



# A century of decline: Loss of genetic diversity in a southern African lion-conservation stronghold

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## Abstract

**Aim:** There is a dearth of evidence that determines the genetic diversity of populations contained within present-day protected areas compared with their historical state prior to large-scale species declines, making inferences about a species' conservation genetic status difficult to assess. The aim of this paper was to demonstrate the use of historical specimens to assess the change in genetic diversity over a defined spatial area.

**Location:** Like many other species, African lion populations (*Panthera leo*) are undergoing dramatic contractions in range and declines in numbers, motivating the identification of a number of lion-conservation strongholds across East and southern Africa. We focus on one such stronghold, the Kavango–Zambezi transfrontier conservation area (KAZA) of Botswana, Namibia, Zambia and Zimbabwe.

**Methods:** We compare genetic diversity between historical museum specimens, collected during the late 19th and early 20th century, with samples from the modern extant population. We use 16 microsatellite markers and sequence 337 base pairs of the hypervariable control region (HVR1) of the mitochondrial genome. We use bootstrap resampling to allow for comparisons between the historical and modern data.

**Results:** We show that the genetic diversity of the modern population was reduced by 12%–17%, with a reduction in allelic diversity of approximately 15%, compared to historical populations, in addition to having lost a number of mitochondrial haplotypes. We also identify a number of “ghost alleles” in the historical samples which are no longer present in the extant population.

**Main Conclusions:** We argue a rapid decline in allelic richness after 1895 suggests the erosion of genetic diversity coincides with the rise of a European colonial presence and the outbreak of rinderpest in the region. Our results support the need to improved connectivity between protected areas in order to prevent further loss of genetic diversity in the region.

## KEYWORDS

conservation, historical DNA, landscape genetics, microsatellites, mitochondrial DNA, *Panthera leo*

## 1 | INTRODUCTION

Globally, mammal wildlife populations are reported to have undergone a 52% decline in the past half-century (McRae, Freeman, & Deinet, 2014), but over longer time periods, the ranges and population declines have been far more severe (Ceballos, 2002; Crees, Carbone, Sommer, Benecke, & Turvey, 2016; Janecka et al., 2014). While such studies focus on losses in population sizes and species' distributions, relatively few have explored temporal losses in genetic diversity (Leonard, 2008), which may have significant impacts on a species' ability to respond to environmental stochasticity and associated conservation interventions (Spielman, Brook, & Frankham, 2004).

Several reviews highlight insufficient genetic data available to decision makers as a major challenge in conservation genetics today (Frankham, 2010; Hoban et al., 2014). Genetic monitoring of individuals and populations over time was identified as one of the main topics in need of urgent attention. This is especially true for species for which ecological data are unavailable or are overly complex to allow extrapolation from modern genetic data alone, for example, due to uneven mating systems, variation in fecundity among females or non-model dispersal patterns (Björklund, 2003). It is crucial to establish baseline genetic diversity measures against which future comparisons can be made to demonstrate decline or recovery (Jackson, Laikre, Baker, & Kendall, 2011). To this effect, the use of ancient museum samples provides an important genetic tool to measure within-species genetic diversity (Díez-del-Molino, Sánchez-Barreiro, Barnes, Gilbert, & Dalén, 2018). This information in turn will be used to the development and implementation of strategies aim at minimizing genetic erosion and safeguarding genetic diversity.

One important flagship species that has undergone a major decline in population size and geographic range is the lion (*Panthera leo*; Bauer, Chapron et al., 2015). Recent assessments of the lion population in Africa estimate between only 16,500 and 35,000 individuals remain (Bauer, Packer, Funston, Henschel, & Nowell, 2015; Riggio et al., 2013), with an estimated decline of 42% over the past 21 years (Bauer, Chapron et al., 2015; Bauer, Packer et al., 2015). Major declines in wildlife populations across the region, however, have also been noted further back in time (Selous, 1908).

The dramatic population decline of the African lion has made the protection of the remaining populations and the improvement of gene flow between populations of the upmost importance and has led to a number of transboundary conservation initiatives (Naidoo, Preez, Stuart-Hill, Jago, & Wegmann, 2012) such as the Kavango–Zambezi transfrontier conservation area (KAZA). The size of the KAZA region and its ability to support a large number of lion prides, results in it being considered one of the few remaining strongholds for the lion population (Cushman, Elliot, Macdonald, & Loveridge, 2015). While this population, and the ability of lions to disperse long distances in the region, may be enough to sustain a robust population (Björklund, 2003), numbers do not necessarily allow us to understand all aspects of population status. Diminished populations are less effective at eliminating deleterious variants through selection (Spielman et al., 2004; Xue et al., 2009) making them vulnerable

to reduced individual fitness, the loss of species' evolutionary potential, and diminished ecosystem function and resilience. There is a risk of overestimating the potential for modern populations to resist the effects of demographic and genetic stochastic events on small populations if genetic factors are not considered. Populations which may be considered stable by contemporary conservation managers may in fact show signs of genetic erosion, thus needing greater conservation attention. However, currently there is no baseline genetic data for lion populations other than from the modern populations, which are likely to have also suffered major losses in genetic diversity (Björklund, 2003). Furthermore, lions do not conform too many of the assumptions required, or the ecological data are not available, to model historical changes in genetic diversity (Björklund, 2003).

At the end of the 19th and beginning of the 20th century, large numbers of animal specimens were being archived in natural history museums around the world (e.g., Dollman, 1921), including lions shot across the KAZA region. With the advent of methods to extract and analyse DNA from historical archival specimens (Higuchi, Bowman, Freiberger, Ryder, & Wilson, 1984; Leonard, 2008), there is the opportunity to assess the genetic diversity of populations pre-dating any significant anthropogenic influence. By comparing genetic data from museum collections with modern wild populations from the same area, one could assess the extent to which current levels of genetic variation have been reduced (Gebremedhin et al., 2009; Wandeler, Hoeck, & Keller, 2007).

To determine whether genetic diversity has declined over time, we compared genetic diversity between historical and modern lion populations from the KAZA region. We extracted DNA from historical lion samples taken from museum specimens in order to compare historical levels of genetic diversity against modern levels from the same region. We used a suite of microsatellite markers as well as sequencing of part of the hypervariable control region (HVR1) of the mitochondrial genome (mtDNA) to assess the degree to which genetic diversity in this population has been lost as a result of regional declines in lion numbers and distribution.

## 2 | METHODS

### 2.1 | Samples

The Natural History Museum of London's collections contain large numbers of lion skins and skulls from across the species range. The labelling of the collection data was of varying quality so specimens were cross-referenced with collector catalogues wherever possible. Twenty-seven lion specimens were sampled, originally collected from within the study region between 1879 and 1935 (Table 1, Figure 1). Scrapes of any tissue remaining on the skulls or skin, or fragments of detached maxilloturbinal bone (thin bones inside the nasal cavities) were collected from each specimen. Modern samples were collected from 204 free ranging wild lions between 2010 and 2013 (Figure 1) 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 system (Karesh,

**TABLE 1** Museum samples from the Natural History Museum of London grouped according to three spatial zones

Sample number	Accession number	Collection date	Time period	Collection location	Approximate longitude	Approximate latitude
Zone I						
1	19.7.15.21	1879	A	Mababe	24.33	-19.12
2	19.7.15.22	1879	A	Mababe	24.19	-18.99
3	19.7.15.23	1879	A	Mababe	24.03	-19.14
4	19.7.15.24	1879	A	Mababe plain	24.36	-18.84
5	19.7.15.25	1879	A	Boteti river	24.37	-20.80
6	19.7.15.27	1879	A	Linyanti-Chobe North bank	23.76	-18.46
7	19.7.15.15	1884	A	Northern Kalahari-Botswana	23.56	-20.43
8	31.2.1.4	1930	B	Mababe flats/Mogogelo river	23.96	-18.89
9	31.2.1.4 <sup>a</sup>	1930	B	Mababe flats/Mogogelo river	23.74	-19.75
10	31.2.1.5	1930	B	Mababe flats/Mogogelo river	24.15	-18.62
11	31.2.1.5 <sup>a</sup>	1930	B	Mababe flats/Mogogelo river	23.87	-19.55
12	31.2.14.6	approx. 1930	B	Kabulubula 60 miles West of Livingstone	24.88	-18.03
Zone II						
13	19.7.15.29	1874	A	Upper tatui river-Zimbabwe/Botswana border near Francetown	27.14	-20.81
14	19.7.15.31	1880	A	Umfuli river-North-central Zimbabwe	28.21	-17.46
15	19.7.15.26	1882	A	Mashonaland-North Zimbabwe approx. 200 miles West of Harare	27.97	-18.42
16	19.7.15.14	1883	A	Mashonaland-approx 200 miles West of Harare	28.23	-18.82
17	19.7.15.30	1886	A	20 miles South of Bulawayo	28.48	-20.76
18	35.3.16.1	Unknown, 1935?	B	North West Rhodesia-Solwezi district	25.84	-13.39
19	35.3.16.2	Unknown, 1935?	B	North West Rhodesia-Solwezi district	26.26	-12.86
Zone III						
20	19.7.15.17	1880	A	Gwabi river Northern Zimbabwe on Zambia border	27.94	-15.89
21	19.7.15.17 <sup>a</sup>	1880	A	Gwabi river Northern Zimbabwe on Zambia border	27.94	-15.89
22	19.7.15.18	1880	A	Gwabi river Northern Zimbabwe on Zambia border	27.94	-15.89
23	93.5.21.1	1893	A	Botswana	Poor data	
24	79.2188	1895	A	Botswana	Poor data	
25	1887.5.16.2	1887	A	Sebakwe River Mashuna Zimbabwe	30.95	-21.19
26	19.7.15.32	1891	A	Hartley hills, near Harare	30.42	-18.07
27	35.9.1.129	1929	B	Karakuwiri Grootfontain	18.42	-19.51

Note. Sample number refers to position on Figure 1. Spatial zones represent (a) samples within the modern sampling area; (b) the samples likely to be within maximum male dispersing distance of the modern sampling area, taken as 200 km; (c) all remaining samples across the region. Time periods represent samples collected between (A) 1874–1895 and (B) 1930–1935. Unclear dates use accession number as date reference. Longitude and latitude are estimated based on location data available for each specimen.

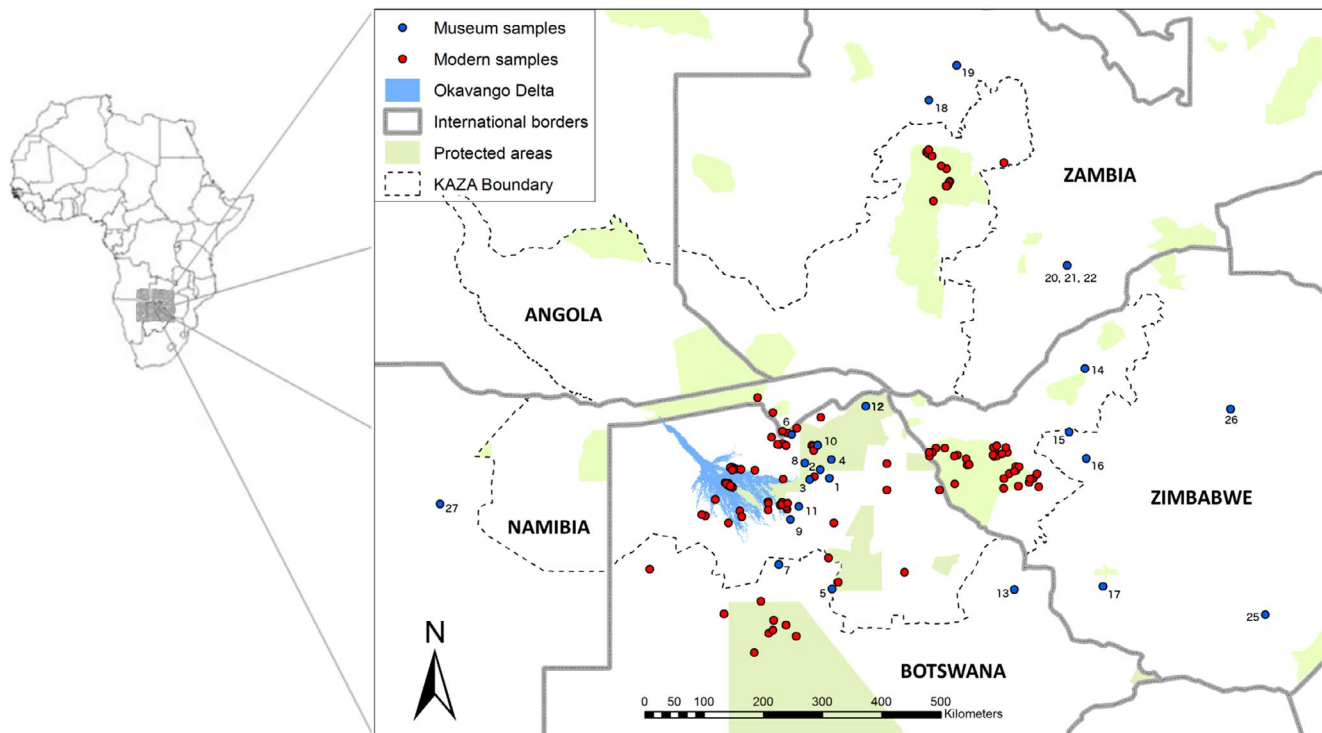
<sup>a</sup>Accession number used a second time for two different samples.

Smith, & Frazier-Taylor, 1987). Hair-pulls and blood were taken from immobilized animals. Dry tissue samples were taken from animals shot by the trophy hunting industry.

## 2.2 | Ancient DNA precautions

All pre-PCR work was performed in a laboratory exclusively devoted to ancient DNA, situated on a different floor from the PCR

amplification laboratory and with an independent air handling system. To avoid sample cross-contamination, a different set of equipment was used for each extraction (e.g., mortar and pestle, scalpel blades). Single-use equipment was immersed in sodium hypochlorite and removed from the working area after use. The working area was cleaned with sodium hypochlorite solution before work on the next sample commenced. All equipment was UV-irradiated overnight prior to further use. Filter tips were used to reduce cross-contamination



**FIGURE 1** Map of Kavango–Zambezi region showing sampling distribution of modern lion samples (red circles) and museum samples (blue triangles and numbered)

(Rohland & Hofreiter, 2007). DNA was extracted from each sample twice and results from each extraction compared for consistency. Two blank extractions containing no tissue or bone were included during both extraction protocols to serve as negative extraction and PCR controls. Each fragment was independently amplified by PCR a minimum of three times following the multi-tube approach (Taberlet, Waits, & Luikart, 1999) in an attempt to detect contamination and genotyping errors. For microsatellite amplification, PCR reactions were repeated until consensus was achieved across at least three independent reactions. For mitochondrial sequencing, PCR reactions were repeated until a minimum of 2× coverage for each base for both the reverse and forward sequences was achieved.

### 2.3 | DNA extraction

Total genomic DNA was extracted from each museum skin sample using approximately 25 mg of tissue using DNeasy® Blood and Tissue kits (Qiagen). We followed the manufacturer's instructions but added a second incubation. To increase tissue lysis, the first incubation was run overnight, and for the second digestion, we added a further 180 µl Buffer ATL and 20 µl proteinase K (600 mAU/ml) and then incubated for a further 3 hr at 56°C.

DNA from bone samples was extracted using approximately 100 mg of bone powder previously ground in a pestle and mortar. A master mix was prepared which, for each sample, comprised of 0.2 ml 10% SDS (Invitrogen), 0.15 ml proteinase K at 15 mg/µl, a 1 × 1 mm piece of DTT at 10 mM and 1.65 ml EDTA of pH 8.0 at 0.5 M. This was warmed at 56°C until all ingredients dissolved and

added to each bone powder sample. Samples were incubated on a rotator at 56°C for 48 hr. Following digestion, tubes were centrifuged for 1 min at 1,300 rpm and supernatant transferred to an Amicon® Millipore Ultra Centrifuge filter which was centrifuged for 30 min at 1,300 rpm. A MinElute purification kit (Qiagen) was used to purify 100 µl of extract following the manufacturer's instructions, washing three times with PE buffer.

Modern DNA was extracted using approximately 25 mg of tissue, 100 µl of raw blood or 5–6 hair follicles using DNeasy® Blood and Tissue kits (Qiagen) according to the manufacturer's instructions. Faecal DNA was extracted using approximately 200 mg of stool using QIAamp® DNA Stool kits (Qiagen) according to the manufacturer's instructions.

### 2.4 | Microsatellite amplification

We used sixteen microsatellite loci previously identified and amplified in the domestic cat (Menotti-Raymond et al., 1999; FCA1, FCA45, FCA69, FCA75, FCA77, FCA96, FCA97, F115, FCA126, FCA129, FCA133, FCA193, FCA205, FCA224, FCA247 and FCA391) which have previously been successfully used in lions (Driscoll, 1992; Driscoll, Menotti-Raymond, Nelson, Goldstein, & O'Brien, 2002; Dubach, Briggs, White, Ament, & Patterson, 2013; Lyke, Dubach, & Briggs, 2013; Spong & Creel, 2001). The nuclear marker primers were divided into multiplex combinations and fluoro-labelled with one of VIC, 6-FAM, PET or NED dyes, according to primer annealing temperatures and non-overlapping allele size range combinations (see Supporting Information Appendix S1). See Supporting Information

Appendix S1 for amplification conditions and sequencing details. The allele sizes and genotypes were scored in GENEMAPPER v4.1 (Applied Biosystems). Each sample was scored and genotyped multiple times until reaching a consensus across a minimum of three independent PCR reactions from the same sample.

## 2.5 | Mitochondrial sequencing

We amplified a 337 bp hypervariable region (HVR1) of the *Panthera leo* mitochondrial control region, using previously published reverse and forward primers (Barnett, Yamaguchi, Barnes, & Cooper, 2006). To improve the quality of the sequencing and avoid the problem of double banding due to the reverse primer being able to bind to multiple reverse sequence repeats, identified previously with these primers, we used a nested reverse primer designed for direct sequencing (Barnett et al., 2006). See Supporting Information Appendix S1 for PCR and sequencing conditions. Consensus sequence for each individual was obtained through alignment of the forward and reverse sequences, resulting from multiple PCR products, with the programme GENEIOUS (Kearse et al., 2012) to yield a minimum of 2× coverage for each base for both the reverse and forward sequences.

## 2.6 | Estimation of change in nuclear diversity

To detect changes in nuclear diversity between the modern and historical populations, using the microsatellite data, we calculated Nei's unbiased estimate of expected heterozygosity ( $H_E$ ), observed heterozygosity ( $H_O$ ), inbreeding coefficient ( $F_{IS}$ ) and mean number of alleles per locus ( $A$ ). This was performed using GENEPOP (Rousset, 2008) using methods documented in previous research on white-tailed eagles (Hailer et al., 2006). GENEPOP was also used to detect significant departures from Hardy-Weinberg equilibrium (HWE) and evidence of linkage disequilibrium within the sample groups. Unique alleles were identified for each time period using CONVERT (Glaubitz, 2004). The mean number of private alleles per locus found in each population was calculated using a rarefaction approach to control for differences in sample size, implemented in ADZE (Szpiech, Jakobsson, & Rosenberg, 2008). DnaSp v.5 (Librado & Rozas, 2009) was used to calculate mtDNA haplotype diversity ( $H$ ) and nucleotide diversity ( $\pi$ ), as well as Tajima's  $D$  and Fu's  $F_s$  to test for deviations from neutral evolution for both the modern and historical populations. To provide an indication of the extent of genetic differences between the historical and modern populations, for example changes in allele frequency,  $F_{ST}$  and Nei's unbiased genetic distance were both calculated as measures of genetic differentiation in GenAlEx 6.5 (Peakall & Smouse, 2012).

## 2.7 | Bootstrap resampling

There is an inherent inability to control the sampling design when using museum collections, including sample size, date and location of their collection. To allow comparisons between modern and historical nucleic diversity, we used a bootstrapping procedure. When

analysing the more rapidly mutating nuclear microsatellite data, we progressively restricted (a) the spatial extent of the historical samples, to match with more certainty the extent of the modern samples; (b) the time period over which the historical samples were collected, to restrict the possible influence of genetic drift with time within the sample set. Thus, we divided our historical data into three spatial zones representing (a) the samples within the modern sampling area; (b) the samples likely to be within male dispersing distance of the modern sampling area, taken as 200 km; (c) all remaining samples across the region (Table 1). We also divided the historical data into two time periods: 1874–1895 (A) and 1929–1935 (B) (Table 1). The results from the historical samples sets were compared against our modern data set using a bootstrapping procedure implemented in POPTOOLS (Hood, 2011). We created 100 populations of equal size to the historical data being used. Furthermore, to account for an apparent lack of historical sampling from within the Okavango Delta bootstrap sampling was repeated both with and without modern Okavango Delta samples. In a species such as lions, where female siblings tend to remain in the same pride or form a neighbouring pride and male siblings commonly forge a coalition, the likelihood of collecting data from close relatives was high. To test for the effects of close relatives, we followed the recommendations of Rodríguez-Ramilo & Wang (2012) and calculated all possible full-sibling and parent-offspring clusters in the programme COLONY (Wang & Scribner, 2014). We then randomly selected just one individual from each close-relative cluster, before re-running the bootstrap procedure on the reduced data set.

## 2.8 | Mitochondrial “ghost” alleles

Following the identification of all haplotypes present in the combined modern and historical data set, we were able to assess private haplotypes only present in one or other time period. Due to the much poorer quality of the museum sample data, many sequences were considerably shorter than the modern counterparts, making direct comparisons of diversity difficult and lacking power. However, we were able to identify haplotypes only present in the historical data, likely to have been lost from the modern population (Leonard, Vilà, & Wayne, 2005).

## 3 | RESULTS

We achieved successful microsatellite amplification of all 27 museum samples and obtained usable mitochondrial sequences from 18 of these. A number of microsatellite loci could not be successfully genotyped across every sample, achieving a mean of  $23.7$  ( $SD \pm 3.5$ ) complete genotypes per locus (Data available on Figshare, <https://doi.org/10.6084/m9.figshare.3514469>). Successful microsatellite amplification was achieved for all 204 modern lion samples, representing 204 distinct genotypes, and usable mitochondrial sequences were obtained from 38 of these. MICROCHECKER 2.2.3 (Van Oosterhout, Hutchinson, Wills, &

**TABLE 2** Genetic diversity for the Kavango–Zambezi African lion population within each spatial scale for 16 microsatellite loci

Sample set	N	$H_E$	SD	$H_O$	SD	$F_{IS}$	A	SD
Zone I–III								
Historical	27	0.7813	–	0.7565	–	0.032	8.50	–
Modern	27	0.6989	(0.014)	0.6541	(0.025)	0.064	6.55	(0.37)
Modern—without Okavango	27	0.7186	(0.013)	0.6688	(0.020)	0.069	7.00	(0.37)
Zone I–II								
Historical	19	0.7807	–	0.7676	–	0.017	7.69	–
Modern	19	0.6928	(0.017)	0.6483	(0.025)	0.064	6.23	(0.35)
Modern—without Okavango	19	0.7169	(0.014)	0.6647	(0.021)	0.073	6.49	(0.35)
Zone I								
Historical	12	0.7945	–	0.7975	–	–0.004	6.75	–
Modern	12	0.6946	(0.023)	0.6523	(0.034)	0.061	5.39	(0.30)
Modern—without Okavango	12	0.7146	(0.021)	0.6606	(0.035)	0.076	5.60	(0.34)

Note. Modern samples represent the average value from 100 bootstrap replications including or excluding the Okavango samples, respectively. N = sample size;  $H_E$  = expected heterozygosity;  $H_O$  = observed heterozygosity;  $F_{IS}$  = inbreeding coefficient; A = mean number of alleles per locus; SD = standard deviation of bootstrap replications.

Shipley, 2004) detected no genotyping error across the microsatellite data, including faecal samples.

No single locus or within-group deviations from HWE were detected, and tests for linkage disequilibrium were not significant after Bonferroni correction. Mitochondrial consensus sequence lengths varied from between 204 and 322 bp, across a 337 bp region (GenBank Accession no. KX661326–KX661331).

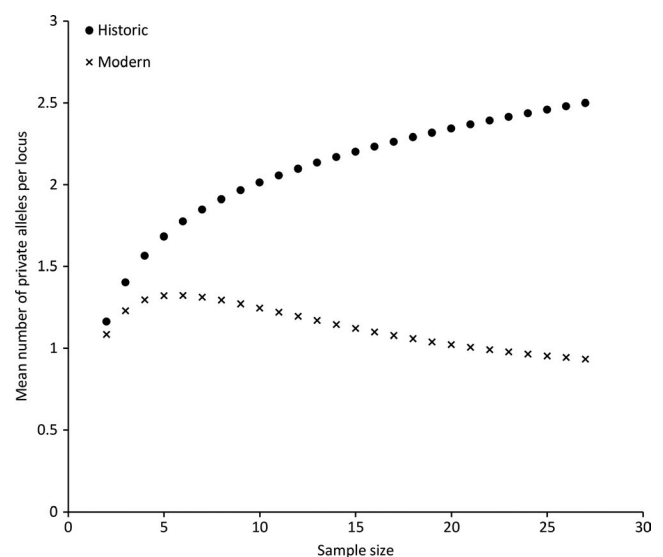
In every bootstrap combination of our microsatellite data, regardless of how many samples were excluded, the historical lion population exhibited a higher heterozygosity, both observed ( $t = 8.75$ ,  $p = 0.006$ ) and expected ( $t = 14.80$ ,  $p = 0.002$ ). The same results for reduced heterozygosity were returned when the Okavango lions were removed from the analysis (observed,  $t = 8.75$ ,  $p = 0.006$ ; expected,  $t = 14.79$ ,  $p = 0.002$ ).

In every iteration of the data, the modern population showed a much greater deficiency in the observed heterozygosity compared to the expected, represented by a significantly larger inbreeding coefficient ( $F_{IS}$ ) for all modern sample sets ( $t = 5.42$ ,  $p = 0.016$ ; Table 2). The reduction in the geographic extent of the historical data resulted in a limited change in the observed heterozygosity from 0.7565 for the broadest sample set up to 0.7975 for the most limited. When we control for differences in sample size ( $n = 27$  vs. 12) using 100 bootstrap replications, the observed heterozygosity for the full sample set of zones I–III increased from 0.7565 to 0.7612, similar to levels observed among the more spatially restricted data encompassing just zones I and II.

Across the data, we identified 29 alleles present only in the historical samples and 54 private alleles only found in the modern data; however, the latter come from a much larger data set. The mean number of private alleles is consistently higher in the historical data than in the modern data when controlling for sample size (Figure 2).

Such “ghost alleles” (Bouzat, Lewin, & Paige, 1998; Groombridge, Jones, Bruford, & Nichols, 2000) were identified in 14 out of the 16 microsatellite markers, only absent from Fca126 and Fca391. Even when reducing the historical data to only those within the most conservative spatial area ( $n = 13$ ), we still found 18 alleles not present in the modern samples, spread across all microsatellite markers except Fca126, Fca129, Fca193 and Fca391.

Analysing historical samples collected between the two time periods returned a negligible difference in their heterozygosity (see Supporting Information Appendix S1); however, it did result in a decrease in the allelic richness from 7.5 in the oldest samples to 5.88.

**FIGURE 2** Mean number of private alleles per locus as a function of standardized sample size for historical and modern microsatellite data



**TABLE 3** Genetic differences between the modern lion population and the lion populations from the two historical time periods (Historical A-1874 to 1895; Historical B-1930 to 1935) as measured by  $F_{ST}$  and Nei's unbiased genetic distance

Modern		Historical A		Historical B		
$F_{ST}$	Nei's unbiased	$F_{ST}$	Nei's unbiased	$F_{ST}$	Nei's Unbiased	
0.000	0.000					Modern
0.039	0.177	0.000	0.000			Historical A
0.019	0.000	0.035	0.068	0.000	0.000	Historical B

When we reduced the data to include only samples collected between 1929 and 1935, the allelic richness (5.88) closely matches that found within the modern samples (6.32).

Comparing the extent of genetic differences between the historical and modern populations resulted in an  $F_{ST}$  and Nei's unbiased genetic distance of 0.025 and 0.098, respectively, suggesting limited differentiation between the time periods. When dividing the historical samples into the two separate time periods (A-1874 to 1895; B-1929 to 1935; Table 3), the difference between the modern samples and the older of the two historical groups (A), as well as both the modern and the more recent historical group, became noticeably more distinct suggesting the greatest differentiation arose between the two historical time periods.

Removing close relatives had a negligible effect on any values. In the full modern data set, the observed heterozygosity increased from 0.6541 to 0.6570, expected heterozygosity from 0.6989 to 0.7039, the inbreeding coefficient from 0.064 to 0.066 and the mean number of alleles from 6.55 to 6.65.

The mtDNA data (Table 4) indicates six haplotypes present within the historical data set ( $H = 0.6993$ ,  $\pi = 0.00065$ ), but three of these appear to be missing from the extant lions ( $H = 0.3257$ ,  $\pi = 0.0007$ ). Tajima's  $D$  and Fu's  $F_s$  for both the historical ( $D = -1.09629$ ,  $p < 0.1$ ;  $F_s = -1.09629$ ,  $p < 0.1$ ) and modern ( $D = -0.53568$ ,  $p < 0.1$ ;  $F_s = -0.521$ ,  $p < 0.1$ ) population are negative but not significant, suggesting no deviation from neutrality. Aside from the three "ghost" haplotypes identified, there may be others present within the same mtDNA region that due to the degradation of the historical DNA remain unidentified. Since two of the "ghost" haplotypes were

identified from single individuals, each only with a single-nucleotide insertion, we must caution that they may be false haplotypes caused by DNA degradation (Wandeler et al., 2007). Even following a more conservative approach, one previously common haplotype remains unrepresented in the modern samples.

## 4 | DISCUSSION

The value of genetic diversity is increasingly recognized for contributing to individual fitness, species' evolutionary potential, and ecosystem function and resilience (Whitham et al., 2008). There is therefore an urgent need for policy-relevant studies to help define sensitive and robust indicators of changes in genetic diversity (Hoban et al., 2013).

Our analysis demonstrates that over the past century, the lion population of the Kavango–Zambezi region has lost genetic diversity. Contemporary observed heterozygosity has been reduced by 12%–17% compared to historical populations. Despite having a number of missing alleles across the samples, genetic diversity was still historically higher than in the contemporary lion population. The decline in heterozygosity is not as dramatic as that seen in some highly threatened or bottlenecked species, for example 57% in the Mauritius kestrel (*Falco punctatus*; Groombridge et al., 2000) or 43% in sea otters (*Enhydra lutris*; Larson, Jameson, Etnier, Fleming, & Bentzen, 2002), it nevertheless represents a worrying reduction in diversity considering this population is one of only six lion strongholds remaining in Africa.

Sample size			Variable nucleotide position <sup>a</sup>				
Modern	Historical	Haplotype	221	343	367	368	378
31	5	i	–	–	T	A	–
	9	ii	T	–	T	A	–
	1	lii	N/A	–	T	A	C
	1	iv	N/A	C	T	A	–
3	1	v	–	–	C	A	–
4	1	vi	–	T	T	G	–

Note. "–" and "N/A" represent a deletion or missing sequence data, respectively, at the specified nucleotide position.

<sup>a</sup>1 corresponds to position 16,176 in the complete *Panthera leo leo* mtDNA sequence (Ma & Wang, 2014).

**TABLE 4** Mitochondrial DNA control region haplotypes from historical specimens and the extant lion population of the KAZA region

While the low sample size of the bootstrapping means caution should be taken before extrapolating to the true  $F_{IS}$ , it is clear that the reduced heterozygosity exposes lions of the region to a higher risk of inbreeding depression than their historical counterparts. As well as clear decline in nuclear diversity, as assessed with the microsatellite analysis, there is also an indication of a loss in mitochondrial diversity. One haplotype detected in multiple historical samples, and two more haplotypes detected in single samples, remain entirely undetected in the modern population. The results are in agreement with previous research which has identified both declining populations and increasing fragmentation in the region (Elliot, Cushman, Macdonald, & Loveridge, 2014; Loveridge, Searle, Murindagomo, & Macdonald, 2007).

Similar to other species, the global decline in lion numbers has largely been driven by human–wildlife conflict and habitat loss (Bauer, Packer et al., 2015; Keyghobadi, Roland, Matter, & Strobeck, 2005). Given the rapid expansion of human activities in the region in the 20th century, the downward trend in genetic diversity we observed is perhaps unsurprising and seemingly confirms the pessimistic observations made in the late 19th century. For example, one account from Frederick Courtney Selous records, “During the twenty years since my first arrival in 1871, I ... had seen game of all kinds gradually decrease and dwindle in numbers to such an extent that I thought that nowhere south of the Great Lakes could there be a corner of Africa left where the wild animals had not been very much thinned out” (Selous, 1908). Interestingly, allelic richness did not differ markedly between the intermediate temporal (1929–1935) and contemporary population samples, suggesting that allelic richness was lost prior to the intermediate sampling period. This is supported by measures of genetic differentiation which are markedly larger between the two historical time periods than between the modern and more recent historical sample sets. A temporal decline in genetic diversity between the two sets of historical samples was not detected through measures of heterozygosity, likely due to changes in allelic richness being detectable before population declines impact upon heterozygosity (Athrey, Lindsay, Lance, & Leberg, 2011); however, it was detected between the modern and historical data. The rapid decline observed in allelic richness and the highest levels of genetic differentiation coincide with the arrival of the first western settlers in 1890 and the subsequent rise of the colonial presence in the region after the end of the Matabele Wars in 1897 (Parsons, 1983). Furthermore, modern firearms became more prevalent following European settlement and predators were often persecuted as vermin (Woodroffe, 2000), which likely contributed to the earlier decline of lions in the study region. While the timing of genetic decline and colonial settlement is compelling enough to suggest causation, the evidence is not conclusive. The epizootic of the rinderpest virus also struck during the late 1890s resulting in the death of vast populations of buffalo, giraffe and wildebeest, as well as domestic livestock (Van den Bossche, de La Rocque, Hendrickx, & Bouyer, 2010). Such an epidemic is very likely to have also had a considerable impact on the predators of the region.

Given the level of habitat loss and fragmentation observed across sub-Saharan Africa (Bauer, Packer et al., 2015; Keyghobadi

et al., 2005), the increased threat of epizootics facilitated by human movements (Butler, Toit, & Bingham, 2004), as well as the impacts of a changing climate (Thomas et al., 2004), it is imperative that efforts are made to conserve genetic diversity. Without such genetic diversity, a species resilience and ability to adapt to future stochastic events becomes greatly compromised (Whitham et al., 2008). This study provides quantitative data on temporal genetic monitoring that is urgently needed to optimize conservation and management efforts. Since KAZA is considered one of the more important populations for lion conservation across Africa, the work presented here should provide motivation for increased conservation action to safeguard against continued loss of genetic diversity of lions and other species across the region (Krofel, Treves, William, Chapron, & López-bao, 2015). In particular, greater connectivity between lion population in protected areas across the region and thus the mixing of genetic material should be supported (Cushman et al., 2015).

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## DATA ACCESSIBILITY

Microsatellite data are available at Figshare, <https://doi.org/10.6084/m9.figshare.3514469>. Mitochondrial sequence data have been submitted to the GenBank database under accession no. KX661326–KX661331.

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## BIOSKETCH

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## SUPPORTING INFORMATION

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

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