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 historic state prior to large-scale species declines, making inferences about a species' conservation genetic status difficult to assess. The aim of this paper is to demonstrate the use of historic specimens to assess the change in genetic diversity over a defined spatial area.

Location

Like 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 historic and modern data.

Results

We show that the genetic diversity of the modern population was reduced by 12% to 17%, with a reduction in allelic diversity of approximately 15%, compared to historic populations, in addition to having lost a number of mitochondrial haplotypes. We also identify reduced allelic diversity and a number of 'ghost alleles' in the historical samples 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, landscape genetics, historic DNA, microsatellites, mitochondrial DNA, *Panthera leo*.

Introduction

Globally, mammal wildlife populations are reported to have undergone a 52% decline in the past half century (McRae et al., 2014), but over longer time periods the ranges and population declines have been far more severe (Ceballos, 2002; Janecka et al., 2014; Crees et al., 2016). While such studies focus on loses 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 et al., 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. It is crucial to establish baseline genetic diversity measures against which future comparisons can be made to demonstrate decline or recovery (Jackson et al., 2011). To this effect the use of ancient museum samples provide an important genetic tool to measure within-species genetic diversity. 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 et al., 2015a). Recent assessments of the lion population in Africa estimate between only 16,500 and 35,000 individuals remain (Riggio et al., 2013; Bauer et al., 2015b), with an estimated decline of 42% over the past 21 years (Bauer et al., 2015a; Bauer et al., 2015b). Major declines in wildlife populations across the region, however, have also been noted further back in time (Selous, 1908).

The dramatic 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

85 number of transboundary conservation initiatives (Naidoo et al., 2012) such as the Kavango-Zambezi
86 transfrontier conservation area (KAZA). The size of the KAZA region and its ability to support a large
87 number of lion prides, results it being considered one of the few remaining strongholds for the lion
88 population (Cushman et al., 2015). While this population, and the ability of lions to disperse long
89 distances in the region, may be enough to sustain a robust population (Björklund, 2003), numbers do
90 not necessarily allow us to understand all aspects of population status. Diminished populations are
91 less effective at eliminating deleterious variants through selection (Xue et al., 2009; Spielman et al.,
92 2004) making them vulnerable to reduced individual fitness, the loss of species' evolutionary
93 potential, and diminished ecosystem function and resilience. There is a risk of overestimating the
94 potential for modern populations to resist the effects of demographic and genetic stochastic events
95 on small populations if genetic factors are not considered. Populations which may be considered
96 stable by contemporary conservation managers may in fact show signs of genetic erosion, thus
97 needing greater conservation attention. However, currently there is no baseline genetic data for lion
98 populations other than from the modern populations, which are likely to have also suffered major
99 losses in genetic diversity (Björklund, 2003).

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

108 To determine whether genetic diversity has declined over time, we compared genetic diversity
109 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 historic 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.

Methods

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 until 1935 (Table 1, Fig. 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 until 2013 (Fig. 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 et al., 1987). Hair pulls and blood were taken from immobilised animals. Dry tissue samples were taken from animals shot by the trophy hunting industry.

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 etc). 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 (Rohland & Hofreiter, 2007). 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 at least three times following the multi-tube approach (Taberlet et al., 1999) in an attempt to detect contamination and genotyping errors.

DNA extraction

Total genomic DNA was extracted from each museum skin sample using approximately 25mg 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, for the second digestion we added a further 180µl Buffer ATL and 20µl proteinase K (600mAU/ml) and then incubated for a further 3 hours at 56°C.

DNA from bone samples was extracted using approximately 100mg of bone powder previously ground in a pestle and mortar. A master mix was prepared which, for each sample, comprised of 0.2ml 10% SDS (Invitrogen), 0.15ml proteinase K at 15mg/µl, a 1x1mm piece of DTT at 10mM and 1.65ml EDTA of pH 8.0 at 0.5M. 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 hours. Following digestion, tubes were centrifuged for one minute at 1300rpm and supernatant transferred to an Amicon® Millipore Ultra Centrifuge filter which was centrifuged for 30 minutes at 1300rpm. A MinElute purification kit (Qiagen) was used to purify 100µl of extract following the manufacturer's instructions, washing three times with PE buffer.

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

161

162 *Microsatellite amplification*

163 We used sixteen microsatellite loci previously identified and amplified in the domestic cat (Menotti-
164 Raymond et al., 1999) (FCA1, FCA45, FCA69, FCA75, FCA77, FCA96, FCA97, F115, FCA126, FCA129,
165 FCA133, FCA193, FCA205, FCA224, FCA247, FCA391) which have previously been successfully used in
166 lions (C. Driscoll, 1992; C. A. Driscoll et al., 2002; Dubach et al., 2013; Lyke et al., 2013; Spong &
167 Creel, 2001). The nuclear marker primers were divided into multiplex combinations and fluoro-
168 labelled with one of VIC, 6-FAM, PET or NED dyes, according to primer annealing temperatures and
169 non-overlapping allele size range combinations (see Supplementary materials). See Supplementary
170 Material for amplification conditions and sequencing details. The allele sizes and genotypes were
171 scored in *GENEMAPPER v4.1* (Applied Biosystems).

172

173 *Mitochondrial sequencing*

174 We amplified a 337bp hypervariable region (HVR1) of the *Panthera leo* mitochondrial control region,
175 using previously published reverse and forward primers (Barnett et al., 2006). To improve the quality
176 of the sequencing and avoid the problem of double banding due to the reverse primer being able to
177 bind to multiple reverse sequence repeats, identified previously with these primers, we used a
178 nested reverse primer designed for direct sequencing (Barnett et al., 2006). See Supplementary
179 Information for PCR and sequencing conditions. Consensus sequence for each individual was

obtained through alignment of the forward and reverse sequences in *GENEIOUS* (Kearse et al., 2012) to yield a minimum of 2x coverage for each base.

Estimation of change in nuclear diversity

To detect changes in nuclear diversity between the modern and historic 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-Weinburg 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* et al., 2008). DnaSp v.5 (Librado & Rozas, 2009) was used to calculate mtDNA haplotype diversity (H) and nucleotide diversity (π), as well as Tajima's D to test for deviations from neutral evolution for both the modern and historic populations.

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 historic nucleic diversity we used a bootstrapping procedure. When analysing the more rapidly mutating nuclear microsatellite data, we progressively restricted; *i*) the spatial extent of the historic samples, to match with more certainty the extent of the modern samples; *ii*) the time period over which the historic samples were collected, to restrict the possible influence of genetic drift with time

204 within the sample set. Thus, we divided our historic data into three spatial zones representing; I) the
205 samples within the modern sampling area; II) the samples likely to be within male dispersing
206 distance of the modern sampling area, taken as 200km; III) all remaining samples across the region
207 (Table 1). We also divided the historic data into two time periods, 1874-1895 (A) and 1929-1935 (B)
208 (Table 1). The results from the historic samples sets were compared against our modern dataset
209 using a bootstrapping procedure implemented in *POPTOOLS* (Hood, 2011). We created 100
210 populations of equal size to the historic data being used. Furthermore, to account for an apparent
211 lack of historic sampling from within the Okavango Delta bootstrap sampling was repeated both with
212 and without modern Okavango Delta samples. In a species such as lions, where female siblings tend
213 to remain in the same pride or form a neighbouring pride and male siblings commonly forge a
214 coalition, the likelihood of collecting data from close relatives was high. To test for the effects of
215 close relatives, we followed the recommendations of Rodríguez-Ramilo & Wang (2012) and
216 calculated all possible full-sibling and parent-offspring clusters in the programme *COLONY* (Wang &
217 Scribner, 2014). We then randomly selected just one individual from each close-relative cluster,
218 before re-rerunning the bootstrap procedure on the reduced data set.

219

220 *Mitochondrial 'ghost' alleles*

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

227

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 (s.d. ± 3.5) complete genotypes per locus (Data available on Figshare, DOI 10.6084/m9.figshare.3514469). 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 to 322bp, across a 337bp region (GenBank Accession no. KX661326 - KX661331).

In every bootstrap combination of our microsatellite data, regardless of how many samples were excluded, the historic 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 historic 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 historic 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 historic data than in the modern data when

controlling for sample size (Fig. 2). Such 'ghost alleles' (Bouzart et al., 1998; Groombridge et al., 2000) were identified in 14 out of the 16 microsatellite markers, only absent from Fca126 and Fca391. Even when reducing the historic 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.

Removing samples collected between 1929-1935 made no difference to heterozygosity across the data (see Supplementary materials), however, it did result in a reduction in the allelic richness from 7.5 to 6.29, the latter being similar to the present day values. When we reduced the data to include only samples collected between 1929-1935, the allelic richness (5.88) closely matches that found within the modern samples.

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 3) indicates six haplotypes present within the historic dataset (H = 0.6993, π = 0.00065), but three of these appear to be missing from the extant lions (H = 0.3257, π = 0.0007). Tajima's D for both the historic (D = -1.09629; $p < 0.1$) and modern (D = -0.53568, $p < 0.1$) 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 historic 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.

276

277 **Discussion**

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

282 Our analysis demonstrates that over the past century the lion population of the Kavango-Zambezi
283 region has lost genetic diversity. Contemporary observed heterozygosity has been reduced by 12%
284 to 17% compared to historic populations. Despite having a number of missing alleles across the
285 samples, genetic diversity was still historically higher than in the contemporary lion population. The
286 decline in heterozygosity is not as dramatic as that seen in some highly threatened or bottlenecked
287 species, for example, 57% in the Mauritius kestrel (*Falco punctatus*) (Groombridge et al., 2000) or
288 43% in sea otters (*Enhydra lutris*) (Larson et al., 2002), it nevertheless represents a worrying
289 reduction in diversity considering this population is one of only six lion strongholds remaining in
290 Africa.

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

299 Similar to other species, the global decline in lion numbers has largely been driven by human-wildlife
300 conflict and habitat loss (Keyghobadi et al., 2005; Bauer et al., 2015b). Given the rapid expansion of
301 human activities in the region in the 20th century, the downward trend in genetic diversity we
302 observed is perhaps unsurprising and seemingly confirms the pessimistic observations made in the
303 late 19th century. For example, one account from Frederick Courtney Selous records, "During the
304 twenty years since my first arrival in 1871, I … had seen game of all kinds gradually decrease and
305 dwindle in numbers to such an extent that I thought that nowhere south of the Great Lakes could
306 there be a corner of Africa left where the wild animals had not been very much thinned out" (Selous,
307 1908). Interestingly, allelic richness did not differ between the intermediate temporal (1929-1935)
308 and contemporary population samples, suggesting that allelic richness was lost prior to the
309 intermediate sampling period. A temporal decline in genetic diversity of the historic samples was
310 not detected through measures of heterozygosity, likely due to changes in allelic richness being
311 detectable before population declines impact upon heterozygosity (Athrey et al., 2011). The rapid
312 decline observed in allelic richness does coincide with the arrival of the first western settlers in 1890
313 and the subsequent rise of the colonial presence in the region after the end of the Matabele Wars in
314 1897 (Parsons, 1993). Furthermore, modern firearms became more prevalent following European
315 settlement and predators were often persecuted as vermin (Woodroffe, 2000), which likely
316 contributed to the earlier decline of lions in the study region. Whilst the timing of genetic decline
317 and colonial settlement is compelling enough to suggest causation, the evidence is not conclusive.
318 The epizootic of the rinderpest virus also struck during the late 1890's resulting in the death of vast
319 populations of buffalo, giraffe and wildebeest, as well as domestic livestock (Van den Bossche et al.
320 2010). Such an epidemic is very likely to have also had a considerable impact on the predators of the
321 region.

322 Given the level of habitat loss and fragmentation observed across sub-Saharan Africa (Keyghobadi et
323 al., 2005; Bauer et al., 2015b), the increased threat of epizootics facilitated by human movements
324 (Butler et al., 2004), as well as the impacts of a changing climate (Thomas et al. 2004), it is

325 imperative that efforts are made to conserve genetic diversity. Without such genetic diversity, a
326 species resilience and ability to adapt to future stochastic events becomes greatly compromised
327 (Whitham et al. 2008). This study provides quantitative data on temporal genetic monitoring that is
328 urgently needed to optimize conservation and management efforts. Since KAZA is considered one of
329 the more stable lion populations in Africa, the work presented here should provide motivation for
330 increased conservation action to safeguard against further loss of genetic diversity of lions and other
331 species across the region (Krofel et al., 2015). In particular greater connectivity between lion
332 population in protected areas across the region and thus the mixing of genetic material should be
333 supported (Cushman et al., 2015).

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477 **Data Accessibility Statement**

478 Microsatellite data is available at Figshare, DOI 10.6084/m9.figshare.3514469

479 Mitochondrial sequence data has been submitted to the GenBank database under accession no.

480 KX661326 - KX661331.

481

Table 1 Museum samples from the Natural History Museum of London grouped according to three spatial zones. Sample Number refers to position on figure 1. Spatial zones represent; I) samples within the modern sampling area; II) the samples likely to be within maximum male dispersing distance of the modern sampling area, taken as 200km; III) all remaining samples across the region. Time periods represent samples collected between; A) 1874 to 1895; B) 1930-1935. Unclear dates use accession number as date reference. Longitude and latitude are estimated based on location data available for each specimen.

	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[a]	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[a]	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 Francestown	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	Mashona land - North Zimbabwe approx. 200miles West of Harare	27.97	-18.42
	16	19.7.15.14	1883	A	Mashona land - approx 200miles 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[a]	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

[a] Accession number used a second time for two different samples.

Table 2 Genetic diversity for the Kavango-Zambzi African lion population within each spatial scale for 16 microsatellite loci. 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; s.d. = standard deviation of bootstrap replications.

	Sample set	*N*	H_E	s.d.	H_O	s.d.	F_{IS}	*A*	s.d.
Zone I-III	Historic	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	Historic	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	Historic	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)

Table 3 Mitochondrial DNA control region haplotypes from historical specimens and the extant lion population of the KAZA region. "-" and "N/A" represent a deletion or missing sequence data, respectively, at the specified nucleotide position.

Sample size		**Haplotype**	**Variable nucleotide position**[a]				
Modern	Historic		221	343	367	368	378
31	5	*i*	-	-	*T*	*A*	-
	9	*ii*	**T**	-	T	A	-
	1	*iii*	N/A	-	T	A	**C**
	1	*iv*	N/A	**C**	T	A	-
3	1	*v*	-	-	**C**	A	-
4	1	*vi*	-	**T**	T	**G**	-

[a] 1 corresponds to position 16,176 in the complete *Pathera leo leo* mtDNA sequence (Ma & Wang, 2014).

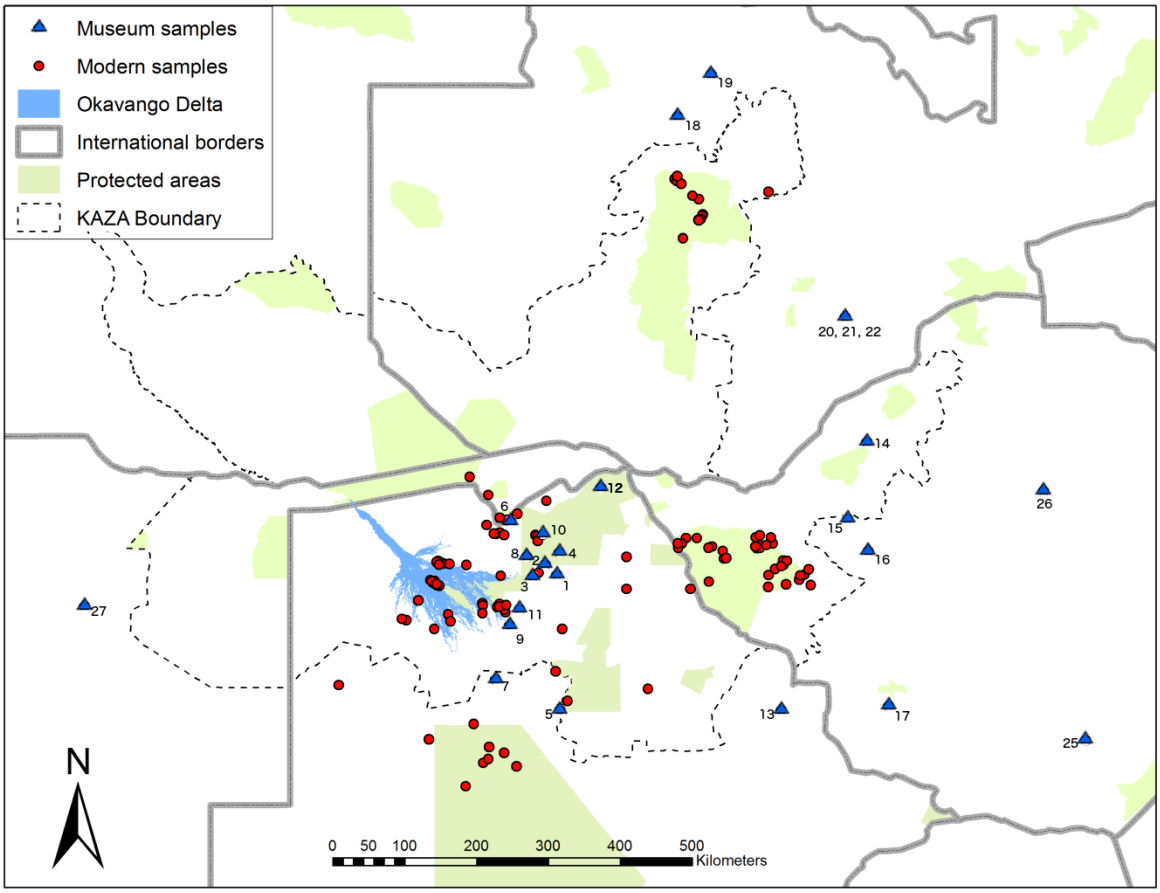
503 **Figure 1** Map of Kavango-Zambezi region showing sampling distribution of modern lion samples (red circles) and museum

504 samples (blue triangles and numbered)

505 **Figure 2** Mean number of private alleles per locus as a function of standardised sample size for historic and modern

506 microsatellite data.

507



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509 **Figure 1**

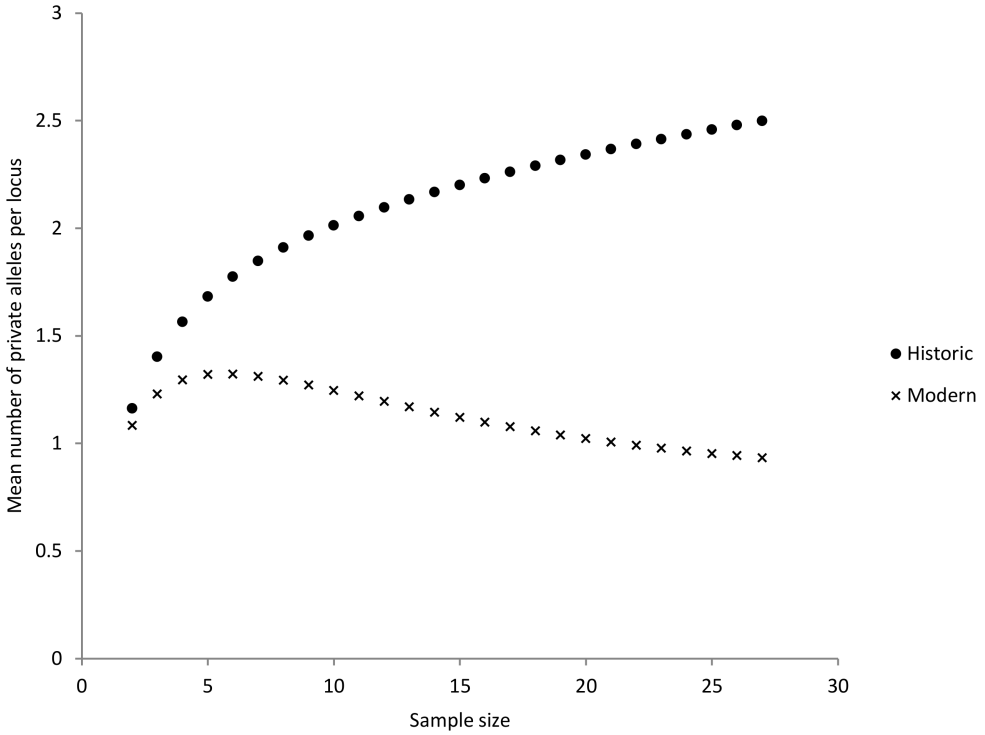

Figure 2