



Perspective

Genetic Evidence for Contrasting Wetland and Savannah Habitat Specializations in Different Populations of Lions (*Panthera leo*)

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Received October 25, 2015; First decision October 29, 2015; Accepted November 17, 2015.

Corresponding Editor: C. Scott Baker

Abstract

South-central Africa is characterized by an archipelago of wetlands, which has evolved in time and space since at least the Miocene, providing refugia for animal species during Pleistocene arid episodes. Their importance for biodiversity in the region is reflected in the evolution of a variety of specialist mammal and bird species, adapted to exploit these wetland habitats. Populations of lions (*Panthera leo*) across south-central and east Africa have contrasting signatures of mitochondrial DNA haplotypes and biparental nuclear DNA in wetland and savannah habitats, respectively, pointing to the evolution of distinct habitat preferences. This explains the absence of genetic admixture of populations from the Kalahari savannah of southwest Botswana and the Okavango wetland of northern Botswana, despite separation by only 500 km. We postulate that ancestral lions were wetland specialists and that the savannah lions evolved from populations that were isolated during arid Pleistocene episodes. Expansion of grasslands and the resultant increase in herbivore populations during mesic Pleistocene climatic episodes provided the stimulus for the rapid population expansion and diversification of the highly successful savannah lion specialists. Our model has important implications for lion conservation.

Subject areas: Population structure and phylogeography

Key words: geomorphic evolution, habitat specialization, lion phylogeography, mitochondrial DNA, Plio-Pleistocene climate change, wetland archipelago

Lions (*Panthera leo*) from the Okavango (BOT-II, northern Botswana) and Etosha (NAM, northwest Namibia) have mitochondrial DNA

(mtDNA) signatures (12S–16S sequences) characterized by a common H1 haplotype and subordinate H9 and H3 haplotypes, respectively.

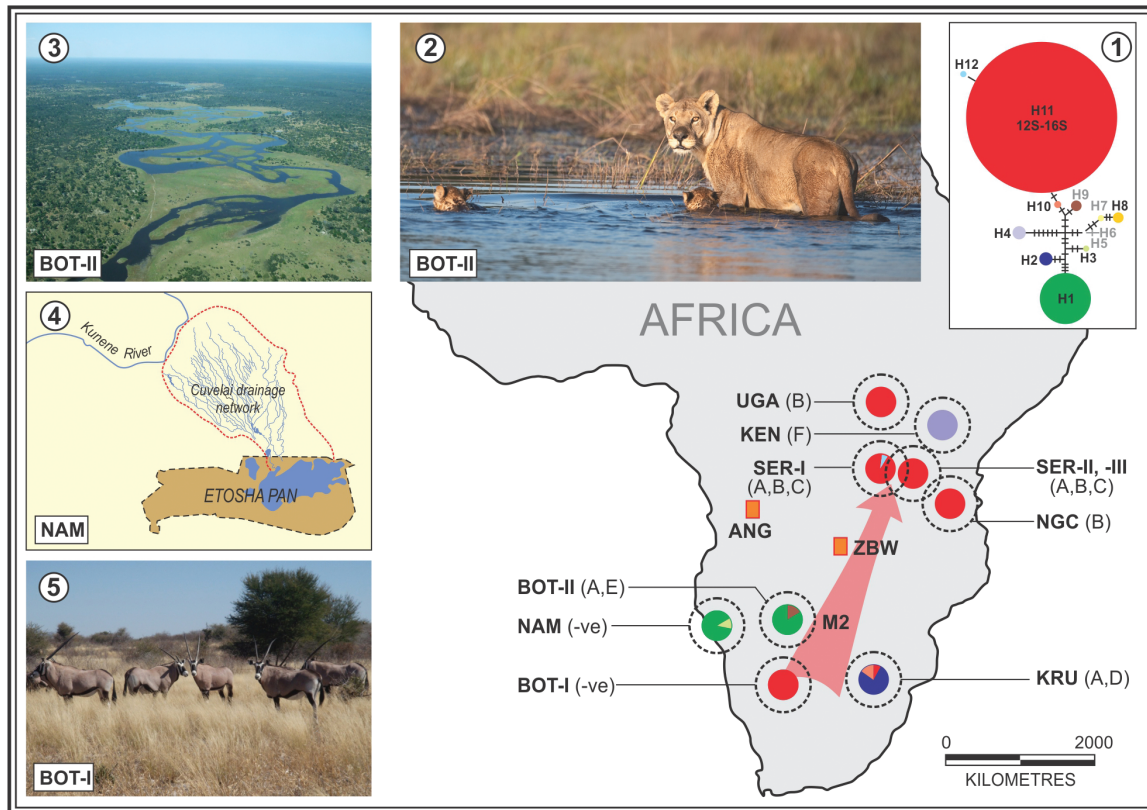


Figure 1. mtDNA haplotypes frequencies in African lion populations, synthesized from data presented in Antunes et al. (2008). Their phylogenetic network (inset 1) is provided primarily to identify the haplotypes documented by these authors. This should be reevaluated using more robust statistical tests, coupled with additional habitat-based genetic data, including populations from further wetland environments. Note that the isolated populations SER-II and SER-III, both characterized by a single mtDNA haplotype (H11), cannot be separated at the scale of the figure. Letters denote the FIV_{ple} subtypes associated with each population. The pink arrow labeled M2 denotes the inferred dispersal path from the southern Kalahari (BOT-I) via the Okavango (BOT-II) into East Africa in response to expanding savannah habitats following a Pleistocene arid glacial episode. Inset 2: Lioness with swimming cubs from the Okavango (BOT-II) seasonal wetland (image courtesy of Matthew Copham of Safari Footprints; Maun, Botswana). Inset 3: Annual flood in the Okavango seasonal wetland (image courtesy of Glynis Humphrey). Inset 4: The intricate network of channels, locally known as oshanas, forming the Cuvetlai seasonal wetland, which drains into Etosha Pan. Inset 5: Gemsbok (*Oryx*) adapted to survive in the arid savannah habitat of the southwest Kalahari (BOT-I), which lacks surface water during the winter months.

In contrast, populations from the south-western Kalahari, straddling Botswana-South Africa (BOT-I), to the south, and a majority from east Africa, to the northeast, are characterized by a single (H11) haplotype (Figure 1) (Antunes et al. 2008). The disparate mtDNA signatures of the Okavango and Etosha populations, relative to those from the southwest Kalahari and East Africa, are supported by an independent genetic study, based on the cytochrome b (*cytb*) mtDNA sequence (Barnett et al. 2014), and thus most unlikely to reflect a statistical sampling artifact. This, coupled with contrasting biparental nuclear DNA (nDNA) signatures of the southwest Kalahari (BOT-I) and Okavango (BOT-II) lions (Antunes et al. 2008, Figure 2c), leads to the remarkable conclusion that there has been a long-term lack of genetic admixture between these 2 populations, despite their separation by only some 500 km.

The wide geographic distribution of the H11 haplotype was ascribed to colonization of East Africa by lions dispersing from the southwest Kalahari (BOT-I) in response to ameliorating Pleistocene climatic changes, and the resultant expansion of grasslands and herbivore populations. This dispersion must have been via the Okavango (BOT-II), as many East African lions carry the FIV_{ple} A virus subtype derived from the Okavango (BOT-II) lion population (Figure 1) (Antunes et al. 2008). However, the absence of the H11 haplotype in the Okavango suggests that transmission of the HIV_{ple} virus was via

territorial conflict rather than sexual interactions. It is thus necessary to explain not only the lack of genetic admixture of these 2 populations but also why the Okavango (BOT-II) lions apparently failed to exploit the favorable habitat provided by expanding grasslands and also colonize East Africa (reflected by the absence of the H1 haplotype in the latter area).

Lions characterized by the H11 haplotype all occupy savannah habitats. In contrast, the Okavango (BOT-II) lions occupy a seasonal wetland habitat (Figure 1, insets 2 and 3), subject to flooding during winter months, controlled by rainfall in the Angola highlands. The population from arid northwest Namibia (NAM) is centered on the Etosha Pan, which is a relict of a long-lived seasonal wetland, which still floods periodically, fed by late summer inflow from the Cuvetlai drainage network to the north (Miller et al. 2010) (Figure 1, inset 4). The Okavango and Etosha populations, both dominated by the H1 haplotype (absent in the savannah lions), thus occupy seasonal wetland habitats. Consequently, there is a 1:1 association between population haplotype signatures and habitat.

South-central Africa is characterized by an archipelago or mosaic of wetlands, which has evolved in time and space since at least the late Miocene (Cotterill 2005, 2006; Moore et al. 2012). Such wetlands would have provided important refugia for plant and animal species, particularly during Pleistocene arid climatic cycles. The distributions

of a number of antelope species (e.g., Sitatunga [*Tragelaphus speki*] and the Lechwe complex [*Kobus leche*]) are tightly congruent with the wetlands, reflecting the evolution of specializations to exploit these long-lived habitats. In contrast, species such as Oryx (*Oryx gazella*) (Figure 1, inset 5), adapted to survive without surface water during dry winter months, exploit surrounding more arid savannah environments. Similarly, bird species typical of arid savannah environments, such as the Tropical Boubou (*Laniarius aethiopicus*) and Pied Babbler (*Turdoides bicolor*), have cogenetic wetland specialists, respectively, the Swamp Boubou (*Laniarius bicolor*) and White-rumped or Hartlaub's Babbler (*Turdoides hartlaubii*) (Leisler and Winkler 2001). These observations highlight the importance of the long-lived wetland archipelago for faunal evolution in south-central Africa.

The disparate mtDNA and nDNA signatures of lions occupying seasonal wetland environments, compared with those from savannah environments, point to the evolution of respective long-term habitat preferences. The single or majority H11 mtDNA haplotype present in many savannah populations has been interpreted to reflect a recent genetic bottleneck, linked to Pleistocene arid climate cycles (Antunes et al. 2008). In contrast, populations occupying wetland habitats would be less susceptible to major climatic deteriorations. We therefore hypothesize that ancestral lions were wetland specialists, but that populations that became isolated during arid Pleistocene cycles experienced severe survival pressure, and adaptation to a savannah habitat, characterized by scarce winter water resources. As a result, only these latter lions exploited the expanding grasslands and associated food resources during more equitable Pleistocene climatic conditions. Pleistocene expansion of open grassland habitats was likely accompanied by diversification and demographic expansions of these highly successful savannah specialists.

Our observations have important implications for conservation and also highlight a number of issues relevant to ongoing research into the natural history of lions:

1. The disparate mtDNA and nDNA signatures of lions from the southwest Kalahari (BOT-I) and those only 500 km to the northeast in the Okavango (BOT-II) have close analogies with genetic differences between nearly contiguous giraffe (*Giraffa camelopardalis*) populations in East Africa (Brown et al. 2007). These authors suggested that this cryptic biodiversity was initiated by contraction and isolation of giraffe populations during Pleistocene arid episodes, highlighting the importance of palaeo-climates in addition to landform evolution for the interpretation of phylogenetic data. Brown et al. (2007) noted that the barriers to genetic interchange between the different giraffe populations are poorly understood but tentatively suggested that pelage imprinting, coupled with systematic differences in reproductive cycles, may play a role in maintaining the integrity of the divergent genetic lineages. Factors responsible for maintaining the disparate genetic signatures of the southwest Kalahari (BOT-I) and Okavango (BOT-II) lions are similarly not understood and represent an important focus for ongoing research.
2. Habitat preference in lion populations will contribute to the poor correlation between their genetic distances and geographic isolation noted by Antunes et al. (2008). Habitat should thus be included as an important variable in planning further phylogenetic studies, with particular attention focused on characterizing the genetic signatures of lions in other major wetlands in south-central Africa.
3. The timing of the divergence of the wetland and savannah lion lineages is not well estimated at present. While beyond the scope of our commentary, this is flagged as an important question for ongoing phylogenetic studies of *P. leo*.

Funding

A.A. was partially supported by the Strategic Funding UID/Multi/04423/2013 through national funds provided by the Portuguese Foundation for Science and Technology (FCT) and European Regional Development Fund (ERDF) in the framework of the programme PT2020 and the FCT project PTDC/AAG-GLO/6887/2014.

Acknowledgments

Roy Siegfried, Peter Apps, Lars Werdelin, and 3 anonymous reviewers are thanked for their constructive comments to earlier versions of this study, Matthew Copham of Safari Footprints (Maun, Botswana) for permission to use his photograph of the swimming lion cubs, Glynis Humphrey for the aerial view of the Okavango flood, and Susan Abraham for her considerable input in drafting and improving the diagram.

References

- Antunes A, Troyer JL, Roelke ME, Pecon-Slattery J, Packer C, Winterbach C, Winterbach H, Hemson G, Frank L, Stander P, et al. 2008. The evolutionary dynamics of the lion *Panthera leo* revealed by host and viral population genomics. *PLoS Genet.* 4:e1000251.
- Barnett R, Yamaguchi N, Shapiro B, Ho SY, Barnes I, Sabin R, Werdelin L, Cuisin J, Larson G. 2014. Revealing the maternal demographic history of *Panthera leo* using ancient DNA and a spatially explicit genealogical analysis. *BMC Evol Biol.* 14:70.
- Brown DM, Brennehan RA, Koepfli KP, Pollinger JP, Milá B, Georgiadis NJ, Louis EE Jr, Grether GF, Jacobs DK, Wayne RK. 2007. Extensive population genetic structure in the giraffe. *BMC Biol.* 5:57.
- Cotterill FPD. 2005. The Upemba lechwe *Kobus anselli*: an antelope new to science emphasizes the conservation importance of Katanga, Democratic Republic of Congo. *J Zool (Lond).* 265:113–132.
- Cotterill FPD. 2006. The evolutionary history and taxonomy of the *Kobus leche* species complex of south-central Africa in the context of Palaeo-drainage dynamics [PhD thesis]. [Stellenbosch (South Africa)]: University of Stellenbosch.
- Leisler B, Winkler H. 2001. Morphological convergence in papyrus dwelling passerines. *Ostrich Suppl.* 15:24–29.
- Miller RMCG, Pickford M, Senut B. 2010. The geology, palaeontology and evolution of the Etosha Pan: implications for terminal Kalahari deposition. *S Afr J Geol.* 113:307–334.
- Moore AE, Cotterill FPD, Eckardt F. 2012. The evolution and ages of palaeo-lakes in the Makgadikgadi Basin: consistent evidence from Kalahari drainage evolution. *S Afr J Geol.* 115:385–413.