Conservation Biology

Contributed Paper

Using Genetic Profiles of African Forest Elephants to Infer Population Structure, Movements, and Habitat Use in a Conservation and Development Landscape in Gabon

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Abstract: *Conservation of wide-ranging species, such as the African forest elephant (*Loxodonta cyclotis*), depends on fully protected areas and multiple-use areas (MUA) that provide habitat connectivity. In the Gamba Complex of Protected Areas in Gabon, which includes 2 national parks separated by a MUA containing energy and forestry concessions, we studied forest elephants to evaluate the importance of the MUA to wide-ranging species. We extracted DNA from elephant dung samples and used genetic information to identify over 500 individuals in the MUA and the parks. We then examined patterns of nuclear microsatellites and mitochondrial control-region sequences to infer population structure, movement patterns, and habitat use by age and sex. Population structure was weak but significant, and differentiation was more pronounced during the wet season. Within the MUA, males were more strongly associated with open habitats, such as wetlands and savannas, than females during the dry season. Many of the movements detected within and between seasons involved the wetlands and bordering lagoons. Our results suggest that the MUA provides year-round habitat for some elephants and additional habitat for others whose primary range is in the parks. With the continuing loss of roadless wilderness areas in Central Africa, well-managed MUAs will likely be important to the conservation of wide-ranging species.*

Keywords: connectivity, conservation outside parks, *Loxodonta cyclotis*, multiple-use areas, noninvasive sampling

Utilización de Perfiles Genéticos de Elefantes Africanos para Inferir su Estructura Poblacional, Movimientos y Uso del Hábitat en un Paisaje con Conservación y Desarrollo en Gabón Resumenfgs

Resumen: *La conservación de especies con distribución amplia, como el elefante africano (Loxodonta cyclotis), depende de áreas completamente protegidas y de áreas de uso múltiple (AUM) que proporcionan conectividad de hábitat. En el Complejo Gamba de Áreas Protegidas en Gabón, que incluye 2 parques*

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Paper submitted August 28, 2012; revised manuscript accepted June 6, 2013.

Conservation Biology, Volume 00, No. 0, 1–12

DOI: 10.1111/cobi.12161

nacionales separados por un área de uso múltiple que contiene concesiones de energéticas y forestales, estudiamos a los elefantes para evaluar la importancia de las AUM para especies con distribución amplia. Extrajimos ADN de muestras de excretas de elefante y utilizamos la información genética para identificar más de 500 individuos en el AUM y los parques. Posteriormente examinamos los patrones en las secuencias de los microsatélites nucleares y de la región de control mitocondrial para inferir la estructura poblacional, los patrones de movimiento y el uso de hábitat por edad y sexo. La estructura poblacional fue débil pero significativa, y la diferenciación fue más pronunciada durante la época de lluvias. En el AUM, los machos están mas fuertemente asociados con los hábitats abiertos, como humedales y sabanas, que las hembras durante el estiaje. Muchos de los movimientos detectados dentro y entre estaciones involucró a los humedales y lagunas circundantes. Nuestros resultados sugieren que el AUM proporciona hábitat todo el año para algunos elefantes y hábitat adicional para otros cuya distribución primaria esta en los parques. Con la pérdida continua de áreas silvestres en África Central, es probable que AUM manejadas adecuadamente sean importantes para la conservación de especies con distribución amplia.

Palabras Clave: áreas de uso múltiple, conectividad, conservación afuera de parques, *Loxodonta cyclotis*, muestreo no invasivo

Introduction

In Africa protected areas are often designed around sites that support species of conservation concern such as large mammals. Today protected areas are increasingly isolated in human-modified landscapes (Newmark 2008). Conservation of wide-ranging species therefore depends on managing multiple-use areas (MUA) to provide protection and connectivity among parks and private and public lands (Western et al. 2009; Ahlering et al. 2012*a*, 2012*b*).

Central African forest elephants (*Loxodonta cyclotis*) declined in abundance by 62%, and their geographic range decreased by 30% from 2002 to 2011 (Maisels et al. 2013). The largest remaining concentration of this species, approximately 53,000 individuals, is in Gabon (Blanc et al. 2007). In 2002 Gabon established 13 national parks that were designed in part to protect biodiversity and threatened wildlife such as elephants and apes (Laurance et al. 2006*a*). Surveys have been conducted to establish baseline abundance estimates for monitoring key species in parks, especially elephants (Maisels et al. 2006; Maisels et al. 2013). However, little scientifically collected wildlife data exist for areas outside parks.

To better understand connectivity between parks for forest elephants and inform land management, we conducted a multipart study in The Gamba Complex of Protected Areas, which covers 4% of Gabon's land area and has been under full or partial protection since 1947. Today it includes 2 national parks separated by a partially protected MUA from which timber and oil are extracted. In the MUA, human factors are stronger determinants of elephant distribution than ecological factors (Buij et al. 2007). Roads depress the abundance of forest elephants, especially in areas where hunting occurs (Laurance et al. 2006*b*). Individuals tracked via GPS collars have small home ranges and restricted movement patterns (Kolowski et al. 2010). Elephants in the MUA do not exhibit elevated fecal glucocorticoid metabolite levels (Munshi-South et al. 2008), and groups are composed primarily of related females and offspring (Munshi-South 2011). This information suggests that extractive-use areas that offer protection from hunting are important habitat for elephants, but that management of habitat and human-wildlife interactions is necessary to maintain their conservation value.

We examined elephant population structure, movement patterns, and habitat use by sex and age group in the MUA during the wet and dry seasons and the national parks during the transition between seasons. Using fecal DNA to identify and track individuals, we tested whether elephants in the MUA were part of a resident population or temporary immigrants whose primary home ranges were in the nearby parks. Our findings may inform land management and extractive industries about elephant conservation management in MUA across central Africa.

Methods

Study Area

The Gamba Complex encompasses Loango National Park (1550 km^2), Moukalaba-Doudou National Park (4500 km^2), and an MUA in between (3585 km^2). The parks include sandy beach, mangrove forest, swamp, coastal scrub forest, open grassland, submontane forest, and large tracts of primary and mature secondary lowland humid forest. Both parks are highly forested. In Loango tree cover is approximately 80% and herbaceous cover is 16%. In Moukalaba-Doudou tree cover is 87% and herbaceous cover is 12% (Hansen et al. 2003). The center of the complex includes parts of the Iguéla and Ngové-Ndogo hunting areas, Setté Cama, and the Ndogo Lagoon. The southern zone includes the town of Gamba (Fig. 1).

We focused on the northern MUA, characterized by hilly terrain (<150 m elevation) with seasonally inundated forest dissecting upland rainforest. In the MUA, petroleum operations have been ongoing since the 1950s and selective logging has occurred since the 1920s. These

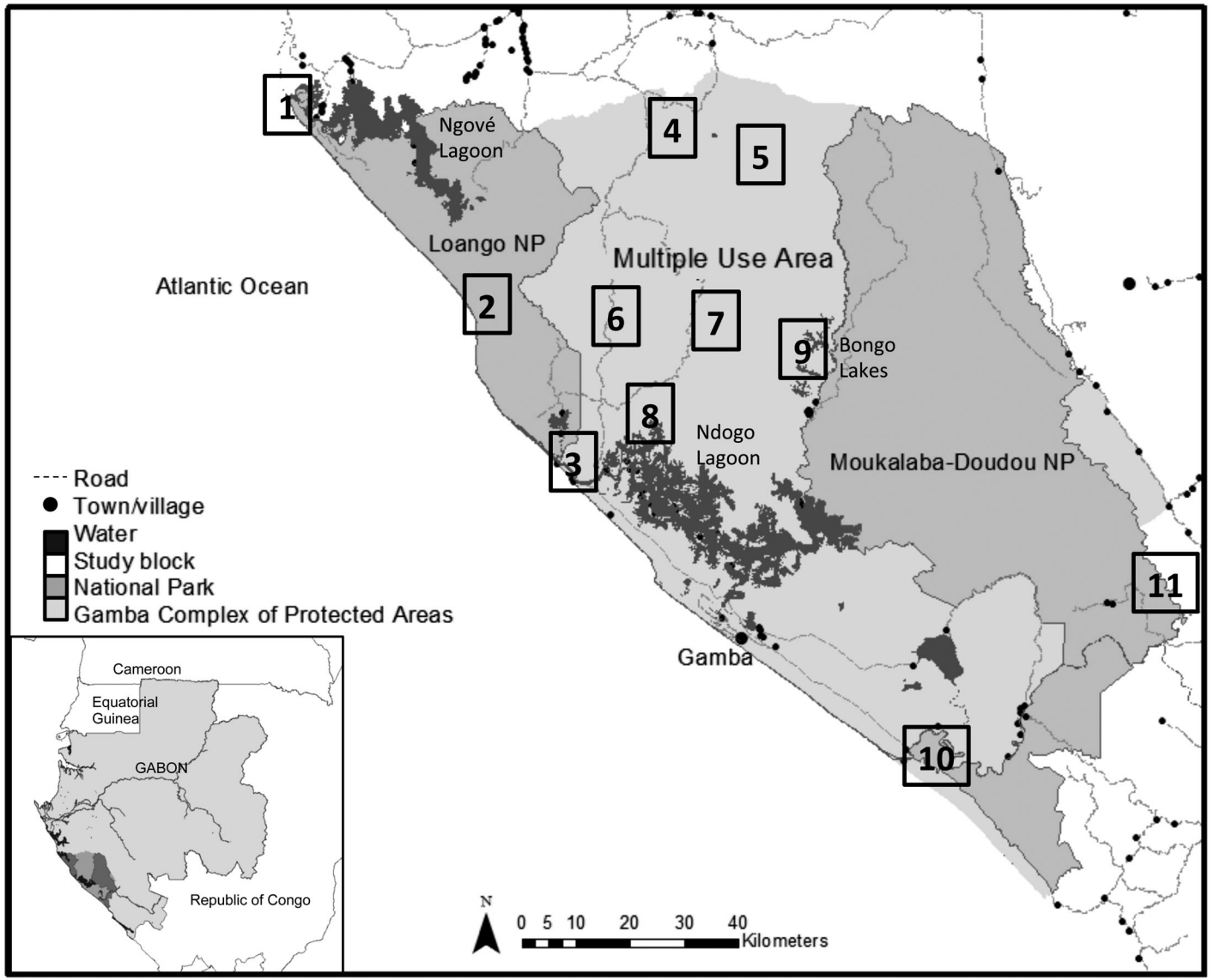

Figure 1. The Gamba Complex of Protected Areas, Gabon, including sampling areas within the 2 national parks and the multiple-use area (1, Iguéla; 2, Loango; 3, Sette Cama; 4, Rabi; 5, Divangui; 6, Echira; 7, Center; 8, Koumaga; 9, Bongo Lakes; 10, Nyanga; and 11, Moukalaba).

operations have increased accessibility, forest clearance, and habitat fragmentation, especially near oilfields. Although remnants of primary forest exist, most is mature secondary forest (Alonso et al. 2006). Only small parts of the MUA are covered in surface water (1.2% [Institut National de Cartographie 2003]) and herbs (6.4%); 90.7% is forested (Hansen et al. 2003). One controlled road mainly used by extractive industries accesses the MUA. At the time of study, the parks had no major roads and relatively little human activity relative to the MUA.

The variety of relatively unaffected areas in the complex contributes to its diverse flora and fauna (Fisher 2004; Alonso et al. 2006; Harris et al. 2012) and makes it especially valuable for species of international conservation concern, such as the leatherback turtle (*Dermochelys coriacea*), western lowland gorilla (*Gorilla gorilla gorilla*), hippopotamus (*Hippopotamus amphibius*), and chimpanzee (*Pan trogolodytes*). The complex has one of the highest concentrations of African forest elephants, estimated at 11,205 (95% CI = 10,236 to 12,174) in 1999 (Thibault et al. 2001). Although historically Gabon had less elephant poaching than nearby countries (Barnes et al. 1995), poaching has become a serious problem leading the Gabonese government to elevate the elephant's conservation status to fully protect and create an antipoaching military unit. Although elephant poaching has been documented in the Gamba Complex (Thibault & Blaney 2003; R.B. and M.L., personal observation) until recently poaching pressure has been relatively low (Blake et al. 2007).

Sampling

We collected dung samples at 6 MUA sites during the dry and wet seasons (June–August and November–December, respectively) in 2004. We sampled each site 1–4 times; visits were separated by at least 1 week (Fig. 1 & Table 1). For comparison, we collected samples at 5 sites in the national parks or their buffer zones during the transition between seasons (October). We collected dung estimated to be <3 d old and recorded GPS locations and circumferences of 1–3 intact boli. Samples were boiled to destroy pathogens and preserved as in Eggert et al. (2008).

Elephants appeared to be concentrated around water sources (Bongo, Echira, Koumaga) in June, and fresh dung samples were relatively rare at drier sites (Center, Divangui, Rabi). Because more samples were collected at wetter sites than planned, we genotyped a subset representing all collection dates, sites, and locations within sites (Table 1).

DNA Laboratory Analyses

We used the method of Eggert et al. (2003) to extract DNA from dung in a separate lab from the one in which DNA was amplified. Extractions were accompanied by controls to ensure that reagents were free of contaminating DNA.

We optimized genetic markers with a preliminary set of 25 samples. We used primers MDL3/MDL5 (Fernando et al. 2000) to sequence 592 bp of the mitochondrial DNA (mtDNA) control region and amplify microsatellite loci FH67, FH19R, FH48R, FH60R, FH94R, LA6R (Comstock et al. 2000, Eggert et al. 2008), FH126 (Comstock et al. 2002), and LafMS02 (Nyakaana & Arctander 1998). In a UV-sterilized hood, we performed the polymerase chain reaction (PCR) in 10 μL volumes containing 0.5 U Ampli*Taq* Gold DNA Polymerase, (Applied Biosystems, Foster City, CA, U.S.A.), 1× Ampli*Taq* Buffer II, 0.4 μM labeled forward primer, 0.4 μM reverse primer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 1× BSA, and 1 μL DNA extract. The profile consisted of denaturation at 95 °C for 10 min, 45 cycles of denaturation at 95 °C for 45 s, annealing at locus-specific temperatures for 35 s, and primer extension at 72 °C for 35 s. Products were separated in an ABI 3100 automated sequencer (Applied Biosystems) and allele sizes were scored with GeneScan 2.7 and Genotyper 2.5 (Applied Biosystems). We used an African savanna elephant sample to standardize scoring and a negative control to detect PCR contamination.

Using the preliminary sample genotypes, we estimated genotyping error rates in RELIOTYPE (Miller et al. 2002). We estimated $P(\mathrm{ID})_{\mathrm{random}}$ (the power to distinguish between random individuals) and $P(\mathrm{ID})_{\mathrm{sibs}}$ (the power to distinguish siblings) (Waits et al. 2001) in GENALEX 6 (Peakall & Smouse 2006). Because elephants are often found in groups of relatives (Archie et al. 2006), we used $P(\mathrm{ID})_{\mathrm{sibs}}$ to assess the power of our panel of loci.

For the remaining field samples, we developed a quality-control check to improve genotyping efficiency. Samples that did not amplify consistently at 5 of 8 loci within 2 attempts were eliminated. Retained samples were genotyped an additional 3 times. We included heterozygous genotypes in the data set after 2 matching scores and homozygous genotypes after 3 matching scores. This resulted in a high-quality data set and reduced costs. Sexes of individuals were determined genetically (Munshi-South et al. 2008). We inferred age class from bolus circumferences (circumference <32cm, juvenile; circumference >32 cm, adult) (Eggert et al. 2003).

We obtained mtDNA sequences from 48 individuals from Loango National Park, 30 individuals from Moukalaba-Doudou National Park, and 95 individuals from the MUA (59 dry season and 36 wet season). Sequences were obtained for both strands in an ABI 3100 automated sequencer (Applied Biosystems) and aligned with Sequencher 4.5 (Gene Codes, Ann Arbor, Michigan).

DNA Data Analyses

We used the Excel Microsatellite Toolkit to compare genotypes (Park 2001). Those that matched at all loci were considered the same individual. When possible,

Table 1. Dung samples collected in 2004 (June–December) and 2005 (January) for genotyping in Loango National Park during the transition between the dry and wet seasons, in the multiple use area of the Gamba Complex of protected areas in Gabon in the dry and wet seasons, and in Moukalaba-Doudou National Park during the transition between dry and wet seasons.

Site no.	*Site name*	*Collection date*	*N*	*No. genotyped (no. successfully genotyped)*	*Collection date*	*n*	*No. genotyped (no. successfully genotyped)*
Loango National Park							
1*	Iguela	8 Oct–13 Oct	25	25 (24)			
2	Loango	19 Oct–20 Oct	32	32 (12)			
3	Sette Cama	19 Oct–20 Oct	8	8 (4)			
		Total	65	65 (40)			
Multiple-use area							
		Dry season			**Wet season**		
4	Rabi	11 Jun–14 Jun	7	6 (4)	3 Nov–13 Nov	5	5 (5)
		25 Aug	3	3 (3)	19 Nov–17 Dec	8	8 (6)
					15 Jan–21 Jan	50	50 (40)
5	Divangui	22 Aug–27 Aug	14	7 (7)	18 Dec–19 Dec	5	5 (4)
					23 Jan	10	10 (1)
6	Echira	22 Jun	2	2 (1)	3 Dec–6 Dec	15	15 (14)
		9 Aug–11 Aug	160	117 (98)	19 Jan–21 Jan	30	30 (7)
		31 Aug	72	58 (50)			
7	Center	11 Jun–28 Jun	13	10 (5)	2 Nov–6 Nov	7	7 (7)
		13 Jul	5	4 (4)	27 Nov–30 Nov	11	11 (11)
		5 Aug	1	1 (0)	11 Jan–15 Jan	62	62 (52)
8	Koumaga	11 Jun	10	10 (7)	30 Sep–4 Oct	14	14 (14)
		10 Jul	16	8 (6)	2 Nov–6 Nov	15	15 (15)
		28 Jul	96	89 (61)	11 Jan	1	1 (1)
		4 Aug	52	46 (33)	24 Jan–26 Jan	49	49 (34)
9	Bongo	17 Jul–23 Jul	112	74 (51)	18 Nov–23 Nov	23	23 (21)
		1 Aug–2 Aug	169	149 (82)	4 Jan–8 Jan	38	38 (33)
		Total	732	584 (412)		343	343 (265)
Moukalaba-Doudou National Park							
10	Nyanga	22 Oct–25 Oct	7	7 (6)			
11	Moukalaba	10 Oct–25 Oct	30	30 (22)			
		Total	37	37 (28)			

**Site numbers correspond to those shown in Fig. 1.*

we compared bolus circumferences and sexes to confirm results. For genotypes that differed at 1–3 loci, we verified the accuracy of the genotype before considering them different individuals. We used MICROCHECKER (van Oosterhout et al. 2004) to test for errors due to stuttering, large allele dropout, and null alleles. We grouped genotypes of unique individuals by collecting site, calculated allelic diversity, and expected and observed heterozygosity values and tested for deviations from expected heterozygosity values under Hardy-Weinberg equilibrium (HWE) and for linkage disequilibrium with GENEPOP (Raymond & Rousset 1997). We evaluated the significance of results after applying a Bonferroni correction for multiple tests.

To test for genetic subdivision at microsatellite loci within and between seasons, we conducted a hierarchical analysis of molecular variance (AMOVA) in ARLEQUIN (Schneider et al. 2000) with data assembled into 4 groups: MUA dry season, MUA wet season, Loango National Park and Moukalaba-Doudou National Park. Individuals sampled at multiple sites ($n = 22$ dry season, $n = 4$ wet season) were included in each site. We calculated pairwise genetic distances (F_{ST}) between MUA sites for each season and between MUA sites and park sites sampled in the transition season in ARLEQUIN and assessed significance with permutation tests after applying a Bonferroni correction. We tested for isolation by distance for each season with IBDWS (Jensen et al. 2005) and assessed significance after applying a Bonferroni correction.

When the AMOVA revealed significant differences among groups, we analyzed the microsatellite data in STRUCTURE 2.3.4 (Pritchard et al. 2000) to investigate genetic and geographic patterns. Using the admixture model with allele frequencies correlated among populations, we performed 10 runs each of $K = 1$–10. The burn-in period was 100,000 repetitions followed by 1,000,000 repetitions. We examined each season separately and included data from the transition zone in the national parks

in both analyses. Individuals sampled at multiple sites were included in each site.

For mtDNA sequence data, we calculated pairwise genetic distances (F_{ST}) between collection sites for each season in ARLEQUIN and assessed significance levels with permutation tests. For each season, we tested for isolation by distance with IBDWS and assessed significance after applying a Bonferroni correction. We performed an AMOVA in ARLEQUIN with the same groupings as the microsatellite data.

Movement Patterns and Habitat Use

Locations of recaptured genotypes were plotted in ARCGIS 9.2 (ESRI, Redlands, CA) and used to evaluate movement distances and habitat use by season, sex, and age. To determine the proximity of samples to water sources, we used field notes, the national hydrological base map, and a digital elevation model (Jarvis et al. 2008). To estimate proximity to water (within 500 m), we used data only from the wet and dry seasons.

To examine habitat use, we assigned dung piles to 1 of 7 habitat types within 2 habitat categories: closed habitats (forest and riverine forest) or open habitats (forest-savanna edge, savanna, swamp, beach, or road). We used chi-square contingency tests in SPSS 19.0 (IBM, Armonk, New York) to examine associations between habitat category and sex and age classes.

Results

We collected 1177 dung samples (Table 1). All 25 preliminary samples had unique genotypes. RELIOTYPE estimated the reliability of these genotypes at a probability of 98.9%. The values of $P(\mathrm{ID})_{\mathrm{random}}$ (4.5×10^{-12}) and $P(\mathrm{ID})_{\mathrm{sibs}}$ (1.7×10^{-4}) indicated that 8 loci provided sufficient power to distinguish individuals. At 5 loci, these values were 2.7×10^{-7} and 5.1×10^{-3}, respectively.

For the remaining samples, 70.5% of dry season, 77% of wet season, and 64% of park samples were successfully genotyped (Table 1). RELIOTYPE estimated the reliability of these genotypes at 98.5%. Of 412 genotypes obtained from dry-season samples, 299 were unique, 64 were recaptures within collecting session, and 49 were recaptures from other collecting sessions or sampling locations. Of 265 genotypes from wet season samples, 215 were unique, 42 were recaptures within collecting session, and 8 were recaptures from other collecting sessions or locations. Eleven individuals were captured in multiple seasons. In Loango, we detected 37 individuals in 40 samples, and in Moukalaba, we detected 27 individuals in 28 samples.

The sex ratio in the MUA was strongly female biased (dry season, $\chi^2_1 = 147.49$, $P < 0.001$; wet season, $\chi^2_1 = 88.58$, $P < 0.001$) (Table 2). In Loango and Moukalaba-Doudou, sex ratio did not differ from 50:50 (Loango: $\chi^2_2 = 0.15$, $P = 0.926$; Moukalaba: $\chi^2_2 = 0.22$, $P = 0.895$), although these results should be viewed with caution because sample sizes were smaller and sexes were not assigned unless confirmed within 3 attempts. We were able to estimate age for 79.9% of individuals in the dry season ($n = 239$), and 36.7% in the wet season ($n = 76$, Table 2). Nearly one-quarter (23.8%) were considered juveniles, although some young females may have been included in this group on the basis of our bolus circumference cutoff (32 cm).

Nuclear Microsatellites

We found no evidence for linkage disequilibrium. Results from MICROCHECKER suggested low-frequency null alleles at 5 of 8 loci in at least one group, but only LafMS02 appeared to have at least one null allele in all groups. Because it had high allelic diversity, this locus was useful in individual identification, and we retained it. When MUA genotypes were analyzed by collecting site, deviations from expected heterozygosity values were confined to a few loci at a few locations (Bongo, Echira, Koumaga for dry season; Center, Bongo, Echira, Koumaga for wet season). We found no deviations at any locus for samples from the parks. When we analyzed the MUA by season rather than collecting site, heterozygosity values deviated from expectations at most loci (Table 3).

We found no evidence for isolation by distance in either season ($r_{\mathrm{dry}} = 0.1603$, $P = 0.2164$, $r_{\mathrm{wet}} = 0.2875$, $P = 0.0870$). The AMOVA results indicated significant subdivision among the 4 groups, among populations within groups, and within populations (Supporting Information) but not between seasons in the MUA ($F_{ST} = 0.0049$, Supporting Information). Pairwise F_{ST} values revealed that Iguéla differed significantly from most sites in the MUA and Moukalaba-Doudou during both seasons and that Moukalaba differed from approximately half of the MUA sites during both seasons (Table 4).

Although STRUCTURE results revealed 3 genetic clusters in the dry season (Supporting Information) and the wet season (Supporting Information), there was no clear association between genetic clusters and geography for either season.

Mitochondrial DNA

Nine of the 16 mtDNA haplotypes we detected matched previously described haplotypes (Supporting Information), whereas 7, including the most common 2 (Gamba1, Gamba4) were unique (GenBank accession #KF638276-638282). The average pairwise difference between haplotypes was 6.46 (3.07) base pairs and nucleotide diversity was 1.12% (0.59).

Results of testing for isolation by distance were marginally significant in the dry season ($r = 0.4790$,

Table 2. Number of elephants by sex and age class identified through genotyping of DNA extracted from dung collected in the multiple-use area of the Gamba Complex of protected areas in Gabon in the dry and wet seasons and in Loango and Moukalaba-Doudou National Parks in the transition season.

			Dry season			*Wet season*		
*Site**		*Sex*	*adult*	*juvenile*	*unknown*	*adult*	*juvenile*	*unknown*
Loango National Park								
1	Iguela	female	5	2	5			
		male	4	0	3			
		unknown	2	1	1			
2	Loango	female	1	1	0			
		male	3	0	0			
		unknown	4	2	0			
3	Sette Cama	female	0	0	0			
		male	1	1	0			
		unknown	1	0	0			
		total	21	7	9			
Multiple-use area								
4	Rabi	female	0	2	2	10	3	22
		male	1	1	0	1	0	4
5	Divangui	female	4	2	0	0	1	4
		male	0	0	0	0	0	0
6	Echira	female	71	8	12	2	2	7
		male	14	1	2	3	1	2
7	Center	female	6	0	1	6	11	39
		male	2	1	0	2	1	0
8	Koumaga	female	19	13	31	8	3	24
		male	4	1	3	7	3	7
9	Bongo	female	61	15	8	5	5	25
		male	13	0	1	1	1	5
		total	195	44	60	45	31	139
Moukalaba-Doudou National Park								
10	Nyanga	female	0	1	0			
		male	0	0	0			
		unknown	1	1	3			
11	Moukalaba	female	3	2	4			
		male	1	2	5			
		unknown	3	0	1			
		total	8	6	13			

Numbers are site numbers and correspond to sites shown in Fig. 1.

$P = 0.0281$) and highly significant in the wet season ($r = 0.5337$, $P = 0.0078$). The AMOVA indicated differentiation among populations within groups and within populations but not among groups (Supporting Information). Pairwise F_{ST} values (Table 4) indicated little differentiation within the MUA during the dry season. During the wet season, Rabi differed from all other MUA sites except nearby Divangui. Iguéla differed from all other sites during both seasons. Other than Iguéla, sites in the MUA differed less from sites in Loango than from those in Moukalaba-Doudou during both seasons.

Movement Patterns and Habitat Use

We detected 34 movements between sites. Twenty-two occurred during the dry season: 6 by adult males (AM), 12 by adult females (AF), and 2 each by juvenile males (JM) and females (JF) (Fig 2a). Most movements were into areas with water sources: 6 into Bongo, 12 into Echira. The only wet-season movement involved a female of undetermined age. Movements within the MUA between seasons were detected for 4 AF, one female of undetermined age, one AM, and 2 JF (Fig 2b). Five individuals detected during the dry season in areas with major water sources were detected during the wet season in areas without them, and 3 individuals moved between areas with water sources. Three longer-distance movements were detected. One AF moved from Iguéla to Koumaga in the wet season (80 linear km), and 2 females (1 AF and 1 of undetermined age) moved from Echira in the dry season to Moukalaba (110 linear km). These females were detected on different days at both sites, and their genotypes suggested they were unrelated.

Most dung was found near large permanent bodies of water at Bongo, Koumaga, and Echira, especially in the dry season (Tables 1 & 2). In the dry season, 72.9% of samples were found near water, whereas in the wet season 16.6% of samples were found near water. Males and females did not differ in proximity to water sources (both seasons: $\chi^2_1 = 0.08$, $n = 81$ males, $n = 435$ females,

Table 3. Genetic diversity of elephants derived from nuclear microsatellite loci for the multiple-use area of the Gamba Complex of protected areas in Gabon in the dry and wet seasons and for Loango and Moukalaba-Doudou National Parks (NP) in the transition season.[a]

		Multiple-use area dry season				*Multiple-use area wet season*				*Loango NP*				*Moukalaba-Doudou NP*			
Locus	*Allele range*	*A*	H_E	H_O	*Freq null*	*A*	H_E	H_O	*Freq null*	*A*	H_E	H_O	*Freq null*	*A*	H_E	H_O	*Freq null*
FH19R	93–121	15	0.890	0.829[b]	0.000	15	0.884	0.854	0.000	10	0.834	0.813	0.000	12	0.850	0.773	0.000
FH48R	73–107	16	0.876	0.768[b]	0.061	16	0.858	0.716[b]	0.083	12	0.863	0.710	0.098	9	0.871	0.905	0.000
FH60R	80–116	15	0.892	0.839[b]	0.000	18	0.906	0.883[b]	0.000	10	0.848	0.839	0.000	12	0.880	0.640	0.117
FH67	75–113	16	0.869	0.787[b]	0.000	14	0.834	0.785[b]	0.000	10	0.795	0.703	0.000	9	0.758	0.662	0.204
FH94R	78–106	12	0.758	0.669[b]	0.064	10	0.745	0.702[b]	0.000	7	0.543	0.459	0.000	6	0.763	0.683	0.000
FH126	83–125	21	0.926	0.781[b]	0.078	19	0.919	0.819[b]	0.054	16	0.911	0.943	0.000	14	0.913	0.750	0.000
LA6R	83–119	15	0.826	0.736[b]	0.054	13	0.845	0.767[b]	0.000	10	0.856	0.824	0.000	7	0.796	0.720	0.000
LafMS02	126–156	15	0.909	0.797[b]	0.061	16	0.904	0.731[b]	0.096	12	0.873	0.682	0.096	9	0.877	0.738	0.179
Average		15.6	0.868	0.776	0.040	15.3	0.862	0.782	0.029	10.9	0.815	0.747	0.024	9.8	0.839	0.734	0.063
SD		2.5	0.053	0.054	0.033	2.9	0.056	0.066	0.042	2.6	0.115	0.145	0.045	2.7	0.058	0.083	0.089

[a]*Abbreviations: A, allelic diversity; H_E, expected heterozygosity; H_O, observed heterozygosity; Freq null, frequency of null alleles estimated in Microchecker*
[b]*Value deviates significantly from Hardy-Weinberg expectations after a Bonferroni correction for multiple tests.*

$P = 0.79$), either separately for each season or when only adults were considered ($\chi^2_1 = 1.18$, $n = 46$ males, $n = 198$ females, $P = 0.28$). We found less dung at upland sites (Center and Rabi) and more dung in the wet than dry season. Divangui, an upland site with a small lake, had similar amounts of dung in both seasons; it was the only site where only females were found. A comparable number of males were sampled in both seasons, whereas more females were sampled in the dry than the wet season (Table 2).

During the dry season, males were more often associated with open habitats (84.6%, $n = 39$ males) than females (62.4%, $n = 255$ females; $\chi^2_1 = 7.40$, $P < 0.01$), but females outnumbered males approximately 5:1 in open habitats. The gender difference in habitat use remained significant when only savannas and swamps were considered open habitats ($\chi^2_1 = 6.00$, $n = 31$ males, $n = 227$ females, $P < 0.05$). We found no evidence for an association between sex and habitat category used during the wet season ($\chi^2_1 = 1.08$, $n = 39$ males, $n = 170$ females, $P = 0.30$) or between adult (seasons combined, $\chi^2_1 = 0.66$, $n = 50$ males, $n = 203$ females, $P = 0.42$) and juvenile elephants (seasons combined. $\chi^2_1 = 2.49$, $n = 13$ males, $n = 62$ females, $P = 0.12$).

Discussion

We detected over 500 elephants in the MUA during both seasons and in the national parks between seasons. Genetic diversity, as measured by haplotype diversity for mtDNA or allelic diversity and heterozygosity for microsatellites, was consistent with results of previous studies (Comstock et al. 2002; Johnson et al. 2007; Okello et al. 2008). Although there were no significant differences among expected or observed heterozygosity values in the MUA or parks, expected heterozygosity values for both seasons in the MUA were higher than in either park. Although the observed deviations from HWE in the MUA might be partially explained by low-frequency null alleles or genotyping error due to degraded DNA, our strict quality control measures minimized the probability of including erroneous genotypes in the data set. When considered with data on movement and population structure, the deviations suggest that elephants in the MUA did not represent a group of randomly mating individuals; rather, they suggest an assemblage of elephants from multiple populations and family groups.

Population Structure and Movements of Individuals

Although we did not detect population structure that correlated with differences between the MUA and the parks, we caution that this should not be interpreted as evidence that there has been no effect of land-use

Table 4. Pairwise genetic distances[a] between collecting sites in the multiple-use area of the Gamba Complex of protected areas in Gabon in the dry and wet seasons and for Loango and Moukalaba-Doudou National Parks (NP) in the transition season.

Season	*Iguela*	*Loango*	*Sette Cama*	*Rabi*	*Divangui*	*Echira*	*Center*	*Koumaga*	*Bongo*	*Nyanga*	*Moukalaba*
Dry											
Iguela	–	0.4635[b]	0.5046[b]	0.6721[b]	0.6437[b]	0.3452[b]	0.6980[b]	0.5529[b]	0.3717[b]	0.7592[b]	0.7108[b]
Loango	0.0219	–	0.0649	0.1331	0.0925	0.0788	0.1515	0.1562[b]	0.1836[b]	0.2854[b]	0.2457[b]
Sette Cama	−0.0481	−0.0413	–	0.0133	−0.0557	−0.0364	0.1971[b]	−0.0455	0.0638	0.0776	0.0532
Rabi	0.0444[b]	0.0425	−0.0076	–	−0.1401	−0.0380	0.4591	0.0677	0.3183[b]	0.2761	0.0473
Divangui	−0.0149	0.0259	−0.1000	0.0087	–	−0.0137	0.1481	−0.0590	0.2285	0.2377	0.0034
Echira	0.0277[b]	0.0359[b]	−0.0270	0.0065	0.0012	–	0.1252	0.0677	0.0687	0.1644[b]	0.1430[b]
Center	0.0556[b]	0.0787[b]	0.0027	−0.0035	0.0481	0.0456	–	0.2119	0.3850	0.7134[b]	0.5314[b]
Koumaga	0.0358[b]	0.0452[b]	0.0031	0.0083	0.0245	0.0089	0.0407	–	0.1197	0.2099[b]	0.0812
Bongo	0.0284[b]	0.0296	−0.0093	0.0174	0.0104	0.0111[b]	0.0336	0.1455[b]	–	0.3712[b]	0.3282[b]
Nyanga	0.0570[b]	0.0434	−0.0155	0.0354	0.0821[b]	0.0580	0.0630	0.0451	0.0599	–	0.1624[b]
Moukalaba	0.0329[b]	0.0369	−0.0504	0.0251	0.0192	0.0297[b]	0.0538	0.0253[b]	0.0293	0.0249	–
Wet											
Iguela	–	0.4635[b]	0.5046[b]	0.5567[b]	0.6417[b]	0.7640[b]	0.6718[b]	0.5028[b]	0.5957[b]	0.7592[b]	0.7108[b]
Loango	0.0291	–	0.0649	0.1118	0.1018	−0.0496	0.1688	0.2581	0.2440[b]	0.2854[b]	0.2457[b]
Sette Cama	0.0489	0.0361	–	0.1039	0.0019	0.1336	0.0225	0.0339	−0.0119	0.0776	0.0532
Rabi	0.0276	0.0403	0.0615	–	−0.1035	0.4442[b]	0.2633[b]	0.3320[b]	0.2889[b]	0.4556[b]	0.3254[b]
Divangui	0.0753[b]	0.0578	0.0893	0.0330	–	0.4248	0.1671	0.2588	0.1717	0.3603	0.1874
Echira	0.0341	0.0251	0.0516	0.0169	0.0391	–	0.2049	0.4139	0.3988	0.5476	0.4179[b]
Center	0.0376[b]	0.0328	0.0422	0.0054	0.0083	0.0064	–	0.2127	0.1882	0.0589	0.2714[b]
Koumaga	0.0371[b]	0.0299	0.0469	0.0070	0.0224	0.0153	0.0044	–	−0.0959	0.3519[b]	0.3144[b]
Bongo	0.0137	0.0170	0.0279	0.0040	0.0270	0.0193	0.0079	0.0076	–	0.2703[b]	0.1844[b]
Nyanga	0.0398[b]	0.0398	0.0367	0.0227	0.0313	0.0428	0.0152	0.0211	0.0120	–	0.1624[b]
Moukalaba	0.0475[b]	0.0411	0.0119	0.0312[b]	0.0328	0.0435	0.0314[b]	0.0353[b]	0.0193	0.0148	–

[a] *Values of F_{ST} calculated from mtDNA control region sequences are shown above the diagonal, and values of F_{ST} calculated from microsatellite genotypes are shown below the diagonal. Negative values indicate distances are not significantly different from zero.*
[b] *Significant in permutation tests in* Arlequin *after a Bonferroni correction for multiple tests.*

changes on forest elephant movements. There is an inherent time lag in genetic signatures of change at the landscape level (Landguth et al. 2010). Assuming a generation time of 25 years for African forest elephants (Blanc 2008), petroleum extraction and logging activity occurred over 2–4 generations. Landguth et al. (2010) found that the lag time to barrier detection with genetic methods can be relatively short (1–15 generations) for mobile animals with large dispersal distances. However, their models do not include the effects of overlapping generations, and they caution that detecting the effects of fragmentation on long-lived species over time scales relevant to conservation may be difficult.

Within the MUA, we found no significant differentiation between populations in the wet and dry seasons, suggesting that this region supports a resident population. The higher levels of differentiation at maternally inherited mtDNA are likely the result of low female dispersal and relatively small female home ranges (Blake et al. 2008). Sites that were most genetically distinct at both mtDNA and biparentally inherited microsatellites were outside the MUA. Elephants from Iguéla differed in mtDNA from elephants at all other sites and in nuclear DNA from elephants at many sites in both seasons. Iguéla samples came from the well-patrolled northern end of Loango, which is isolated by a large lagoon. Telemetry studies of elephants in Iguéla reveal highly localized movements (Blake et al. 2008; Schuttler et al. 2012). Although both telemetry and dung data were derived from a small number of individuals (6 and 23, respectively), they suggest elephants in Iguéla may be resident to that area. Other sites in Loango or its buffer zone (Loango, Sette Cama) did not differ consistently from sites in the MUA, which suggests some movement and gene flow. Because these sites were sampled only in the transition season, we cannot rule out the possibility that differentiation might be detectable during the wet or dry seasons. However, results of Blake et al.'s (2008) studies over multiple seasons and parks showed that home ranges of forest elephants are generally small, suggesting that seasonality plays a role in short but not longer-distance movements (i.e., between parks).

During the dry season, elephants at Moukalaba (58–107 km from MUA sites, separated by mountains) did not differ at mtDNA or nuclear microsatellite DNA from elephants at most MUA sites, but during the wet season the Moukalaba elephants differed at maternally inherited mtDNA from most MUA elephants and at nuclear microsatellite DNA from elephants at Rabi, Center, and Koumaga. This suggests that elephants move into the MUA during the dry season and return to the Moukalaba region during the wet season. A similar pattern was observed for Nyanga, but the level of differentiation between elephants at Nyanga and elephants in the MUA was greater than that of elephants at Moukalaba at both

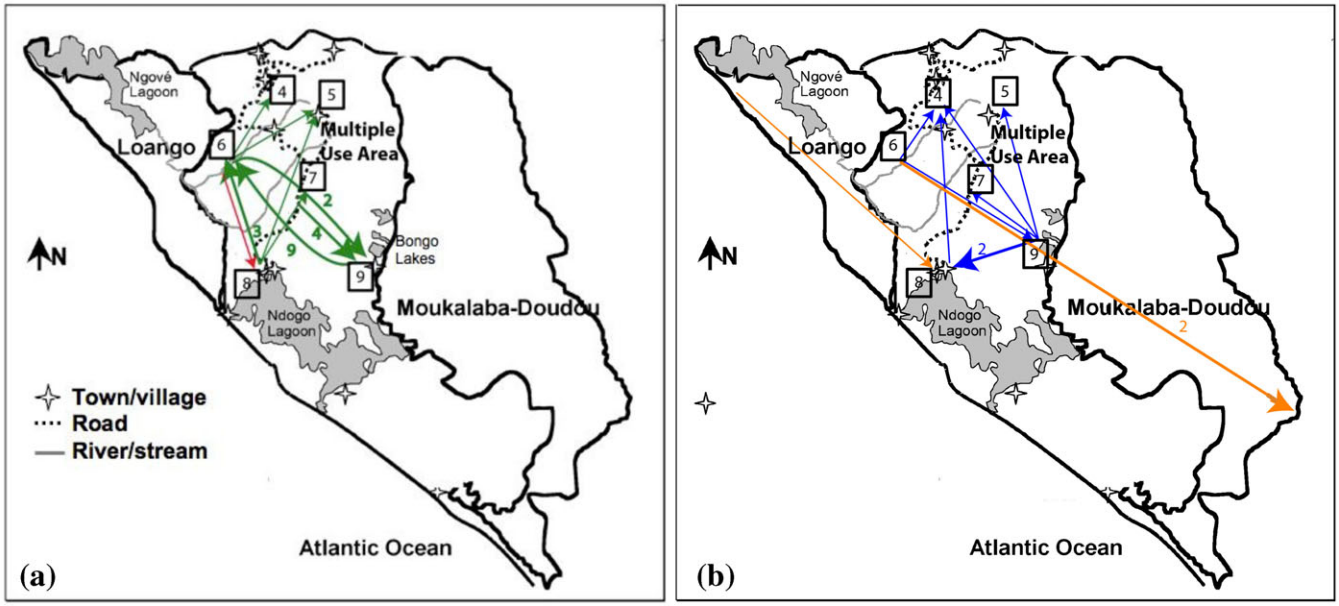

Figure 2. Movements of individual elephants detected in the Gamba Complex of Protected Areas, Gabon (thin lines, movement of a single elephant in the direction of the arrow; thick lines, movement by multiple elephants [number shown]). In (a) green lines indicate movements in the dry season and the single red line indicates movement during the wet season. In (b) blue lines indicate movements between the dry and wet seasons within the multiple-use area and orange lines indicate movements between sites in the national parks and the multiple-use area.

mitochondrial and nuclear DNA in the dry season. This pattern is supported by observations and GPS tracks of elephants moving along the coast from Nyanga through Setté Cama in the wet season (Kolowski et al. 2010) and congregating in the papyrus swamps around the Nyanga River in the dry season (M.L., personal observation). We speculate the Nyanga River swamps may sustain elephants from a different subgroup than those gathering around MUA water sources such as Bongo or Koumaga.

Movement and Site Visitation

Although movement data were limited to 34 individuals, most movements were over short distances (approximately 30 km), mostly within the MUA. Only 3 covered longer distances: 1 AF moved from Iguéla to Koumaga (80 linear km), and 2 unrelated AF moved from Echira to Moukalaba (110 linear km). The other 91% of movements were limited to the MUA, most involved individuals moving into or between major water sources in the dry season (Koumaga-Echira, 30 linear km; Bongo-Echira, 45 linear km). We also detected most dung near large, permanent water sources (Bongo, Koumaga, Echira), which suggests high visitation, especially in the dry season. Wetlands are abundant in high-quality browse (Blake 2002), and seasonally flooded grasslands fringing wetlands may provide important grazing for forest elephants (Tchamba & Seme 1993), especially during the dry season (Buij et al. 2007). Our results further showed that males may be particularly attracted to wetlands and savannas, which could serve as important gathering spots in a resource-limited season and appear to play an important role in directing elephant movements to particular sites in particular seasons.

Implications for Wildlife Management

We estimate that the MUA contains approximately one-tenth of the global population of African forest elephants (approximately 100,000 in 2011 [Maisels et al. 2013]). Our genetic and movement data suggest the MUA provides year-round habitat for some individuals, particularly females, and additional habitat for elephants whose primary range is in the parks. The wetlands, lagoons, and lakes in the MUA provide critical, year-round resources and may serve as congregation points for elephants, providing an avenue for gene flow. Increasing infrastructure development, poor management of existing infrastructure, hunting pressure or forest fragmentation could impede such gene flow. We therefore recommend that critical water and food resources located outside the parks be fully protected. Although Bongo Lakes and Echira are in the process of being incorporated into Loango and Moukalaba-Doudou national parks, the Koumaga wetlands are not. We recommend protection for Setté Cama (in the buffer zone of Loango) and Divangui (designated by the logging company as a conservation zone), which appear to support elephant movements and are located on water sources.

At the time of our study, protection for elephants and other wild species in the MUA may have been as good or better than the national parks, which were just being set up. Access to the MUA had been controlled for years by companies that prohibited hunting or transport of wildlife products (Laurence et al. 2006*b*). Since then, most of the MUA has been zoned for logging. Although logging operations hold government-approved sustainable management plans and are certified by the Forest Stewardship Council, the nature of their extractive operations, which include expanding road networks, will inevitably affect one of Gabon's last tracts of mature sedimentary-basin rainforest and its globally important population of elephants. Today, the MUA is in the process of having its partial-protection status removed except along park borders, including Bongo Lakes and Echira. Scientific evidence supports the fact that roads, and the size of roadless wilderness, are the most important variables defining the distribution and movement patterns of forest elephants in the MUA (Buij et al. 2007) and throughout the Congo Basin (Blake et al. 2008). If the MUA's protection status must be removed, replacing it with a new, legally enforceable mechanism, such as sustainable-development guidelines, should be a top priority.

Acknowledgments

We thank Shell Gabon and the Smithsonian Conservation Biology Institute for funding and in-kind support. We thank the National Center for Research and Scientific Technology, National Parks Gabon, and the Directorate of Wildlife and Hunting (DFC) for permits and the DFC and Wildlife Conservation Society (WCS)/Operation Loango for dedicating agents to our study. Logistics were facilitated by Satram, Sodexho, and WCS/Operation Loango. C. McIntosh and A. Garrison provided assistance with genotyping. This is publication 120 of the Gabon Biodiversity Program.

Supporting Information

The results of AMOVA tests for differentiation among groups of elephants based on nuclear microsatellite genotypes and mitochondrial DNA control region sequences (Appendix S1), the distribution of mitochondrial DNA haplotypes among collecting sites (Appendix S2), and the genetic clusters detected in Structure 2.3.4 (Appendix S3) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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