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Incipient signs of genetic differentiation among African elephant populations in fragmenting miombo ecosystems in south-western Tanzania

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Abstract

Habitat fragmentation can play a major role in the reduction of genetic diversity among wildlife populations. The Ruaha-Rungwa and Katavi-Rukwa ecosystems in south-western Tanzania comprise one of the world's largest remaining African savannah elephant metapopulations but are increasingly threatened by loss of connectivity and poaching for ivory. To investigate the genetic structure of populations, we compared the genotypes for nine microsatellite loci in the western, central and eastern populations. We found evidence of genetic differentiation among the three populations, but the levels were low and mostly concerned the younger cohort, suggesting recent divergence probably resulting from habitat loss between the two ecosystems. We identified weak isolation by distance, suggesting higher gene flow among individuals located less than 50 km apart. In a long-lived species with overlapping generations, it takes a long time to develop genetic substructure even when there are substantial obstacles to migration. Thus, in these recently fragmented populations, inbreeding (and the loss of heterozygosity) may be less of an immediate concern than the loss of adults due to illegal hunting.

Résumé

La fragmentation des habitats peut jouer un rôle majeur dans la réduction de la diversité génétique des populations sauvages. Les écosystèmes de Ruaha-Rungwa et de Katavi-Rukwa, au sud-ouest de la Tanzanie, abritent une des dernières grandes métapopulations d'éléphants de savane africains, mais elle est de plus en plus menacée par la perte de connectivité et le braconnage pour l'ivoire. Pour étudier la structure génétique des populations, nous avons comparé neuf loci microsatellites des génotypes des populations de l'ouest, du centre et de l'est. Nous avons trouvé des signes de différenciation génétique dans les trois populations, mais le niveau était bas et touchait plutôt les plus jeunes, ce qui laisse penser à une divergence récente résultant probablement de la perte d'habitat entre les deux écosystèmes. Nous avons identifié un faible isolement par la distance, ce qui suggère un plus grand flux génétique entre individus situés à moins de 50 km d'écart. Chez une espèce à longue durée de vie, où les générations se chevauchent, il faut du temps pour développer une sous-structure génétique même lorsqu'il existe de sérieux obstacles à la migration. Donc, dans ces populations fragmentées depuis peu, l'inbreeding (et la

perte de caractère hétérozygote) peut être moins préoccupant dans l'immédiat que la disparition des adultes à cause de la chasse illégale.

KEYWORDS
African elephant, genetic isolation, habitat fragmentation, microsatellites

1 | INTRODUCTION

Human population growth is one of the main drivers of natural habitat loss and increased isolation of natural landscapes (Jones et al., 2012; Pereira et al., 2010; Rands et al., 2010). Habitat loss and fragmentation is a conservation problem not only because of the direct loss of range and increased edge effects (Hanski, 2011; Lamb, Balmford, Green, & Phalan, 2016), but also because of the potential for inbreeding depression through genetic drift (Hedrick & Kalinowski, 2000), making restoration and conservation of wildlife corridors increasingly important in times of unprecedented habitat fragmentation (Graham, Douglas-Hamilton, Adams, & Lee, 2009; Jones et al., 2012). Of particular, concern is the speed and scale at which fragmentation is happening (Haddad et al., 2015; Hansen et al., 2013), because few migration routes are entirely within protected areas (Bartlam-Brooks, Bonyongo, & Harris, 2011; Harris, Thirgood, Hopcraft, Cromsigt, & Berger, 2009; Tucker et al., 2018). A recent study conducted across five continents indicates that fragmentation of natural habitat reduces biodiversity by 13%–75% with effects being greatest in the smallest and most isolated fragments (Haddad et al., 2015).

African elephant (*Loxodonta africana*) populations were historically distributed across the continent (Douglas-Hamilton, 1987), with very little or no genetic structure among populations (Georgiadis et al., 1994). But in recent years, fragmentation has escalated across their range largely restricting many mega-herbivores to protected areas (Graham et al., 2009; Jenkins & Joppa, 2009), which represent fragments of the once continuous historic ranges (Ripple et al., 2015). Habitat fragmentation and illegal hunting for ivory may lead to inbreeding depression (Allendorf, Luikart, & Aitken, 2013; Ishida, Gugala, Georgiadis, & Roca, 2018) and loss of genetic variation (Gobush, Kerr, & Wasser, 2009; Wasser et al., 2015), especially when the oldest individuals (who are often the target) are involved (Archie et al., 2008). This poses a question of whether populations that once ranged across the continent are becoming genetically isolated because of ongoing habitat destruction, fragmentation and illegal killings. While it is important to recognize that there is a time lag between changes to habitats and the time when the full implications of those changes are experienced by wildlife species (Bennett, 1998, 2003), it is desirable to understand early signs of variation among populations using measures of genetic differentiation (Paule, Krajmerová, Romšáková, & Schlosserová, 2012; Taylor, Walker, Goldingay, Ball, & Van Der Ree, 2011). Information contained in a series of individual genotypes can quantify the extent to which isolated populations have lost genetic diversity over time, making it a relevant tool for assessing differences in structure within and among populations of the same species in fragmenting habitats (Taylor et al., 2011).

The past 20 years have seen widespread deforestation of the miombo woodlands in areas between Katavi-Rukwa and Ruaha-Rungwa ecosystems in south-western Tanzania, with about 17.5% of the woodlands and forests modified or removed to make way for agricultural development, threatening connectivity between these ecosystems (Lobora et al., 2017). The area has one of the world's largest remaining African elephant populations (Chase et al., 2016; TAWIRI, 2014, unpublished data) and of high conservation priority because it (a) joins two large, well-protected elephant populations and (b) forms the principle link between the central and western African elephant populations in Tanzania. Because fragmentation in this landscape is relatively recent (Lobora et al., 2017), and because elephants are long-lived (generation time of 25 years; Armbruster & Lande, 1993; Blanc, 2008) and show large population sizes, previous studies carried out in this area found little genetic structure among adults (Epps, Wasser, Keim, Mutayoba, & Brashares, 2013). We thus expect there to be little or no genetic structure among adults. However, Wasser et al. (2015) show that there is sufficient differentiation across the continent to allow identification of source populations of poached ivory from genetic data. Given the potential for recent disruption of gene flow, testing for developing spatial genetic structure among individuals from the younger age classes could be informative. If adult movement has recently become restricted due to the recent fragmentation of the Katavi-Rukwa and Ruaha-Rungwa ecosystems, we expect to see incipient signs of genetic structure, particularly among the younger cohorts within these populations, and particularly given that male movement and dominance patterns are already known to drive age-related population structure in African elephants (Archie et al., 2008).

2 | MATERIALS AND METHODS

2.1 | Study area

The study area covers about 109,050 km^2 and lies between latitude 6°15′59.38″ and 8°10′23.78″ S and between longitude 30°45′13.29″ and 35°28′34.44″ E. The area comprises the Katavi-Rukwa ecosystem in the west, a contingent of Game Reserves (henceforth "GRs"), Game Controlled Areas (GCAs) and Open Areas (OAs) in the central part, as well as the Ruaha-Rungwa ecosystem in the east (Figure 1). About 45,961 km^2 of this area is designated as Fully Protected Areas (two National Parks-NPs, seven GRs where no human settlements are permitted), and 34,196 km^2 designated as Lesser Protected

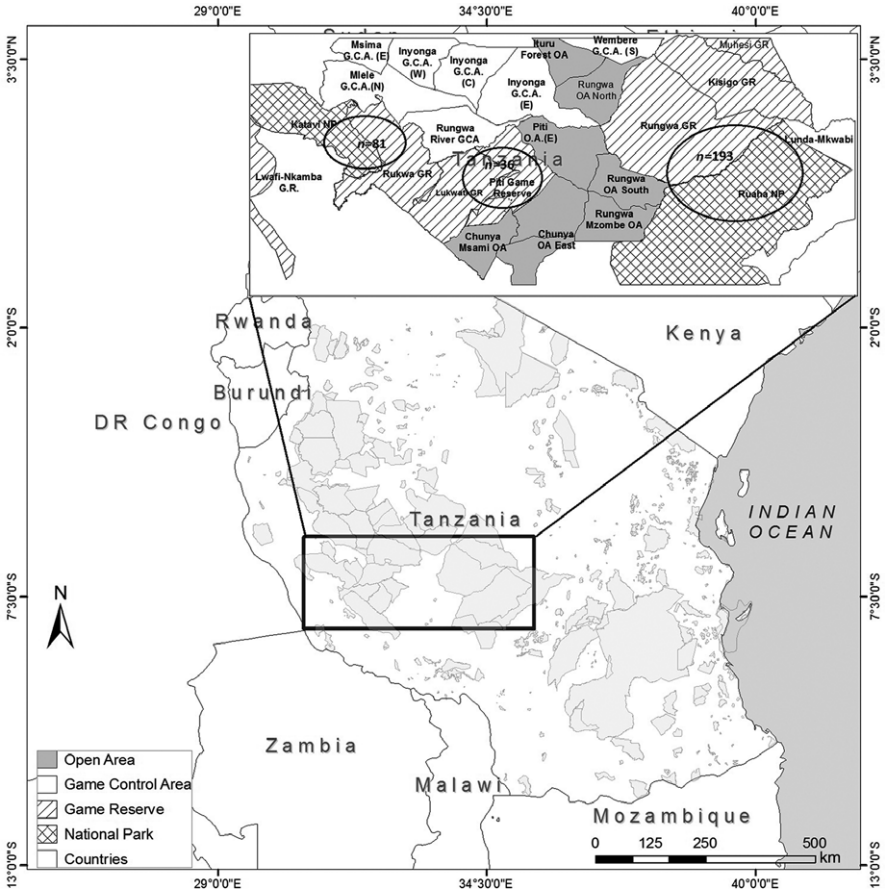

FIGURE 1 Sampling locations across the study area

Areas (eight GCAs and eight OAs where human settlements are permitted alongside wildlife conservation). A further 28,893 km^2 of land within the study area is unprotected and includes towns and highly populated regions north and south of Katavi National Park, and to the north-east and south of Ruaha National Park (Figure 1).

2.2 | Methods

2.2.1 | Sample collection

We collected 380 fresh dung samples between July and November 2015 in Katavi-Rukwa ecosystem (henceforth "western population"), Lukwati and Piti Game Reserves (henceforth "central population") and Ruaha-Rungwa ecosystem (henceforth "eastern population"). An opportunistic random sampling strategy was used to obtain samples from different parts of the study area while avoiding samples from closely related individuals (e.g., in the event that a group of fresh samples were encountered in the same location, we only collected one sample). For each sample, we placed approximately 10 g of the external region of the dung bolus surface with genetic content (<12 hr old) in 40-ml polypropylene tubes and boiled them for 15 min in the field to stall microbial activity and then preserved in Queens College Buffer (20% DMSO, 100 mM Tris pH 7.5, 0.25 M EDTA, saturated with NaCl; Amos et al., 1992). Samples were initially kept in the dark at room temperature in the field station and later moved to a laboratory at the Nelson Mandela African Institution for Science and Technology (NM-AIST) for postfield storage and subsequently shipped to the University of Missouri-Division of Biological Sciences under USDA permit number 128686 for subsequent DNA extraction and analyses.

2.2.2 | DNA extraction, PCR, sexing and microsatellite genotyping

The QIAamp mini stool extraction kit (Qiagen, Valencia, CA) was used to extract DNA from samples following earlier published protocols (Archie, Moss, & Alberts, 2006). The extraction process took place in a laboratory designated exclusively for the extraction of DNA from noninvasively collected samples to minimize the possibility of contamination (Ahlering, Hailer, Roberts, & Foley, 2011; Okello et al., 2008). We genotyped all samples at 11 dinucleotide microsatellite loci developed for the African elephant (FH1, LaT24, FH60, LA5, FH19, LafMS06, LA6, LaT08, LafMSO2, FH48 and FH67), using published primers (Archie et al., 2006, 2008; Eggert, Patterson, & Maldonado, 2008; Kongrit et al., 2008; Nyakaana, Abe, Arctander, & Siegismund, 2001; Okello et al., 2008) with fluorescent labels. Multiplex PCRs (Ahlering et al., 2011) were performed using Platinum Multiplex PCR Master Mix (Applied Biosystems, Foster City, CA) following the manufacturer protocols, but in 8 μl volumes with 0.8X BSA and GC enhancer solution added to a final

concentration of 10%. The PCR profile included an initial denaturing step at 95°C for 2 min, followed by 40 cycles of 95°C denaturing for 30 s, annealing at locus-specific temperatures for 90 s and 72°C extension for 60 s; and a final 30-min extension at 60°C. A negative control was included in each PCR plate to detect contamination of the PCR reagents, and a positive control sample was included to standardize scoring. We genotyped all samples on an ABI 3730XL capillary sequencer and subsequently analysed with GeneMarker v2.6.7 (Soft Genetics LLC). To minimize the probability of genotyping error, we repeated our genotyping three times or until were able to obtain at least three confirmations of each genotype (Ahlering et al., 2011; Frantz et al., 2003; Hansen, Ben-David, & Mcdonald, 2008).

We used the Excel Microsatellite Toolkit (Park, 2001) to identify potential genotyping errors, create input files for population genetic analysis programmes, find genetically identical samples and calculate allele frequencies and diversity statistics. Because DNA extracts from noninvasively collected samples are dilute and contain degraded DNA, we rechecked each pair of genotypes that differed at three or fewer loci for possible problems with allelic dropout and considered genotypes to represent the same individual if they differed at two or fewer alleles but matched in sex and had very similar bolus circumferences (Ahlering et al., 2011). This conservative approach was taken to avoid scoring samples as different individuals when they are actually erroneous genotypes (Ahlering et al., 2011).

To determine individual sex, we followed Ahlering et al. (2011). The PCR was performed in 25 μl reactions containing 1 U AmpliTaq Gold DNA Polymerase (Applied Biosystems), 1X PCR Gold Buffer (Applied Biosystems), 0.2 mM dNTPs, 0.4 mM SRY1 forward primer, 0.4 mM SRY1 reverse primer, 0.4 mM AMELY2 forward primer, 0.4 mM AMELY2 reverse primer, 0.4 mM PLP1 forward primer, 0.4 mM PLP1 reverse primer, 0.4 mM MgCl2, 0.8X BSA and 1 μl DNA extract. The PCR profile consisted of an initial denaturation at 95°C for 10 min, followed by 45 cycles of 95°C denaturing for 30 s, annealing at 59°C for 30 s and extension at 72°C for 45 s, with a final extension of 10 min at 72°C. Each PCR plate contained a negative (no DNA) and positive control to detect possible contamination of the PCR reagents and consistency of the amplification, respectively (Ahlering et al., 2011). Five microlitres of PCR product was subsequently electrophoresed at 80 V for 40 min on a 2% Agarose gel. As the restriction site is on the Y-chromosome, we scored single bands (PLP1-191 bp) as females and three bands (SRY1-71 bp, AMELY2-122 bp, PLP1-191 bp) as males and repeated the process once for each sample to confirm sex (Ahlering et al., 2011).

2.3 | Genetic analysis

We analysed the set of unique genotypes within and among populations using GenePop 4.2 (Raymond & Rousset, 1995; Rousset, 2008) to test for deviations from expected heterozygosity values under Hardy–Weinberg equilibrium (HWE), for linkage disequilibrium, and to determine the number of alleles at each locus (*A*), the observed (H_O) and expected (H_E) heterozygosity values and the coefficient of inbreeding (F_{IS}) as estimated by Weir & Cockerham, 1984. Because sample sizes were unequal, we used rarefaction in HP-Rare (Kalinowski, 2005) to estimate allelic richness, that is, the mean number of alleles at a sample size of 36 (the smallest sample size of any population). We compared rarefied allelic richness among populations using a Kruskal–Wallis test with loci treated as replicates. We estimated genetic distances (fixation index-F_{st}) between pairs of populations in Arlequin version 3.5.1.3 (Excoffier & Lischer, 2010) and evaluated the significance of these F_{st} values using a permutation test (1,000 permutations).

We tested for genetic differentiation using pairwise F_{st} across all individuals in the three populations (Text S1). To test for the influence of age class on genetic differentiation, we also estimated global F_{st} for each age and then compared F_{st} across groups of different age cohorts, that is, young age 0–9, subadult age 10–19 and adult age 20+. We obtained the age structure of the three populations through dung bolus measurements following Morrison, Chiyo, Moss, and Alberts (2005). To determine whether observed levels of genetic differentiation across age cohorts were significant, we randomly permuted age cohorts among individuals and computed a theoretical global F_{st} under the hypothesis of no age structure in the data set. The permutation procedure was repeated 1,000 times, and, for each age cohort, we computed the mean and the 95% quantiles of the obtained theoretical distributions. Global F_{st} estimates were calculated using the hierfstat (Goudet, 2005) and adegenet (Jombart, 2008) R-packages.

To investigate possible patterns of isolation by distance (IBD), we used an individual-based approach. We computed a pairwise matrix of interindividual genetic distances using the Bray–Curtis percentage dissimilarity measure (function *diss.dist* from the R-package *poppr*; Kamvar, Tabima, & Grünwald, 2014) that we compared to the corresponding pairwise matrix of interindividual Euclidean distances using a simple Mantel test with 10,000 permutations (function mantel.randtest from the R-package ade4: Dray & Dufour, 2007). Additionally, based on the geographic coordinates of sample locations, we investigated spatial patterns of IBD using a spatial autocorrelogram in GenAlEx (Peakall & Smouse, 2006) using the matrix of Bray–Curtis percentage dissimilarity measures as the response variable. Euclidean distance classes were defined every 50,000 m (up to 50 km). Mantel spatial autocorrelograms were also computed for each sex separately.

To test for genetic structure on an evolutionary timescale, we analysed genotypes in STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000), a Bayesian model-based clustering algorithm. We programmed the length of the burn-in period to 10,000 and the number of Markov chain Monte Carlo reps after the burn-in to at least 100,000 steps. We further programmed STRUCTURE to run 10 times for each value of *K* from 1 to 10, with the use of prior information about the location (LOC_PRIOR) from which the sample was collected, under the admixture model with correlated allele frequencies among populations. We used spatial principal component analysis (Jombart, Devillard, Dufour, & Pontier, 2008) to reveal possible cryptic genetic structures, stemming from the specific life-history

traits of this long-lived species. The sPCA seeks principal components that optimize the variance of individual allelic frequencies while taking spatial autocorrelation of data into account. It disentangles global structures stemming from strong genetic similarity or positive autocorrelation between neighbours, from local ones, stemming from strong genetic differences or negative autocorrelation between neighbours. Interindividual spatial autocorrelation was modelled according to a distance-based neighbourhood network with a distance threshold consistent with results from the spatial autocorrelogram: All individuals located more than 50 km apart were not considered as neighbours in the spatial network (see results for details). Global and local Monte Carlo tests were carried out with 10,000 permutations to evaluate the significance of detected global and local patterns (Jombart et al., 2008).

3 | RESULTS

Of the 380 samples collected across the landscape, 376 (98.9%) were successfully genotyped and 310 individuals identified by their unique genotypes. The remaining 66 samples were recaptures within the same populations and therefore discarded from subsequent analyses. The age class distribution by sex of the three populations is presented in Table 1. We were unable to determine sexes for some individuals due to repeated failure to discriminate bands as either males or females (Table 1).

Two of the 11 loci (LafMS02 and LafMS06) did not conform to expectations under HWE in any of the three populations after applying Bonferroni correction for multiple tests (Rice, 1989). These loci had significant excesses of heterozygosity that could not be resolved through reanalysis of the genotypes and hence were removed from the analyses. Other than these loci, LA5 and FH19 deviated from expectations in the western population, FH60 deviated in the central population and LA5 and FH48 deviated in the eastern population. Because there were no consistent patterns of deviation across populations, these loci were retained in the analyses.

3.1 | Genetic diversity within populations

We found high levels of genetic diversity in all populations, with allelic diversity ranging from an average of 8.7 (±1.9 *SD*) alleles per locus in the eastern population to 6.2 (±2.3 *SD*) alleles per locus in the central population (Table S1). When these values were corrected with a standard sample size of 36 (the size of the smallest sample) using rarefaction (Kalinowski, 2004), there was no significant difference among populations in the number of alleles (Kruskal–Wallis $K = 5.208$, $df = 8$, $p = 0.735$) or private alleles (Kruskal–Wallis, $K = 5.865$, $df = 8$, $p = 0.662$).

3.2 | Genetic differentiation among populations

We found the three populations to be significantly different, although the level of differentiation was small, with F_{st} values ranging from 0.006 between the eastern and central populations to 0.011 between the western and central populations (Table 2). We identified weak (but statistically significant) IBD (Mantel test, $r = 0.09$, $p = 0.045$), suggesting higher gene flow among nearer individuals. Samples within 50 km of each other were more likely to be genetically similar, but beyond this distance, there was no remaining spatial autocorrelation (Figure 2). No significant spatial autocorrelation pattern could be identified when considering males and females separately (data not shown).

Analyses in STRUCTURE detected no significant genetic clustering among populations across the study landscape ($K = 1$) (Table S2), suggesting that while there is significant differentiation over a recent, ecological timescale, individuals represent a single genetic population over an evolutionary timescale. Nevertheless, we identified significant cryptic genetic structures when using sPCA. The global Monte Carlo test performed in sPCA was significant (max (t) = 0.007, $p = 0.007$), indicating the presence of a significant global genetic structure. On the contrary, the local Monte Carlo test did not detect any significant local structure (max(t) = 0.013, $p > 0.05$). Scores of individuals along the first sPCA axis distinguished the western population from the central and eastern populations (Figure 3). Along the second axis though, individuals showed a spatial pattern characterized by a longitudinal alternation of genetic clusters, roughly delimited every 50 km, highlighting the influence of a continuous IBD in this species (Figure 3).

3.3 | Genetic differentiation among age cohorts

As expected, global F_{st} estimates were higher in young cohorts (F_{st} = 0.055) than in subadults and adult's cohorts (F_{st} = 0.008 in both cohorts). The 95% confidence intervals around mean expected F_{st} values under the hypothesis of an absence of age structuration indicated that young individuals from different populations were significantly more genetically distinct than older individuals from different populations (Figure 4).

TABLE 1 Age class distribution by sex

Sex	Adult	Subadult	Young	Juvenile	Unknown
Female	68	41	11	0	
Males	90	39	11	2	
Unknown					48
	158	80	22	2	48

TABLE 2 Genetic distance measures among populations

	Western	Central	Eastern
Western	—	0.011*	0.007**
Central	0.011*	—	0.006**
Eastern	0.007**	0.006**	—

Note. Significance levels are indicated as *$p < 0.05$, **$p < 0.01$.

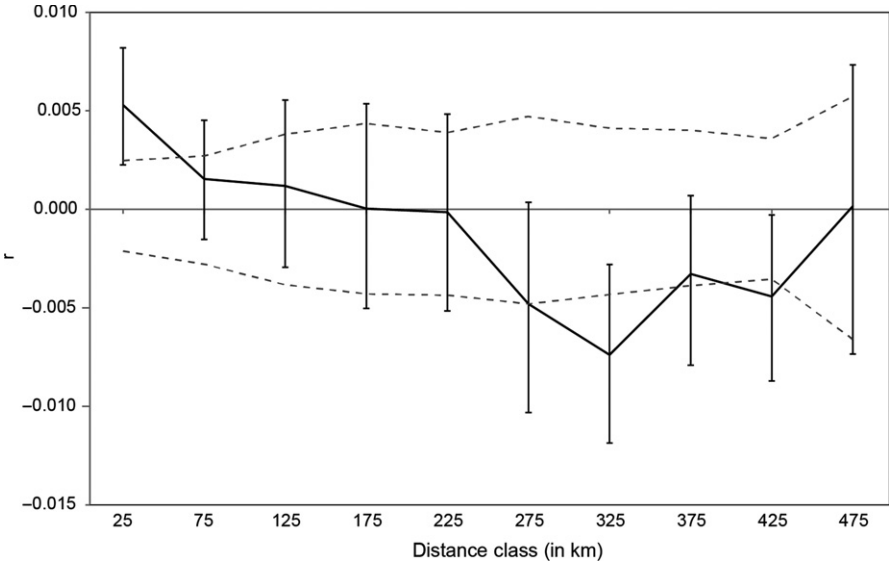

FIGURE 2 Correlogram showing spatial genetic autocorrelation (*r*) among individuals as a function of Euclidean distance. Distance classes were defined every 50 km. Dotted lines indicate the 95% CI about the null hypothesis of no genetic structure. The error bars about *r* represent the 95% CI, as determined by bootstrapping (1,000 iterations)

4 | DISCUSSION

We found evidence of weak but significant genetic differentiation among the three recently divided populations, particularly between younger elephants, suggesting that the recent loss of natural habitat (Lobora et al., 2017) may be starting to generate population-level differences. Isolation was weakly but significantly associated with distance, consistent with early stages of population fragmentation. Landguth et al. (2010) found the lag time to barrier detection with genetic methods to be relatively short (1–15 generations) for wide-ranging species but cautioned that detecting the effects of fragmentation on long-lived species (with overlapping generations) over ecological time scales may be difficult. Thus, we are not surprised that STRUCTURE did not detect significant genetic clustering. Although this programme works well when population structure is relatively weak (Hubisz, Falush, Stephens, & Pritchard, 2009), it may fail to detect structure when differentiation levels are as low as those in this study (Duchesne & Turgeon, 2012). This is also consistent with our prediction that there would be no significant structure across adult individuals in these populations at evolutionary timescales because habitat fragmentation is a recent phenomenon.

Nevertheless, sPCA revealed subtle global hierarchical genetic structure, with eastern and central populations (white squares) showing higher genetic relatedness than the western population (black squares) at the higher level of the hierarchy (Figure 3). This is unsurprising because habitat loss/fragmentation due to anthropogenic activities is higher between western and central populations than between central and eastern populations (Lobora et al., 2017). At the lower level of the hierarchy, it appeared that genetic structuring mostly stemmed from a longitudinal IBD pattern, with a lag distance of about 50 km, suggesting that IBD is an important driver of genetic differentiation in this system.

The historical large extent of miombo woodland linking these three populations appears to have facilitated broadscale connectivity, at least until recently (Epps et al., 2013). Our recent analysis on the broad area extending from the Ruaha-Rungwa ecosystem to the Katavi-Rukwa ecosystem indicates that these areas retained approximately 73% of miombo woodland cover up until 1990s and continuous connectivity may only have been impaired recently (Lobora et al., 2017). Despite large areas of natural woodland remaining between the two ecosystems, even now habitat loss has limited movement between the two ecosystems to a very narrow region (corridor), including some areas heavily used by people and a main road that links the northern and southern regions of Tanzania (Caro, Jones, & Davenport, 2009; Jones, Caro, & Davenport, 2009; Riggio & Caro, 2017).

The low level of genetic differentiation among populations could partly be explained by the fact that, in the absence of long-standing habitat fragmentation, the average distance between farthest populations (about 200 km) is within the dispersal capabilities of the African elephant (Blanc et al., 2007). The measure of population subdivision across all populations (F_{st}) was low suggesting many successful migrants entering each population per generation (approximately 25 years for African elephant, Blanc, 2008) assuming an island model of migration (Frankham, Ballou, & Briscoe, 2002). Nevertheless, without substantial levels of gene flow, habitat fragmentation and other anthropogenic disturbances can lead to extensive genetic differentiation among populations (Dixon et al., 2007), even among populations that are geographically close (Vos, Jong, Goedhart, & Smulders, 2001), as suggested by higher genetic differentiation in the young cohort.

Our genetic data did not suggest that there has been significant inbreeding in these populations, highlighting the importance of management actions (such as protection of the remaining potential habitat for connectivity) to maintain migration corridors that reinforce gene flow. This is particularly important because conservation of wide-ranging species depends not only on protected areas but also dispersal areas to provide connectivity (Ahlering et al., 2012; Caro & Riggio, 2014; Epps et al., 2013; Western, Russell, & Cuthill, 2009). Our analysis indicates fragmentation signs to be affecting the genetic structure of young individuals born when movement became increasingly restricted after 1990 and that genetic variation observed

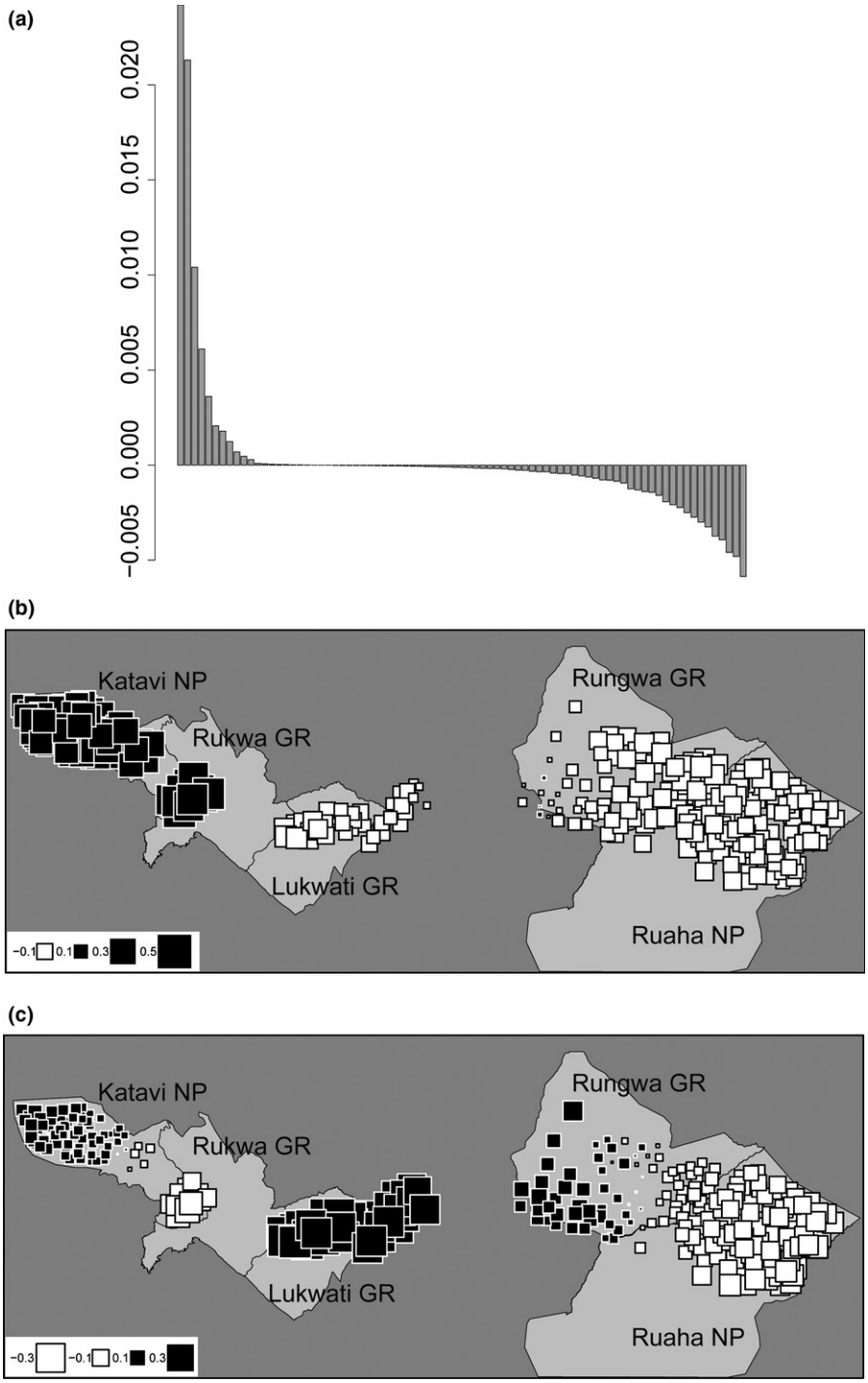

FIGURE 3 Analyses of individual genetic data using sPCA. (a) Positive and negative sPCA eigenvalues; only the two-first positive (global) axes, corresponding to patterns of positive spatial autocorrelation among genotypes, were considered here. (b) Map of the first global sPCA scores for each individual. (c) Map of the second global sPCA scores for each individual. Large white and black squares stand for highly negative and positive individual scores, respectively. Small squares stand for low individual scores. White and black squares allow identifying distinct genetic clusters along each global axis, whereas the size of squares indicates how similar a genotype is from its neighbours (here located less than 50 km apart)

between adults and the young age could be precursors of what can be expected in the future. These changes will persist for at least a generation (even if connectivity was completely resurrected today), but appropriate management could restore a fully panmictic population in the future.

Overall, the results obtained in our analysis are consistent with the suggestion that habitat fragmentation and loss will soon constitute a threat to African elephant populations across their range (Comstock et al., 2002). As demonstrated in other taxa such as large carnivores (Johnson, Eizirik, Roelke-Parker, & O Brien, 2001), African elephants are also susceptible to losses in genetic variation due to habitat fragmentation, despite long generation times (Blanc, 2008). The incipient signs of genetic differentiation detected in our analysis indicate increasing conservation challenges in human-dominated landscapes (Newmark, 2008), calling for deliberate efforts and political will to save remaining dispersal areas for continued gene flow.

4.1 | Management implications

A species' ability to cope with the changing selective forces resulting from anthropogenic disturbance may be partially determined by the

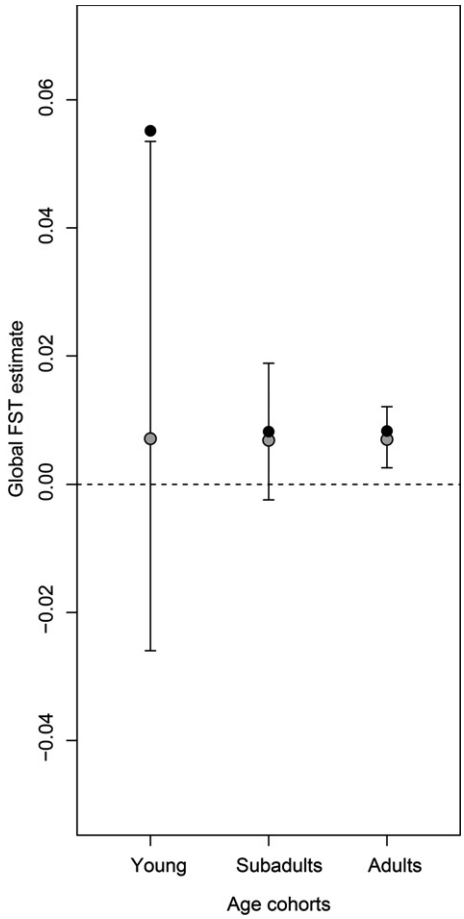

FIGURE 4 Comparison of global F_{st} estimates across age cohorts. For each cohort, observed F_{st} values are in black, whereas expected mean values under the hypothesis of an absence of age structure are in grey. Error bars indicate 95% confidence intervals around mean expected values, as computed from the random permutation of age cohorts among individuals (1,000 iterations)

amount of genetic variability in populations as well as the way that variation is structured within and between populations (Archie, Fitzpatrick, Moss, & Alberts, 2011; Ishida et al., 2016). Evidence for recent emergence of genetic structure within the three studied elephant populations suggests that habitat loss and fragmentation in the areas between Ruaha and Katavi are starting to alter population connectivity. At present, a narrow corridor of natural habitat persists between the two systems, but heavy human use likely reduces the suitability of this corridor for elephant movements. The remaining potential habitat for connectivity between the two ecosystems falls under the multiple landuse categories (OAs), and we call for deliberate and timely actions to upgrade the protection status of this area to ensure continued gene flow between these populations. One of these efforts may include transforming these OAs (Piti east & Rungwa south) to a Wildlife Management Area, a new landuse category that promotes local community-driven conservation allowing greater local community buy-in (USAID, 2013; WWF, 2014), or establishing Game Reserves that restricts multiple uses on a case by case basis.

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