. (2013a; 52 King colobus samples, genotyped for 11–14 loci, with quality index of 0.84; 72 Western red colobus, genotyped for 10–13 loci, with quality index of 0.77).

3.2 | Genetic diversity

Despite the smaller TNP sample size, both species showed higher genetic diversity in TNP when compared to CFNP. In addition, King colobus showed a lower genetic diversity than Western red colobus in TNP as had been found in CFNP by Minhós et al. (2013a). The King colobus and the Western red colobus from TNP yielded a higher average number of alleles per locus than in CFNP (Table 1; 5.5 and 10.1 alleles per locus compared to 4.2 and 5.2, respectively). The genetic and genotypic diversity measured as H_E and H_O, respectively, were much higher in TNP than in CFNP (Table 1). The intraspecific difference in the genetic diversity between protected areas was higher for the Western red colobus than for the King colobus (Table 1). F_{IS} values were slightly positive in TNP and slightly negative in CFNP, but not significant.

3.3 | Genetic structure

The STRUCTURE analysis performed for the Western red colobus in TNP did not show any pattern of genetic structure (Supporting Information: Figure S1). Although ΔK peaked at $K = 2$, the individual membership coefficients showed equal posterior probability of belonging to either cluster. Indeed, the highest likelihood of K values was found at $K = 1$ (Western red colobus: $P(K1) = 0.999$, $P(K2) = 0.001$), confirming the lack of strong differentiation between these samples. The PCA corroborates this result (Supporting Information: Figure S2). The PCA for the King colobus of TNP also does not show any clear pattern of genetic structure (Supporting Information: Figure S3).

3.4 | Demographic analyses

Posterior estimates of the time (T) of the start of putative population size changes varied across species and we observed bimodal posterior distributions in the case of TNP populations, whereas no such bimodal posteriors had been observed in CFNP (Figure 2). The mode corresponding to the oldest times appears to be unrealistically large (>10^6 to 10^9 years). Indeed, if a population size change had taken place that far back in time, the populations would have reached a new equilibrium and no population size change could have been detected. We had allowed for priors with large T values to avoid favoring recent times, as has been done in previous studies. However, as such ancient T values would be meaningless, we discarded these old times and did not consider T values that were that large for the inference of the other parameters. In other words, all posteriors for N_0 and N_1 presented here were computed by conditioning on T values being smaller than an arbitrary threshold (chosen as 10^5; see Section 4). Before providing results for the T

TABLE 1 Genetic diversity measures from the present study and other African colobus available in the literature

Taxon	Species/subspecies	Park	Origin	Nsam	n_a	n_e	H_O	H_E	F_{IS}	AR	Source
King colobus	C. polykomos	TNP	Ivory Coast	8	5.5	5.00	0.69	0.79	0.14	5.08	This study
	C. polykomos	CFNP	Guinea-Bissau	52	4.2	-	0.48	0.42	−0.14	2.06	Minhós al. (2013a)
Western red colobus	P. badius badius	TNP	Ivory Coast	29	10.1	5.21	0.79	0.81	0.03	9.96	This study
	P. badius temminckii	CFNP	Guinea-Bissau	72	5.2	-	0.54	0.51	−0.05	1.63	Minhós et al. (2013a)
Tana river red colobus	P. rufomitratus	-	Uganda	78–85	7.7	4.32	0.70	0.72	0.04	-	Allen et al. (2012)
	P. rufomitratus[(1)]	-	Uganda	24–28	5.7	3.93	0.64	0.71	0.10	-	Miyamoto et al. (2013)
				31–36	6.4	3.90	0.72	0.71	−0.01	-	
	P. rufomitratus	-	Kenya	72	5.6	-	0.71	0.70	−0.02	1.68	Mbora and McPeek (2015)
Udzungwa red colobus	P. gordonorum	-	Tanzania	121	4.9	3.12	0.67	0.65	−0.02	4.42	Ruiz-Lopez et al. (2016)

Note: The first four lines show genetic diversity summaries comparison between Western red colobus and King colobus species at the Taï National Park (TNP; this study) and Cantanhez Forests National Park (CFNP; Minhós et al., 2013a) using the same set of microsatellite loci.

Abbreviations: AR, allelic richness; F_{IS}, inbreeding coefficient; H_E, expected heterozygosity; H_O, observed heterozygosity; n_a, number of different alleles; n_e, number of effective alleles; Nsam, number of successfully genotyped samples.

[(1)] Values measured within two social groups, in contrast to measures in other studies which represent summaries per population.
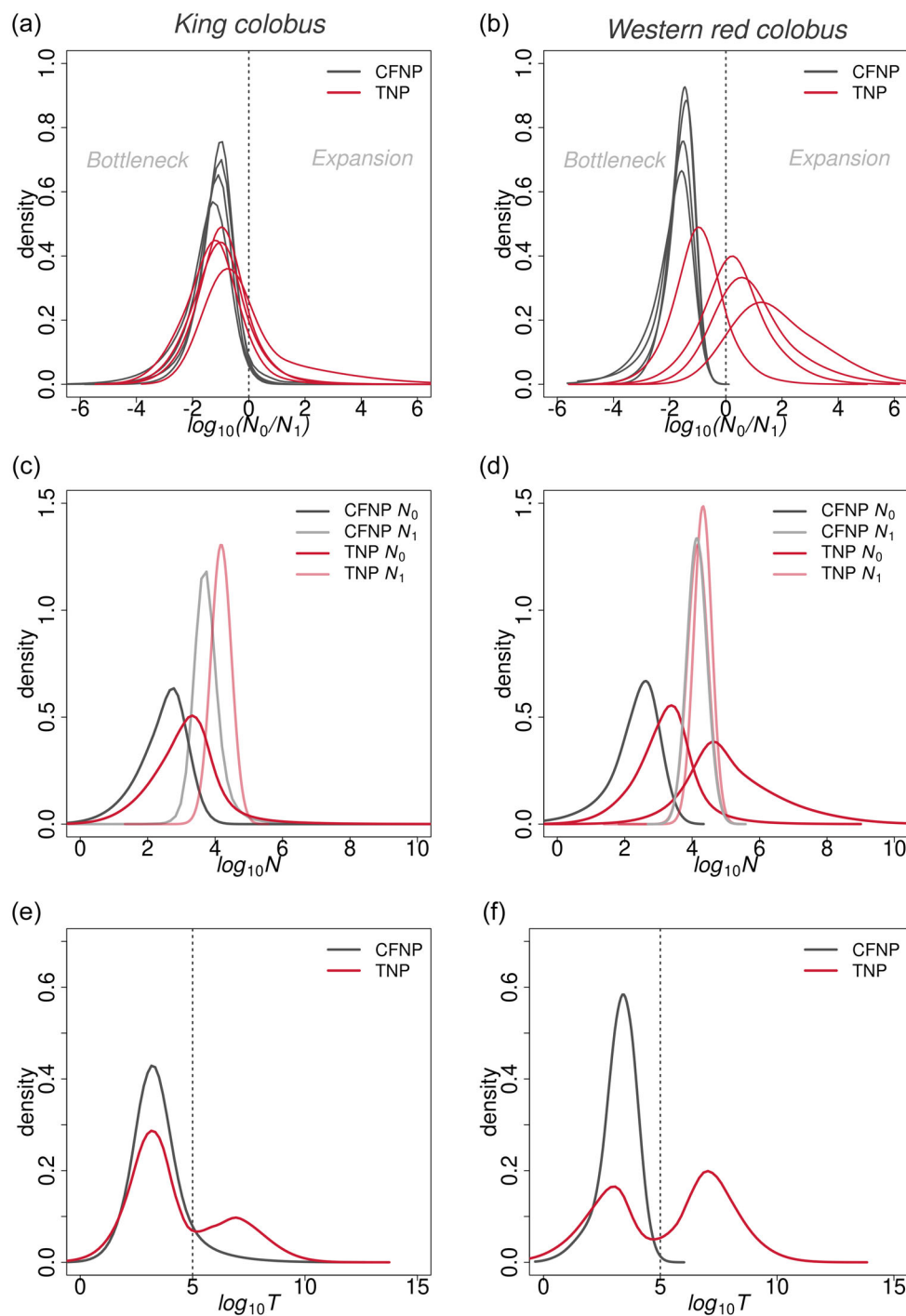
FIGURE 2 Posterior distributions of effective population size change (N_0/N_1), estimated present (N_0) and past (N_1) effective population size and time (T) since demographic change. Each panel shows results obtained for Taï National Park (TNP: red lines) and Cantanhez Forests National Park (CFNP: black/gray lines, as obtained in Minhós et al., 2016). In the left column: King colobus *Colobus polykomos* (also named black-and-white colobus in other studies, including Minhós et al., 2016); in the right column: Western red colobus *Piliocolobus badius*. a and b: Posterior distributions of the ratio of present to past population size (N_0/N_1). A value equal to zero (log_{10} scale), represented by the dashed vertical line, corresponds to the absence of population size change. The N_0 and N_1 posteriors are conditioned to posterior $log_{10}(T) < 5$ (100,000 years before present). Each curve shows results obtained from one independent run. c and d: Posterior distributions of past (N_1) and present (N_0) population sizes. The posterior distributions of present size are represented by dark colors and past population sizes are represented by lighter colors. e and f: Time (in years before present) since population started to decline/expand. The dashed vertical line at $log_{10}(T)=5$ (100,000 years) corresponds to the time below which we conditioned all posterior values. Note that (c–f) show results from one single run for King colobus (run 3 corresponding to the priors favoring a severe expansion model; see Supporting Information: Table S1). For Western red colobus in TNP (red lines), two estimated posterior are shown (runs 2 and 3) as results were not consistent among runs. For all the results, see Supporting Information Figure S4.

posterior distributions, we first turn to the results for N_0 and N_1 and to their robustness.

In the King colobus sampled in TNP, we found that the four priors tested using MSVAR1.3 did not lead to major differences in the posteriors of T, N_0, and N_1 (Supporting Information: Table S2 and Figure S4), suggesting that King colobus results are robust to prior specifications. Also, the fact that prior means of N_0 and N_1, the present and past population sizes, differed from each other by one order of magnitude suggests that there is significant information in the data leading to the same or very similar posteriors for the King colobus (Supporting Information: Table S2 and Figure S4). For the Western red colobus the results changed with the priors, sometimes suggesting a stationary, slightly increasing or decreasing population (Supporting Information: Table S2 and Figure S4). However, the inferred changes were never strong and thus we find no clear signal of population size change in the TNP Western red colobus population.

In a previous study on the same two species sampled in CFNP, we identified significant differences between the posteriors obtained for present (N_0) and past (N_1) population sizes (Minhós et al., 2016; black posteriors in Figures 2a,b), suggesting either major population decreases or the confounding effect of population structure (Chikhi et al., 2010). The new results obtained for TNP contrast with the ones from CFNP, as there is either partial overlap between N_0 and N_1 posteriors in the Western red colobus with no clear signal of size change (Figure 2b,d) or a possible signal of limited decrease in the King colobus (Figure 2a,c). More specifically, in the King colobus from TNP, the posteriors identified a possible decrease in population size, with posteriors median N_0/N_1 between 0.05 and 0.26 across all scenarios. This could correspond to a ~4 to 20-fold decrease but could also be compatible with no size change, due to the variance of the estimates.

Indeed, Figure 2 shows that the distributions of present (N_0) and past (N_1) King colobus population sizes in TNP have a large overlap, with medians between ~770 and 4,170 for N_0 and ~14,450 and 15,100 for N_1. Compared to the decreases detected in CFNP, this corresponds to a less evident decrease (CFNP posterior N_0/N_1 medians between 0.04 and 0.08, corresponding to a minimum 12.5 to 25-fold population size decreases, with no overlap between N_0 and N_1 estimates). In the Western red colobus data set, the posterior N_0/N_1 median was between ~0.11 and 39, thus indicating a change that could correspond to a ~9-fold population decrease, through no demographic changes (~1) to a ~39-fold population increase, supporting either a decreasing, stationary or an increasing population for this species depending on the priors. The estimates of current and past population sizes were respectively between ~1,700 and 870,000 (median N_0) and between ~14,500 and 21,900 (median N_1). The large variance in N_0 estimates explains the contradictory inferred demographic histories. However, in all cases they reveal that present-day populations (N_0) are likely much larger for Western red colobus than for King colobus.

Note that overall posterior distributions were wide and therefore the 95% HPD extended over a large range of values (Supporting Information: Table S2). For Western red colobus, we found estimates of T to be between ~550 and 1,500 years before present (Figure 2 and Supporting Information: Table S2). The BF analysis allowed us to determine the most likely period of time during which a putative demographic event would have taken place. We found that the BF tended to favor recent events with the last 2,000 recent years exhibiting the highest BF values (Supporting Information: Figure S5). In particular, there is positive evidence for the hypothesis that the population decrease took place in the last century in both King colobus and Western red colobus (BF ≥3 in all runs, including significant BF ≥7 in runs 1 and 2).

3.5 | Patterns of mtDNA diversity

After controlling for data quality, we obtained 7 sequences trimmed to 444 bp for the King colobus and 28 sequences trimmed to 463 bp for the Western red colobus. We detected five different haplotypes within the King colobus population, a haplotype diversity (Hd) of 0.8514 (±0.0161), and a nucleotide diversity (π) of 0.0381 (±0.0005). The mtDNA diversity found for the King colobus from TNP was substantially higher when compared with the population from CFNP (Hd of 0.17 and π of 0.0004) (Table 2). Contrary to CFNP, we found highly divergent haplotypes in the TNP King colobus' population, with five of the seven individuals showing a private mtDNA variant. Western red colobus showed 24 different haplotypes out of the 28 individuals, with an Hd of 0.9868 (±0.0001) and a π of 0.0792 (±0.0016). The Western red colobus' TNP population also exhibits higher levels of mitochondrial diversity but the difference between this and the CFNP population was smaller (Hd of 0.82 and π of 0.0370). Similarly to the King colobus, the Western red colobus from TNP also exhibit highly divergent mtDNA lineages with most of the individuals showing unique haplotypes. The figures of haplotype richness, which allow controlling for different sample sizes among datasets, confirm these results and show higher levels of diversity for both species in TNP. King colobus show a much higher difference in this statistic (5.00 in TNP and 1.55 in CFNP) than Western red colobus (6.73 in TNP and 4.27 in CFNP), which is interestingly the opposite of what we saw with the number of alleles in microsatellites. Consistent with the pattern found in CFNP, the TNP Western red colobus population is more diverse than the King colobus for mtDNA, in agreement with the microsatellite results (Table 2). As for structure, TNP colobines seem to show some genetic clustering at the mtDNA genome (Table 2, Supporting Information: Figures S6 and S7).

4 | DISCUSSION

In this study, we aimed at quantifying the genetic diversity, population structure, and population size changes of colobine populations inhabiting a large and continuous forest in TNP, Ivory Coast. We compared the results obtained in TNP to those previously

found in closely related colobine monkey populations inhabiting a fragmented forest in CFNP, Guinea-Bissau (Minhós et al., 2016; Minhós et al., 2013a). We found that the two species have contrasting patterns of genetic diversity but that both lack conspicuous nuclear genetic structure in TNP, as had also been found in CFNP. The Western red colobus showed higher levels of genetic diversity in TNP when compared with the sympatric King colobus at both nuclear and mitochondrial markers. This was again in agreement with the results of CFNP, suggesting that population sizes of Western red colobus are larger than those of King colobus in both national parks.

4.1 | The effect of habitat fragmentation on genetic diversity and population structure

Both the Western red colobus and King colobus sampled in TNP exhibited high levels of nuclear genetic diversity when compared with the other few studied African colobine populations and species

(Table 1). In contrast, at CFNP both species showed the lowest nuclear genetic diversity of all African colobines studied so far (H_E = 0.51 and 0.42 compared to 0.81 and 0.79 in TNP for Western red and King colobus, respectively, Table 1). It has been suggested that, for certain species, populations located at the margins of distributions should be less genetically diverse than those present in more central areas, and this has been described in some species (Eckert et al., 2008). The fact that CFNP populations are located at the western margin of the colobine distribution might contribute, in part, to the lower genetic diversity in these populations. This has been suggested for Cross river gorillas (*Gorilla gorilla diehli*), which represent the westernmost subspecies of gorillas, using microsatellite loci. Central subspecies exhibit significantly higher levels of genetic diversity than the western and the eastern ones (Bergl et al., 2008). This also seems to be confirmed by the lower mtDNA diversity in CFNP compared to TNP.

We also found extremely high levels of genetic diversity for the mtDNA of the two TNP colobines, with most of the individuals showing unique haplotypes in both species. However, the difference

TABLE 2 Mitochondrial DNA results in Taï National Park (TNP) and Cantanhez Forests National Park (CFNP)

		TNP (this study)	CFNP (Minhós et al., 2013a)
King colobus (*C. polykomos*)	Genetic diversity	Nsam = 7 H =: 5 Hd 0.85 π = 0.03810 HR = 5.000	Nsam = 56 H = 3 Hd = 0.17 π = 0.00038 HR = 1.548
	Haplotype network		
Western red colobus (*P. badius*)	Genetic diversity	Nsam = 28 H = 24 Hd = 0.99 π = 0.07923 HR = 6.733	Nsam = 79 H = 9 Hd = 0.82 π = 0.03700 HR = 4.267

TABLE 2 (Continued)

TNP (this study)	CFNP (Minhós et al., 2013a)

Haplotype network
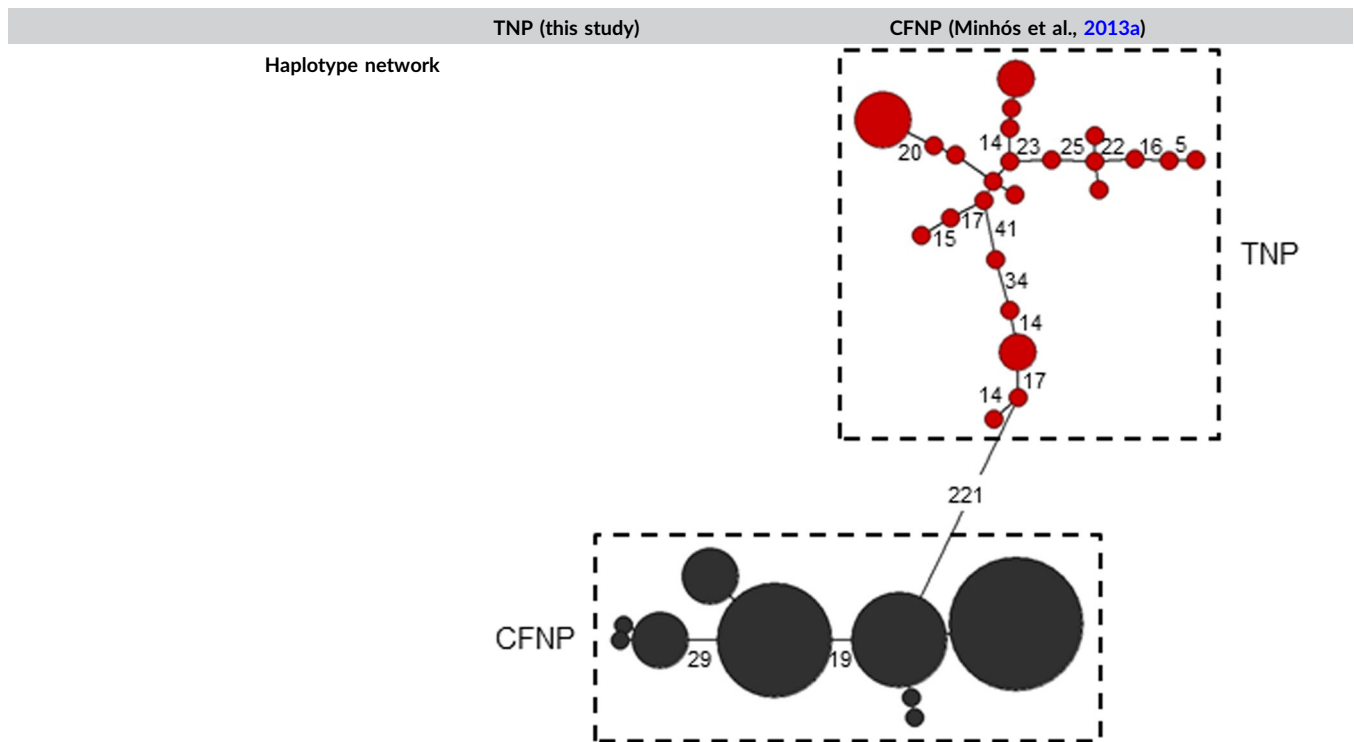
Note: Circles represent haplotypes whereas numbers between circles correspond to the nucleotide differences between haplotypes (in bp). In TNP, the area of each circle is proportional to its frequency. The CFNP data set used in this figure is a subset of the original data set and contains a total number of haplotypes equal to that of TNP (maintaining the original haplotype proportions) so as to make the visualization of the TNP haplotype frequencies easier. HR was calculated using a rarefaction size of 7, the number of sequences in the smallest data set.

Abbreviations: H, number of haplotypes; Hd, haplotype diversity; HR, haplotype richness; *Nsam*, number of successfully sequenced samples; π, nucleotide diversity.

in mtDNA diversity levels between TNP and CFNP is not as marked for the Western red colobus as it is for the King colobus. Since these comparisons would need to be accompanied by simulations to better quantify the expected levels of genetic diversity and thus determine whether the "margins" hypothesis is valid, we suggest at this stage that geography alone may not fully explain the strong difference found in nuclear genetic diversity. Another indication that caution is needed comes from the fact that the endemic red colobus population from Tana River, Kenya, exhibits higher levels of genetic diversity than CFNP, despite being at the eastern end of the red colobus distribution (Table 1; Mbora & McPeek, 2015) suggesting that the edge distribution or margin effect is unlikely to be the only factor explaining the low diversity in CFNP. In addition, the fact that the Western red colobus is a complex of subspecies (*P. b. temminckii* and *P. b. badius*) with distinct evolutionary histories may also play a role in their genetic differences. However, again, it should not fully explain the differences as the King colobus are the same taxon in both protected areas and exhibit the same patterns of genetic diversity as the Western red colobus.

In any case one should be very cautious before applying this kind of "theory" when moving from populations within a species to a loosely connected but complex set of closely related subspecies, that

may have had time to accumulate genetic diversity in the newly colonized regions, even when these regions are at the "edge" or margin. Indeed, a subspecies located in an edge today could harbor important levels of diversity if it is less threatened than central populations facing habitat loss in the recent past.

The lower nuclear genetic diversity of CFNP colobines, compared to TNP colobines, might be better explained by recent events and changes in their habitat and population sizes. Another research that has studied the effects of forest fragmentation on forest dwelling primates yielded results similar to those of CFNP compared to TNP. Bergl et al. (2008) used microsatellite loci to study four populations of gorillas (*Gorilla* spp.) exposed to varying levels of habitat disturbance and fragmentation, and found reduced diversity in the populations living in the most fragmented environments. For example, individual heterozygosity was significantly higher for *Gorilla gorilla gorilla* in the large, continuous and presumably relatively undisturbed population, when compared to three other more fragmented populations. Another study, analyzing microsatellite diversity of Brazilian pied tamarins (*Saguinus bicolor*) at sites recently fragmented by human activity concluded that, although genetic diversity had not been drastically reduced, samples collected before fragmentation were more genetically diverse than present-day ones

(Farias et al., 2015). Yet another example can be seen in a similar analysis of snub-nosed monkeys (*Rhinopithecus roxellana*) in three populations in China (Chang et al., 2012). The authors found the lowest levels of genetic diversity in the population located at the eastern limit of the species distribution in the country (Shennongjia Nature Reserve), which was also the smallest population in size and was present in the most highly fragmented habitat. These examples highlight the power of genetic data to differentiate populations among sites with varying levels of habitat fragmentation and the effect of forest degradation eroding the genetic diversity and the putative evolutionary potential of forest dwelling primates.

We detected no significant differences in the nuclear genetic structure of both King colobus and Western red colobus populations within each of the two national parks. Indeed, colobine monkeys from TNP and CFNP do not appear to be genetically structured across the landscape. While this was perhaps not a surprising finding in TNP as both species occupy a large, continuous and well-preserved block of forest, it was not expected in CFNP. We note however that although Minhós et al. (2016) did not detect genetic structure in the fragmented forests of CFNP, they detected fine-scale genetic structure when assessing isolation-by-distance and spatial auto-correlation patterns. It may be too early to conclude but these previous results may indicate an ongoing fragmentation process restricting the dispersal between groups across the increasingly fragmented landscape of CFNP. If this interpretation is correct, this suggests that TNP can still be seen as a continuous habitat, for colobine monkeys at least, and should be preserved as such.

Our analyses of genetic structure for the mtDNA suggest some level of haplotype clustering (Table 2). However, since we did not have GPS data for the TNP individuals, we cannot know at this stage whether the divergent haplotypes correspond to individuals from different locations (or social groups) or from a large and well-mixed population. The high mtDNA diversity found for the King colobus in TNP contrasts with the very low diversity found for this primate in CFNP. This was not the case for the Western red colobus for which mtDNA diversity was high in both protected areas. These results suggest that the diversity and structure of the mitochondrial diversity of these colobines are better explained by a complex interplay between the demography of the populations with the evolutionary history of the species and their socio-ecological features (e.g., dispersal mode) and cannot be simply attributed to one of these factors alone, or a simple "edge theory."

4.2 | Are TNP colobine populations still large and stationary?

The results obtained for the demographic histories of colobines from TNP and CFNP are also contrasting. Whereas CFNP Western red colobus and King colobus exhibited genetic signals of a severe population bottleneck, estimated to have occurred over the last 200 years, most likely as a result of human activities (e.g., human population growth in the region and subsequent overexploitation of

natural resources; Minhós et al., 2016), the TNP colobines were inferred to have suffered less (for King colobus) or to have not suffered at all (for Western red colobus) from a similarly important demographic event. We did find a signal for a very recent event in King colobus but its importance was much more limited and within statistical error. As for Western red colobus, there was no strong data going against the idea that they had been living in large and stationary populations during historical times. Thus, our genetic data suggest that the large and continuous forest of TNP has provided beneficial conditions to either protect the two species from human activities or to maintain large, stationary and well-connected colobus monkey populations.

This should not however be taken as suggesting that TNP colobines are not under threat. Other studies suggest that they are also under increasing hunting pressure (Refisch & Koné, 2005a). Refisch and Koné (2005a) have estimated a total annual harvest from TNP of 66,121 kg of Western red colobus and of 45,490 kg of King colobus, which makes these two primates the most hunted species in the area, with over 110 tons (tens of thousands of individuals) being harvested every year. The fact that the TNP forest is still large, apparently continuous and that the canopy structure is well-preserved may have been protecting these colobus monkeys from being highly exposed to hunters, but it is clearly not enough to prevent intense harvest rates. The level of forest preservation in TNP may not only provide the ecological conditions for these primates' subsistence but also limit the level of accessibility to poachers and provide a certain degree of protection against intensive hunting. It is also known that the continuous and long-term presence of researchers in this protected area has acted as a buffer towards the increase of the hunting pressure on nonhuman primates (Campbell et al., 2011; Hoppe-Dominik et al., 2011). Therefore, it is likely that primate hunting is more limited in TNP than in CFNP, as a result of the combined effect of forest preservation and long-term research. Nonetheless, the human populations living in the neighborhood of the TNP have been increasing in the past 20 years and population growth has led to increasing preassure for land for cultivation. The buffer zone of the TNP forest has been replaced by cash and food crops and is now fragmented and severely degraded, in the peripheral limits of the park new roads were built and gold panning is carried out in the central region. In addition, Ebola fever has been affecting wildlife in West Africa (Osterholm et al., 2015) and chimpanzees specifically in TNP (Formenty et al., 1999).

In Guinea-Bissau, colobus monkeys are among the most hunted primates (Minhós et al., 2013b). Although harvest rates are unknown for CFNP, six different primate species are traded in two Bissau bushmeat markets at an estimated minimum of 1,550 individuals per dry season, which includes 183 Western red colobus and 34 King colobus (Minhós et al., 2013b). These animals, traded and consumed in urban areas, do not represent the total harvest from the source forests. The impact of hunting on primates in Guinea-Bissau is thus certainly much larger than these minimum estimates. Therefore, it is not yet possible to have a direct comparison between hunting pressure in TNP and CFNP, but the numbers are clearly not low in

both regions. The critical conservation status of the colobines in CFNP is most likely the result of not only the forest destruction and fragmentation but also of the increased hunting that is facilitated by the easier accessibility to the forests (Robinson, 1996; Turner & Corlett, 1996). Nonetheless, hunting pressure results from a combination of factors, such as human population density, accessibility to the forests (by road), domestic and international trade, availability of prey animals and alternative protein sources (including livestock production), cultural practices and the resilience of species to hunting, and not only from the size and connectivity of the forest, making the motivation underlying its occurrence overly complex to measure.

Our genetic data cannot exclude the possibility of ongoing population decrease in TNP as a result of recent intense hunting, due to the unavoidable limitations of genetic data in detecting very recent events in large populations. The posterior distribution of T was indicating a recent but not necessarily very recent event for the King colobus. Interestingly the BF analysis tended to favor the most recent times. At this stage we should be cautious regarding the dating of an event that is quantified as mild. We also note that many tropical forest dwelling species have been suffering increased hunting pressure or habitat loss, yet many show no strong signal of population decrease in their genomes, at least thus far (Ferreira da Silva et al., 2021a; Sgarlata et al., 2016, 2018; Storz et al., 2002; Wang et al., 2019). This may be misinterpreted as indicating that such anthropogenic pressures have no effect in many primate species. Demographic changes may take a few generations for their genetic signature to become detected. Population genetic methods may fail to detect demographic changes occurring in the very recent past of species (<2–10 generations) (Peery et al., 2012), although there are cases in which severe population declines are detected in less than 10 generations, as was likely the case for the Bornean orangutans (Goossens et al., 2006). For instance, in Guinea baboons (*Papio papio*) in Guinea-Bissau, population genetic methods failed to detect a signal of population decline (Ferreira da Silva et al., 2021b) despite (i) evidence of commercial hunting (Minhós et al., 2013b), (ii) hunters reporting extensive pressure and perceiving baboon populations to be decreasing and even disappearing from certain areas in the last 30 years (Ferreira da Silva 2021b) and (iii) the intensity of hunting practices in Guinea-Bissau toward baboons that may have impacted dispersal of this population to the extent of disrupting the female-biased gene flow pattern that is thought to be characteristic of the species (Ferreira da Silva et al., 2018). Other mammals with similar generation times may also present a discrepancy between documented population declines and levels of genetic diversity and detection of bottlenecks events using molecular data. Moura et al. (2014) found high levels of microsatellite heterozygosity in European wolves (*Canis lupus*) in Bulgaria, despite intense unregulated hunting. Altogether, these studies show that population declines due to recent intensification of hunting may not be easily detected using a relatively limited panel of microsatellite loci in slow reproductive species. Thus, we cannot exclude the possibility of ongoing population decrease in the TNP because of recently increased hunting pressure.

4.3 | Genetics applied to species conservation

We should stress at this stage of the discussion that we are aware of the difficulty in properly and unambiguously estimating and dating population size changes when populations are structured (Beaumont, 2004; Chikhi et al., 2010, 2018; Goossens et al., 2006; Mazet et al., 2016; Quéméré et al., 2012; Wakeley, 1999). We thus do not take the estimated population size changes at face value. We call for the use of complex modeling and demographic inference. If we wish to clarify the role of anthropogenic activities and climate change in changes in diversity patterns in endangered species, we will need to account for population structure, habitat fragmentation, changes in connectivity, and in population size, as was for instance done in lemurs by Salmona et al. (2017) or Teixeira et al. (2021a, 2021b) (see also Arredondo et al., 2021). While genetic data may be more difficult to interpret than is usually believed, we strongly defend the idea that genetic information obtained from threatened species can and should contribute to the decision making of stakeholders involved in biodiversity conservation.

Our study uses different types of molecular markers to study and quantify the genetic consequences of habitat fragmentation and stresses the importance of maintaining large and well-preserved forests to ensure these primates long-term survival. Despite bushmeat hunting in both national parks, the fact that colobine populations from TNP inhabit a large and continuous forest has likely allowed for the maintenance of high levels of genetic diversity and thus of evolutionary potential. We do not, however, dismiss the very likely chance of the TNP colobus populations being negatively impacted by the intense hunting and probably declining in numbers (Bitty et al., 2015; Kouassi et al., 2017; Refisch & Koné, 2005a). We worry that the genetic consequences of the recent increase in the harvest rates might only be unraveled in the next few decades, when it might be too late. The fact that TNP colobine populations still display high levels of genetic diversity leaves us optimistic. If hunting is controlled and overexploitation prevented, the colobines from TNP have the potential to persist in the forest in the long-term and be an important source of genetic diversity through dispersing individuals to other areas if connectivity is maintained or restored. If no measure is taken to control deforestation and hunting, the population size may decrease too rapidly, before being detected by genetic-based population size estimations, and the populations may reach critically low numbers. The small population size and low genetic diversity of CFNP colobines illustrate how persistent habitat degradation and overexploitation can lead populations to the brink of extinction. This was probably the case of Miss Waldron's red colobus (*Piliocolobus waldroni*) originally found in West African forests. This species was not observed in the field after 1978 and indirect signs (e.g., skins, photographs) were last seen in 2002. It is believed that only a few individuals are left in the forests, illustrating the challenges faced by colobine monkeys in West African anthropogenic landscapes (Oates et al., 2000, 2020). It is urgent that targeted conservation measures are taken for the CFNP colobines so as to prevent their extinction in the park, which would greatly compromise the persistence of both species in Guinea-Bissau (Gonedelé et al., 2020; Minhós et al., 2020; Linder et al., 2021).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The complete genotype microsatellite data set is available at the Zenodo Digital Repository: https://doi.org/10.5281/zenodo.7182015.

ETHICS STATEMENT

This research abided by the American Society of Primatologists guidelines for the ethical treatment of nonhuman primates. All the samples were obtained in collaboration to the Taï National Park local authorities and in compliance with the legislation of Ivory Coast and following international laws.

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SUPPORTING INFORMATION

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