Genetic diversity in the lion (panthera leo (Linnaeus 1758)) : unravelling the past and prospects for the future

Bertola, L.D.

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Author: Bertola, Laura Diana
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Summary

Insights into the spatial distribution of genetic diversity contribute to the understanding of evolutionary histories which have shaped these patterns. But they also provide us with guidelines on how to effectively conserve this diversity. This may serve to rationalize prioritization of populations for conservation, aiming to minimize the loss of genetic diversity and to preserve genetically distinct lineages. In this thesis, the intraspecific genetic diversity of the lion (*Panthera leo*) is assessed. Top predators are known to fulfill a crucial role in the ecosystem, contributing to both species richness and resilience. However, many large carnivores, including the lion, have shown recent declines in population numbers, as a result of anthropogenic pressures. The importance as a keystone species and the vulnerability of large carnivores are arguments to target them as a model to develop conservation plans.

Currently, only two subspecies of lions are officially recognized by the IUCN: the African lion (*Panthera leo leo*), distributed throughout sub-Sahara Africa with the exception of dense rain forest, and the Asiatic lion (*Panthera leo persica*), confined to a single population in India. However, other species with a similar distribution throughout the African continent, show a basal dichotomy, distinguishing populations from West/Central African and populations from East/Southern Africa. This dichotomy is often reflected in their taxonomy. Morphological data of lions, originally leading to the distinction of up to eight “subspecies”, seem to confirm the existence of different African lineages, although results of these studies should be interpreted with caution since they have not always adequately treated age- and sex-related variation. Later studies, including genetic data, have also confirmed that the genetic variation in the lion is greater than the taxonomy implies, and that several genetic lineages can be recognized within the African subspecies. Since taxonomy is often reflected in the conservation strategy, it is important to ensure that the existing genetic variation is thoroughly documented.

Previous studies on lion phylogeny have mainly included lions from East and Southern Africa, and focused on the position of the Asiatic subspecies in relation to these African populations. At the same time, populations in West and (parts of) Central Africa have shown exceptionally strong declines in lion numbers and other wildlife species. This has resulted in the West African lion to be classified as ‘Regionally Endangered’ on the IUCN Red List, with the recent suggestion to uplist it to ‘Critically Endangered’. The urgent conservation need in this region, together with the notion that West and Central Africa may harbour unique genetic lineages, are the main reasons why there is a focus on the West and Central African lion in this thesis. Samples were included from every West and Central African LCU with a recently confirmed resident lion population.

In Chapter 2 the knowledge gap for the West and Central African lion is filled by analysing populations which had not been previously included in phylogeographic studies. By selecting cytochrome *b* and part of the control region to assess the phylogeography of the lion, it was possible to include data from two previous studies. Resulting phylogenetic trees show differentiated lineages in East/

Southern Africa and a strong genetic relationship between the West/Central African lion and the Asiatic subspecies. This may be the result of severe droughts in the western part of the lion range, leading to a strong bottleneck or local extinction of these populations. Subsequent recolonization, possibly from North Africa or the Middle East would explain the strong genetic relationship with the Asiatic subspecies. Main conclusion is that the current taxonomy does not adequately reflect the genetic diversity of the African lion and that options for a taxonomic revision, notably with regard to the position of the West/Central African lion, should be explored.

Phylogenies based on mitochondrial markers may not reflect the complete genomic complexity due to differences in the mode of inheritance and coalescence time compared to autosomal markers. Therefore, a revision of the taxonomy, with potentially far-reaching ramifications for management, should be based on a combination of unlinked genetic markers. In Chapter 3 the congruence between phylogenetic patterns based on mitochondrial and autosomal markers is assessed by including data of 20 microsatellite loci and mtDNA for 15 lion populations. Results show that four genetic clusters can be distinguished: 1) West/Central Africa, 2) East Africa, 3) Southern Africa, and 4) the Asiatic subspecies. Although microsatellites are a suitable marker to infer population structure, they do not give much insight into phylogenetic relationships. The reduced genetic diversity of the Asiatic population, as a result of severe bottleneks, led to a clear distinction of the Asiatic subspecies which does not necessarily reflect a long evolutionary distance. Therefore, it is not possible to use microsatellite data to confirm the close evolutionary relationship between populations in West/Central Africa and the Asiatic subspecies, as was observed in mtDNA data. There is no indication of reduced genetic diversity in West/Central African populations, as was hypothesized based on recent population histories in this region. Possibly the reduction in lion numbers is too recent to be visible as a genetic signature. However, examples of intensively managed lion populations show how fast inbreeding may arise in small and isolated populations. Management interventions may therefore be needed in the future, to safeguard these populations against further declines of genetic diversity and subsequent fitness effects.

Since autosomal data do not contradict phylogenetic patterns based on mtDNA, this dataset was expanded with data from more sampling localities in Chapter 4. Specimens from natural history collections were included for areas where lions are extinct (i.e. North Africa and Middle East) and for areas from which it was not possible to include modern samples of wild lions. An ancient DNA approach was used for genetic analyses. A total of 194 samples from 22 countries were included and complete mitogenomes were analysed for 14 individuals, covering the main phylogeographic groups. Resulting phylogenetic trees reveal a strongly supported basal dichotomy, distinguishing lions from the northern part of the range, including the Asiatic subspecies (North group), and populations from the southern part of the range (South group). Six main haplogroups are identified: three in the North Group (1) West Africa, 2) Central Africa, 3) North Africa/Asia) and three in the South Group (4) North East Africa, 5) East/Southern Africa, and 6) South West Africa). The basal split into two main phylogenetic groups and the distinction of the other haplogroups are reiterated in several other savannah mammals. This points towards environmentally driven evolution in which similar forces have shaped the phylogeographic patterns of co-occurring species. For the lion, the most recent common ancestor of these major lineages is estimated at ~300 thousand years ago, and radiation of the haplogroups probably occurred during the last ~100 thousand years. It is hypothesized that this is the result of cyclical expansion of the rain forest and desert, which may have hampered gene flow between populations. The temporal contraction to local refugia may have led to clearly distinguishable clades due to fast coalescence of mtDNA markers. The proposed scenario is further confirmed by results of bioclimatic envelop models published in a previous study, which predicts refugia in line with the haplogroups we distinguish in the lion. The degree of divergence between the North and the South group, and especially the nested position of the Asiatic subspecies within the West and Central African lion, supports the notion that the current taxonomy is not in line with the evolutionary history of the lion, and that therefore a taxonomic revision is warranted.

In Chapter 5 we develop a new lion-specific genetic marker by sequencing the complete genome of 10 lions, covering the main phylogenetic groups. The genome data were mined for variable positions and a total of ~18,000 lion-specific SNPs was identified. Phylogenetic analyses based on these SNPs result in a tree with a hierarchical structure in which no reciprocally monophyletic clades can be identified. However, the Asiatic subspecies again shows a nested position with the populations in West and Central Africa, which confirms earlier findings based on mtDNA. The SNPs identified in this chapter provide a source for the generation of a SNP panel which can be used for cost-effectively genotyping a larger number of lions. This may serve high-throughput genetic analysis of free-ranging lions as well as the assessment of genetic lineages present in captive stocks, for the design of breeding programmes.

The datasets presented in this thesis consistently illustrate that the Asiatic subspecies has a nested position within the African lion. Both mtDNA and microsatellite datasets show that the West and Central African lion can be distinguished as a separate clade. In East and Southern Africa there is a geographical discrepancy between the clades identified by mtDNA and microsatellites. This may be the result of male-mediated gene flow, as lions are known to exhibit sex-biased dispersal, with males showing stronger dispersal than females. The fast coalescence time of mtDNA further contributes to clearly recognizable monophyletic clades based on these data. The topology of the tree based on the SNPs indicates a continent-wide pattern of gene flow. Differences in mutation rates between bi-allelic SNP markers and multi-allelic microsatellite markers suggest that the SNP data reflect a more ancient pattern of gene flow, before populations were isolated in local refugia.

To translate these patterns into conservation recommendations, it is advisable to recognize units below the subspecies level, such as ESUs or MUs. Requiring reciprocally monophyletic groups for both mtDNA and autosomal markers may be overly restrictive. However, a degree of nuclear allele divergence should be present to avoid misclassification of units which are linked by nuclear gene flow only, and not by organellar gene flow. In addition, divergence based on mtDNA data may represent a relic of historic isolation, only sustained by strong female philopatry. Therefore, it is suggested to treat the monophyletic mtDNA clades as ESUs and not elevate them to a subspecies status. To ensure that the taxonomy reflects the evolutionary history of the lion, it is suggested to revise the current taxonomy and recognise the following units:

Panthera leo leo (Linnaeus, 1758)
Unit 1: Asia (+ North Africa & Middle East, extinct)
Unit 2: Central Africa
Unit 3: West Africa

Panthera leo melanochaita (Hamilton Smith, 1848)
Unit 4: North East Africa
Unit 5: East/Southern Africa
Unit 6: South West Africa

It is advisable to follow a pragmatic approach, keeping in mind the scale-dependency of the problems and possible solutions related to the management of these units, both for free-ranging lions as well as for captive stocks. The data presented in this thesis give a more complete overview of the distribution of genetic diversity in the lion, by including data from more populations, notably from West and Central Africa, and by analysing a variety of genetic markers. The more detailed phylogeography of the lion gives insight into evolutionary drivers that have shaped this genetic makeup, but will also contribute to the development of effective management plans to conserve the full genetic diversity of the lion.