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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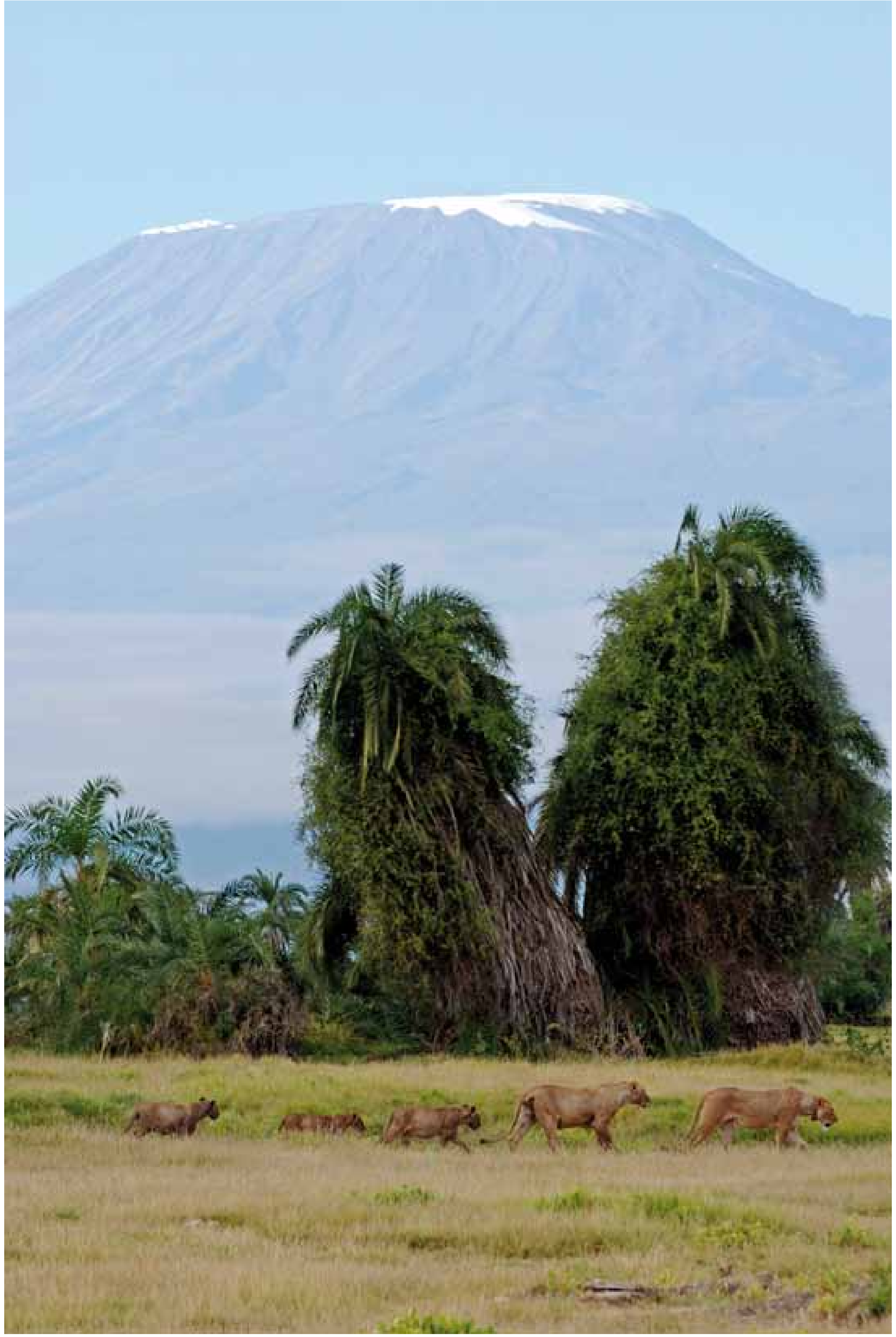

Chapter 1

General Introduction

Global biodiversity is rapidly declining, largely due to human-induced processes, such as land conversion, habitat degradation and overexploitation (Pimm *et al.* 1995, 2014; Pimm & Raven 2000; Butchart *et al.* 2010; Dirzo *et al.* 2014). The conservation of biodiversity is crucial since biodiversity contributes to stability and resilience in ecosystems, following the diversity-stability hypothesis (McNaughton 1977; Pimm 1984; Tilman & Downing 1994; Peterson *et al.* 1998; McCann 2000; MacArthur 2008). We depend on environmental services, provided by well-functioning ecosystems (Millenium Ecosystem Assessment 2005). The scale at which biodiversity can be studied, ranges from entire ecosystems to intraspecific diversity at the genetic level (UNEP-CBD 2006). Insights into the spatial distribution of this diversity provide us with information regarding evolutionary processes which have shaped these patterns, and may provide us with guidelines on how to conserve it.

Genetic diversity at the intraspecific level further contains the evolutionary potential, and therefore, to some degree, the resilience of a species. The goal to preserve species as dynamic entities with the potential to adapt to changing environmental factors, illustrates why recognizing the genetic diversity contributes to conservation planning. This follows from the notion that conservation should not focus on preserving particular objects (species or ecosystems), but rather on preserving the processes of life (Bowen 1999). In addition, genetic diversity may also reflect other types of diversity of importance for conservation, e.g. ecological, behavioural, morphological and demographical diversity, and provides natural resources for breeding.

The aim of this thesis is to gain insight into the intraspecific genetic diversity of an ecological and cultural umbrella species: the lion (*Panthera leo* (Linnaeus, 1758)). The distribution of this diversity reflects the evolutionary history on one hand, but may also provide guidance on how to retain this for the future on the other. The lion serves as a model for illustrating general phylogeographic patterns on the African continent, and provides us with a framework in which we can make recommendations for conservation practices.

The importance of conserving carnivores

Many carnivores have suffered from population declines and range contractions in the past two centuries (Woodroffe 2000; Ceballos & Ehrlich 2002; Ray *et al.* 2005b; Morrison *et al.* 2007; Ripple *et al.* 2014). At the same time, large carnivores are known to play an important role in maintaining rich and resilient ecosystems (Fretwell 1987; Miller *et al.* 2001; Terborgh *et al.* 2001; Sala 2006; Johnson *et al.* 2007; Bruno & Cardinale 2008; Letnic *et al.* 2009, 2012; Beschta & Ripple 2009; Schmitz *et al.* 2010; Strong & Frank 2010; Estes *et al.* 2011). Elimination of top predators from a community, may lead to the reorganization of trophic webs and biodiversity loss, following from trophic cascade theory (Hairston *et al.* 2010) and mesopredator release (Crooks & Soulé 2010). Downstream extinctions of other species have been observed both in community models (Borrvall & Ebenman 2006) and in natural situations (Johnson *et al.* 2007; Letnic *et al.* 2009).

Top predators typically have large home ranges, which they require to fulfil their ecological needs (Schaller 1972). Therefore, they are often considered umbrella species, following the notion that protection of species with large ranges indirectly encompasses populations of co-occurring species (Noss 1990). In addition, large predators are especially sensitive to human activity and have been actively persecuted in most regions of the world, since their ecological role often conflicts with that of local people (Woodroffe 2000; Treves & Karanth 2003; Patterson *et al.* 2004; Woodroffe *et al.* 2005; Bauer *et al.* 2010; Sogbohossou *et al.* 2011; Yirga *et al.* 2012; Tumenta *et al.* 2013). Because of these characteristics, they are more strongly affected by edge effects, often occurring at the borders of protected areas (Woodroffe 1998). Due to the ecological importance and the demanding requirements of large carnivores, it is generally advocated that ensuring that an ecosystem can sustain populations of top predators, this is likely to also benefit other species. This makes carnivores suitable model species for defining and testing conservation strategies.

The African continent is home to a diverse assemblage of carnivores. The African large carnivore guild is made up of seven species: African wild dog (*Lycaon pictus*), spotted hyena (*Crocuta crocuta*), striped hyena (*Hyaena hyaena*), brown hyena (*Hyaena brunnea*), cheetah (*Acinonyx jubatus*), leopard (*Panthera pardus*) and lion (*Panthera leo*) (Dalerum *et al.* 2008). All seven species show decreasing trends and only the spotted hyena is attributed with a Least Concern status according to the IUCN global Red List (IUCN 2014). Despite the fact that all these species fulfil their role as a top predator, they exhibit a high functional diversity as a result of different ecological preferences and different prey spectra (Hayward & Kerley 2008). A study using data from the Serengeti ecosystem, collected over 40 years, has shown that predation on populations is not only affected by the abundance, but also by the diversity of predators, concluding that the loss of this diversity could disrupt important interactions (Sinclair *et al.* 2003). The conservation of ecological interactions has been stressed since the introduction of the key-stone species concept, according to which certain species have a disproportionately large effect on their environment relative to their abundance (Paine 1966, 1969). Targeting species-rich assemblages of large carnivores, which cover high phylogenetic and high functional diversity (Dalerum 2013), follows and expands this notion. The largest species generally represents an important aspect of unique functional diversity, and the lion in particular shows the largest contribution to functional diversity in the global assemblage (Dalerum 2013). These results provide justification for prioritizing this species for conservation efforts.

There are few studies with empirical data on the effect of extirpation of lions on the rest of the ecosystem. Long term data from six protected areas in Ghana show that after extinction of lions and leopards in three of the included areas, the landscape was opened up for olive baboons (*Papio anubis*), which strongly increased in number and range (Brashares *et al.* 2010). Although baboons are only moderately predated by lions and leopards, the absence of an apex predator had a large influence on both the behaviour and population numbers of olive baboons. This negatively affected population numbers of small primates and ungulates, and diet analysis showed that in the absence of a top predator, baboons shift towards a more carnivorous diet (Brashares *et al.* 2010). It illustrates that the presence of a predator, even though it might occur in low densities, may have a profound effect on an ecosystem. The potentially far-reaching effects of large predators was previously shown for wolves (*Canis lupus*) in Yellowstone National Park, also including behavioural changes in prey species, termed “the ecology of fear” (Brown *et al.* 1999). Similar forces may act on the African savannah ecosystem, although monitoring studies in a range of lion habitats could provide more detailed data on the effect of losing this species from the system.

Above mentioned arguments illustrate the need for conservation actions for top predators in general, and the suitability of the lion as a model species in particular. The lions continent-wide distribution and its extension into Asia provides a framework which can be used to study phylogenetic relationships on a large geographic scale. Finally, the lion, generally perceived as the "king of the beasts", is a true cultural flagship for carnivore conservation.

The lion

The lion (*Panthera leo* (Linnaeus, 1758)) is one of the five big or "roaring" cats in the genus *Panthera*. In the current taxonomy, two subspecies are officially recognized: the African lion *Panthera leo leo* (Linnaeus, 1758) and the Asiatic lion *Panthera leo persica* (Meyer, 1826). The species is classified as 'Vulnerable' on the basis of criterion A2abcd on the IUCN Red List of Threatened Species (Bauer *et al.* 2012), defined as "Population reduction observed, estimated, inferred, or suspected in the past where the causes of reduction may not have ceased OR may not be understood OR may not be reversible." (IUCN 2012). For distinct geographic regions other categories are more suitable, e.g. West Africa where lions are categorized as 'Regionally Endangered' (Bauer & Nowell 2004) with the recent suggestion to uplist the West African lion as 'Critically Endangered' (Henschel *et al.* 2014). The Asiatic subspecies is categorized as 'Endangered', based on criterion D (number of mature individuals < 250) (Breitenmoser *et al.* 2008).

Although extinct subspecies of the lion ranged as far as North America and the northern part of South America during the Middle and Late Pleistocene, the range of the modern lion was restricted to Africa, near Asia and the Southern part of Europe (Turner & Antón 1997; Yamaguchi *et al.* 2004; Schnitzler 2011). Lions went extinct in Europe between 3000 and 1000 BC, likely to be the result of human persecution. Later, they disappeared in the Near East, Arabian Peninsula, Trans-Caucasia and the north of Afghanistan around the 12th and 13th century AD, and in all North African countries and the Middle East between the end of the 19th century and the first part of the 20th century (Guggisberg 1961; Schnitzler 2011). The last remaining population outside of Africa is located in the Gir forest National Park in India, being the sole representatives of the Asiatic subspecies (Breitenmoser *et al.* 2008; Bauer *et al.* 2012).

Several studies have aimed to estimate the number of remaining African lions, using a scope of different methods (Table 1). IUCN/SSC Cat Specialist Group members made "guesstimates" of 30,000 – 100,000 wild African lions in the early 1990s (Nowell & Jackson 1996a). The African Lion Working Group (ALWG), which is also affiliated with the IUCN/SSC Cat Specialist Group, conducted a mail survey, which resulted in an estimation of 23,000 lions in protected areas in Africa, with a range of 16,500–30,000 (Bauer & Van der Merwe 2004). Chardonnet (2002) based his estimates on the extrapolation of known populations estimates into areas where lion status was unknown, resulting in an estimation of 39,000 lions, with a range of 29,000–47,000 (Chardonnet 2002). Other studies used a GIS-based model to predict the range and numbers of the African lion (Ferreras & Cousins 1996; Riggio *et al.* 2012). Following the most recent estimate, it is expected that there are 32,000 - 35,000 free-ranging African lions, in 67 areas (Riggio *et al.* 2012). Because of methodological differences between the estimates, a direct comparison of these figures is bothersome. However, a group exercise led by the IUCN/SSC Cat Specialist Group estimated a decline in lion numbers for 42% of the major lion populations (IUCN SSC Cat Specialist Group 2006a; b). Also according to the IUCN Red List data the African lion is indicated as having a decreasing population trend (Bauer *et al.* 2012). The Asiatic populations numbers around 350 individuals and the populations trend is stable (Breitenmoser *et al.* 2008).

Table 1. Studies estimating the number of free-ranging African lions.

Year of prediction	Authors	Number of African lions	Method
1980	Ferreras and Cousins (1996)	75,800	GIS-based model
Early 1990s	Nowell and Jackson (1996a)	30,000-100,000	"Guesstimate"
2002	Chardonnet (2002)	39,000 (range: 29,000-47,000)	Extrapolation from known populations
2004	Bauer & Van der Merwe (2004)	23,000* (range: 16,500-30,000)	Mail survey
2012	Riggio *et al.* (2012)	32,000-35,000	GIS-based model

* protected areas only

The decrease in lion numbers is paralleled by the decline of suitable lion habitat, as is the case for many other species (Riggio *et al.* 2012; Ripple *et al.* 2014). The current range of the African lion is estimated to comprise only 17% of its historical range (Ray *et al.* 2005a; Ripple *et al.* 2014). Comparing the current state to the situation in 1960, using human population density and land conversion data, it was calculated that, at best, 3,390,821 km^2 or about 25% of the original savannah area remain as potential range of free-ranging lions (Riggio *et al.* 2012). The sub-Saharan human population is estimated to have increased nearly four-fold, from 229 million in 1960 to 863 million in 2010 (CIESIN & CIAT 2005). Human population projections further predict this number to double to 1.753 billion in 2050 (CIESIN & CIAT 2005), suggesting that human encroachment of habitat suitable for the lion and other wildlife will be a major challenge conservationists are facing. Apart from land conversion, decline of natural prey base and increased conflict between carnivores and humans are the main threats for the lion (Ferreras & Cousins 1996; Chardonnet 2002; Ray *et al.* 2005b; Winterbach *et al.* 2012). A modelling study has shown that human population and reserve size together accounted for 98% in the variation of extinction of 41 mammal species between reserves in West Africa (Brashares *et al.* 2001). Extinctions rates in six reserves were 14-307 times higher than those predicted by species-area models and reserve size alone, indicating the impact of human demography on local wildlife (Brashares *et al.* 2001). Particularly alarming is that data from the Masai Mara Reserve and its surroundings show that land conversion outside the protected area has resulted in approximately equal declines in wildlife both inside and outside the reserve (Ottichilo *et al.* 2000; Newmark 2008).

A total of 86 Lion Conservation Units (LCUs) was determined on the African continent (IUCN SSC Cat Specialist Group 2006a; b) by expert-defined criteria which classified these areas as important for lion conservation, following an approach previously applied to the jaguar (*Panthera onca*) (Sanderson *et al.* 2002) (Figure 1). The total area of these LCUs was calculated as being 3,163,260 km^2 and is in line with the estimate of suitable lion habitat, being 3,390,821 km^2 (Riggio *et al.* 2012). An estimated 588,000 km^2 are designated as hunting zones, where lions can be harvested as trophies (Lindsey *et al.* 2013). Although hunting zones may act as corridors and avert land conversions into e.g. agricultural land, they have also shown disturbed lion population structure, possibly extending the effect into the adjacent protected area (Loveridge *et al.* 2007; Croes *et al.* 2011; Lindsey *et al.* 2013). In addition to the decline in suitable habitat, remaining areas are often poorly protected and increasingly fragmented (Newmark 2008). This fragmentation and human activities, such as hunting, may lead to the creation of sinks (i.e. sites with unusually high mortality rates for wildlife) in the human-dominated matrix surrounding the protected areas, possibly affecting the populations within the protected area (Loveridge *et al.* 2007; Newmark 2008).

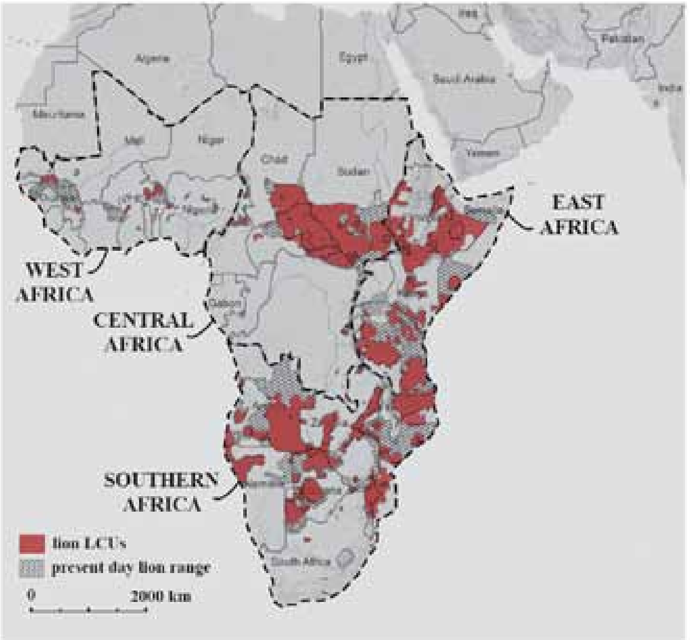

Figure 1.
Map showing delineation of the regions according to the Lion Conservation Strategies (IUCN SSC Cat Specialist Group 2006a;b) and the recognized LCUs in red (Panthera). Lion range data are from IUCN (2014).

It must be noted that only a relatively small part of the total lion range is located in West and (the western part of) Central Africa. The habitat in this region is generally smaller and more isolated, compared to East and Southern Africa. Also the declines in habitat are the strongest in West (39%) and Central Africa (18%), compared to East (16%) and Southern Africa (9%) (Chardonnet 2002). This is paralleled with exceptionally strong declines in wildlife in West Africa (Craigie *et al.* 2010) and some parts of Central Africa (Bouché *et al.* 2012). After recent surveys, resident lion populations could only be confirmed in four out of 16 LCUs in West Africa (Henschel *et al.* 2014) and were absent in all three Central African LCUs included in an earlier study (Henschel *et al.* 2010). This means that, at least for this region, the indicated lion range in Figure 1 may be an overestimation. On the African continent Riggio *et al.* (2012) identified ten lion strongholds, meeting the necessary requirements for long-term viability of the lion population. This entails that 1) the area contains at least 500 individuals, 2) the area is located within protected areas or designated hunting areas, and 3) the numbers of lions is stable or increasing as assessed by the IUCN Cat Specialist Group (Riggio *et al.* 2012). Of these strongholds four are located in East Africa, six in Southern Africa and none have been identified in West or Central Africa (Riggio *et al.* 2012).

Despite the recent and ongoing contraction of lion habitat, the species still exhibits a vast range, covering almost the entire sub-Saharan continent (with the exception of dense rain forest and dry desert), and one location in India. For many large mammals with a similar range, numerous subspecies are recognized and for several phylogenetic data are available (for an overview see Chapter 4). Distributions of diversity, either described in the taxonomy or as a phylogeographic pattern, are often congruent (Hewitt 2004; Lorenzen *et al.* 2012). This leads to the notion that current lion nomenclature, considering all African lion populations to belong to a single subspecies, should be questioned, as it may not reflect the full underlying diversity.

Phylogeography

The term phylogeography was defined in 1987 by Avise *et al.* (1987), aiming to bridge the gap between population genetics and systematics. Demographic events, like population expansions and bottlenecks, as well as migration patterns leave their traces in the genetic makeup of species and populations. Environmentally driven evolution entails that similar forces have determined the evolutionary histories and thereby shaped analogous phylogeographic patterns of multiple species, depending on their ecological requirements. Studying the patterns of co-distributed taxonomic groups, often termed “comparative phylogeography”, may lead to previously unrecognized biogeographic patterns and contribute to guiding conservation decisions (Bermingham & Moritz 1998; Moritz & Faith 1998; Arbogast & Kenagy 2001). Distinct genetic clades may be the result of retraction and subsequent expansion of populations into and from refugia during the cyclical climatic events (Hewitt 2000, 2004). Combining genetic data with climatic data and ecological niche modelling provides us with insights into evolutionary forces acting upon the species. This type of information may also contribute to recognizing risks for long term conservation of a species or population.

Although the species is the general unit for conservation practices (Mace 2004), the general aim to protect the full diversity embedded in the species leads to the notion that it is important to also include units below the species level. This is particularly important for species with a large range. Conserving the maximum of genetic diversity within a species, also entails that the evolutionary potential is maintained. Safeguarding the adaptability of species is of utmost importance in a changing environment, for example as a result of shifting climate conditions (Visser 2008). However, subspecies are generally delimited as geographic variants and do not necessarily reflect information on adaptive genetic diversity.

Phylogeographic data can be used to define such sub-specific units of importance for species conservation, such as Evolutionary Significant Unit (ESU) and Management Units (MU). The term Evolutionary Significant Unit was first proposed by Ryder (1986) and aimed at finding a rational basis for prioritizing units “possessing genetic attributes significant for present and future generations”. Although the precise definition of ESU is disputed (for reviews see: De Guia & Saitoh, 2007; Fraser

& Bernatchez, 2001), the concept has widely been used for conservation studies (Randi *et al.* 2003; Alpers *et al.* 2004; Duriez *et al.* 2006; Hansen *et al.* 2009; Hu *et al.* 2011; Höglund *et al.* 2013) and applied in legal and management contexts (Waples 1991). In general, it is suggested that ESUs ought to be geographically discrete and should display concordant divergence in both molecular and non-molecular traits (Ryder 1986; Dizon *et al.* 1992; Vogler & DeSalle 1994). Adaptive variation as well as divergence, as a reflection of the evolutionary history, are typically included in the definition of ESU, but genetic criteria range from significant divergence of allele frequencies (Waples 1991), to some level of genetic distance (Ryder 1986), to congruently structured phylogenies of genes (Avise & Ball 1990). Common criticism is that the concept is subjective, by aiming to conserve a group of populations which is e.g. "substantially reproductively isolated from other conspecific populations", and "represents an important component in the evolutionary legacy of the species" (Waples 1991), which are guidelines that are difficult to implement in practice.

A later, and commonly used, definition included that an ESU contains a group of populations that is "reciprocally monophyletic for mtDNA alleles" and "shows significant divergence of allele frequencies at nuclear loci" (Moritz 1994). To avoid an overly restrictive definition and to be able to address the cases with less separation than reciprocal monophyly, Moritz (1994) also proposed the term Management Unit (MU), defined as "populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles.". These populations represent groups that are connected with such low levels of gene flow that they are functionally independent and although they show divergence in their allele frequencies, it is not necessarily the case that there is a phylogenetic distinctiveness of the alleles. Following these general definitions, ESUs are concerned with historical population structure, phylogeny and long-term conservation needs. In contrast, MUs address current population structure, allele frequencies and short-term management issues.

Despite numerous definitions and attempts to create a unifying concept (e.g. Fraser & Bernatchez, 2001), the role of neutral genetic markers versus adaptive diversity, concordance with other sources of information such as ecological, morphological and physiological data, and the applicability to differentiate units along the evolutionary continuum of populations, are still under debate (Crandall *et al.* 2000). All definitions that have been proposed are characterized by strong points on one hand, and inherent uncertainties on the other. The applicability of these concepts should be assessed at a case-by-case basis, and conservationists should aim to apply a flexible and integrative approach (Fraser & Bernatchez 2001).

As was previously mentioned, the African lion (*P. l. leo*) and the Asiatic lion (*P. l. persica*) are the only officially recognized subspecies according to the IUCN (Bauer *et al.* 2012). However, differentiation within Africa, distinguishing between lions from East/Southern Africa and West/Central Africa, and the close relationship of the latter to North Africa/Asia, was noted based on a range of morphometric data (Hemmer 1974). Results of a more recent study on craniometric data corroborated the close relationship of West African, North African and Asiatic lions (Mazák 2010). Historically, up to eight lion "subspecies" have been recognized based on intraspecific morphological variation and up to 24 synonyms circulate (Haas *et al.* 2005; Dubach *et al.* 2005; ISIS 2014). Initial descriptions of subspecies have not always adequately treated age- and sex-related variation, and conclusions should therefore be treated with caution (Dubach *et al.* 2005). Two of these eight subspecies , *P. l. leo* and *P. l. melanochaita*, are considered to be either extinct or a con(sub)specific with and extant subspecies (Barnett *et al.* 2006a). Additionally, Hemmer (1974) suggests to combine the four subspecies traditionally recognized in East/Southern Africa into two subspecies, representing East and Southern Africa. This classification leads to three main "subspecies" in Africa: *P. l. senegalensis* (West and Central Africa), *P .l nubica* (East Africa) and *P. l. melanochaita* / *krugeri* (Southern Africa), in addition to the Asiatic subspecies *P. l. persica* (also see Chapter 6).

Several phylogeographic studies have been conducted over the years, aiming to gain insight into the level of distinctiveness between populations and corroboration for the subspecies status of the Asiatic lion. The phylogenetic studies support the single-African-origin model, as was originally proposed based on morphology, distribution and parietal art (Yamaguchi *et al.* 2004). Long branches and the position of the outgroups point the evolutionary cradle of the lion towards East and Southern Africa (Barnett *et al.* 2006b; Antunes *et al.* 2008), which is further supported by the oldest fossil evidences that were found in this region (Petter 1973; Lewis 1997; Werdelin *et al.* 2010). The distinctiveness of the Asiatic subspecies was addressed by O'Brien *et al.* (1987), using allozyme separation data, although genetic distances between the subspecies was low and led to the conclusion that African and Asiatic lions shared a relatively recent common ancestor. Moreover, studies from which the subspecies distinction could be inferred, only included lions from India and from East and Southern Africa and therefore suffered from incomplete sampling of the full species' diversity (O'Brien *et al.* 1987; Burger *et al.* 2004). On the African continent, three major refugial areas have been proposed, deduced from current phylogeographic patterns in large mammals: West/Central Africa, East Africa, and Southern Africa (Hewitt 2004; Lorenzen *et al.* 2012). This may imply that lions from West/Central Africa also represent a different genetic clade, compared to their East and Southern African counterparts. Due to the knowledge gap for West and Central African lions, and the urgent conservation need in this region, these populations are of particular importance (Bauer 2003).

Most studies done so far have been based on mitochondrial DNA (mtDNA). Initially, within East and Southern Africa four distinct groups were distinguished: 1) South-West, 2) East of the Rift Valley, 3) West of the Rift Valley and 4) the Sabi Sands population (Dubach *et al.*, 2005). These four groups are discussed in the light of future conservation interventions, such as translocations to mimic gene flow, and are therefore proposed as distinct ESUs sensu Crandall (2000) (Dubach *et al.* 2005). Barnett and colleagues used a different part of the mtDNA for their phylogenetic studies and included several populations from West and Central Africa, as well as currently extinct populations from North Africa and the Middle East (Barnett *et al.* 2006b). They concluded that five main clades can be distinguished in the modern lion: 1) North Africa–Asia, 2)West Africa, 3) Eastern Sahel (steppe/savannah areas immediately south of the Sahara), 4) Eastern–Southern Africa, and 5) South- Western Africa. According to the authors, these groups could be interpreted both as ESUs or as MUs (sensu Moritz, 1994), although the authors also stress the scale-dependency of identifying units crucial for conservation. Barnett *et al.* (2006b) proposed basal geographic distinctions between lions from 1) North Africa-Asia, 2) Middle Africa and 3) Southern Africa. This pattern may have been shaped and maintained by natural barriers for lion dispersal, such as the Sahara desert, dense tropical rain forest and the Rift Valley (Nowell & Jackson 1996b; Burger *et al.* 2004; Dubach *et al.* 2005), as well as historic events, such as climatological changes (Barnett *et al.* 2006b, 2014). Later phylogeographic studies by these groups continuously added data to the existing dataset and were able to assess the intraspecific genetic diversity of the lion on a finer scale and with higher certainty. Dubach *et al.* (2013) confirmed the distinct position of West & Central African lions, and their close genetic relationship with the Asiatic subspecies. However, they were not able to fully resolve the phylogenetic relationships in this clade, and branches obtained low support in the more diverse East and Southern African branches (Dubach *et al.* 2013). Although Dubach *et al.* (2013) also include microsatellite data for all sampled populations, these were analysed to detect admixture and gene flow, rather than phylogeographic origin (based on the number of identified clusters). Barnett *et al.* (2014), again including samples from currently extinct populations, were the first to describe a basal split in the lion phylogeny, although the branch of the Southern clade does not show significant support and the position of the Asiatic subspecies remains unresolved. Five ESUs (sensu Moritz, 1994) are proposed in absence of conflict with the limited morphological or nuclear DNA data: 1) North Africa/Asia, 2) West Africa, 3) Central Africa, 4) South Africa and 5) East-South Africa. Antunes *et al.* (2008) also included nuclear genetic markers and seroprevalence data of Feline Immunodeficiency Virus (FIV_{Ple}) to infer the evolutionary dynamics of the lion. In this study no free-ranging populations from West and Central Africa had been included and the distinct position of the Asiatic subspecies receives limited support. Interestingly, in the nuclear data there is a clear split observed between East and Southern African lions, whereas in the mtDNA haplotypes this split is less distinct. Although Antunes *et al.* (2008) do not extensively address the conservation implications of these results and the applicability of ESUs or MUs, they do state that due to the differentiation within the African lion “a bottom-up perspective that prioritizes populations, rather than large-scale units (e.g. all African lions), might preserve and maintain lion diversity and evolutionary processes most efficiently.”

The consensus of the phylogeographic studies is that intraspecific diversity of the African lion is greater than the current taxonomy implies, and that different lineages on the African continent can be recognized. In addition the distinct status of the Asiatic subspecies is questioned, regarding the partially unresolved phylogeny of the northern lion populations. The phylogenetic position of the West and Central African lion is of particular interest, notably because of the vulnerable position of populations in this region. As several of the studies discussed in this section were published during the course of this project, the relationship between these studies and the datasets that were generated for this thesis are discussed in the individual chapters.

Conservation genetics and Inbreeding

In the field of conservation genetics, genetic methods are applied to guide conservation and restoration practices. Compared to phylogeography, conservation genetics is typically applied on a lower geographic scale, namely on that of a (meta)population. Levels of genetic diversity on this geographic scale are not so much related to evolutionary potential, but rather to direct fitness measures.

The loss of genetic variability is strongest in small, isolated populations, where genetic drift plays a relatively large role and deleterious mutations accumulate faster as a result of increasing levels of relatedness. Reduction in population size may ultimately lead to inbreeding with consequent fitness effects, such as lower fecundity and higher mortality, termed inbreeding depression (Wright 1977). The effect of inbreeding has been illustrated in several laboratory settings and showed that inbred populations display decreased fitness and are particularly vulnerable to environmental stress, thereby suffering from an increased extinction risk (Bijlsma *et al.* 2000; Reed & Frankham 2003; Armbruster & Reed 2005). In addition, the phenomenon has been studied in domestic and captive-bred wild populations (Ralls & Ballou 1986; Ralls *et al.* 1988; Lacy *et al.* 1993). Although these situations cannot be directly compared to free-ranging, wild populations, Ralls *et al.* (1988) conclude that “the total costs of inbreeding in natural populations are probably considerably higher than our estimates”, which would make the effect of genetic diversity or inbreeding an important driver in natural populations.

Despite of this, the role of genetic factors in the process of extinction in wild populations has long been questioned, following the argument that environmental and demographic stochastic processes, as well as catastrophes, would drive small populations to extinction before the effects of inbreeding would become visible (Caughley 1994; Caro & Laurenson 1994). An extensive review of wild populations of mammals, birds, poikiloterms (snakes, fish and snails) and plants compared 169 estimates of inbreeding depression for 137 traits, and concluded that wild populations under natural conditions frequently exhibit moderate to high levels of inbreeding depression (Crnokrak & Roff 1999). More publications stressed the detrimental effects of low genetic diversity in natural populations and the risk of genetic erosion to long term survival (Keller & Waller 2002; Frankham 2005, 2010). The fact that the extinction risk significantly increased with decreasing heterozygosity, an indication of inbreeding, was shown in a field study on butterflies (Saccheri *et al.* 1998). A comparison of genetic diversity between 170 threatened and taxonomically related, non-threatened species showed that average heterozygosities were lower in the threatened species for 77% of comparisons (Spielman *et al.* 2004). Since reduced fitness often reveals itself as impaired fertility (Keller & Waller 2002), sperm abnormalities and motility were assessed in 20 mammal species (Fitzpatrick & Evans 2009). Species with reduced mean heterozygosity also showed impaired ejaculate quality, with a stronger positive correlation for endangered populations/(sub)species (Fitzpatrick & Evans 2009).

In the fertility study by Fitzpatrick & Evans (2009), three lion populations were assessed, including two populations well-known for their low levels of genetic diversity: the lions of the Ngorongoro crater in Tanzania and the lions of the Gir forest in India. Both inbred lion populations, and the outbred population from the Serengeti plains, had been subjected to a physiological study, which showed that reduced genetic diversity correlates well with an increased incidence of abnormal sperm and with decreased testosterone levels, a hormone crucial for normal spermatogenesis (Wildt *et al.* 1987). Other studies have shown that lion populations with reduced genetic diversity are more susceptible to disease, making them more vulnerable to extinction (Kissui & Packer 2004; Trinkel *et al.* 2011).

The well monitored lions of the Ngorongoro crater in Tanzania illustrate how fast inbreeding can play a role in a natural population. An outbreak of the biting fly *Stomoxys calcitrans* in the early sixties reduced the population from around 70 to 10 individuals, consisting of nine females and one male (Packer *et al.* 1991). Seven males immigrated into the crater in the late 50s and early 60s, but apart from this event, no immigration occurred in the following 25 years. The population recovered, and in 1975 a number of 75-125 lions was estimated to be in the Ngorongoro crater. However, all animals were descendants of only 15 founder individuals (seven females and eight males) and genetic diversity in this population proved to be much lower than in the neighbouring Serengeti ecosystem (O’Brien *et al.* 1987; Yuhki & O’Brien 1990; Packer *et al.* 1991), as well as above mentioned reproductive measures (Wildt *et al.* 1987).

The isolated lion population in the Gir forest, India, the sole representative of the Asiatic subspecies, is also characterized by extremely low heterozygosity levels. The Gir forest population may have suffered from isolation even before the extinction of populations connecting the Asiatic to the African subspecies (Driscoll *et al.* 2002). In addition, the population underwent a severe bottleneck as a result of sport hunting and habitat encroachment and as few as 20 individuals may have been the only survivors in the early twentieth century (see Driscoll *et al.*, 2002, and references herein). Several genetic markers, including allozymes (O'Brien *et al.* 1987), MHC-RFLP (Yuhki & O'Brien 1990), minisatellites (Gilbert *et al.* 1990) and microsatellites (Driscoll *et al.* 2002), confirmed that the Gir forest lions are genetically further impoverished than the Ngorongoro crater lions and even unrelated individuals showed identical minisatellite DNA fingerprints (Gilbert *et al.* 1990). This strong reduction in genetic variability can be explained by long-term geographic isolation and bottlenecks, exacerbated recently by human encroachment (Driscoll *et al.* 2002).

Apart from these two case studies, inbreeding and its potentially detrimental fitness effects are mainly known for lions in captivity. The occurrence of disturbed behaviour and malformations in cubs, possibly resulting from inbreeding depression, has been witnessed in captive lion populations, but according to our knowledge the prevalence was never assessed (Wensing, pers. comm.). A semi-captive setting is represented by the numerous, small and fenced reserves with intensively managed lion populations, in which changes in genetic diversity may be monitored and demographic parameters can be studied. During the past decades lions have been reintroduced into over 40 small (<1000 km^2), fenced reserves in RSA, currently comprising over 500 individuals (Hayward *et al.* 2007; Trinkel *et al.* 2010; Miller & Funston 2014). The lion population of the small and fenced Hluhluwe-iMfolozi Park, RSA, has originated from five founder individuals in the 1960s (Trinkel *et al.* 2008). Subsequent translocations have been conducted to reinforce the population and counteract inbreeding depression. Litter size and cub survival was higher for pairings including a translocated parent, compared to pairings between native lions (Trinkel *et al.* 2008). One of the two sources for the Hluhluwe/iMfolozi population, is the lion population in the Madikwe Game Reserve. These lions were monitored and results further illustrate how fast inbreeding coefficients rise if a small population is completely closed to gene flow (Trinkel *et al.* 2010). This leads to the notion that continuous supplementation of existing small and isolated populations may be needed and that a meta-population based management plan should be implemented for small reserves with no or reduced gene flow (Trinkel *et al.* 2010; Miller & Funston 2014). This is an important aspect that needs to be taken into account in the current debate about fencing of populations for conservation (Hayward & Kerley 2009; Packer *et al.* 2013a; b; Creel *et al.* 2013; Woodroffe *et al.* 2014).

Above mentioned cases have illustrated how genetic data can contribute to the understanding of the distribution of the genetic diversity in a species, and how this information can be applied in the field of conservation. With new technical and computational developments, genomic data are becoming readily available to conservation geneticists. Recently developed Next Generation Sequencing (NGS) techniques open up possibilities to target new magnitudes of genome-wide genetic markers, also in non-model organisms (Ekblom & Galindo 2011; Gayral *et al.* 2013). The versatile character of such datasets allow to tackle diverse questions of importance for conservation practices, ranging from the identification of management units, to insights into demographic histories (McCormack *et al.* 2013; McMahon *et al.* 2014). The entire genome of a single individual already provides enough data for many kinds of analyses, which may lead to a shift from "one gene, many individuals" to "few individuals, several genes", as is suggested by McMahon *et al.* (2014). A shift from genetics to genomics may therefore open new possibilities in the field of conservation biology.

Research questions and thesis outline

In this thesis the intraspecific genetic diversity of the lion is explored. Current taxonomy, only distinguishing African lion (*P. l. leo*) and Asiatic lion (*P. l. persica*), does not reflect the diversity within the African subspecies. Considering the indications for a unique position of populations in West and Central Africa, the urgent conservation needs and the knowledge gap in this region, this research aims to clarify the position and status of the West and Central Africa lion.

A number of approaches is applied, targeting different genetic markers, to solve the following questions:

1) How is the intraspecific genetic diversity in the lion distributed, and what is the phylogenetic position of the West and Central African lion in particular?
2) How can the phylogeographic pattern of the lion give insight into the evolutionary history of the species, and how does that relate to the phylogeographic patterns of other large African savannah mammals?
3) How does the genetic diversity in West and Central African lions compare with Southern and Eastern African lions, and Asiatic lions? Is there evidence that populations went through bottlenecks?
4) How do different genetic markers with different modes of inheritance and different coalescence times contribute to an insight into the genomic complexity underlying the intraspecific genetic diversity in the lion?
5) Is a revision of lion taxonomy justified and advisable? What are the implications for management of the species, especially with respect to defining ESUs and MUs?

This thesis is structured as follows: Chapter 1 is a general introduction, providing a background on the status of the world's carnivores in general, and the lion in particular. Further it frames the field of phylogeography and conservation genetics. Most relevant studies on lion phylogenetics and population genetics are shortly reviewed. In Chapter 2 data of the two main phylogeographic studies on lions (Dubach *et al.* 2005; Barnett *et al.* 2006b) are combined and further extended by addition of new populations, mainly from West and Central Africa. Since mtDNA data may not represent the true underlying structure in a biogeographic context, nuclear markers were assessed for a number of representative populations in Chapter 3. In addition, genetic diversity measures were calculated to gain insight into the levels of genetic diversity on a population level, and detect traces of bottleneck events. Since microsatellite data, presented in Chapter 3, did not contradict the main genetic clades suggested in Chapter 2, the mtDNA dataset was expanded by more sampling locations to obtain a more fine scale picture of phylogeographic groups. Chapter 4 presents this dataset, including fourteen complete mitochondrial genomes from representative populations and aDNA data, along with newly published data from Barnett *et al*. (2006+2014). The phylogeographic pattern of the lion is compared to a range of large savannah species and its evolutionary history is assessed. Chapter 5 describes the development of a new lion-specific genetic marker by SNP discovery from whole genome data of ten lions. These data are analysed in a phylogeographic framework and compared to previously described scenarios in Chapter 2, 3 and 4. Chapter 6 is a general discussion, aiming to get a complete overview of available lion data and unraveling the phylogenetic relationships of the different lion clades and their evolutionary histories. It sums up with a look into the future and general recommendations on how to apply these data in the field of lion conservation.

References

Alpers DL, Van Vuuren BJ, Arctander P, Robinson TJ (2004) Population genetics of the roan antelope (Hippotragus equinus) with suggestions for conservation. *Molecular ecology*, **13**, 1771–84.

Antunes A, Troyer JL, Roelke ME *et al.* (2008) The evolutionary dynamics of the lion *Panthera leo* revealed by host and viral population genomics. *PLoS genetics*, **4**, e1000251.

Arbogast B, Kenagy GJ (2001) Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography*, **28**, 819–825.

Armbruster P, Reed DH (2005) Inbreeding depression in benign and stressful environments. *Heredity*, **95**, 235–42.

Avise JC, Ball RM (1990) Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Surveys in Evolutionary Biology*, **7**, 45–67.

Barnett R, Yamaguchi N, Barnes I, Cooper A (2006a) Lost populations and preserving genetic diversity in the lion Panthera leo: Implications for its ex situ conservation. *Conservation Genetics*, **7**, 507–514.

Barnett R, Yamaguchi N, Barnes I, Cooper A (2006b) The origin, current diversity and future conservation of the modern lion (Panthera leo). *Proceedings of the Royal Society B: Biological Sciences*, **273**, 2119–2125.

Barnett R, Yamaguchi N, Shapiro B *et al.* (2014) Revealing the maternal demographic history of Panthera leo using ancient DNA and a spatially explicit genealogical analysis. *BMC Evolutionary Biology*, **14**, 70.

Bauer H (2003) Research needs for lion conservation in West and Central Africa. *Comptes Rendus Biologies*, **326**, 112–118.

Bauer H, Iongh HD, Sogbohossou E (2010) Assessment and mitigation of human-lion conflict in West and Central Africa. *Mammalia*, **74**, 363–367.

Bauer H, Van der Merwe S (2004) Inventory of free-ranging lions Panthera leo in Africa. *Oryx*, **38**, 26–31.

Bauer H, Nowell K (2004) Endangered Classification for West African Lions. *Cat News*, **41**, 35–36.

Bauer H, Nowell K, Packer C (2012) IUCN Red List of Threatened Species: Panthera leo. *Version 2013.2*.

Bermingham E, Moritz C (1998) Comparative phylogeography: concepts and applications. *Molecular ecology*, **7**, 367–369.

Beschta RL, Ripple WJ (2009) Large predators and trophic cascades in terrestrial ecosystems of the western United States. *Biological Conservation*, **142**, 2401–2414.

Bijlsma R, Bundgaard J, Boerema AC (2000) Does inbreeding affect the extinction risk of small populations?: predictions from Drosophila. *Journal of Evolutionary Biology*, **13**, 502–514.

Borrvall C, Ebenman B (2006) Early onset of secondary extinctions in ecological communities following the loss of top predators. *Ecology letters*, **9**, 435–42.

Bouché P, Nzapa Mbeti Mange R, Tankalet F *et al.* (2012) Game over! Wildlife collapse in northern Central African Republic. *Environmental monitoring and assessment*, **184**, 7001–11.

Bowen BW (1999) Preserving genes, species, or ecosystems? Healing the fractured foundations of conservation policy. *Molecular ecology*, **8**, S5–10.

Brashares JS, Arcese P, Sam MK (2001) Human demography and reserve size predict wildlife extinction in West Africa. *Proceedings. Biological sciences / The Royal Society*, **268**, 2473–8.

Brashares JS, Prugh PR, Stoner CJ, Epps CW (2010) Trophic Cascades: Predators, Prey, and the Changing Dynamics of Nature. In: *Trophic Cascades: Predators, Prey, and the Changing Dynamics of Nature* (eds Terborgh J, Estes JA), pp. 221–240. Island Press, Washington D.C., U.S.A.

Breitenmoser U, Mallon DP, Ahmad Khan J, Driscoll C (2008) Panthera leo persica. In: *IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2.*

Brown JS, Laundre JW, Gurung M (1999) The ecology of fear: Optimal foraging, game theory, and trophic interactions. *Journal of Mammalogy*, **80**, 385–399.

Bruno JF, Cardinale BJ (2008) Cascading effects of predator richness. *Frontiers in Ecology and the Environment*, **6**, 539–546.

Burger J, Rosendahl W, Loreille O *et al.* (2004) Molecular phylogeny of the extinct cave lion Panthera leo spelaea. *Molecular phylogenetics and evolution*, **30**, 841–849.

Butchart SHM, Walpole M, Collen B *et al.* (2010) Global Biodiversity : Indicators of recent Declines. *Science*, **328**, 1164–1168.

CIESIN, CIAT (2005) Gridded Population of the World Version 3 (GPWv3): Population Density Grids.

Caro TM, Laurenson MK (1994) Ecological and genetic factors in conservation: a cautionary tale. *Science (New York, N.Y.)*, **263**, 485–6.

Caughley G (1994) Directions in conservation biology. *Journal of Animal Ecology*, **63**, 215–244.

Ceballos G, Ehrlich PR (2002) Mammal population losses and the extinction crisis. *Science (New York, N.Y.)*, **296**, 904–7.

Chardonnet P (2002) *Conservation of the African Lion : Contribution to a Status Survey*. International Foundation for the Conservation of Wildlife, France and Conservation Force, U.S.A., Paris.

Craigie ID, Baillie JEM, Balmford A *et al.* (2010) Large mammal population declines in Africa's protected areas. *Biological Conservation*, **143**, 2221–2228.

Crandall KA, Bininda-emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Science*, **15**, 290–295.

Creel S, Becker MS, Durant SM *et al.* (2013) Conserving large populations of lions - the argument for fences has holes. *Ecology letters*.

Crnokrak P, Roff DA (1999) Inbreeding depression in the wild. *Heredity*, **83 (Pt 3)**, 260–70.

Croes BM, Funston PJ, Rasmussen G *et al.* (2011) The impact of trophy hunting on lions (Panthera leo) and other large carnivores in the Bénoué Complex, northern Cameroon. *Biological Conservation*, **144**, 3064–3072.

Crooks KR, Soulé ME (2010) Mesopredator release and avifaunal extinctions in a fragmented system. *Nature*, **400**, 563–566.

Dalerum F (2013) Phylogenetic and functional diversity in large carnivore assemblages. *Proceedings. Biological sciences / The Royal Society*, **280**, 20130049.

Dalerum F, Somers MJ, Kunkel KE, Cameron EZ (2008) The potential for large carnivores to act as biodiversity surrogates in southern Africa. *Biodiversity and Conservation*, **17**, 2939–2949.

Dirzo R, Young HS, Galetti M *et al.* (2014) Defaunation in the Anthropocene. *Science (New York, N.Y.)*, **345**, 401–6.

Dizon AE, Lockyer C, Perrin W, Demaster D, Sisson J (1992) Rethinking the Stock Concept: A Phylogeographic Approach. *Conservation Biology*, **6**, 24–36.

Driscoll CA, Menotti-Raymond M, Nelson G, Goldstein D, O'Brien SJ (2002) Genomic microsatellites as evolutionary chronometers: a test in wild cats. *Genome research*, **12**, 414–423.

Dubach JM, Briggs MB, White PA, Ament BA, Patterson BD (2013) Genetic perspectives on "Lion Conservation Units" in Eastern and Southern Africa. *Conservation Genetics*, **1942**.

Dubach J, Patterson BD, Briggs MB *et al.* (2005) Molecular genetic variation across the southern and eastern geographic ranges of the African lion, Panthera leo. *Conservation Genetics*, **6**, 15–24.

Duriez O, Sachet J-M, Ménoni E *et al.* (2006) Phylogeography of the capercaillie in Eurasia: what is the conservation status in the Pyrenees and Cantabrian Mounts? *Conservation Genetics*, **8**, 513–526.

Ekblom R, Galindo J (2011) Applications of next generation sequencing in molecular ecology of non-model organisms. *Heredity*, **107**, 1–15.

Estes JA, Terborgh J, Brashares JS *et al.* (2011) Trophic downgrading of planet Earth. *Science (New York, N.Y.)*, **333**, 301–6.

Ferreras P, Cousins SH (1996) *The use of a Delphi technique with GIS for estimating the global abundance of top predators*. Cranfield, UK.

Fitzpatrick JL, Evans JP (2009) Reduced heterozygosity impairs sperm quality in endangered mammals. *Biology letters*, **5**, 320–3.

Frankham R (2005) Genetics and extinction. *Biological Conservation*, **126**, 131–140.

Frankham R (2010) Inbreeding in the wild really does matter. *Heredity*, **104**, 124.

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular ecology*, **10**, 2741–2752.

Fretwell SD (1987) Food chain dynamics: The central theory of ecology? *Oikos*, **50**, 291–301.

Gayral P, Melo-Ferreira J, Glémin S *et al.* (2013) Reference-free population genomics from next-generation transcriptome data and the vertebrate-invertebrate gap. *PLoS genetics*, **9**, e1003457.

Gilbert DA, Packer C, Pusey AE, Stephens JC, O'Brien SJ (1990) Analytical DNA fingerprinting in lions: parentage, genetic diversity, and kinship. *The Journal of heredity*, **82**, 378–86.

Guggisberg CAW (1961) *Simba, the life of the lion*. Howard Timmins, Capetown.

De Guia APO, Saitoh T (2007) The gap between the concept and definitions in the Evolutionarily Significant Unit: the need to integrate neutral genetic variation and adaptive variation. *Ecological Research*, **22**, 604–612.

Haas SK, Hayssen V, Krausman PR (2005) Mammalian species - Panthera leo. *Mammalian Species*, 1–11.

Hairston NG, Smith FE, Slobodkin LB (2010) The American Society of Naturalists Community Structure , Population Control , and Competition. *The American Naturalist*, **94**, 421–425.

Hansen BD, Harley DKP, Lindenmayer DB, Taylor AC (2009) Population genetic analysis reveals a long-term decline of a threatened endemic Australian marsupial. *Molecular ecology*, **18**, 3346–3362.

Hayward MW, Adendorff J, O'Brien J *et al.* (2007) Practical Considerations for the Reintroduction of Large, Terrestrial, Mammalian Predators Based on Reintroductions to South Africa's Eastern Cape Province. *The Open Conservation Biology Journal*, **1**, 1–11.

Hayward MW, Kerley GIH (2008) Prey preferences and dietary overlap amongst Africa ' s large predators. *South African Journal of Wildlife Research*, **38**, 93–108.

Hayward MW, Kerley GIH (2009) Fencing for conservation: Restriction of evolutionary potential or a riposte to threatening processes? *Biological Conservation*, **142**, 1–13.

Hemmer H (1974) *Untersuchungen zur Stammesgeschichte der Pantherkatzen (Pantherinae) 3 Zur Artgeschichte des Löwen Panthera (Panthera) leo (Linnaeus 1758).* Veröffentlichungen der Zoologischen Staatssammlung München, München, Germany.

Henschel P, Azani D, Burton C *et al.* (2010) Lion status updates from five range countries in West and Central Africa. *CATnews*, **52**, 34–39.

Henschel P, Coad L, Burton C *et al.* (2014) The Lion in West Africa Is Critically Endangered (M Hayward, Ed,). *PLoS ONE*, **9**, e83500.

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–13.

Hewitt GM (2004) The structure of biodiversity - insights from molecular phylogeography. *Frontiers in zoology*, **1**, 4.

Hu Y, Guo Y, Qi D *et al.* (2011) Genetic structuring and recent demographic history of red pandas (Ailurus fulgens) inferred from microsatellite and mitochondrial DNA. *Molecular ecology*, **20**, 2662–75.

Hunter LTB, Pretorius K, Carlisle LC *et al.* (2007) Restoring lions Panthera leo to northern KwaZulu-Natal, South Africa: short-term biological and technical success but equivocal long-term conservation. *Oryx*, **41**, 196.

Höglund J, Wang B, Axelsson T, Quintela M (2013) Phylogeography of willow grouse (Lagopus lagopus) in the Arctic: taxonomic discordance as inferred from molecular data. *Biological Journal of the Linnean Society*, **110**, 77–90.

ISIS (2014) Species Holdings - Panthera leo.

IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. Second edition.

IUCN (2014) The IUCN Red List of Threatened Species. Version 2014.1.

IUCN SSC Cat Specialist Group (2006a) *Conservation strategy for the lion in West and Central Africa*. IUCN, Gland, Switzerland.

IUCN SSC Cat Specialist Group (2006b) *Conservation strategy for the lion (Panthera leo) in Eastern and Southern Africa*. IUCN, Gland, Switzerland.

Johnson CN, Isaac JL, Fisher DO (2007) Rarity of a top predator triggers continent-wide collapse of mammal prey: dingoes and marsupials in Australia. *Proceedings. Biological sciences / The Royal Society*, **274**, 341–6.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends in ecology & evolution*, **17**, 19–23.

Kissui BM, Packer C (2004) Top-down population regulation of a top predator: lions in the Ngorongoro Crater. *Proceedings. Biological sciences / The Royal Society*, **271**, 1867–74.

Lacy RC, Petric A, Warneke M (1993) Inbreeding and Outbreeding in Captive Populations of Wild Animals. pdf. In: *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (ed Thornhill NW), pp. 352–374. Chicago University Press, Chicago, U.S.A.

Letnic M, Koch F, Gordon C, Crowther MS, Dickman CR (2009) Keystone effects of an alien top-predator stem extinctions of native mammals. *Proceedings. Biological sciences / The Royal Society*, **276**, 3249–56.

Letnic M, Ritchie EG, Dickman CR (2012) Top predators as biodiversity regulators: the dingo Canis lupus dingo as a case study. *Biological reviews of the Cambridge Philosophical Society*, **87**, 390–413.

Lewis ME (1997) Carnivoran paleoguilds of Africa : implications for hominid food. *Journal of Human Evolution*, **32**, 257–288.

Lindsey PA, Balme GA, Funston P *et al.* (2013) The Trophy Hunting of African Lions: Scale, Current Management Practices and Factors Undermining Sustainability (M Hayward, Ed,). *PLoS ONE*, **8**, e73808.

Lorenzen ED, Heller R, Siegismund HR (2012) Comparative phylogeography of African savannah ungulates. *Molecular ecology*, **21**, 3656–70.

Loveridge AJ, Searle AW, Murindagomo F, Macdonald DW (2007) The impact of sport-hunting on the population dynamics of an African lion population in a protected area. *Biological Conservation*, **134**, 548–558.

MacArthur R (2008) Fluctuations of Animal Populations and a Measure of Community Stability. *Ecology*, **36**, 533–536.

Mace GM (2004) The role of taxonomy in species conservation. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **359**, 711–9.

Mazák JH (2010) Geographical variation and phylogenetics of modern lions based on craniometric data. *Journal of Zoology*, **281**, 194–209.

McCann KS (2000) The diversity-stability debate. *Nature*, **405**, 228–33.

McCormack JE, Hird SM, Zellmer AJ, Carstens BC, Brumfield RT (2013) Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular phylogenetics and evolution*, **66**, 526–38.

McMahon BJ, Teeling EC, Höglund J (2014) How and why should we implement genomics into conservation? *Evolutionary Applications*, n/a–n/a.

McNaughton SJ (1977) Diversity and Stability of Ecological Communities : A Comment on the Role of Empiricism in Ecology. *The American Naturalist*, **111**, 515–525.

Millenium Ecosystem Assessment (2005) *Ecosystems and Human Well-Being*.

Miller B, Dugelby B, Foreman D *et al.* (2001) The Importance of Large Carnivores to Healthy Ecosystems. *Endangered Species*, **18**, 202–210.

Miller SM, Funston PJ (2014) Rapid growth rates of lion (Panthera leo) populations in small , fenced reserves in South Africa: a management dilemma. *South African Journal of Wildlife Research*, **44**, 43–55.

Moritz C (1994) Defining “Evolutionarily Significant Units” for conservation. *Trends in ecology & evolution (Personal edition)*, **9**, 373–375.

Moritz C, Faith DP (1998) Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular ecology*, **7**, 419–429.

Morrison JC, Sechrest W, Dinerstein E, Wilcove DS, Lamoreux JF (2007) Persistence of Large Mammal Faunas as Indicators of Global Human Impacts. *Journal of Mammalogy*, **88**, 1363–1380.

Newmark WD (2008) Isolation of African protected areas. *Frontiers in Ecology and the Environment*, **6**, 321–328.

Noss R (1990) Indicators for monitoring biodiversity: a hierarchical approach. *Conservation Biology*, **4**, 355–364.

Nowell K, Jackson P (Eds.) (1996a) *Wild Cats, Status Survey and Conservation Action Plan*. IUCN, Gland.

Nowell K, Jackson P (1996b) *Wild Cats*. IUCN, Gland, Switzerland.

Ottichilo WK, Leeuw JD, Skidmore AK *et al.* (2000) Population trends of large non-migratory wild herbivores and livestock in the Masai Mara. *African Journal of Ecology*, **38**, 202–216.

O’Brien SJ, Martenson JS, Packer C *et al.* (1987) Biochemical Genetic Variation in Geographic Isolates of African and Asiatic Lions. *National Geographic Research*, **3**, 114–124.

Packer C, Loveridge a., Canney S *et al.* (2013a) Conserving large carnivores: dollars and fence (N Haddad, Ed,). *Ecology Letters*, n/a–n/a.

Packer C, Pusey AE, Rowley H *et al.* (1991) Case Study of Population Bottleneck: Lions of Ngorongoro. *Biological Conservation*, **5**, 219–230.

Packer C, Swanson a., Canney S *et al.* (2013b) The case for fencing remains intact (J Chase, Ed,). *Ecology Letters*, n/a–n/a.

Paine RT (1966) Food Web Complexity and Species Diversity. *The American Naturalist*, **100**, 65–75.

Paine RT (1969) A Note on Trophic Complexity and Community Stability. *The American Naturalist*, **103**, 91–93.

Patterson BD, Kasiki SM, Selempo E, Kays RW (2004) Livestock predation by lions (Panthera leo) and other carnivores on ranches neighboring Tsavo National ParkS, Kenya. *Biological Conservation*, **119**, 507–516.

Peterson G, Allen CR, Holling CS (1998) Ecological Resilience , Biodiversity , and Scale. *Ecosystems*, **1**, 6–18.

Petter G (1973) *Fossil vertebrates of Africa: Carnivores Pléistocènes du Ravin d’Olduvai* (LSB Leakey, RJG Savage, SC Coryndon, Eds,). Academic Press, London, UK.

Pimm SL (1984) The complexity and stability of ecosystems. *Nature*, **307**, 321–326.

Pimm SL, Jenkins CN, Abell R *et al.* (2014) The biodiversity of species and their rates of extinction, distribution, and protection. *Science*, **344**, 1246752–1246752.

Pimm SL, Raven P (2000) Extinction by numbers. *Nature*, **403**, 843–845.

Pimm SL, Russell GJ, Gittleman JL, Brooks TM (1995) The future of biodiversity. *Science (New York, N.Y.)*, **269**, 347–50.

Ralls K, Ballou J (1986) Captive breeding programs for populations with a small number of founders. *Trends in ecology & evolution*, **1**, 19–22.

Ralls K, Ballou JD, Templeton A (1988) Estimates of Lethal Equivalents and the Cost of Inbreeding in Mammals. *Conservation Biology*, **2**, 185–193.

Randi E, Tabarroni C, Rimondi S, Lucchini V, Sfougaris a. (2003) Phylogeography of the rock partridge (Alectoris graeca). *Molecular Ecology*, **12**, 2201–2214.

Ray JC, Hunter L, Zigouris J (2005a) *Setting conservation and research priorities for larger African carnivores*. New York, U.S.A.

Ray J, Redford . H, Steneck R, Berger J (2005b) *Large Carnivores and the Conservation of Biodiversity*. Island Press, Washington D.C., U.S.A.

Reed DH, Frankham R (2003) Correlation between Fitness and Genetic Diversity. *Conservation Biology*, **17**, 230–237.

Riggio J, Jacobson A, Dollar L *et al.* (2012) The size of savannah Africa: a lion’s (Panthera leo) view. *Biodiversity and Conservation*, **22**, 17–35.

Ripple WJ, Estes JA, Beschta RL *et al.* (2014) Status and Ecological Effects of the World’s Largest Carnivores. *Science*, **343**, 1241484–1241484.

Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. *Trends in ecology & evolution*, **1**, 9–10.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **45**, 1996–1999.

Sala E (2006) Top predators provide insurance against climate change. *Trends in ecology & evolution*, **21**, 479–80.

Sanderson EW, Redford KH, Chetkiewicz C-LB *et al.* (2002) Planning to Save a Species: the Jaguar as a Model. *Conservation Biology*, **16**, 58–72.

Schaller GB (1972) *The Serengeti Lion - A study of predator-prey relations*. University of Chicago Press, Chicago, U.S.A.

Schmitz OJ, Hawlena D, Trussell GC (2010) Predator control of ecosystem nutrient dynamics. *Ecology letters*, **13**, 1199–209.

Schnitzler AE (2011) Past and present distribution of the North African-Asian lion subgroup: a review. *Mammal Review*.

Sinclair ARE, Mduma S, Brashares JS (2003) Patterns of predation in a diverse predator – prey system. *Nature*, **425**, 288–290.

Sogbohossou EA, de Iongh HH, Sinsin B, de Snoo GR, Funston PJ (2011) Human–carnivore conflict around Pendjari Biosphere Reserve, northern Benin. *Oryx*, **45**, 569–578.

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. *PNAS*, **101**, 15261–15264.

Strong DR, Frank KT (2010) Human Involvement in Food Webs. *Annual Review of Environment and Resources*, **35**, 1–23.

Terborgh J, Lopez L, Nuñez P *et al.* (2001) Ecological meltdown in predator-free forest fragments. *Science (New York, N.Y.)*, **294**, 1923–6.

Tilman D, Downing JA (1994) Biodiversity and stability in grasslands. *Nature*, **367**, 363–365.

Treves A, Karanth KU (2003) Human-Carnivore Conflict and Perspectives on Carnivore Management Worldwide. *Conservation Biology*, **17**, 1491–1499.

Trinkel M, Cooper D, Packer C, Slotow R (2011) Inbreeding depression increases susceptibility to bovine tuberculosis in lions: an experimental test using an inbred-outbred contrast through translocation. *Journal of wildlife diseases*, **47**, 494–500.

Trinkel M, Ferguson N, Reid a. *et al.* (2008) Translocating lions into an inbred lion population in the Hluhluwe-iMfolozi Park, South Africa. *Animal Conservation*, **11**, 138–143.

Trinkel M, Funston P, Hofmeyr M *et al.* (2010) Inbreeding and density-dependent population growth in a small, isolated lion population. *Animal Conservation*, **13**, 374–382.

Tumenta PN, Visser HD, van Rijssel J *et al.* (2013) Lion predation on livestock and native wildlife in Waza National Park, northern Cameroon. *Mammalia*, **77**, 247–251.

Turner A, Antón M (1997) *The big cats and their fossil relatives*. Columbia University Press, New York.

UNEP-CBD (2006) Convention on Biological Diversity, Framework for monitoring implementation of the achievement of the 2010 target and integration of targets into the thematic programmes of work, COP8 Decision VIII/15.

Visser ME (2008) Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings. Biological sciences / The Royal Society*, **275**, 649–659.

Vogler AP, DeSalle R (1994) Diagnosing Units of Conservation Managment. *Conservation Biology*, **6**, 170–178.

Waples RS (1991) Pacific Salmon, Oncorhynchus spp ., and the Definition of “Species” Under the Endangered Species Act. *Marine Fisheries Reviews*, **53**, 11–22.

Werdelin L, Yamaguchi N, Johnson WE, O’Brien SJ (2010) Biology and Conservation of Wild Felids. In: *Biology and Conservation of Wild Felids* (eds Macdonald DW, Loveridge AJ), pp. 59–82. Oxford University Press, Oxford.

Wildt DE, Bush M, Goodrowe KL *et al.* (1987) Reproductive and genetic consequences of founding isolated lion populations. *Nature*, **329**, 328–331.

Winterbach HEK, Winterbach CW, Somers MJ, Hayward MW (2012) Key factors and related principles in the conservation of large African carnivores. *Mammal Review*, **43**, 89–110.

Woodroffe R (1998) Edge Effects and the Extinction of Populations Inside Protected Areas. *Science*, **280**, 2126–2128.

Woodroffe R (2000) Predators and people: using human densities to interpret declines of large carnivores. *Animal Conservation*, **3**, 165–173.

Woodroffe R, Hedges S, Durant SM (2014) To Fence or Not to Fence. , **344**, 46–48.

Woodroffe R, Thirgood S, Rabinowitz A (2005) *People and Wildlife, Conflict or Co-existence*. Cambridge University Press, Cambridge, U.K.

Wright S (1977) *Evolution and the Genetics of Populations: Volume 3 - Experimental Results and Evolutionary Deductions*. Chicago University Press, Chicago, U.S.A.

Yamaguchi N, Cooper A, Werdelin L, Macdonald DW (2004) Evolution of the mane and group-living in the lion (Panthera leo): a review. *Journal of Zoology*, **263**, 329–342.

Yirga G, Iongh HHD, Leirs H *et al.* (2012) The ecology of large carnivores in the highlands of northern Ethiopia. *African Journal of Ecology*, **51**, 78–86.

Yuhki N, O’Brien SJ (1990) DNA variation of the mammalian major histocompatibility complex reflects genomic diversity and population history. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 836–840.