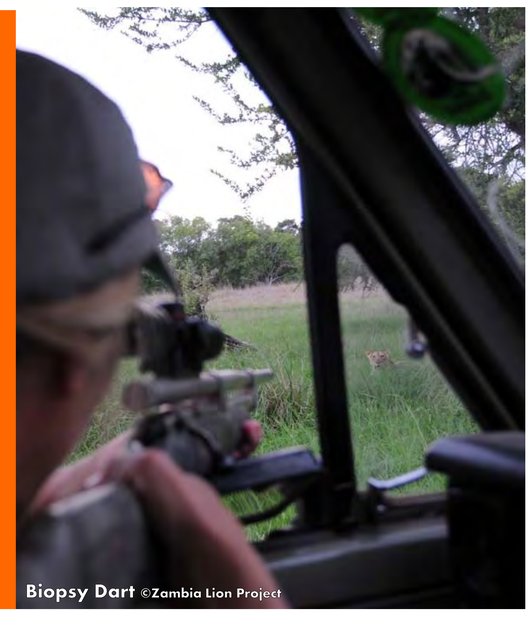
Diversity and Distribution of the Lion Across Zambia

Caitlin J Curry[1], Paula A White[2], James N Derr[1]

[1] Interdisciplinary Program of Genetics, Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA
[2] Center for Tropical Research, Institute of the Environment and Sustainability, University of California, Los Angeles, Los Angeles, CA, USA


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 mtDNA of 165 lions found in five main areas in Zambia (*Figure 1*).

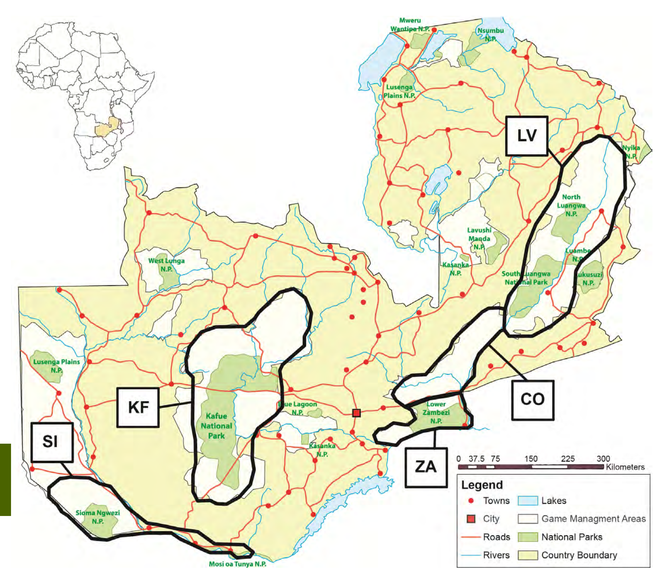


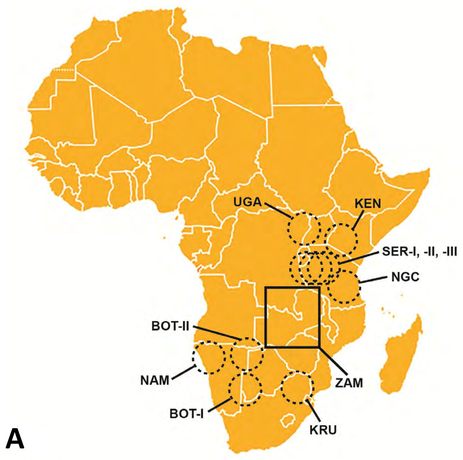
Figure 1: ***Map of five main areas of Zambia*** sampled: LV (Luangwa Valley); CO (Corridor); ZA (Lower Zambezi); KF (Kafue); and SI (Sioma Ngwezi).

Methods

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.

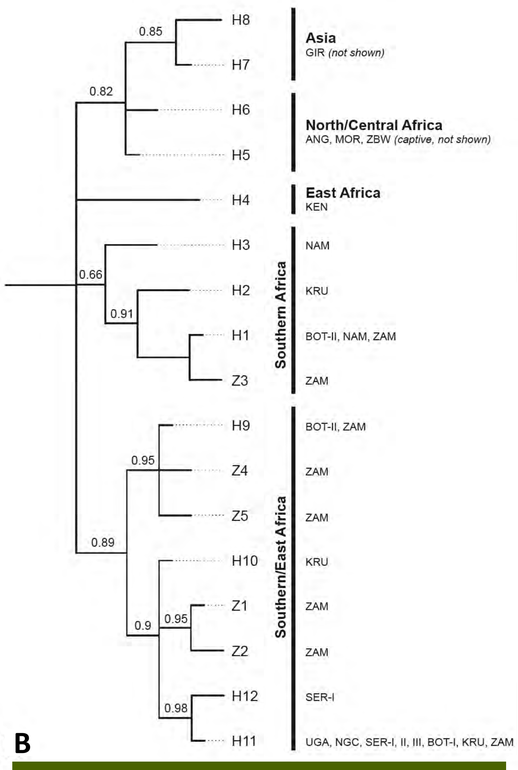
DNA isolation, PCR and DNA sequencing and analysis were completed using standard laboratory techniques in the DNA Technologies Laboratory at Texas A&M University in College Station, TX. Genetic diversity calculations were implemented using Arlequin v3.5. Phylogenetic analysis was performed using Bayesian inference methods with MrBayes v3.2.2 (*Figure 2*) and a median-joining network was produced using Network v4.6.1.3 (*Figure 3*).

Results



A

Figure 2: **(A)** ***Range-wide map of lions sampled.*** Zambia (ZAM) is denoted by a square. Circles indicate geographic locations for populations determined and established by Antunes et al (2008): UGA, (Uganda); KEN (Kenya), SER (Serengeti National Park, Tanzania); NGC (Ngorongoro Crater, Tanzania); KRU (Kruger National Park, South Africa); BOT-I, (Southern Botswana and Kalahari, South Africa); BOT-II (Northern Botswana); NAM (Namibia); (Gir Forest, India); ANG (Angola); ZBW (Zimbabwe); and MOR (Morocco).
(B) ***Bayesian analysis*** with posterior probability values on the nodes. Posterior probability values suggest good support with >60% for all nodes.



B

Gene Diversity:	0.7319 +/- 0.0174
Nucletide 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 1: ***Molecular Diversity Indices and Nucleotide Composition*** calculated as a single population.

- Genetic diversity throughout the population is high at 0.7319 +/- 0.0174
- AMOVA analysis resulted in a high F_{ST} of 0.47 for regional sub-populations.
- Eight haplotypes were found; three haplotypes previously described by Antunes et al (2008) and five previously unseen haplotypes (*Table 3*).
  - 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 3*).
- 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

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation	p-value
Among Populations	1	18.966	0.22785 Va	47.50	<0.001
Within Populations	163	41.052	0.25185 Vb	52.50	<0.001
Total	164	60.018	0.47971		
Fixation Index	*FST:*	0.47499			

Table 2: ***AMOVA results with FST***. Lions were divided into regional sub-populations, Eastern and Western, for intra-population calculations of the coefficient of differentiation (FST) and hierarchical analyses of molecular variance (AMOVA).

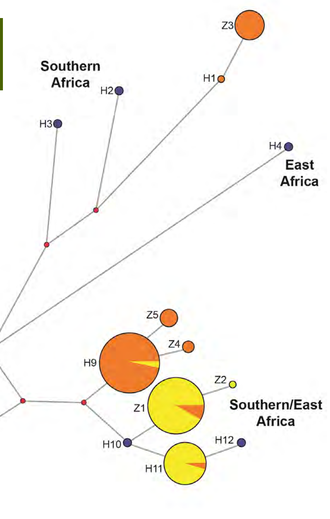


Figure 3: ***Median-Joining Network.***
Orange = Western Sub-population
Yellow = Eastern Sub-population
Red circles = Median Vectors
Blue circles = Not Found in Zambia
Circle sizes are proportional to haplotype frequency for haplotypes found in Zambia.

Haplotype	Eastern Region									Western Region						n	ƒ	s.d.
	LV			CO			ZA			KF			SI					
	♀	♂	?	♀	♂	?	♀	♂	?	♀	♂	?	♀	♂	?			
H1										0	1	0				1	0.0061	0.0061
H9	0	1	0							18	41	0	0	1	0	61	0.3697	0.0377
H11	6	13	0	3	5	0	1	0	0	0	1	0				29	0.1758	0.0297
Z1	7	32	1	0	6	0	1	0	0	0	4	0				51	0.3091	0.0361
Z2							0	1	0							1	0.0061	0.0061
Z3										5	10	0				15	0.0909	0.0224
Z4										0	2	0				2	0.0121	0.0085
Z5										4	1	0				5	0.0303	0.0134
n	13	46	1	3	11	0	2	1	0	27	60	0	0	1	0	165		
	60			14			3			87			1					
A	3			2			3			7			1			8		

Table 3: Number of males (♂), females (♀), and with unknown gender (?) for each haplotype is indicated for all areas sampled in Zambia along with the haplotype frequencies. n = sample size, for ♂, ♀ and ? for each area and by area, haplotype and total; A = number of haplotypes; ƒ = frequency; s.d. = standard deviation. For region abbreviations see Figure 1.

Conclusions

While genetic diversity is high, gene flow within the population is 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. AMOVA analysis, however, suggests there is little to no gene flow between the populations within Zambia (*Table 2*). 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, 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.

Continued Research

Further research including the addition of microsatellite analysis is being done to better quantify the level of overall genetic diversity within the population and calculate effective population size. The combination of mtDNA with nuclear markers will give a clearer picture to identify evolutionarily distinct populations and calculate effective population size. These results will be part of a larger, dissertation study which will encompass the entire range of the African lion over time.

Acknowledgements

Travel Awards Provided By:

References

Antunes, A., Troyer, J.L., Roelke, M.E., Pecon-Slattery, J., Packer, C., Winterbach, C., … and Johnson, W.E. 2008. The Evolutionary Dynamics of the Lion Panthera leo Revealed by Host and Viral Population Genetics. PLoS Genetics 4(11): e1000251. DOI:10.1371/journal.pgen.1000251