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Who are the elephants living in the hybridization zone? How genetics may guide conservation to better protect endangered elephants

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ABSTRACT

After a long-standing debate, African elephants are now considered by the IUCN as two distinct species: savannah elephants (*Loxodonta africana*), listed as endangered on the IUCN Red List of Threatened Species, and forest elephants (*Loxodonta cyclotis*), critically endangered. Both are severely threatened by forest loss, fragmentation and degradation due to agriculture expansion, as well as by illegal ivory trade. Although the two species have different habitat preferences, their range overlaps in some ecotones; despite an ancient separation between these two species, hybrids have been reported in five locations. The main hybrid hotspot is located on the Democratic Republic of Congo-Uganda border and still remains understudied. Using 15 microsatellites, we investigated this hybridization zone by determining the species and hybrid status of 177 fecal samples collected in the area of Sebitoli, at the extreme North of Kibale National Park. Surprisingly for a forest area, no pure forest elephants were detected. Out of the 91 individuals sampled, a very large proportion (81.3%) were hybrid individuals mainly from a second generation or more. Only 18.7% of pure savannah elephants were detected, all originating from the DRC-Uganda border. Further analyses are necessary to assess the age of this hybridization zone. Our results emphasize that hybrids and savannah elephants can successfully range in forested area. They also show that forest elephants are rare even in their native habitat. In the current context of high threat faced by African elephant species, it is crucial to strengthen conservation efforts for these species before it is too late.

1. Introduction

After a long-standing debate between researchers considering African elephants as two distinct species (Groves and Grubb, 2000; Grubb et al., 2000; Roca et al., 2001, 2005, 2007; Comstock et al., 2002; Eggert et al., 2002; Rohland et al., 2010; Mondol et al., 2015), and those considering them as two subspecies belonging to a single species (Debruyne, 2005; Johnson et al., 2007), the International

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Union for Conservation of Nature (IUCN) has shifted its position and now considers for the first time the African elephants as two distinct species: the African savannah elephant (*Loxodonta africana*) and the African forest elephant (*Loxodonta cyclotis*) (Hart et al., 2021). Previously, the African Elephant was listed as "vulnerable" on the IUCN Red List of Threatened Species while now savannah elephants are "endangered" (Gobush et al., 2021a) and forest elephants "critically endangered" (Gobush et al., 2021b). Approximately 415 000 African elephants remain in 37 countries and only 9% of them would be forest elephants (Thouless et al., 2016). The latest Elephant Status report 2016 from IUCN is the first in 25 years that has reported a continental-wide decline in African elephant numbers.

Many phenotypic and behavioral differences between savannah elephants and forest elephants have been reported. Forest elephants are smaller, with small rounded ears, and long thin tusks directed toward the ground, while savannah elephants are larger, with large triangular ears, and their tusks are often thicker and curved forward (Grubb et al., 2000; Morgan and Lee, 2003). Forest elephants appear to be more frugivorous (Merz, 1981; Short, 1981, 1983; White et al., 1993; Grubb et al., 2000; Turkalo and Fay, 2001; Morgan and Lee, 2007) than savannah elephants, which have a grass or browse diet (Napier Bax and Sheldrick, 1963; Field, 1971; Codron et al., 2011). Forest elephants are also found in small groups of one to four individuals with a core social structure thought to be that of a mother-calf pair (White et al., 1993; Grubb et al., 2000; Turkalo and Fay, 2001), unlike savannah elephants which live in large family groups of ten to 15 individuals and above (Buss, 1961; Douglas-Hamilton, 1973). The age at first birth among forest elephants is also nearly double that of savannah elephants (Turkalo et al., 2018). Even communication differs, with a significative difference in call structure between savannah and forest elephants (Pardo et al., 2019). Genetic studies have also shown a large genetic variation between the two species (F_{ST}=0.94 in Roca et al., 2001; R_{ST}=0.90 in Comstock et al., 2002).

Despite the above differences, hybrid elephants have been reported in some forest-savannah ecotones including Garamba National Park (Roca et al., 2001, 2005; Comstock et al., 2002; Mondol et al., 2015; Kim and Wasser, 2019); the northern Central African Republic (CAR) (Mondol et al., 2015; Kim and Wasser, 2019); along the Democratic Republic of Congo (DRC)-Uganda border (Mondol et al., 2015; Kim and Wasser, 2019); along the Pendjari-Arli complex on the Benin-Burkina Faso border (Mondol et al., 2015; Kim and Wasser, 2019), and the Gourma region in Mali (Mondol et al., 2015; Kim and Wasser, 2019). To date, a small proportion of individuals have been confirmed to be hybrids: 171 samples out of 2122 have been identified as belonging to hybrid individuals, all located in only 14 of the 411 input zones were samples have been collected (Kim and Wasser, 2019). Of these 171 samples, 107 (62.6%) were collected along the DRC-Uganda border, which made it the main hybridization zone in Africa (Kim and Wasser, 2019). Despite the ancient separation between the two species, estimated to be between 2.6 and 5.6 million years ago (Barriel et al., 1999; Roca et al., 2001; Eggert et al., 2002; Rohland et al., 2010), Mondol et al. (2015) detected numerous second-generation hybrids, indicating that hybrids

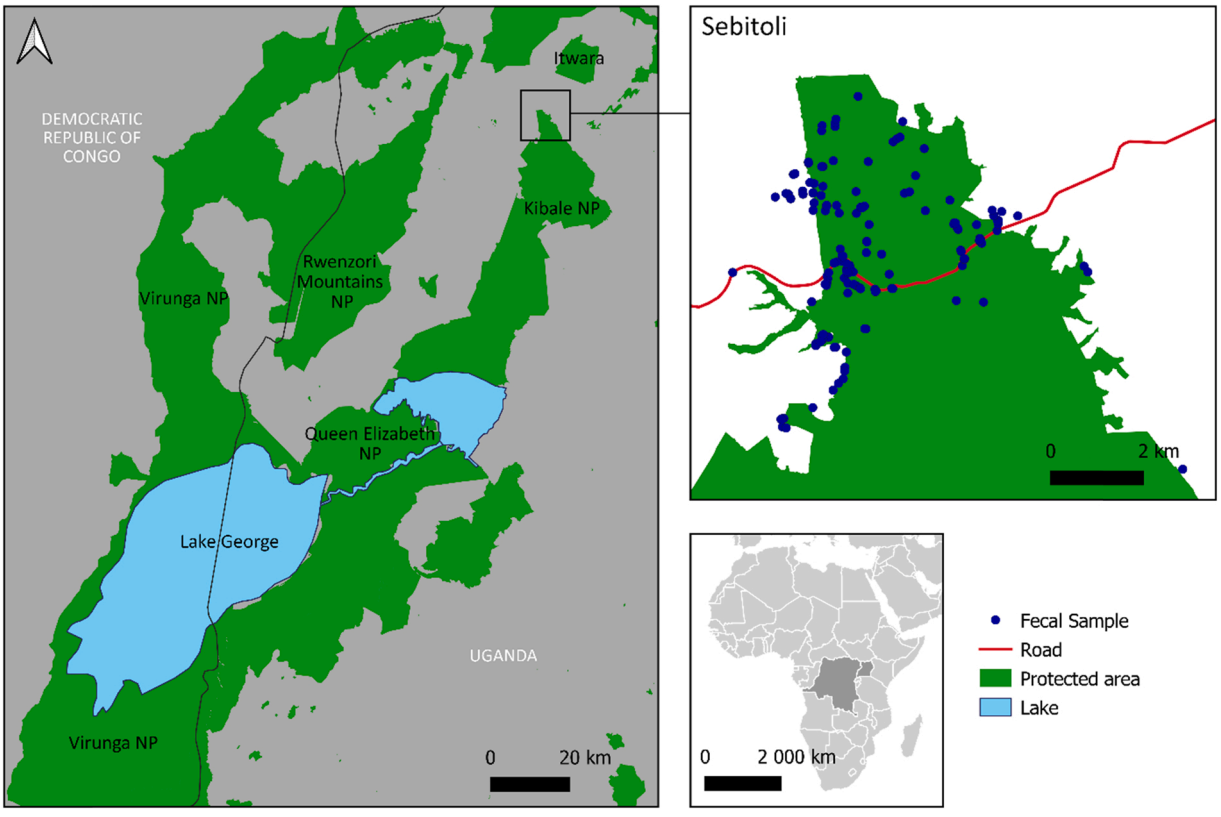

Fig. 1. Map of the Democratic Republic of Congo-Uganda border, showing National Parks (NP), including Kibale National Park (Uganda), and the Sebitoli area, where the fecal samples of elephants were collected.

are fertile.

In this study, we aim to better determine the composition of the elephant population living in a forested hybridization area in terms of species. Our goal is to provide a better knowledge of the main hybrid hotspot described by Kim and Wasser (2019) and Hart et al. (2021), to support efficient conservation plans, and especially to protect the critically endangered forest elephants.

We conducted our survey in a forested area in Uganda, at the border between the Democratic Republic of Congo and Uganda (DRC-Uganda), which was already molecularly identified as a hybridization zone by Mondol et al. (2015). This previous survey collected and analyzed 209 samples from both size of the DRC-Uganda border, of which only 42 were collected in Kibale National Park, in both savannah and forest habitat and found a low proportion (6.8%) of forest elephants (data from Mondol et al., 2015 and additional unpublished data). This new genetic study, focused on the forested Sebitoli area located at the extreme north of Kibale National Park at about 60 km as the crow flies from the savannah area of the Queen Elizabeth National Park, with no other forest connections but the South of the park, can shed light on the population dynamics at play in this particular hybridization zone, thanks to an extensive sample collection over a smaller area. As it is a forested area connected to savannah habitat, we expect to find both forest and savannah elephants, as well as F1, F2 and F2 + generation hybrids, as we assume they are fertile.

Using a panel of 15 microsatellites, we determined the species and the hybrid status of 177 fecal samples collected in the Sebitoli area, expanding the knowledge regarding this hybridization zone.

2. Material and methods

2.1. Study site

This study was conducted in the Sebitoli area in the extreme north of Kibale National Park (0°13' to 0°41'N and 30°19' to 30°22'E), southwestern Uganda (Krief et al., 2014). The park occupies 795 km^2 of mid-altitude moist forest, secondary forest, grassland, swamps and plantations of eucalyptus and pines (Chapman and Lambert, 2000). The Sebitoli area, approximately 25 km^2, commercially logged in the 1970's, is now composed of 70% regenerating forests and only 14% old growth forest (Bortolamiol et al., 2014).

The north of Kibale National Park is separated by 15 km of anthropized areas from Itwara forest, the nearest forest fragment, making Sebitoli area a dead end for forest wildlife (Fig. 1). Moreover, the border of Kibale National Park is highly populated (Hartter, 2010), and agricultural lands surround the forest: tea and eucalyptus plantations, as well as crops which attract wildlife, including elephants (Naughton-Treves, 1998). The only passage outside of Kibale National Park is to the South, where it connects with a savannah area, Queen Elizabeth National Park. The latter is also connected on the West side with Virunga National Park in DRC, which is a forest area. Migration of elephants from Virunga National Park to Queen Elizabeth National Park has been observed on several occasions since the 1960 s, much of which appears to be largely unidirectional from DRC into Uganda (Keigwin et al., 2016).

2.2. Samples

From November 2016 to January 2019, 187 fecal samples were collected inside the forest, and into cropfields at the border of the forest (Fig. 1). Given the difficulty of finding fresh feces, we collected all the fresh samples we found and then intensified the search in areas of the forest where no collection had been made. However, we avoided collecting feces of the same size, at the same location, on the same day, to limit resampling the same individual. All fresh feces found outside of the forest were collected. A quantity of 10–15 g of feces were placed in 70% ethanol for 24–48 h. After pouring the supernatant, feces were stored in a gauze placed on silica gel beads. Samples were stored at ambient temperature.

For each sample, we recorded the date and hour of collection, and the location. Samples were transported to France in compliance with the Memorandum of Understanding MNHN/UWA/Makerere University SJ 445-12.

2.3. DNA extraction, microsatellite locus amplification and genotyping

DNA extraction and amplification were performed at the "Plateau de Paléogénomique et Génétique Moléculaire P2GM" (UMR 7206) from the French National Museum of Natural History (MNHN) at the Musée de l'Homme in Paris.

After removing the largest vegetation parts, between 150 and 200 mg of dried feces were extracted with the Power Fecal DNA Isolation Kit from MoBio (Carlsbad, CA, USA).

DNA extracts were amplified at 15 microsatellite loci developed for African elephants (FH19, FH39, FH40, FH48, FH60, FH67, FH71, FH94, FH102, FH103, FH126, FH127, FH129, FH153, Laf MS04) (Nyakaana and Arctander, 1998; Comstock et al., 2000, 2002). We divided the set of microsatellites into 4 multiplex mixes (Mix1: FH67, FH94 and FH129; Mix2: FH60 and FH126; Mix3: FH39, FH102, and Laf MS04; Mix4: FH48 and FH103) and 5 simplexes (FH19, FH40, FH71, FH127 and FH153).

Polymerase-Chain Reaction (PCR) amplification was carried out using the Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany) in a 15.5 μL reaction volume containing 8.0 μL of 2x Type-it Microsatellite PCR Master Mix buffer, 4.27 μL of 10x primer mix (1.5–2.5uM of each primer), 1.73 μL of RNase-free water and 1.5 μL of template DNA.

The amplification conditions started with an initial step of denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, annealing at a specific temperature depending on the multiplex set (at 58 °C or 60 °C) for 90 s, and 30 s at 72 °C and lastly a final extension at 72 °C for 45 min. Negative controls were included in all PCR reactions to enable detection of cross contamination of the samples, and a known positive control was also amplified. Between 2 and 6 independent amplifications were performed on each sample.

Then three PCR simplexes were incorporated into the previous multiplex mix and two of them (FH71 and FH127) formed a fifth multiplex for the genotyping analysis. The 5'-end of the forward primer was fluorescently labeled (FAM, YakimaYellow, ATTO565, ATTO550). Amplification products were diluted with water, 2 μL of the diluted amplification product was added to 0.12 μL of 600 LIZ size standard (Applied Biosystems, Foster City, USA) and 9.88 μL of formamide. Genotyping was performed on an ABI 3130 capillary sequencer (Applied Biosystems, Foster City, USA) at the "Service de Systématique Moléculaire" (UMS 2700) of the French National Museum of Natural History (MNHN). Allele sizes were scored using the program Peakscanner v1.0 (Applied Biosystems, Foster City, CA, USA).

To minimize error due to allelic dropout or spurious alleles, all heterozygotes were scored at least twice and homozygotes were scored at least three times (Wasser et al., 2004, 2015; Chiyo et al., 2011). Ten samples did not amplify well or were run only once because of low quantity of DNA. They were excluded from further analysis, leaving a total of 177 samples genotyped.

Species identification for the Sebitoli samples was performed using the reference samples from Wasser et al. (2015), which were genotyped at the Center for Conservation Biology at the University of Washington, Seattle, USA. In order to match those reference samples, 20 of the Sebitoli samples were also amplified and genotyped by the Center for Conservation Biology on a capillary sequencer ABI 3730 (Applied Biosystems, Foster City, CA, USA). Then we determined the correction factor for each locus, as needed, to assure that the allele calls of the genotyping performed in the two laboratories corresponded to one another.

2.4. Identification of the individuals

As the elephants were not seen during sample collection, several samples could have been collected from the same individual. We thus used CERVUS software (Marshall et al., 1998; Kalinowski et al., 2007) and RELPAIR software (Epstein et al., 2000) to create composite genotypes for paired samples determined to be from the same individual.

We calculated the probability of identity (PID), which is the probability that a pair of individuals will match at a specific number of loci, to determine the minimum number of loci required to reliably discriminate between genetic samples collected from different individuals. Previous studies have identified a PID threshold of 0.0001 as sufficient for discriminating between genotypes of different individuals (Waits et al., 2001; Creel et al., 2003). We sought to identify the number of loci that would provide a similar threshold for our studied population. We calculated the PID from allele frequency using the formula provided by Waits et al. (2001). We treated two genotype samples as coming from the same individual if 9 or more loci were identical. We also allowed for a mismatch at a maximum of 3 additional loci for pairs that were identical at all other loci that we typed to further minimize possible genotyping error. With this method, we obtained 91 individuals from our 177 genotyped samples.

Molecular sexing was previously carried out on the same set of samples using a novel TaqMan-MGB qPCR technique described in Aznar-Cormano et al. (2021).

2.5. Species and hybrid assignment analyses

The reference sample set consists of 2407 samples gathered by the Center for Conservation Biology, including 1692 savannah elephants and 715 forest elephants (Mondol et al., 2015). The individuals identified in the present study were combined with those 2407 reference samples to run EBhybrids software (Mondol et al., 2015). We then obtained for each individual i) a probability of being a hybrid or a pure species and ii) probabilities of being each of five possible hybrid categories: 1) First generation (F1) which is the result of the reproduction of a pure savannah and a pure forest elephant, 2) Second generation (F2) which is the result of the reproduction of two F1, 3) backcrossed savannah which is the result of a reproduction of a F1 and a pure savannah elephant, 4) backcrossed forest which is the result of the reproduction of a F1 and a pure forest elephant, and 5) unassigned hybrids which are samples that fall outside the previous four categories. We used cutoffs of 50%, 80% and 95% hybrid probability. The 95% threshold allows us to be very confident about not including pure forest or savannah elephants in the hybrid group. Conversely, a threshold of 50% allows us to be sure not to include hybrids in the pure forest and savannah elephant groups. Since this area is a main hybridization zone and the presence of hybrids has already been demonstrated, we will use the 50% threshold to accurately determine the presence and proportion of pure forest and savannah elephants.

2.6. Geographic origin assignment

We used SCAT2 version 2.2 software (Wasser et al., 2004) to assign a geographic origin to pure individuals. This software uses an isolation-by-distance model to create continuous allele frequencies for forest and savannah elephants across their respective ranges, which enables allele frequencies to be estimated for areas that lack reference samples based on allele frequencies from nearby populations. Applying the continuous assignment technique to these data enables SCAT to assign locations to areas with few or no reference samples. SCAT2 was run 9 times for each pure sample using the appropriate set. We used custom species range files which exclude ocean, but include areas along the forest/savannah border in which either savannah or forest elephants might possibly be found. We then ran VORONOI version 1.1 software (Wasser et al., 2007) to improve inference of the sample origins, using the same custom map file used in SCAT2.

3. Results

Of the 187 samples collected un the Sebitoli area, 177 were assigned to 91 individuals. Among them, 74 (81.3%) were assigned to

hybrids, 17 (18.7%) to the savannah species, and none to the forest species, using the 50% threshold (Table 1A).

Among hybrids, using the same threshold, we get one F1 (1.4% of the hybrids), 23 F2 (31.1%), 41 backcrossed savannahs (55.4%), and six backcrossed forest (8.1%) (Table 1B). Three hybrid individuals (4.1%) were unassigned hybrids (i.e. fell outside one of the first four categories) although their probability of being a hybrid was greater than 99%. By considering higher thresholds, we still retain a large proportion of hybrids: with the 80% threshold, we obtained 70 hybrids (76.9%) and with the 95% threshold, 66 hybrids (72.5%) (Table 1A).

Out of the 91 individuals, 26 individuals (18 hybrids and 8 savannah elephants) were sampled at least twice, between several days and two years apart.

Among the savannah elephants, 14 were males and three were females. The set of hybrids is composed of 48 males and 26 females (Table 2).

Analyses carried out with SCAT2 software showed that the origin of only 6 of the 17 savannah elephants could be localized with precision, all of them being from the DRC-Uganda border (Fig. 2A). For the others, the region of possible origin is very large (Fig. 2B). However, when this set of individuals is considered as a group in the VORONOI analyses, all seem to originate from the DRC-Uganda border as well (Fig. 3).

4. Discussion

This study, based on 177 analyzed elephant fecal samples collected in the forested area in the north of Kibale National Park, shows that 81% of the 91 different individuals are hybrids, the remaining being savannah elephants likely coming from the Uganda-DRC border. No individuals of the critically endangered species of forest elephant were detected. Among the 74 hybrids, only one elephant was a first generation hybrid, the majority (55.4%) being backcrossed savannah elephants. Our results confirm that hybrids are fertile with 98.6% of hybrids belonging to second generation or more.

Mondol et al. (2015) previously analyzed 209 samples, each presumed from individually unique elephant collected in a vast area along the DRC-Uganda border in both savannah and forest ecosystem potentially including Sebitoli area. They reported at least 38 hybrids (18.2%), using a 95% hybrid threshold. Using the same threshold, we identified a much higher proportion of 72.5% of hybrids. Contrary to what would have been expected from a sampling in a forest ecosystem and to previous survey by Mondol et al. in 2015 in which seven (16.7%) of the 42 elephants sampled in Kibale forest were assigned to forest elephants, our survey did not detect any forest elephants. Also, the low proportion of F1 (only one individual found in the current survey) is similar to the results of Mondol et al. (2015) where no F1s were detected. The three individuals not assigned to a class of hybrids would likely be products of several generations of hybrid crosses.

The high proportion of males sampled could be due to the sampling method. Females would have been sampled less because only one fecal sample of the same size was collected even when there were several to avoid repeatedly sampling the same individual. Thus, only a small proportion of the adult females were probably sampled, compared to the more solitary males.

Despite the ancient separation between the two African elephant species (Barriel et al., 1999; Roca et al., 2001; Eggert et al., 2002; Rohland et al., 2010), our results confirm those of Mondol et al. (2015) on the ability of hybrids to reproduce. This result contrasts with that of Roca et al. (2007), who supported a lack of reproductive success among hybrid males, which would make them an evolutionary dead end. However, the Roca et al. (2007) study was based on a very small number of hybrids. Roca et al. (2007) suggested that competition between males for access to reproductive females might have caused this disparity (i.e., large savannah males could have much greater reproductive success than their smaller forest counterparts, with savannah elephant males sometimes reaching twice the size of forest elephant males). However, Mondol et al. (2015) used mtDNA and Y-STRs to show that paternity included both forest and savannah elephants among their samples. The samples collected by Roca et al. (2007) came from the Garamba region (DRC), a mainly open environment with vast savannahs interspersed with gallery forests (De Merode et al., 2000), while the north of Kibale National Park is a mountainous equatorial forest which was logged in the 20th century, leading to a bushy undercover. This closed environment, in addition to possibly favoring smaller phenotypes, could help to reduce this competition by at least visually isolating the individuals, and thus allow access to reproduction for smaller males such as forest or hybrid males.

There is little literature that specifies whether forest elephants were found in the region near the Uganda-DRC border prior to the

Table 1
A) Distribution of the 91 elephants in the different species categories, using three thresholds (50%, 80% and 95%). S: pure savannah elephants, F: pure forest elephants, H: hybrid individuals, Species_unk: unassigned individuals. B) Distribution of the hybrid individuals in the different hybrid categories, using three thresholds (50%, 80% and 95%). F1: F1 hybrid; F2: F2 hybrids; BXS: Backcrossed savannah; BXF: Backcrossed forest; Hybrid_unk: unassigned individuals.

Probability Threshold	Species (n=91)				Probability Threshold	Number of hybrids	Hybrids				
	S	F	H	Species_unk			F1	F2	BXS	BXF	Hybrid_unk
50%	17 (18.7%)	0	74 (81.3%)	0	50%	74	1 (1.4%)	23 (31.1%)	41 (55.4%)	6 (8.1%)	3 (4.1%)
80%	16 (17.6%)	0	70 (76.9%)	5 (5.5%)	80%	70	1 (1.4%)	11 (15.7%)	24 (34.3%)	0	34 (48.6%)
95%	13 (14.3%)	0	66 (72.5%)	12 (13.2%)	95%	66	0	5 (7.6%)	12 (18.2%)	0	49 (74.2%)

Table 2
Distribution of each sex in the different species/hybrid categories using the 50% threshold. S: Pure savannah elephants; F1: F1 hybrid; F2: F2 hybrids; BXS: Backcrossed savannah; BXF: Backcrossed forest.

	S	Hybrids					*Total*
		F1	F2	BXS	BXF	Unassigned	
Probability threshold		80%	80%	80%	50%		
Male	14	0	15	27	4	2	62
Female	3	1	8	14	2	1	29
Total	17	1	23	41	6	3	91

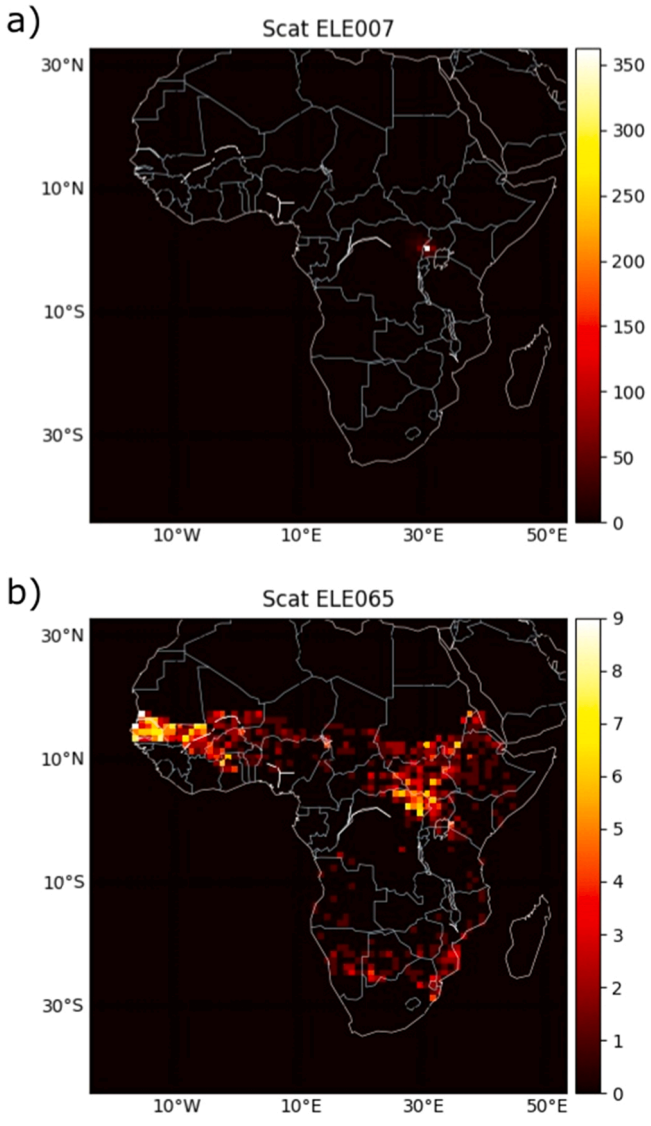

Fig. 2. Maps illustrating the SCAT2 results for two elephant samples (a) ELE007 and (b) ELE065, showing their respective most probable geographical origins in colored squares of one degree of latitude by one degree of longitude. The values corresponding of the color scale for each square are the number of SCAT inferences out of 900 that fell within that square. The individual ELE007 appears to originate from the Democratic Republic of Congo-Uganda border region (over 350/900 inferences). The individual ELE065 have a very large possible region of origin (9/900 inferences), suggesting some degree of undetected hybridization.

immigration caused by poaching. Elephants observed in savannah areas such as Queen Elizabeth National Park or Murchison Falls National Park could be savannah elephants (Laws, 1966; Field and Laws, 1970). However, the species originally present in Kibale National Park is not known with certainty. Elephants present in other forest parks in western Uganda have been reported by locals, and

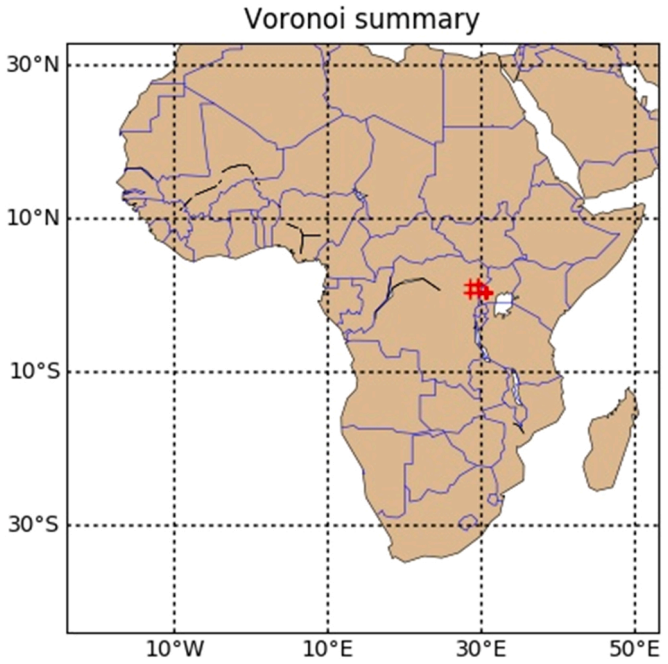

Fig. 3. Africa's map showing a probable historical geographical origin of 17 savannah elephants sampled in the Sebitoli area, indicated by red crosses, based on the Voronoi results that recognizes all of them as originating from the Democratic Republic of Congo-Uganda border.

tourism and conservation NGO actors as forest elephants (Kaganda Julius and Maate Dezi in Rwenzori Mountains National Park, Okimat John Paul in Budongo Forest, personal communications). However, the morphological phenotype of hybrids has not yet been studied, and descriptions report a gradient of intermediate phenotypes observed in the hybridization zones (Groves and Grubb, 2000). It is possible that the forest elephants described by local informants are in fact populations containing hybrids as they are present in the DRC-Uganda border hybridization zone.

The 17 putative savannah elephants sampled in this study appear to originate, as a group, in proximity to the DRC-Uganda border. This geolocation, close to the collection site, could be due to the fact that reference samples were collected recently in this area, and would therefore prevent the real geographical origin of the pure savannah elephants from being obtained. However, without using grouping information, many of the putative savannah elephants have large uncertainties in their origins, which might suggest that the savannah elephants sampled in this study would still possess some degree of admixture.

Although our findings do not allow us to estimate the age of the hybridization zone, some clues should be reported. Despite numerous observations of migration between Virunga National Park in DRC and Queen Elizabeth National Park in Uganda (Plumptre et al., 2007; Keigwin et al., 2016), the vast majority of the hybrids were found on the Uganda side of the border: 55 hybrids were sampled on the Uganda side and nine on the DRC side (Mondol et al., 2015 and additional unpublished data). Yet, if this hybridization zone was ancient, we would also expect a large number of hybrids in the forest-woodland mosaic of North-East DRC, which does not appear to be the case. Moreover, the average age at first reproduction for savannah and forest elephants is respectively around 14 years old (Moss, 2001) and 23 years old (Turkalo et al., 2018). The presence of second generation or later hybrids, along with pure forest elephant, pure savannah elephant and F1 hybrids, is compatible with the hypothesis that the hybridization zone would be recent and that population movements would have been influenced by the poaching pressures that took place in the 20th century during the civil war under the mandate of Idi Amin (Stapenhurst and Sahr, 1999), as well as by intensive poaching in Uganda and the DRC (Eltringham and Malpas, 1980; Mondol et al., 2015; Wasser et al., 2015). The Kibale forest, which became a national park in 1993, could then serve as a refuge for threatened individuals (Struhsaker, 1997). However, it is very surprising that no forest elephants were sampled in this favorable ecosystem.

Further genetic analyses, using a large number of SNP markers distributed across the genome, would allow a better estimation of the age of this hybridization zone (Tonzo et al., 2020). These analyses could be combined with the study of ancient samples of known geographical origin, such as museum specimens or old ivory seizures, in order to determine the distribution and spread of the two species in this area before the migrations at the end of the 20th century.

Population census and monitoring in forested areas are challenging: whereas an overflight over savannah areas allows a direct population census, in most cases, one can only carry out an indirect census in the forest by counting fecal boluses (Barnes and Jensen, 1987). It is surprising to have sampled 91 different individuals ie 18.6% of the total population estimated at 487 elephants in just over two years and in only 3% of the surface of the Kibale National Park (Thouless et al., 2016). Given the difficulties involved in monitoring populations in forest environments, it is possible that the elephant population of Kibale National Park has been underestimated during the survey carried out by the Uganda Wildlife Authority in 2010. Conversely, estimating the size of a population using microsatellites

can lead to overestimation (Creel et al., 2003). However, the 15 microsatellites used in this study provide us with a PID smaller than the recommended threshold (Waits et al., 2001; Creel et al., 2003), which makes us confident that these 91 genotypes sampled in the Sebitoli area are indeed unique individuals. It is also possible that elephants move extensively within Kibale National Park, and that a large proportion of this population seasonally visit the Sebitoli area which has abundant water and food. These movements may be even wider and involve elephant populations from Queen Elizabeth National Park, which is inhabited by about 3 000 elephants (Thouless et al., 2016). These methodological difficulties hinder estimation of population size and thus influence the conservation status of the forest elephant. In addition, the dense forest makes it difficult to carry out conservation actions in the field, such as preventive patrols.

Forest elephants have suffered a decline of more than 80% of their population in less than two generation (Maisels et al., 2013). They are also scarcely present in hybridization zones, even in forested areas as shown by Kim and Wasser (2019), and in this study. The reduction in number of forest elephants can have a significant effect on the ecology of forest environments by reducing or modifying the seed dispersal pattern and forest regeneration (Poulsen et al., 2018). Indeed, forest elephants are highly frugivorous (Short, 1981; White et al., 1993; Turkalo and Fay, 2001) compared to the grazer-browser diet of savannah elephants (Codron et al., 2011). Moreover, even in a different environment, elephants may keep food preferences close to their original diet (Seydack et al., 2000). The role of “mega gardeners of the forest” (Campos-Arceiz and Blake, 2011) played by forest elephants, may not be as well performed by their savannah counterparts, particularly with regard to seed dispersal. Ethological and ecological studies are needed to find out how savannah elephants and hybrids interact with forest environment.

With the IUCN’s recent decision to recognize two distinct species of African elephants the question arises as to the status of hybrid individuals, which has not yet been determined. However, more than 80% of the individuals sampled in this study were found to be hybrids. For conservation purposes, it is important to study the hybridization zones, in order to know the number and proportion of hybrid individuals, the dynamics of these areas and their evolution, particularly in relation to the degradation of their natural habitat and human pressures such as poaching. Moreover, forest elephants, now considered critically endangered, deserve a special attention. Our results show that their presence is very likely declining even in forested areas where they were previously cohabiting with savannah elephants, supplanted by hybrids. Our results may provide useful information to wildlife authority to adapt their conservation plans to make sure all species and hybrids are benefitting from efficient measures to protect them from extinction in a near future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2021.e01917.

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