CONTRIBUTED PAPER

Patterns of genetic diversity in African forest elephants living in a human-modified landscape in southwest Gabon

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Abstract
We investigated the patterns of genetic diversity and structure of African forest elephants in a human-modified landscape in the Gamba complex of protected areas (GCPA), a tropical wilderness area along the southwest coast of Gabon. We collected 298 elephant fecal samples from four sites (Sette Cama, Gamba area, Vera Plains, and Mayonami), along approximately 80 km of coastline from north to south. We used microsatellites and mitochondrial DNA (mtDNA) to successfully genotype 295 of the 298 fecal samples, and identified 213 individuals. Using sex markers, we identified 84 males and 118 females; we could not determine the sex of the remaining 11 individuals. We also characterized the sex, group size, and social status of crop-raiding elephants and did not find characteristics distinguishing them from nonraiders. Overall, our mtDNA and microsatellite markers revealed that elephants in the research area maintain high levels of genetic variation and low levels of subdivision. Gene flow appears to be mostly mediated by male dispersal away from natal herds. Our structure analysis revealed two highly admixed genetic clusters, attributable to high connectivity among the protected areas. However, forest areas within the GCPA have become increasingly fragmented by human-induced habitat modification. We detected a pattern of isolation by distance, accentuated by the presence of the town of Gamba between Sette-Cama and Mayonami. We found a high degree of connectivity among sampling locations within the GCPA. This study emphasizes the importance of conducting baseline monitoring of demographic data, genetic diversity, and structure to enable future comparisons to assess the long-term impact of human-induced habitat fragmentation.

KEYWORDS
crop raids, forest elephant, genetics, land cover change

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INTRODUCTION

Previous studies have demonstrated that industrial concessions that implement proper conservation management strategies may not be directly detrimental to endangered forest elephants that occupy their adjacent areas. However, they can create indirect impacts with infrastructure expansion, settlements, and agriculture (Blake et al., 2008; Eggert et al., 2013; Kolowski et al., 2010; Schuttler, Blake, & Eggert, 2012; Vanthomme, Kolowski, Korte, & Alonso, 2013). The Gamba complex of protected areas (GCPA) in Gabon is an example of a place where human-induced landscape alterations are occurring at a rapid rate, potentially indirectly impacting elephants in these areas. The GCPA includes two national parks (NP), Moukalaba-Doudou NP (4,500 km²) to the east, and Loango NP (1,550 km²) to the west, with an industrial corridor (3,585 km²) between the parks, where oil is extracted and timber is harvested (Alonso, Dallmeier, Korte, & Vanthomme, 2014). The GCPA has vast areas of tropical wilderness and supports forest elephants as well as other endangered species, including gorillas, chimpanzees, and hippos (Alonso, Lee, Campbell, Pauwels, & Dallmeier, 2006). Despite a small human population, logging, hunting, and habitat destruction for agriculture, oil exploration, and human settlement are serious threats to biodiversity in this region (Dallmeier et al., 2006). Our research area extends approximately 891 km² along the Atlantic Ocean coastline of Gabon, from the village of Sette Cama (STC) in the north, to Mayonami (MAY) on the Nyanga River in the south (Figure 1). The study area also includes the town of Gamba with an oil producing area sector in the southwest coastal area where elephants live in close vicinity with people, increasing human–elephant conflicts (Graham & Ochieng, 2010). Prior to the discovery of a major oil field in 1967, the town had only a few families. Alonso et al. (2014) noted that with immigration of workers from other parts of the country, the town boomed to about 10,000 people (DGS, 2015).

At the GCPA, female forest elephants within operational oilfields have small home ranges and localized movement patterns (Kolowski et al., 2010), perhaps because elephants...

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**FIGURE 1** Map of sampling locations southwest of the industrial corridor of the Gamba complex of protected areas including sample size of individuals, mitochondrial DNA haplotype frequencies for each site, and frequencies of STRUCTURE clusters.
find year-round resources and do not need to move long distances. Eggert et al. (2013) and Munshi-South (2011) studied elephants from the northern part of the industrial corridor, a hilly landscape with wetlands, lagoons, and lakes. They suggested that the area serves as a corridor, facilitating gene flow north of the Ndougo lagoon, and connecting Loango and Moukalaba-Doudou NP. However, the continuous expansion of Gamba town in the southwest could cut off elephant movement corridors between northern and southern, and/or eastern and western locations. Previous studies have determined that poaching and habitat loss associated with human development can limit gene flow and result in significant genetic differentiation of elephant populations (Nyakaana & Arctander, 1999).

The main objective of this study was to evaluate the patterns of genetic diversity and structure of forest elephants in the research area across a forested landscape that is bisected by areas that have recently experienced human-induced fragmentation. We hypothesized that the expansion of human infrastructures around Gamba would limit movement and levels of gene flow between the elephants living north and south of the town. We also aimed to provide baseline demographic data about African forest elephant populations living near human settlements. Specifically, we sought to identify the sex, group size, and social status of elephants that have crop-raided plantations and compare these raiders to the genetic profiles of non-raider elephants across the GCPA. As their natural habitat has recently been intensively transformed to agricultural land (Lahm, 1996), the probability for elephants to crop raid plantations has increased (Barnes, Asika, & Asamoah-Boateng, 1995), leading to an increasing risk of conflict between humans and elephants. Lahm (1996) confirmed that elephants in Gabon seasonally raid plantations at crop maturity, primarily during the wet season. It has been proposed that differences in life history, social status, and sex are important factors that can influence the propensity of elephants to raid crops. Chiyo, Moss, and Alberts (2012) suggested that raiding is acquired through social learning from older males that are already raiders; Hoare (1999) and Sukumar and Gadgil (1988) proposed that solitary male elephants are primarily involved in conflicts as they are willing to take more risks to increase their reproductive success. To our knowledge, this is the first study to use noninvasive genetic analysis to test the hypothesis that elephants with more predilection for repeatedly raiding crops (or raiders) have specific profiles regarding levels of genetic relatedness, sex, and group size composition that can distinguish them from nonraidars. We also tested the hypothesis that solitary males were more likely to raid crops.

### 2 METHODS

#### 2.1 Study design

In order to characterize the patterns of genetic structure of the elephants in the GCPA, we systematically sampled elephant dung from four sites along an 80-km stretch of coastal, savanna, and forest habitats ranging from the village of STC in the north, through the Gamba area, including the Yenzi Camp (YNZ), to MAY, to the Nyanga River in the south (Figure 1). We also sampled a savanna-forest mosaic east of Gamba known as the Vera Plain (VER), near the buffer zone of Moukalaba-Doudou NP and Mbouda village on Catchimba Lake.

We generated a sampling grid of 100 regularly spaced points (3-km intervals) over the entire research area with ArcGIS 10.1 (Environmental Systems Research Institute, 2012). We collected dung samples at 61 and 82 points of the grid during the dry (June–August 2013) and wet (October 2013–March 2014) seasons, respectively (Figure 1). Each sampling day, three to four points were randomly chosen. A team of three people navigated toward the most accessible point and searched for fresh dung for 1 km (500 m each side of an elephant trail), walking at 1 km/hr with the aid of a compass and global positioning system, and with minimal opening of vegetation (recce transects, Walsh & White, 1999). In addition, the team followed recent trails within a 500-m radius from the point for about 10 min. Fresh dung piles estimated to be less than 24 hr old, that is, wet and shiny with surface covered with mucus, (Goossens, Anthony, Jeffery, Johnson-Bawe, & Bruford, 2003) were collected. For each sample, we collected approximately 10 g of the external layer of dung and stored it in a 50-mL polypropylene tube. The samples were then transported to the Gabon Biodiversity Program lab, where they were boiled in water for at least 15 min. This was done to kill pathogens in order to comply with requirements from the US Department of Agriculture for the legal importation of samples into the United States. After boiling, we immediately dried the samples using silica gel at room temperature and shipped them for genetic analysis to the Genomics lab at the Smithsonian Conservation Biology Institute in Washington, DC.

In addition to systematic sampling over the study area, we also collected fresh dung piles left by raiders in seven plantations during the dry and wet seasons periods mentioned above (Table 1). Three of the plantations were located north (Mbassu [MBA], Matsiegui [MAT], Totou [TOT]) and four (Guittiguibatu [GUI], Bagdad [BAG], Apollinaire [APO], Edouard [EDO]) south of the town of Gamba. We visited each plantation once a week between July 2013 and March 2014. Plantations were separated by a minimum of 3 km, were similar in size (700–1,600 m perimeter) and type of crop (cassava, banana, taro, and/or sugar cane), and were
| Sampling locations | Dry season | Wet season | Total F:M:U across seasons | Total unique individuals |
|--------------------|------------|------------|---------------------------|-------------------------|
| Area               | Site       | Plantations Inside/outside | Successfully genotyped n/N<sup>a</sup> | Unique individuals<sup>b</sup> | F:M:U<sup>c</sup> | Successfully genotyped n/N<sup>a</sup> | Unique individuals<sup>b</sup> | F:M:U<sup>c</sup> |
| North Gamba (NGax)| Sette Cama | Mbassu      | 0/0                        | 0                         | 00:00:00           | 1/1                        | 1                             | 00:01:00 |
|                    |            | Matsiegui   | 1/1                        | 1                         | 00:01:00           | 34/36                      | 22                            | 10:09:03 |
|                    |            | Totou       | 0/0                        | 0                         | 00:00:00           | 0/1                        | 0                             | 00:00:00 |
|                    |            | Subtotal plantations | 1/1                        | 1                         | 00:01:00           | 35/38                      | 23                            | 10:10:03 |
|                    |            | Outside     | 21/21                      | 18                        | 10:08:00           | 43/43                      | 32                            | 20:08:04 |
| Total North        |            |             | 22/22                      | 19                        | 10:09:00           | 78/81                      | 55                            | 30:18:07 |
| South Gamba (SGax)| Verra Plaine| Apolinaire (APO) | 6/6                        | 6                         | 05:00:01           | 20/20                      | 4                             | 01:03:00 |
|                    |            | Bagdad      | 0/0                        | 0                         | 00:00:00           | 7/7                        | 5                             | 03:02:00 |
|                    |            | Edouard     | 0/0                        | 0                         | 00:00:00           | 0/0                        | 0                             | 00:00:00 |
|                    |            | Guituguiabatu | 6/6                       | 6                         | 01:04:01           | 2/2                        | 2                             | 00:02:00 |
|                    |            | Outside     | 9/9                        | 7                         | 06:01:00           | 34/34                      | 29                            | 18:11:00 |
|                    |            | Subtotal plantations | 12/12                     | 12                        | 06:04:02           | 29/29                      | 11                            | 04:07:00 |
| Total South        |            |             | 57/57                      | 41                        | 08:04:00           | 64/67                      | 34                            | 14:17:03 |
| Total plantations  |            |             | 13/13                      | 13                        | 06:05:00           | 64/67                      | 34                            | 14:17:03 |
| Total (North + South)|          |             | 79/79                      | 60                        | 38:20:02           | 216/219                    | 153                           | 80:64:09 |

<sup>a</sup>n/N is the number of successfully genotyped samples (n) out of the total samples (N) collected for each site.

<sup>b</sup>Values of unique individuals do not include recaptures.

<sup>c</sup>F:M:U is the sex ratio representing the number of unique individual females (F), males (M), and those of unknown gender (U) whose sex failed to be genetically determined.
protected with various traditional deterrent techniques by farmers, including fences, noise, smoke, light, and human presence. We defined a raiding event in a collecting session as when one or more fresh dung piles were found within the perimeter of the plantation.

### 2.2 Data collection

Genomic DNA was extracted from each dung sample using a QIAamp Fast DNA Stool mini kit (QIAGEN, cat. no 51604). In order to control for contamination, DNA extractions were conducted in a dedicated room with a separate air handling system from the main lab, where polymerase chain reaction (PCR) reactions are conducted. We also used a blank sample for each extraction batch. For the mitochondrial DNA (mtDNA) analysis, we amplified and sequenced a 600-bp segment of the mtDNA control region from all samples \((n = 298)\) using primers MDL3 and MDLS (Fernando, Pfenrader, Encalada, & Lande, 2000). PCRs were performed with a QIAGEN Multiplex PCR Kit following the manufacturer's protocol with 20-μL reaction volumes, 1-μL DNA template added, and a 63°C annealing temperature for 32 cycles.

We also genotyped all samples \((n = 298)\) using a panel of eight microsatellite loci (FH60R, LA6R, FH94R, FH67, FH48R; Comstock, Wasser, & Ostrander, 2000), LA4, LA5 (Eggert, Ramakrishnan, Mundy, & Woodruff, 2000), and FH126 (Comstock et al., 2002). The PCR amplification was carried out in a 10-μL volume containing 1 μL of DNA template and following QIAGEN® Multiplex PCR Kit instructions. Annealing temperatures were optimized for each multiplex (Supporting Information; Appendix S1). We included in each batch of amplifications an extraction blank and PCR negative control to detect PCR contamination. Each PCR was repeated three times for homozygotes and twice for heterozygotes following a modified multitube approach to reduce genotyping error and ensure reliability of genotypes. (Dutta et al., 2012). For sexing, we used primers that amplify two short Y-specific fragments (SRY1 and AMELY2) and one longer X-specific fragment (PLP1) in one multiplex PCR following protocols by Ahlering, Haier, Roberts, and Foley (2011).

DNA sequencing and microsatellite fragment analyses were performed using capillary electrophoresis on a DNA 3730XL analyzer (Applied Biosystems™) at the Center for Conservation Genomics, Smithsonian Institution in Washington, DC, USA. Microsatellites were run with a GeneScan–500 ROX Size Standard (Applied Biosystems, Carlsbad, CA) and scored using GeneMapper 4.1 (Applied Biosystems). We sequenced forward and reverse strands of the mtDNA control region using MDL3 and MDLS (Fernando et al., 2000) for each individual and the consensus sequences for all individuals were aligned using SEQUENCER (Gene Codes Corporation 1998, version 3.1.1) and edited by eye.

### 2.3 Statistical analyses

We used MICRO-CHECKER 2.2.1 (van Oosterhout, Hutchinson, Wills, & Shipley, 2004) to identify genotyping errors including null alleles, large allele dropout, and scoring of stutter peaks. We estimated the mean number of alleles per locus and the observed (Ho) and expected (He) heterozygosity per site and per locus using ARLEQUIN 3.11 (Excoffier, Laval, & Schneider, 2005). We tested for departure from Hardy–Weinberg equilibrium (HWE) with a global test of heterozygote deficiency using the Markov chain method in GENEPOP 3.4 (Raymond & Rousset, 1995). Genotypic linkage disequilibrium (LD) was measured using the correlation coefficient for each pair of loci per population and across all populations also using GENEPOP 3.4 (Raymond & Rousset, 1995).

We used GenAIEx to identify unique individuals based on matching genotypes all eight loci. We also identified multiple copies of samples from a single individual and recaptures (Peakall & Smouse, 2006). Recaptures are those individuals sampled at least twice on different days and/or at different sites. We used the method described in Ahlering et al. (2011) to determine the sex and to identify individuals.

We calculated mtDNA variation, using only unique individuals, by estimating haplotype \((h)\) and nucleotide \((\pi)\) diversities as implemented in DNASP 5.10 (Librado & Rozas, 2009). We also generated a median-joining network based on weighted maximum likelihood (ML) distances, using NETWORK 4.1.1.1 (Bandelt, Forster, & Rohl, 1999) to visualize the relationships among haplotype sequences.

We evaluated the significance of the genetic structure separately for nuclear and mitochondrial markers using an analysis of molecular variance (AMOVA) with 10,000 permutations, among and within sites, with ARLEQUIN (Schneider, Roessli, & Excoffier, 2000). A permutation was applied to determine the significance level \((p < .05)\) to detect admixture between groups of populations and/or population structure (Paff et al., 2001; Pritchard & Wen, 2003).

We identified population subdivision with a Bayesian clustering approach derived from assignment tests with the assumption of random mating individuals with STRUCTURE 2.3.1 (Pritchard, Stephens, & Donnelly, 2000) and STRUCTURE HARVESTER (Earl & vonHoldt, 2012). Together, these programs determine the most likely number of clusters \((K)\). The method assigns individuals of unknown origin to a theoretical population under the assumption of admixture model with correlated allele frequencies. We performed 10 runs for each value of K ranging from 1 to 8. We
used a burn-in period of 100,000 steps, followed by 1,000,000 recorded steps.

Finally, we used the program ML-Relate (Kalinowski, Wagner, & Taper, 2006) to calculate ML estimates of relatedness. The method implemented in ML-Relate results in lower variance than other approaches (Milligan, 2003), and the program is able to accommodate loci with null alleles, which are more prevalent in noninvasive samples (Wagner, Creel, & Kalinowski, 2006). We estimated the levels of relatedness among elephants-raiding plantations using the highest natural logarithm of likelihood values (LnL) among all possible relationships. We assumed that a single dung found within a plantation was indicative of a single individual raiding the plantation and was defined as a “solitary” animal. When two or more related males were detected during the same collecting session they were defined as “Related males.” When closely related females with one or more offspring (male and/or female) were detected during the same collecting session, they were defined as a “Family group.” When a combination of related and unrelated individuals was detected during the same collecting session, these individuals were defined as “Family group plus.” Finally, when two or three unrelated individuals (males and/or females) were detected during the same collecting session, they were defined as “Unrelated.”

3 | RESULTS

We collected a total of 298 dung samples and successfully genotyped 295, from which we identified 213 unique individuals (74 from the north and 139 from the south); of these, 164 were captured once and 49 twice or more (Table 1; Supporting Information; Appendix S2). We determined the sex of 202 individuals; 11 were undetermined (Table 1). The sex ratio of the general population (all age classes included) was significantly different from 1:1 in favor of females (118 F; 84 M; $p < .05$).

The microsatellite analysis revealed high levels of genetic diversity across sampled localities north and south of the town of Gamba. All loci were polymorphic with an average 10.9 alleles across samples ($n = 213$), ranging from 6 to 16. The average observed (Ho) and expected (He) heterozygosities were 0.76 and 0.79, respectively. Neither average allelic diversity nor Ho or He differed significantly among localities (Table 2). We noted the potential presence of null alleles at locus LA4, which also showed a deviation from HWE at two sampling sites (STC and MAY). Significant LD after Bonferroni corrections ($p = .0018$) was found for 9 out of 28 pairwise comparisons of each locus pair across all localities.

The results of the AMOVA on the entire population showed that 98% of the genetic variation was within localities, with low, but significant differentiation among the four localities ($F_{ST} = 0.015$; $p < .05$). Elephant mtDNA sequences showed an average nucleotide diversity ($\pi$) of 0.011 ($SD = 0.00075$), and a mean haplotype diversity ($h$) of 0.712 ($SD = 0.018$). Six mtDNA control region haplotypes were defined (Figure 1), with higher diversity in STC and YNZ, and lower diversity VER and MAY (Figure 1; Table 3). In VER, haplotype H4 was carried by a single male plantation raider also found once at YNZ. Haplotype H3 was carried by two males in MAY, and H5 was only detected in STC in two females (a mother and her daughter) and one male (Figure 1).

All pairwise comparisons of genetic differentiation between localities revealed low $F_{ST}$ values with both microsatellite and mitochondrial markers (Table 3); all but one, between YNZ and STC in mtDNA, were statistically significant ($p < .05$). Overall, elephants from YNZ showed the

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**TABLE 2** | Genetic diversity averaged across eight microsatellite loci and sampled sites north and south of Gamba town

| Locus | Global ($N = 213$) | Sette Cama ($N = 74$) | Yenzi ($N = 41$) | Verra Plains ($N = 59$) | Mayonami ($N = 39$) |
|-------|-------------------|----------------------|-----------------|------------------------|-------------------|
|       | $A$ | $H_o$ | $H_e$ | $A$ | $H_o$ | $H_e$ | $A$ | $H_o$ | $H_e$ | $A$ | $H_o$ | $H_e$ |
| FH60R | 12  | 0.85 | 0.85 | 12  | 0.86 | 0.86 | 9  | 0.86 | 0.86 | 10  | 0.84 | 0.84 |
| LA6R  | 10  | 0.78 | 0.74 | 9   | 0.71 | 0.72 | 6   | 0.71 | 0.76 | 9   | 0.84 | 0.77 |
| FH94R | 10  | 0.72 | 0.65 | 8   | 0.73 | 0.65 | 8   | 0.73 | 0.71 | 10  | 0.67 | 0.61 |
| FH126 | 16  | 0.81 | 0.88 | 13  | 0.85 | 0.85 | 16  | 0.83 | 0.88 | 14  | 0.75 | 0.87 |
| FH67  | 6   | 0.75 | 0.76 | 6   | 0.72 | 0.73 | 6   | 0.63 | 0.75 | 6   | 0.88 | 0.81 |
| LA4   | 12  | 0.7  | 0.85 | 12* | 0.69 | 0.85 | 9   | 0.74 | 0.87 | 12  | 0.79 | 0.84 |
| FH48R | 13  | 0.77 | 0.81 | 8   | 0.66 | 0.79 | 10  | 0.8  | 0.8  | 11  | 0.82 | 0.84 |
| LA5   | 8   | 0.68 | 0.77 | 6   | 0.65 | 0.77 | 7   | 0.71 | 0.75 | 7   | 0.65 | 0.76 |
| Average | 10.88 | 0.76 | 0.79 | 9.25 | 0.73 | 0.78 | 8.88 | 0.76 | 0.78 | 9.88 | 0.78 | 0.79 |

Note: Loci deviated from HWE in different localities are marked with *. $A$ is the allelic diversity, the average number of alleles across loci. $H_o$ is the observed heterozygosity. $H_e$ is the expected heterozygosity.
least genetic distance with all other sampled localities, while elephants detected in STC showed the most genetic distance with VER and MAY. Combining areas to test for differentiation north and south of Gamba, we found an $F_{ST}$ value of 0.0078 ($p < .05$). The Mantel test coefficient suggested a small but significant ($R_{xy} = .096; p < .05$) correlation between pairwise genetic and geographic distance. In the south, despite their close geographic distance, VER and MAY presented, in both genetic markers, $F_{ST}$ values as large as between STC and MAY.

STRUCTURE HARVESTER revealed that the maximum value of the log likelihood of the data was at $K = 2$, and the $\Delta K$ approach showed greatest support for two distinct genetic clusters with no clear association with geographic locations (Figure 1; Supplementary information; Appendix S3). STRUCTURE assigned 70% (150/213) of individuals (with $q \geq 0.8$; Randi, Tabarroni, Rimondi, Lucchini, & Sfougaris, 2003) to cluster 1 or 2. The remaining 30% were admixed individuals. Whereas most sites had nearly half of their elephants in clusters 1 and 2, VER grouped 79% of its individuals in cluster 2 (Figure 1).

We recorded 21 elephant-raiding events (Table 4) and collected 78 dung samples from five of the seven plantations sampled. The sex ratio of elephants found inside plantations is not different from 1:1 (20:22; $p = .377$). We did not find genetic differences between elephants-raiding plantations and elephants outside plantations ($p = .432$). We detected every level of relatedness within groups of elephants-raiding plantations (Table 4). Besides groups of related or unrelated raiders, we documented the presence of a putative solitary male within each plantation except at BAG. APO had a solitary female, which may have been part of a group of elephants not detected because they may not have defecated at that collecting session. MAT had the greatest number of raids ($N = 8$) and the largest group of raiders ($N = 9$). One elephant, “male Y,” was part of the largest group of raiders in MAT, and male Y was detected in a single raid in MBA (Supporting Information; Appendix S2). Male Y moved between plantations in the north of Gamba, and male Q moved between APO and YNZ in the south Gamba, but we never detected the same individual moving between the north and south of Gamba. The longest distance recorded between recaptures was 28 km traveled by male Q between YNZ and APO, and we observed an average distance traveled of 6 km for males and 3 km for females.

### DISCUSSION

Our study documented low levels of genetic structure in African forest elephants living in a human-modified landscape that has roads, plantations, and petroleum infrastructure. We found that the nucleotide and haplotype diversities of the 213 individuals genetically identified from four areas sampled for elephant dung in the GCPA were similar to those in other central African forest elephant populations (Johnson et al., 2007). To explain the level of nucleotide and haplotype diversities, Johnson et al. (2007) suggested the absence of genetic barriers between the sampled areas.

#### Table 3

| STC | YNZ | VER | MAY |
|-----|-----|-----|-----|
| STC | 1.45 | 0.0060 | 0.0138 | 0.0168 |
| YNZ | 0.0356 | 1.33 | 0.0122 | 0.0069 |
| VER | 0.3020 | 0.2431 | 0.77 | 0.0165 |
| MAY | **0.2515** | 0.0977 | **0.2611** | 0.93 |

*Note: Significant $F_{ST}$ values are in bold.*

**Abbreviations:** MAY, Mayonami; mDNA, mitochondrial DNA; STC, Sette Cama; VER, Vera Plain; YNZ, Yenzi Camp.
existence of at least two different refugia in the central African region harbouring distinct elephant populations that diverged allopatrically during Pleistocene glacial maxima. Moreover, the repeated retraction of forest cover into refugial zones was followed by re-expansion of isolated populations and secondary contact. Our results of the STRUCTURE analysis showing signatures of two genetic clusters in all of the localities support this hypothesis of expansion and secondary contact of two previously isolated populations.

Even though our sampling localities are a maximum of 80 km apart, within the known recorded dispersal distance of forest elephants, we detected low but significant \( F_{ST} \) values, with higher values observed with mtDNA than microsatellites. This suggests that the differentiation we observed is driven by lower mobility of females from their natal location. In species such as elephants, patterns of female philopatry are strong because females rarely disperse, unlike males, which prevent population differentiation through male-mediated gene flow (Okello et al., 2008). In addition, we found a stronger geographic pattern in mtDNA haplotype distribution, also supporting a hypothesis of lower female dispersal/higher levels of female philopatry, particularly in the VER elephants. VER elephants likely find year-round resources, as suggested by the small home ranges reported by Kolowski et al. (2010). Males might be moving north and south along the coastline, as also proposed by Kolowski et al. (2010).

For both mtDNA and microsatellite markers, we found low pairwise \( F_{ST} \) values between sampling localities, which suggests either that barriers to movement and gene flow between these sites do not yet exist, or that they have not been in place long enough to leave a strong signature in our genetic markers. Previous studies looking at elephant movement in the area revealed that most observed movements were over short distances of ca. 30 km, but a few were as long as 110 linear km (Eggert et al., 2013). Our recapture data did not find any individual moving from STC to MAY or from MAY to STC. Maintaining connectivity in human-dominated landscapes such as the GCPA is vital for the long-term survival of populations (Ahlering, Hedges, et al., 2011; Dutta et al., 2012).

The fact that we did not detect strong signatures of population genetic structure between sampling sites in the north (STC) versus south (MAY) of the town of Gamba should not be interpreted as though the impacts on the forest habitat by human activity, including clearing land for plantations and infrastructure, have not affected elephant movements across the landscape. It has been well-documented that these human activities are a major determinant of African forest elephant distribution in the GCPA (Buij et al., 2007; Eggert et al., 2013; Laurance et al., 2006; Vanthomme et al., 2013).

It has also previously been determined that there is an expected time-lag in detection of genetic signatures of change at the landscape level for large mobile animals with long dispersal distances (Eggert et al., 2013; Landguth et al., 2010). Assuming a generation time of 25 years for African forest elephants (Blanc et al., 2007), the habitat modifications caused by humans around the town of Gamba may have only started approximately two elephant generations ago. Landguth et al. (2010) cautioned that it may be very difficult to detect the effects of fragmentation on long-lived species over the short time scales that are relevant for conservation management. In this case, the effects of habitat fragmentation on this landscape are likely to continue to increase in the future by the growing impacts by people living in the expanding town of Gamba and this study can be used as a baseline for monitoring future changes in genetic diversity and structure.

The loss of elephant habitat increases the probability of elephants encountering human settlements, likely increasing risks of crop raiding (Barnes et al., 1995). We found that in the Gamba town area there were as many male as female elephants among the raiders and that they were not genetically different from nonraiders. No specific elephant profile was depicted for raiders. We found that both solitary males and females, as well as groups of related and unrelated individuals, devastated plantations in our study areas, in contrast to Hoare (1999), who reported incidents caused primarily by solitary males in Zimbabwe. Our study showed that a different solitary male was found in all sampled plantations except BAG, which supports the observations of farmers who report that solitary males scout the fields and return with groups when the crop is ripe. We found that raids occurred during the wet season, close to harvest time, from mid-February to the end of March, as also reported by Lahm (1996) for other parts of Gabon. Similarly, in Botswana and Zimbabwe, elephant crop raiding occurred as crops ripened toward the end of the rainy season (Hoare, 1999; Jackson, Mosojane, Ferreira, & van Aarde, 2008). In contrast, Inogwabin, Mbende, Bakanza, and Bokika (2013) showed that in the Democratic Republic of Congo, elephants come across plantations and destroy crops when searching for permanent water in the dry season.

Despite our efforts in the field to differentiate dung piles from different individuals, we collected an average of 2 to 3 samples at a single site from the same elephant. Farmers that encounter multiple shapes and numbers of dung piles may think that the number of individuals raiding their crops is greater than it is, and use this conclusion along with the extent of crop damage to rationalize their retaliation.

Our results showing elephant movements in this landscape can be used to inform local farmers about areas where they can establish plantations and infrastructure while
minimizing human-elephant conflict. They could build locally made mobile fences, for example, and increase plantation guarding only during the crop harvest time, and support current initiatives for effective conservation and management of elephants of the GCPA (Blanc et al., 2007). Based on ecological data and modeling, Vanthomme, Nzamba, Alonso, and Todd (2018) recently suggested that the elephant population in our study site likely consists of migrant animals moving from Moukalaba-Doudou National Park to the south to Loango National Park to the north, but also resident elephants exploiting locally available resources such as fruits, water, human rubbish, and plantations in the Gamba area. The genetic information analyzed in the present study concurs with the conclusions in Vanthomme et al. (2018), and identifies male elephants as the probable dispersers. The lack of strong signatures of genetic differentiation between elephants in northern and southern localities found in this study supports current initiatives for conservation and management of resident elephants of the GCPA (Blanc et al., 2007) and calls for the protection of corridors that will support the elephant connectivity identified in Vanthomme et al. (2018). These models could guide project development managers to maintain adequate elephant habitat in the Gamba area, along with preserving landscape connectivity for this flagship species.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTIONS

All authors contributed to research design, interpreting data, and writing the manuscript. M.B.J., H.V., L.T., and L.K. organized and led the field data collection; M.B.J., L.D.P., and J.E.M. carried out the analyses; M.B.J. led the manuscript preparation, to which all authors contributed.

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

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