Genetics and Genomics: Powerful Tools for Wildlife Conservation

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Biopsy Dart ©Zambia Lion Project

Introduction

The African lion (*Panthera leo*) is one of the most iconic species in the world, representing an entire continent as a member of Africa's "Big 5". The true conservation status of the African lion is in question due to a lack of knowledge regarding genetic diversity and conflicting estimates of population size. For example, in Zambia, although the lion has a large distribution spanning over 167,000 km^2 of habitat in managed areas, there are limited estimates of both population size and genetic sub-structure. This lack of reliable information compromises conservation decisions, some of which, such as the banning of trophy hunting, could have a profound impact on both the long-term security of the species as well as Zambia's economy.

Levels of genetic diversity are directly proportional to a species' ability to adapt, survive and thrive. Therefore, loss of genetic diversity is detrimental to overall population health and long-term survival because it decreases its potential to adjust to an ever changing environment. Mitochondrial DNA (mtDNA) has a relatively fast mutation rate resulting in significant variation in mtDNA sequences allowing us to investigate gene flow and distribution. For this study, we calculated the extent of genetic diversity in Zambian lion populations through the analysis of mitochondrial DNA (mtDNA) of 165 lions found in five main areas in Zambia (Figure 1).

Methods

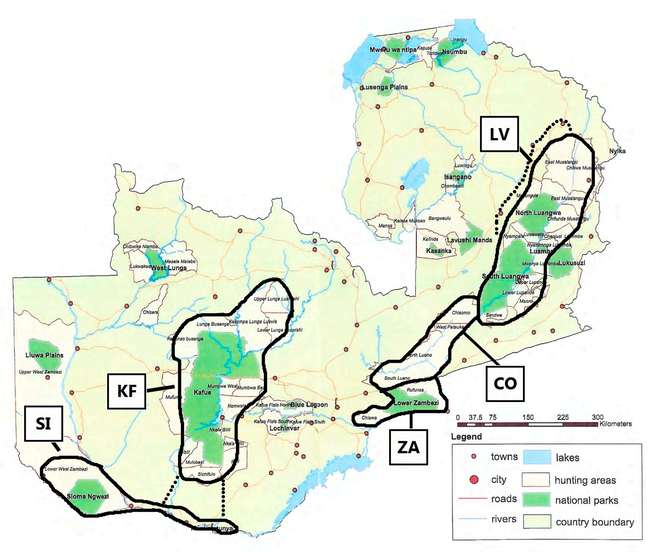

Figure 1: ***Map of five main areas of Zambia*** sampled: LV (Luangwa Valley); CO (Corridor); ZA (Lower Zambezi); KF (Kafue); and SI (Sioma Ngwezi).

African lion DNA samples were provided in the form of hair, skin, bone and/or tissue through the collections of Dr. Paula White and the Zambia Lion Project. The 12S and 16S genes were analyzed from sequences successfully amplified from 165 lions found in five main areas in Zambia (Figure 1). To allow for a direct comparison with previously published data, we used the same maternal sequence (mtDNA) assessed by Antunes et al (2008), whose analysis did not include this region of Africa.

DNA isolation, PCR and DNA sequencing and analysis were completed using standard laboratory techniques in the DNA Technologies Laboratory at Texas A&M University in College Station, TX. Genetic diversity calculations were implemented using Arlequin v3.5. Phylogenetic analysis was performed using Bayesian inference methods with MrBayes v3.2.2 (Figure 2) and a median-joining network was produced (Figure 3).

Results

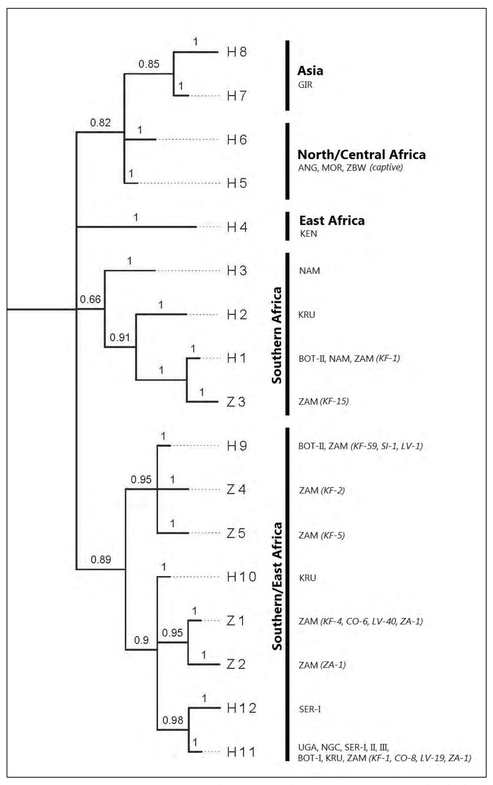

Figure 2: ***Bayesian analysis*** with posterior probability values on the nodes. Location abbreviations: ANG (Angola); BOT-I, (Southern Botswana and Kalahari, South Africa); BOT-II (Northern Botswana); GIR (Gir Forest, India); KEN (Kenya); KRU (Kruger National Park, South Africa); MOR (Morocco); NAM (Namibia); NGC (Ngorongoro Crater, Tanzania); SER (Serengeti National Park, Tanzania); UGA, (Uganda); ZBW (Zimbabwe); ZAM (Zambia; *location - # of samples with haplotype*)

Genetic diversity throughout the population is high at 0.7319 +/- 0.0174. AMOVA analysis resulted in a high FST of 0.44 for regional sub-populations. Eight haplotypes were found; three haplotypes previously described by Antunes et al (2008) and five previously unseen haplotypes (Table 1). H1 and H9 were previously found in northern Botswana and Namibia while H11 was found throughout eastern Africa spanning from Uganda across the Serengeti to the Ngorongoro Crater in Tanzania and southern Botswana. Of the five new haplotypes, three were considered rare with frequencies below 5% (Table 1).

Bayesian analysis indicates four clusters which can be grouped regionally – Asia/Central/Northern Africa, East Africa, Southern Africa and Southern/East Africa (Figure 2). The Southern/East Africa group consists of two clusters, Southern and Southern/East. Posterior probability values suggest good support at >60% for all nodes.

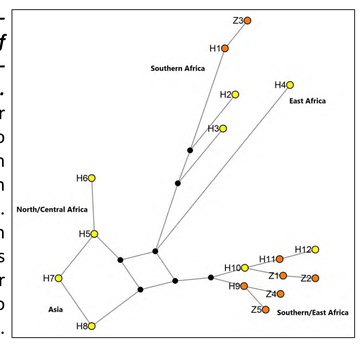

Figure 3: ***Median-joining network of African lion 12S-16S haplotypes.*** Haplotypes cluster according to region which coincides with Bayesian tree. Zambian haplotypes (orange) occur within the two southern clusters.

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation	p-value
Among Populations	4	20.009	0.19788 Va	44.18	0.00000
Within Populations	160	49.010	0.25006 Vb	55.82	0.00000
Total	164	60.018	0.44794		
Fixation Index	***FST:***	0.44175			

Table 3: ***AMOVA results with FST***. Lions were divided into regional sub-populations for intra-population calculations of the coefficient of differentiation (FST) and hierarchical analyses of molecular variance (AMOVA).

Haplotype	Frequency	s.d.
H1	0.006061	0.006061
H9	0.369697	0.037694
H11	0.175758	0.029721
Z1	0.309091	0.036085
Z2	0.006061	0.006061
Z3	0.090909	0.022448
Z4	0.012121	0.008545
Z5	0.030303	0.013386

Table 1: ***Haplotype Frequencies*** for 12S-16S haplotypes found in Zambian lion populations.

Gene Diversity:	0.7319 +/- 0.0174
Nucleotide Sites:	1882
Polymorphic Sites:	16
Transitions:	13
Transversions:	1
Indels:	2
Nucleotide Composition	
C:	22.11%
T:	22.67%
A:	36.64%
G:	18.58%

Table 2: ***Molecular Diversity Indices and Nucleotide Composition*** as calculated using Arlequin calculated as a single population.

Conclusions

While genetic diversity is high, gene flow within the population appears to be low. The Bayesian analysis suggests the Zambian population may act as a bridge connecting the lions in southern Africa to eastern Africa. The haplotypes present in the Zambian population link the Southern Africa lineage described by Antunes et al (2008) with the Southern/East Africa lineage. This grouping agrees with studies done on HVR1[2] and Cytochrome b[3] mtDNA sequences as well. AMOVA analysis, however, suggests there is little to no gene flow between the populations within Zambia. The western population, Kafue and Sioma Ngwezi, is isolated from the eastern population, Luangwa Valley connected by the Corridor to the Lower Zambezi, with an expanse of cities and roads separating them.

The determination of regional sub-populations could be the first step to the creation of conservation programs and proper legislation to focus on saving specific, at risk populations. With translocation becoming a well-practiced technique to prevent inbreeding within populations closed to dispersal or immigration[4], it must be determined whether there needs to be a focus on maintaining genetic diversity throughout the entire population or if there needs to be a more narrowed focus to prevent the loss of genetic diversity between populations.

Future Research

Further research including the addition of microsatellite analysis is needed to better quantify the level of overall genetic diversity within the population. These results are the first of a larger study which will also include the analysis of nuclear markers (nDNA; 15 microsatellites) and encompass the entire range of the African lion over time.

References

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Acknowledgements

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