

Population connectivity across a transboundary conservation network: potential for restoration?

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Abstract

Context: There has been limited research identifying large-scale functional connectivity of wildlife populations across sub-Saharan Africa, despite the increased focus on transboundary conservation networks.

Objectives: This study set out to assess the functional connectivity of a highly mobile predator of conservation concern across the Kavango-Zambezi transboundary conservation area (KAZA) and the northern Central Kalahari Game Reserve (CKGR), covering almost 300,000km² of Botswana, Namibia, Zambia and Zimbabwe.

Methods: We analysed the nuclear diversity of 204 individual lions from across the metapopulation using Bayesian and multivariate statistics to assess population structure and recent migration. A maximum-likelihood method was used to determine average male dispersal distances to determine the potential for functional connectivity across the region.

Results: The results are consistent with work identifying the existence of ecotypic differences between wetland and dryland lions, but also indicate hierarchical population structure further dividing the population into four geographic clusters; the Okavango Delta, the Central Kalahari, Kafue National Park, and the Hwange-Chobe complex. Analysis of dispersal distances of males suggests that reconnecting the clusters through conservation intervention should be successful in improving gene flow and connectivity across the region.

Conclusions: While trans-boundary conservation areas may currently have limited gene flow and connectivity, there is potential for the restoration of functional connectivity via the natural dispersal of highly mobile species. However, the matrix of habitat through which such dispersing animals must traverse must be conducive to their movement and survival, highlighting the importance of land outside protected areas for the preservation of highly mobile animals such as lions.

Introduction

As global landscapes and habitats around the world become increasingly fragmented and are placed under increased pressure from anthropic development (Fischer & Lindenmayer, 2007) a better understanding of species responses to such change and the degree of connectivity between populations is essential (Fahrig, 2007; Kool, Moilanen & Treml 2012). While protected areas are clearly of utmost importance for conservation (Bruner, Gullison, Rice & da Fonseca, 2001), the habitat matrix between protected areas through which organisms may disperse, or even reside in, is also of equal importance to achieve the greatest conservation returns (Ewers & Didham, 2006; Franklin & Lindenmayer, 2009). The unprotected matrix habitat is even more important in fragmented landscapes, and doubly so with wide ranging species, as movement across these habitats facilitates connectivity (Woodroffe & Ginsberg, 1998; Sharma et al. 2013b). Such reasoning has led to priority-setting exercises for large, wide roaming carnivores that has shifted traditional conservation away from a focus on discreet populations towards a

wider consideration of how aggregated populations can contribute to species preservation as a whole (Rabinowitz & Zeller, 2010). However, such priority setting must first understand the level of functional connectivity that already exists. In such contexts, functional connectivity refers to the behavioural response of individual animals to the landscape and the extent to which ecological processes such as movement and gene flow are facilitated (Sharma, Dutta, Maldonado, Wood, Panwar, & Seidensticker, 2013; Tischendorf & Fahrig, 2000). This contrasts with structural connectivity, which only recognises the physical relationships between patches without direct consideration of attributes relating to the organism(s) of interest (Kadoya, 2008; Tischendorf & Fahrig, 2000).

One region for which there has been limited genetic literature assessing the large-scale functional connectivity of wildlife populations is sub-Saharan Africa. Flamingh et al. (2014) and Roever et al. (2013) looked at connectivity of elephant populations across this region, however, this is a species that has vastly different movement constraints to other animals. Addressing this lack of knowledge regarding contemporary landscape-level gene flow between the existing reserve networks is of great conservation importance. This is especially so in light of emphasis being placed on the development of large, transboundary, conservation areas; encompassing large areas of the current reserve network (Hayward & Kerley, 2009; van Aarde & Jackson, 2007; Winterbach, Winterbach, Somers, & Hayward, 2013). Without understanding current functional habitat connectivity, it will be very hard to predict how well such parks are likely to perform in maintaining population persistence, or what management actions may be needed for them to improve their conservation function.

As large, wide-ranging predators that have suffered dramatic range contractions and an apparent loss of structural connectivity between populations (Bauer et al., 2015; Simon G. Dures et al., 2019), lions (*Panthera leo*) are well suited for investigating functional habitat connectivity from a conservation perspective. Lion populations that are believed to be of ecological and conservation importance have been identified in 66 Lion Conservation Units (LCU's) encompassing 2.37 million km² fragmented throughout much of Sub-Saharan Africa (Dubach, Briggs, White, Ament, & Patterson, 2013). This represents less than 20% of their historical range 150 years ago (IUCN, 2006) and of the remaining populations only 32% are considered stable.

This study encompasses four LCU's (Kafue; Khaudum-Capriivi; Okavango-Hwange; Kgalagadi) (IUCN, 2006) covering a spatially heterogeneous metapopulation that is large enough to contain a sustainable and viable population of lions (Björklund 2003). Politically, the study area is largely contained within the region incorporated into the Kavango-Zambezi Transfrontier Conservation Area (KAZA). KAZA covers almost 300,000km² and encompasses parts of Angola, Botswana, Namibia, Zambia and Zimbabwe and is intended, among other things, to connect a number of large national parks, game reserves and other protected areas all within the context of sustainable development. In an effort to look at connectivity within this region Morandin et al. (2014) examined the genetic structure and immigration of lions across Hwange National Park, highlighting the need for further studies to, "focus on detecting genetic structure at a larger scale and try to identify putative corridors of connectivity". This was followed by a series of studies that used the movement behaviour of lions in and around Hwange to extrapolate possible levels

of connectivity under different landscape change scenarios (Cushman et al., 2018; Cushman, Elliot, Macdonald, & Loveridge, 2015; Elliot, Cushman, Macdonald, & Loveridge, 2014). These studies argue that the most effective way to maintain connectivity in the lion population is by maintaining the current protected area network, augmented with protected dispersal corridors. However, while these studies point towards an expectation of genetic structuring and fragmentation of the wider meta-population by anthropogenic factors there is currently limited empirical evidence confirming the extent of gene flow, and thus the amount of dispersal leading to successful mating. There is evidence that ecological barriers influence gene flow in northern Botswana (Dures et al. 2020; Moore et al. 2015), though anthropogenic fragmentation of the landscape is a significant factor in the wider transboundary meta-population (Cushman et al. 2018).

We use nuclear markers to assess the functional connectivity, in the form of gene flow, across the KAZA lion population and test for genetic structuring that may indicate impediments to dispersal and therefore connectivity between populations. We hypothesise that the KAZA lion population is fragmented into smaller sub-populations, corresponding to the current protected area network, with limited gene flow between them due to ecotypic differences and anthropogenic disturbance. Using within population genetic relatedness, we utilise the philopatric nature of female lions to estimate male dispersal capabilities (Spong & Creel, 2001). These dispersal capabilities are then used to assess the capacity for functional population connectivity across the KAZA network should structural links be restored through management and restoration of the habitat between protected areas.

Methods

The study was conducted on the lion population centred on the Kavango-Zambezi Transfrontier Conservation Area (KAZA), but also incorporating lions from the northern Central Kalahari Game Reserve (CKGR) (Fig. 1). No lions from the Angolan portion of KAZA were included as only occasional reports of extant lions have emerged from this region in recent decades. Samples were collected from 204 free ranging wild lions in the form of blood ($n = 23$), fresh tissue ($n = 113$), dry tissue ($n = 13$), faecal ($n = 14$) and hair-pulls ($n = 41$). Fresh tissue samples were collected using a remote biopsy dart delivery system (Karesh, Smith, & Frazier-Taylor, 1987). Hair pulls and blood were taken from immobilised animals. Dry tissue samples were taken from animals shot by trophy hunters. DNA was extracted from each sample using approximately 25mg of tissue, 100 μ l of raw blood or 5–6 hair follicles using DNeasy® Blood and Tissue kits, or using approximately 200mg of stool using QIAamp® DNA Stool kits (Qiagen) according to the manufacturer's instructions.

Microsatellite amplification

The same twenty microsatellite loci used in Dures et al. (2020) were used in this study (Fca1, Fca6, Fca8, Fca31, fca45, fca69, fca75, fca77, Fca96, Fca97, F115, Fca126, Fca129, Fca133, Fca193, Fca205, Fca224, FCA247, FCA391, FCA506) (Menotti-Raymond, David, & Lyons, 1999). DNA extraction,

polymerase chain reaction (PCR) and sequencing were performed following the procedures described in Dures et al. (2020).

Each sample was independently amplified at least three times to rule out any genotyping errors resulting from allelic dropout, false alleles or contamination, following the recommendations of Bonin et al. (2004). Two DNA samples of known genotypes were included on each PCR as positive controls and to calibrate allele size across runs. Each sample and locus was checked for null-alleles and possible scoring errors using Microchecker 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004)

Sex determination

Field notes were taken on the sex of each individual sampled, and this was verified using a molecular method. Felid specific primers, ZFX/ZFY, have been developed for sex identification on the zinc finger region that has a three base pair deletion in males (Pilgrim, Mckelvey, Riddle, & Schwartz, 2005). This deletion results in a single PCR fragment in females (163bp) compared to two PCR fragments in males (163bp and 160bp). The forward primer was fluoro-labelled (6-FAM) and the PCR product run through the ABI 3130 capillary sequencer. DNA Amplification took place in 6µl PCR reactions using 1µl DNA (1:5 dilution), 3.5µl Qiagen® Multiplex PCR Master Mix and 1.5µl of forward and reverse primer mix (10 pM/µl). Thermal cycling was performed in a Gene Amp® PCR system 9700 (Life Technologies) and consisted of denaturing and hot-start enzyme activation for 15 min at 95°C then 35 repeating cycles of 30s denaturing at 94°C, 1min at 56°C and 30s extension at 70°C. The final extension period was for 20 min at 72°C.

Analysis of genetic diversity

Genetic diversity was measured by the number of alleles per locus (M), inbreeding coefficient (F_{IS}), and observed and expected heterozygosity (H_O & H_E) under Hardy-Weinberg assumptions. Allelic richness (AR) was also calculated as a measure of observed number of alleles irrespective of samples size as well as a way to calculate levels of genetic diversity among populations. Tests for departure from Hardy-Weinberg equilibrium were performed using exact tests (Guo & Thompson, 1992) with Bonferroni correction. These analyses were performed in *GENEPOP 4.2*, *FSTAT 2.9.3.2* and *GENETIX 4.0*. Given the expectation of genetic structure, *CoDiDi* (Wang, 2015) was used to test if any deviations from Hardy-Weinberg equilibrium were likely to be due to gene flow or mutation.

Analysis of genetic structure

To increase robustness one multivariate method, the discriminant analysis of principal components (*DAPC*) (Jombart, Devillard, & Balloux, 2010), and two Bayesian clustering analyses, *STRUCTURE* (Pritchard, Stephens, & Donnelly, 2000) and *TESS* (Chen, Durand, Forbes, & François, 2007), were used to infer population structure across the study region. *DAPC* uses principal component analysis to ensure variables are completely uncorrelated and, due to its lack of reliance on a population genetics model, is not constrained by assumptions regarding Hardy-Weinberg or linkage disequilibrium. In contrast, *STRUCTURE* clusters samples by minimizing deviations from Hardy-Weinberg and linkage disequilibrium

without *a priori* spatial information. *TESS* is similar to *STRUCTURE* but is a spatially explicit model that incorporates geographic coordinates *a priori* and takes account of spatial autocorrelation and admixture zones.

In *STRUCTURE*, K was tested from 1–10 using 500,000 iterations with a burn-in period of 50,000 iterations and 10 replicated runs for each K value. An admixture model was used with correlated allele frequencies and without *a priori* information regarding the origin of the samples. The most likely number of clusters was determined using the ΔK method (Evanno, Regnaut, & Goudet, 2005) in *STRUCTURE HARVESTER* (Earl & von Holdt, 2012). This method was also re-run using only those individuals with less than a 95% membership to the Okavango Delta cluster to test if the regional pattern of genetic clustering withstood the influence of the ecotypic division previously identified (Dures et al. 2020; Moore et al. 2015).

In *TESS*, K was tested from 2–10 using the conditional autoregressive admixture model (CAR), using 120,000 iterations with a 20,000 iteration burn in period and replicated 100 times for each K . The most likely number of clusters was selected by averaging, for each value of K , the replicates with the lowest 10% Deviance Information Criterion values (DIC). This average was then plotted against K and the value at which the plot plateaus out selected, similar to the ΔK method described by Evanno et al. 2005.

DAPC was performed using the *adegenet* package (Jombart et al. 2010) in *R*. We first used a K-means method to assess the likely number of clusters up to a maximum of 10 using the lowest Bayesian Information Criterion value. A linear discriminant analysis was then performed on the principal components, with a view to minimizing within cluster variation and maximizing between cluster variations. As recommended by Jombart et al. (2010), we used the *a*-score to assess the optimal number of principal components and adjusted accordingly to avoid over-fitting.

Based on the results of the clustering analysis, Jost's D_{EST} (Jost, 2008) was calculated in the software SMOGD version 1.2.5. (Crawford, 2010) for each genetic cluster. D_{EST} is considered a better estimator than F_{ST} for population genetic differentiation in microsatellite markers (Gerlach et al. 2010; Heller & Siegismund 2009; Meirmans & Hedrick 2011; Meirmans 2012). F_{ST} for each cluster was calculated in *FSTAT* (Goudet, 2001) to allow comparison with other studies and 95% confidence intervals calculated with 1000 bootstrap replications. Genetic diversity for each cluster was calculated in the same way as for the metapopulation (F_{IS} , H_D , H_E , AR) Nei's unbiased expected heterozygosity was used to take account of unequal sample sizes. The number of alleles (NA) and the number of private alleles (PA) were calculated in *CONVERT* (Glaubitz, 2004), and we used a rarefaction method in *ADZE* to correct for sample sizes (Szpiech, Jakobsson, & Rosenberg, 2008).

Recent Migration

To assess the level of recent migration likely to have occurred between sampling regions we used two Bayesian approaches. The first, implemented in *BayesAss 3.0* (Wilson & Rannala, 2003), was used to calculate the probability of an individual belonging to the population in which they were sampled, or

having migrated from another population. We ran the analysis using 10 million iterations with a burn in of 1 million iterations, sampling every 1000 iterations. The size of the adjustments allowable for allele frequencies, inbreeding coefficients and migration rates were fine tuned to ensure acceptance rates remained within the suggested range ($0.30 < x < 0.60$).

The second approach used the *USEPOPINFO* function of *STRUCTURE* to infer likelihood for each individual of being a non-migrant, or a first, second or third generation migrant from one of the other sampling regions. Providing *a priori* population sampling information for each individual lion allows posterior calculation of probability of membership to that sampling population. As we do not have good data on dispersal rates across the entire landscape, the analysis requires a sensitivity analysis and so multiple runs were performed using a range of migration rates (*MIGPRIOR* = 0.5, 0.1, 0.05, 0.005) to estimate the extent to which the rate affects the predictions.

Dispersal distances

To calculate the maximum, minimum and average dispersal distances for lions in this region we adapted a method first described by Spong & Creel (2001). To enable the results to translate into management recommendations, we measured distances between sampled lions in kilometres, instead of territories as used by Spong & Creel. To ensure population structuring does not bias the signal of dispersal, a single genetic cluster was used for this analysis. The most appropriate and widely distributed genetic cluster was chosen based on the previous analysis of population structure. A maximum-likelihood method implemented in *MLrelate* (Kalinowski, Wagner, & Taper, 2006) was used to calculate a pairwise estimate of relatedness (r) from every male to every female across the population and discriminate between relationship classes of unrelated (U), half-siblings (HS), full-siblings (FS), and parent-offspring (PO). Confidence intervals were measured using 10,000 bootstrap samples of genetic relatedness. Given that males holding tenure over a pride tend to be unrelated to females within the pride (Packer et al., 1991; Spong & Creel, 2001) we calculated pairwise relatedness of every unrelated (U) male sampled within 30km of a female. This was under the assumption that pride members are unlikely to roam further than 30km from each other during the period of sampling. This gave us an indication of the likely relatedness value (r) of breeding pride males to the females in a pride. Pairwise Lynch and Ritland relatedness values for female-female pairs, calculated in *MLrelate* (Kalinowski, 2006), were then regressed against pairwise geographic distances using a least squares regression. Due to female phylopatriy and adopting the principles outlined by Spong & Creel (2001), the regression line can then be used to infer how far on average males holding pride tenure were likely to have dispersed from their natal pride before settling. As a second measure of male dispersal from the natal pride we used geographic Euclidian distance between 1st order relatives (full-siblings and parent-offspring) of the opposite sex. The mean and 95% confidence intervals were also calculated. Since the data used to calculate average dispersal distances also averages any differences in landscape resistance between individuals, the mean represents an estimate for potential dispersal across landscapes of a similar type. To put dispersal distances into a conservation context we used the IUCN & UNEP World Database on Protected Areas (2010) and extracted national parks and game reserves. These regions were then buffered with the mean male lion dispersal distances

to assess the possibility of dispersal from one protected area population to another, assuming a suitable dispersal corridor is available.

Results

Genetic data was obtained from 204 individual lions. The number of alleles per locus ranged from 5 to 18 alleles, with a mean per locus of 10.75. The population showed a similar mean observed (H_O) and expected heterozygosity (H_E) of 0.662 and 0.707 respectively (See Appendix A in Supporting Information); similar to that reported in other lion populations (Antunes et al. 2008). An inbreeding coefficient (F_{is}) of 0.069 suggests there is no inbreeding across the population. One loci (Fca6) tested significant for deviation from Hardy-Weinberg equilibrium ($p < 0.01$), which is likely to be due to population substructure (Lyke, Dubach, & Briggs, 2013). The lack of significant correlation between single locus genetic differentiation and diversity, as identified in *CoDiDi*, suggests gene flow not mutation accounts for deviations observed (Wang, 2015).

Genetic structure

Genetic clustering analysis in *STRUCTURE* identified high levels of structuring as well as significant hierarchical structuring. The ΔK (Evanno et al., 2005) method of identifying the number of clusters identified two main population groups, but with internal hierarchical structure identifying four groups (Fig. 2a). Visual inspection of the strongest structuring signal ($K = 2$) supports previous work indicating that the Okavango Delta is genetically separated from the remainder of the regional lion population (Dures et al. 2020; Moore et al. 2015) (Fig. 2b). The hierarchical structure ($K = 4$) appears to indicate structure corresponding to 4 primary populations; 1) the Okavango Delta; 2) the Central Kalahari; 3) Kafue; 4) Hwange-Chobe complex (Fig. 2b). To assess the degree to which an apparent ecotypic population structure might be overshadowing more discreet patterns at the lower levels of hierarchical structure, any sampling groups with $> 95\%$ membership to the Okavango cluster were removed from the analysis. The results showed $\Delta K = 3$ as the most likely number of genetic clusters. This confirms the initial analysis and also serves to highlight the strength of the bifurcated dryland/wetland pattern which overshadows any lower level hierarchical structure present across the 'dryland' population (Dures et al. 2020; Moore et al. 2015). Repeating the analysis using *TESS* confirms these results (Fig. 2c).

DAPC also identified four populations (Fig. 2d) however, the individual assignment to those four populations differed from both *TESS* and *STRUCTURE*. Where the latter identified the northern Kafue population as being separate from the Hwange-Chobe population, *DAPC* clustered them as a single population. In contrast, *DAPC* identified substructure within the Okavango population, splitting the central Okavango island from the remainder of the Okavango.

All results generated based on the clustering analysis were based on the *STRUCTURE/TESS* output which produced results more consistent with likely patterns of human induced fragmentation. The *DAPC* output,

however, demands increased scrutiny of any analysis of cluster separation, particularly for the Kafue cluster.

Genetic differentiation

D_{EST} ranged from 0.0641–0.1648 (Table 1), with the greatest variation between the Kalahari and the Kafue population ($D_{EST} = 0.1648$), and between the Okavango and Kafue populations ($D_{EST} = 0.1528$). The Hwange/Chobe cluster appears least differentiated from all other clusters, suggesting it shares some degree of commonality with them all. F_{ST} between all pairs showed significant differentiation at $p < 0.01$ but had lower and less varied scores ($F_{ST} = 0.0512–0.1$). We report F_{ST} to allow some comparison with past literature; however reiterate that D_{EST} should be viewed as the more robust measure of population genetic differentiation for microsatellite markers (Gerlach, Jueterbock, Kraemer, Deppermann, & Harmand, 2010; Jost, 2008).

Table 1
Pairwise F_{ST} (bottom) and D_{EST} (top in bold) between populations

		D_{EST}			
–		Hwange/ Chobe	Okavango	Kalahari	Kafue
F_{ST}	Hwange/Chobe	–	0.0641	0.0645	0.0916
	Okavango	0.0541*	–	0.1107	0.1528
	Kalahari	0.0523*	0.1000*	–	0.1648
	Kafue	0.0512*	0.0962*	0.0958*	–
* Significant at $p < 0.01$					

The Hwange-Chobe, Okavango and Kalahari population clusters all had low or slightly negative inbreeding coefficient (F_{IS}) with little deviation of the observed from the expected heterozygosity (Table 2). In contrast, the Kafue population appears to have an elevated level of inbreeding ($F_{IS} = 0.245$) with far less heterozygosity across the population than expected (see Appendix B in Supporting Information). In addition, the Kafue population has a very high number of private alleles ($n = 50$), more than double that of the Okavango ($n = 20$) and Hwange-Chobe areas ($n = 19$). The CKGR in contrast has only a single private allele (see Appendix C in Supporting Information)

Table 2

Genetic diversities for each population inferred by STRUCTURE. N , number of individuals; F_{IS} , inbreeding coefficient; H_O , observed heterozygosity; H_E , Nei's expected heterozygosity; NA , mean number of alleles; AR , mean allele richness; PA , total number of private alleles. 95% confidence intervals and standard deviation in parenthesis when appropriate.

	N	F_{IS}	H_O	H_E	NA	AR	PA
Hwange/ Chobe	75	0.053 (0.0085– 0.086)	0.6714 (0.13)	0.7085 (0.1318)	7.3 (1.95)	4.87 (1.29)	19
Okavango	84	0.003 (-0.041– 0.021)	0.6388 (0.17)	0.6366 (0.1713)	6.2 (1.85)	4.26 (1.16)	20
Kalahari	28	0.013 (-0.084– 0.026)	0.6375 (0.14)	0.6297 (0.1334)	4.8 (1.58)	3.97 (1.15)	1
Kafue	14	0.245 (0.062–0.317)	0.5844 (0.59)	0.7648 (0.1229)	6.9 (2.07)	6.05 (1.54)	50

3. 3. 3. Recent migration

Bayesian population assignment suggests that gene flow is generally low between the 4 clusters but with the Hwange/Chobe cluster acting as a source for both the Kalahari and Kafue population and the Okavango acting as a source for the Hwange-Chobe cluster (Table 3).

Table 3

Fraction of individuals from each study population likely to be migrants derived from another population per generation as calculated in *BayesAss*. Shaded values highlight those pathways greater than 0.05 that are significant at the 5% confidence interval. Significance values in brackets.

SOURCE		Hwange/Chobe	Okavango	CKGR	Zambia
RECIEVER	Hwange/Chobe	0.9248 (0.0198)	0.0642 (0.0186)	0.0068 (0.0066)	0.0042 (0.0042)
	Okavango	0.0436 (0.0144)	0.9486 (0.0152)	0.0040 (0.0040)	0.0038 (0.0038)
	Kalahari	0.1140 (0.0459)	0.0177 (0.0163)	0.8580 (0.0440)	0.0103 (0.0099)
	Kafue	0.1152 (0.0422)	0.0202 (0.0190)	0.0208 (0.0194)	0.8438 (0.0446)

Similarly, migrant analysis in *STRUCTURE* suggests that with the migration rate set low ($MIGPRIOR=0.005$) the proportion of membership of each pre-defined population in each of the 4 clusters was very high (0.0996–0.0999). Even when dramatically increasing the expected migration rate ($MIGPRIOR=0.5$) the proportion of membership to each pre-defined population remained high (0.883–0.935), with levels of assignment to a different cluster greater than 0.05 only detected for Okavango sampled lions assigned to the Hwange-Chobe cluster (0.083), Hwange-Chobe sampled lions assigned to the Okavango (0.058) and Kalahari sampled lions assigned to the Hwange/Chobe cluster (0.053). Even at the higher migration rate, only five individual lions have a greater than 50% probability of being 1st generation migrants. Four of these possible 1st generation migrants were from the Okavango into the surrounding Hwange-Chobe, and one was the reverse. Similarly, of individuals with more than 50% probability of being 2nd generation migrants, three individuals in the Hwange-Chobe are likely to have Okavango ancestry and a single animal the reverse pattern.

3.3.4. Dispersal distances

As the largest and most contiguous genetic cluster and the one appearing to have the closest links to other clusters, the Hwange-Chobe cluster was chosen for an assessment of dispersal distances. Mean dispersal distance between 1st order related male-female pairs was 79km with a 95% confidence interval of 44km. The maximum detected dispersal distance was 663km. However, the male of this pair was a trophy hunted animal with relatively poor location data, which may be erroneous. The maximum distance between individuals with accurate location data was 471km. A mean Lynch and Ritland relatedness value of $r = -0.01$ was calculated for males assigned a relationship status of unrelated (U) and within 30km of a female. Extracting the dispersal distance at which $r = -0.01$ along the regression line gave us a value of 90km, with minimum and maximum 95% confidence intervals of 69km and 125km respectively (Figs. 3 & 4).

Discussion

Across the KAZA landscape we detected moderately high levels of genetic diversity, consistent with previous studies (Dubach et al., 2013; Morandin et al., 2014). However, the northern Kafue population revealed a high inbreeding coefficient ($F_{IS}=0.245$) that has not been identified previously, possibly due to the limited low allelic richness of the markers used previously (Dubach et al. 2013). Given the recent population estimate of just 200 lions in northern Kafue (Midlane, Justin O’Riain, Balme, & Hunter, 2015), most likely far less than the 50 prides necessary to prevent inbreeding (Björklund 2003), indicating the worrying possibility of future inbreeding depression and the need for urgent conservation action to facilitate the ingression of new genetic material into this park.

Once the previously detected ecotypic divide between wetland and dryland lions (Dures et al. 2020) is accounted for, Bayesian clustering analysis suggests that the lions of this region are broadly divided into four separate genetic clusters. As expected, these largely coincide with protected areas and, aside from the Okavango wetland cluster, separation is likely to be due to anthropogenic disturbance restricting movement between clusters. These four groups are broadly comprised of the Okavango Delta, the Central

Kalahari Game Reserve (CKGR), Kafue National Park and the Hwange-Chobe Protected Area Complex, incorporating the dryland regions of northern Botswana outside of the Okavango and the CKGR. Differentiation between the four clusters measured by D_{EST} indicates the structure identified is accurate and, when compared to similar landscape genetic studies, F_{ST} values align closely with what can be expected between differentiated populations of large carnivores (Haag, Santos, & Sana, 2010; Mcrae, Beier, Dewald, Huynh, & Keim, 2005). Importantly, the structure we identified using genetic methods largely corroborate the findings of previous work by Cushman et al. (2018) who, apart from the Okavango group, predicted the same regions of connectivity using landscape connectivity models parameterised with satellite collar data from dispersing sub-adult males. The fact that the Okavango group was not identified by Cushman et al., (2018) is unsurprising given this structuring is generated by ecological barriers (S. G. Dures, Carbone, Savolainen, Maude, & Gottelli, 2020) which could not be predicted based on extrapolations from movement data.

The population structure is largely supported by *DAPC* analysis but with two differences. Firstly, *DAPC* separates the Okavango into two populations; the central Okavango island (Chiefs Island) and the remainder of the Okavango. This result is in line with previous studies that have highlighted *DAPC* as being more sensitive to substructure (Viricel & Rosel, 2014) and can be replicated in *STRUCTURE* if the Okavango cluster is analysed in exclusion (see Appendix D in Supporting Information). Secondly, *DAPC* combines the Kafue and Hwange populations into a single genetic cluster. Due to the amount of human presence between these two areas this is surprising, however, if we consider the statistical methods this pattern makes more sense. *DAPC* works by minimizing within cluster variation and maximizing between cluster variations. In contrast *STRUCTURE* and *TESS* work by minimizing deviations from Hardy-Weinberg and linkage disequilibrium. When we examine at the Kafue samples, we see that heterozygosity is lower than that of the other populations and the F_{IS} is higher, indicating significant deviation from random mating and Hardy-Weinberg equilibrium. This suggests that while the Kafue population may be genetically similar to the Hwange population, they are exposed to different population pressures and gene-flow between these areas has been disrupted.

Despite the differentiation between the four clusters, there appears to be low-level gene flow, indicating some areas are acting as a source for others. Our data suggests that the Hwange-Chobe population may be acting as a source for both the Kafue and CKGR population. The Hwange-Chobe population on the other hand appears to benefit genetically from immigrants originating from the Okavango Delta, although to a smaller degree. This latter movement is unsurprising given the high resource availability afforded by the rich Okavango wetlands producing young itinerant male emigrants looking to claim a territory. The asymmetrical movement between the other regions is perhaps more surprising and merits further investigation, but may be a result of perturbation due to persecution resulting from human-wildlife conflict resulting in an ecological trap for lions dispersing from relatively high lion-density areas into areas of low lion-density (Loveridge et al. 2007; Pitman et al. 2015). It should be noted that gene flow in all cases was low and, in the case of Kafue, not enough to prevent isolation and increased F_{IS} .

Our methods indicate a mean dispersal within the dry areas of Northern Botswana and Zimbabwe of 90km (95% CI 69-125km), consistent with the findings of Elliot et al. 2014 using satellite GPS telemetry fitted to dispersing males. We would suggest that these results represent the optimum minimum distance over which effective gene flow can be expected between lion populations occupying similar habitat. While we detected a number of dispersal events greater than this, these are likely to be much rarer events that should not be relied upon to provide functional gene flow. However, this highlights the need to better understand the frequency distribution of long-distance dispersal events.

Incorporating these dispersal distances onto a protected area map of the KAZA Transfrontier Conservation Area (Fig. 4) indicates that from a structural perspective there should be connectivity between these areas with the exception Kafue, which would require introduction of conservation interventions to ensure connectivity to Chobe. Such connectivity is contingent upon the intervening habitat matrix being conducive to dispersal and having little or no interference to movement through anthropogenic processes. When we consider the genetic evidence for current functional gene flow and dispersal across the region this is clearly not the case and indicates that while these protected areas are suitably positioned to facilitate a functional lion metapopulation, there are active impediments to dispersal. This evidence supports previous work in the region identifying anthropogenic limitations to dispersal (Elliot et al., 2014), and predictions regarding management of landscapes to optimise lion population connectivity (Cushman et al., 2018, 2015), but takes it one step further by identifying the actual population-level genetic fragmentation and dispersal in operation. Crucially, the convergence in the results of this research and that of Cushman et al. (2018) act to corroborate the methodologies and findings of both. While satellite collaring can identify potential dispersal corridors at a finer resolution, genetic methods are able to identify broad scale landscape structure as well as indicate more cryptic barriers to dispersal, such as that found between the Okavango delta and the surrounding dryland areas. Interestingly, the Hwange/Chobe genetic cluster extends beyond the boundaries of the strictly protected areas and into the 'wildlife management areas' found between the Okavango Delta and Hwange National Park. Until recently this land was largely used for commercial trophy hunting and highlights that utilisation by humans may not always be detrimental to population connectivity provided the habitat and prey populations are protected. In fact, if strict protection is not financially enforceable, such low impact utilisation, if correctly managed, may be more beneficial than alternative land uses such as agriculture (Di Minin, Leader-Williams, & Bradshaw, 2015). For instance, there have been anecdotal suggestions from residents of a loss in connectivity due to the presence of cattle farming, where the resulting human-wildlife conflict frequently results in lion mortality (Schiess-Meier, Ramsauer, Gabanapelo, & König, 2007), and many residents will openly admit to shooting lions on sight if they are seen in these areas (pers. com.). In any case it is clear that areas that are not under strict protection, but nevertheless protect natural habitat, are still vital for preserving gene flow and diversity in the region and suggests fencing protected areas (Packer et al. 2013), may be detrimental to the regional lion metapopulation (Cushman et al. 2015). While the protected area network is fragmented, based on the male dispersal potential in northern Botswana and Zimbabwe, preserving, and where necessary re-establishing, functional connectivity via areas of safe movement is likely to be an effective management strategy.

This work has demonstrated that, while transfrontier conservation areas may not currently have fully connected populations and free gene flow, there may be the potential for natural dispersal of more mobile species to restore lost functional connectivity. While it may well be possible for such functional connectivity to be restored, it will require a concerted effort from conservation managers to ensure the matrix of habitat through which animals must traverse during dispersal is conducive to their movement and survival. There has been a lot of work highlighting the importance of land outside of protected areas for biodiversity conservation (Balme et al. 2010; Western et al. 2009) and the work we present highlights the importance of such areas for the preservation of large carnivores such as lions, and most likely for other highly mobile animals. Furthermore, from a methodological perspective, this research compliments previous work using remotely sensed data (Cushman et al., 2018, 2015; Elliot et al., 2014) and demonstrates how alternative methodologies can both validate findings and fill knowledge gaps to provide a rigorous picture of landscape use by mobile species. Our results reinforce the importance of maintaining population connectivity, particularly for species that live at low population densities such as apex predators. Further, the work provides more evidence of the need to ensure the current protected area network is augmented with protected corridors as has been highlighted by Cushman et al. (2018). We encourage management authorities tasked with ensuring the long-term sustainability of highly mobile species to provide structural connectivity within any protected area network, as is happening across the KAZA landscape (Botswana Ministry of Land Management, 2018), but also to ensure that such connectivity is functional with regards to dispersal and gene flow.

Declarations

Funding – This research was supported by a BBSRC CASE-studentship (BB/F017324/1).

Conflicts of interest/Competing interests – The authors declare they have no conflict of interest.

Ethics approval - The work carried out in this study was approved by both the Zoology Ethical Review Committee, a subsidiary of Oxford Universities Animal Care and Ethical Review (ACER) Committee (License GFA/0628), and by the Botswana Department of Wildlife and National Parks (permit (EWT 8/36/4 XIII [35])).

Consent to participate - Not applicable.

Consent for publication – Not applicable.

Availability of data and material - Microsatellite data are available at Figshare, (<https://doi.org/10.6084/m9.figshare.3514469>).

Code availability – Not applicable.

Authors' contributions – SGD, CC, and DG contributed to the conceptualization and design of the project. SGD, AJL, GM, and NM collected the data; DD and SGD performed the lab work; SGD performed the data

analysis and led the writing of the text. All authors critiqued the manuscript and gave final approval for submission.

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Figures

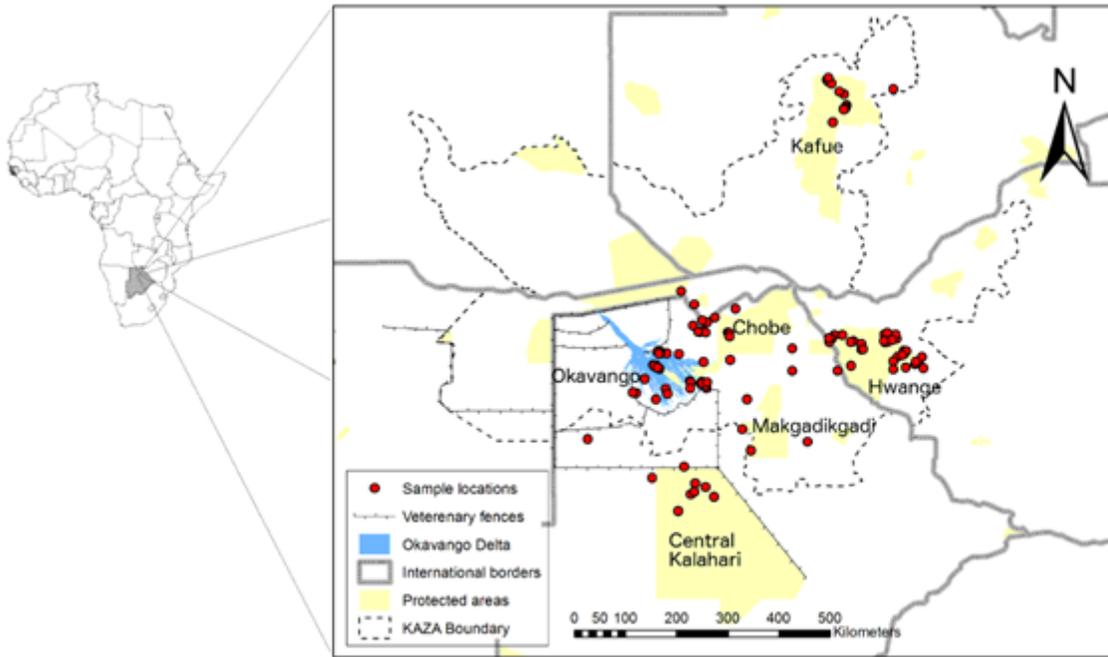


Figure 1

Map of study area showing the Kavango-Zambezi transfrontier conservation area (KAZA) boundary, the protected areas relating to this study, as well as sampling locations.

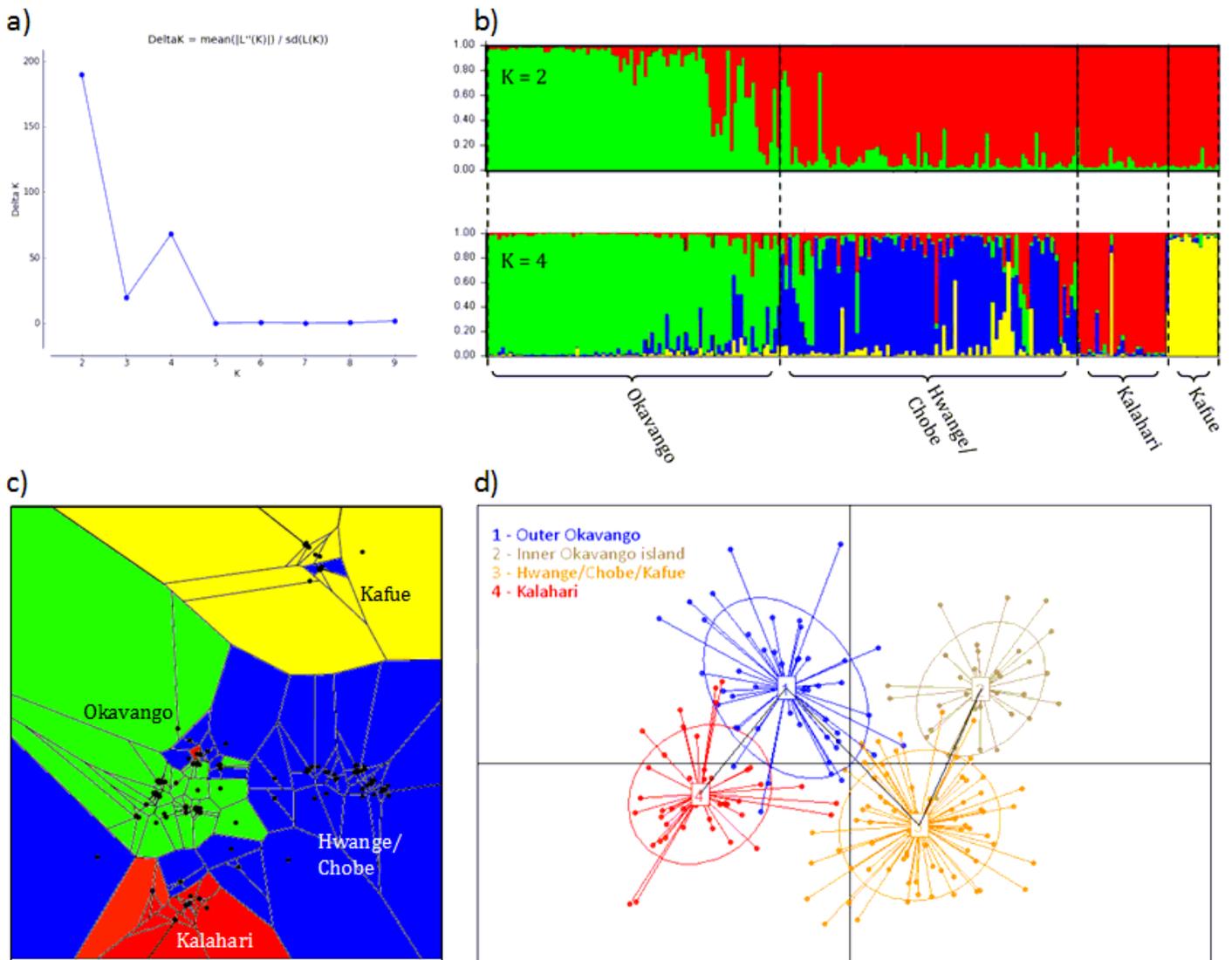


Figure 2

Genetic structure of the KAZA lion population using two Bayesian and one multivariate method. a) STRUCTURE output of Evano plot of Delta K showing likelihood of 2-9 genetic clusters. b) Individual membership proportions for K = 2 & 4, indicating geographic clusters. c) TESS output of K=4 displaying geographic distribution of the genetic clusters. d) Discriminant Analysis of Principal Components (DAPC) plot showing the lion population divided into 4 distinct clusters with limited movement across clusters.

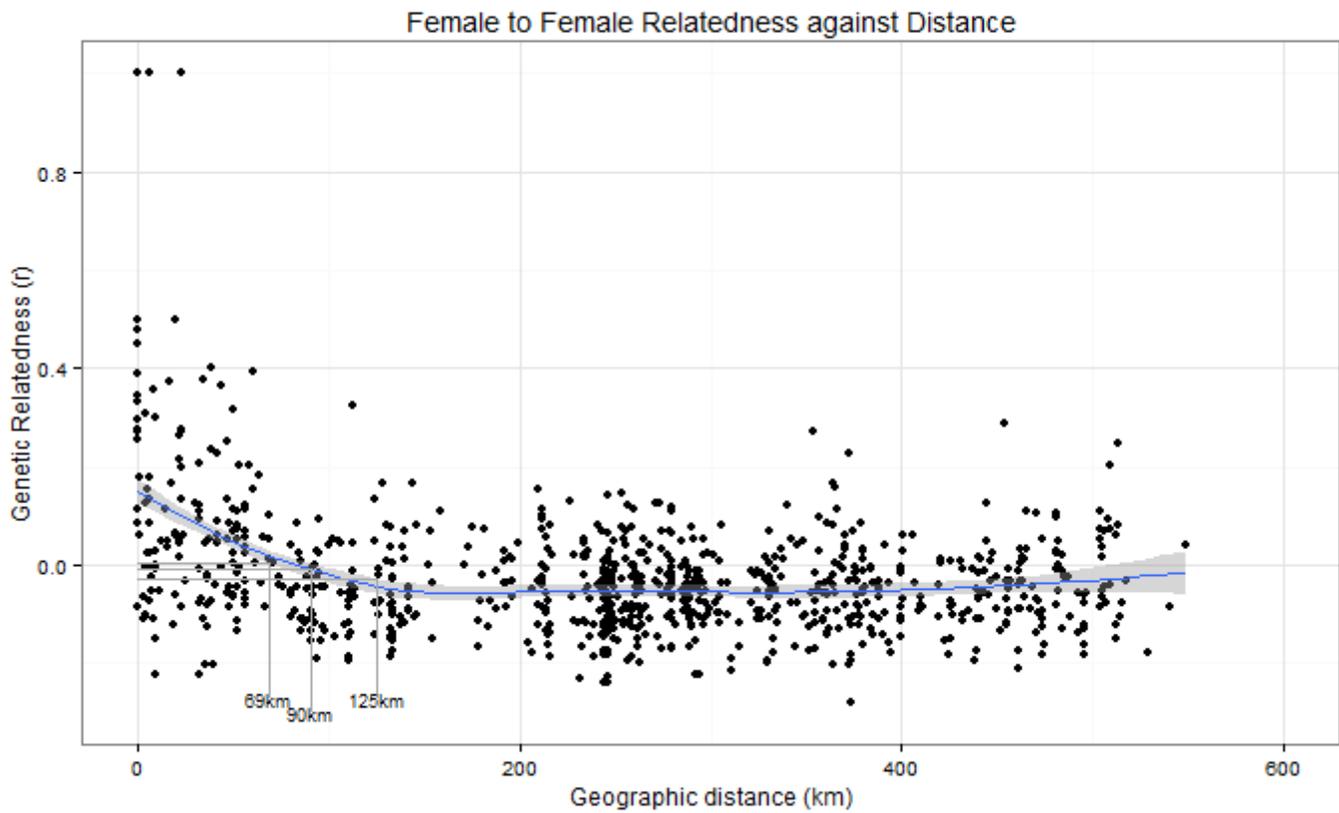


Figure 3

Least-square regression estimates of mean pairwise r for females plotted against pairwise distance. The shaded grey curves show the 95% confidence intervals derived by bootstrapping. The vertical lines identify the mean extrapolated dispersal distance (90km) and overall 95% confidence intervals (69-125km).

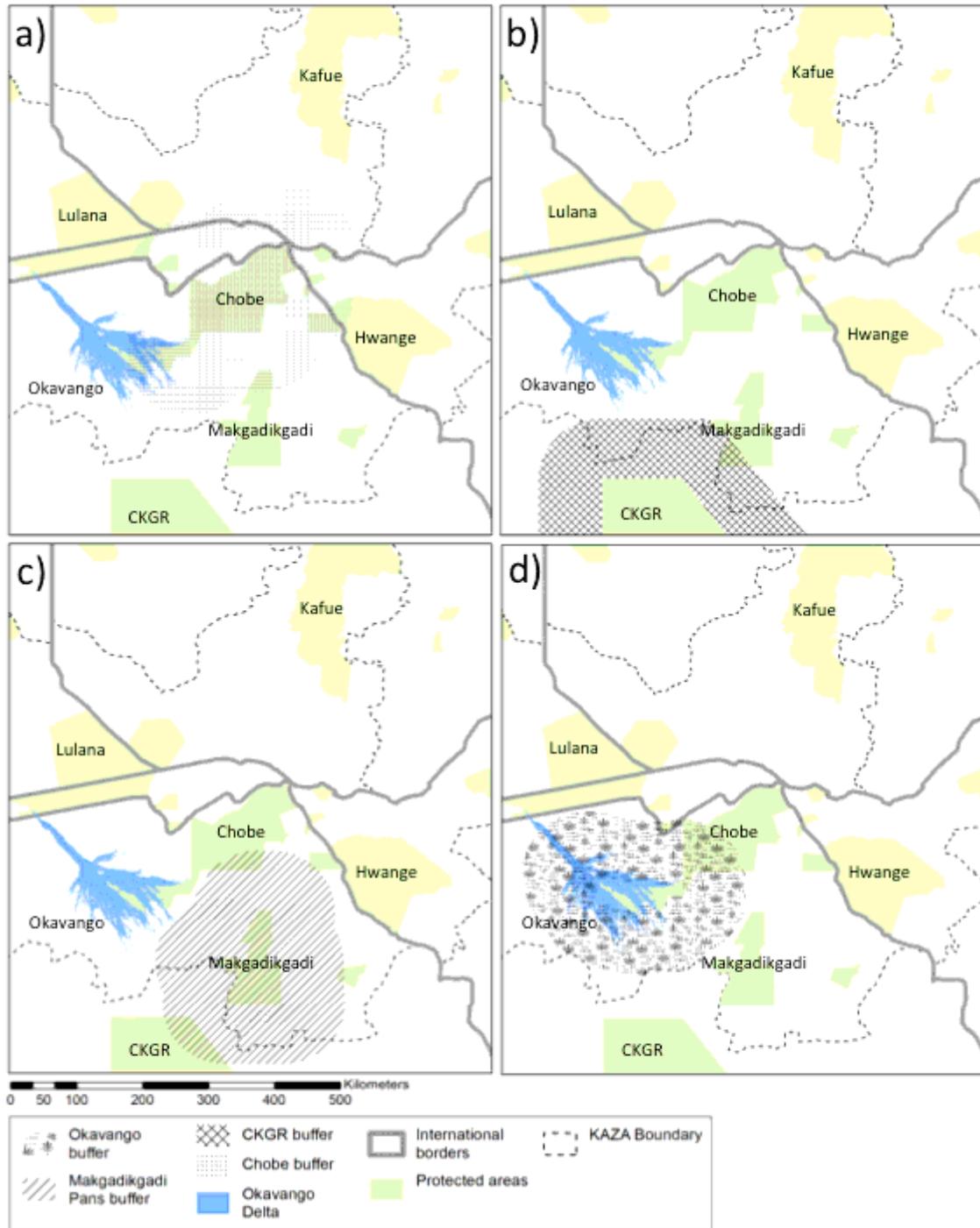


Figure 4

Map of KAZA protected areas showing projected average dispersal range of lions (90km) from a) Chobe National Park; b) the Central Kalahari Game Reserve (CKGR); c) Makgadikgadi and Nxai Pan National Park; d) Moremi Game reserve / Okavango Delta.

Supplementary Files

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