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Molecular genetic variation across the southern and eastern geographic ranges of the African lion, *Panthera leo*

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Abstract

We examined sequence variation in the mitochondrial cytochrome *b* and NADH dehydrogenase subunit 5 genes (2,360 bp total) for 26 lions from eleven locations throughout sub-Saharan Africa. Six distinct haplotypes were observed in the combined sequences, forming two clades: the eastern and the western savannas. The Uganda-Western Kenya haplotype grouped at a basal position with the eastern clade of lions from Tsavo south to the Transvaal and Natal regions. The phylogenetic position of the haplotype from Sabi Sands in the southern part of Kruger National Park remained poorly resolved. The haplotypes found in Namibia and Botswana formed the western clade. The modest genetic variation documented here argues against taxonomic distinctions among living African lions.

Introduction

Until historic times, the lion (*Panthera leo*) was one of the most broadly distributed terrestrial mammals. Lions ranged over most of non-Saharan Africa, the Middle East, and southwest Asia into the eastern Balkans in Europe and western India (Guggisberg 1961, Coheleach 1982). During the Pleistocene, the same or similar species ranged over much of northern and western Eurasia, and into North America (Vereshchagin 1971; Hemmer 1974; Guthrie 1990). The collapse of the Pleistocene megafauna in the Holarctic and expanding human populations have paced the range contraction of *P. leo* on all fronts (Smuts 1978; Hanby & Bygott 1979). Range collapse during historic times seems to have eliminated several of the more peripheral populations (O'Brien et al. 1987; Kingdon 1997). The distinctive Barbary and Cape lions, often treated as separate subspecies, are now extinct (Mazák 1970; 1975), as are all southwest Asian populations, leaving only the highly endangered Gir Forest lions as the last remnant Asian population (O'Brien et al. 1987).

Like other large-bodied, wide-ranging species, lions show great morphological variation (Hallgrimsson & Maiorana 2000). Variation is particularly marked in size, coat thickness and color, retention of juvenile spots, and in male secondary characters (Hollister 1917). The mane can vary considerably in color, density and distribution within and among populations (Patterson 2004; West & Packer 2002).

Previous analyses of intraspecific variation in morphology of lions have been plagued by inadequate treatment of age- and sex-related variation. This is especially true for East Africa where nine different subspecies have been named and substantial morphological variation has been noted (Elliot 1897; Patterson 1927; Wolffe 1955; Schaller 1972). Twenty-four different names have been proposed for geographic races of African *P. leo* (Allen 1939; Meester & Setzer 1971 (as revised 1977)), but most taxonomic authorities recognize only two subspecies of extant lions, *P. leo leo* and *P. leo persicus*, for African and Asian populations, respectively (Ellerman et al. 1953; Meester & Setzer 1971 (as revised 1977); O'Brien et al. 1987).

Here, we evaluate sequence data from two mitochondrial genes, cytochrome *b* and NADH dehydrogenase subunit 5, to examine the genetic diversity among geographically distant populations of sub-Saharan lions across the southern and eastern parts of their range. Patterns of genetic variation should help to identify significant evolutionary units within *P. leo* and clarify their biogeographic history.

Figure 1. Current lion distribution in shaded areas (Kingdon 1997). Location of sample sites are indicated.

Materials and methods

Sample collection

Blood samples were obtained from wild lions following the method described by Blood et al. (1979). Peripheral whole blood and buffy coats were preserved frozen in liquid nitrogen or in long-term storage buffer (100 mM Tris–HCL, 100 mM EDTA, 2%SDS, pH 8.0, mixed 1:1 blood to buffer), and brought to the Brookfield Zoo genetics lab. Dried skin samples from Kenyan lions were hydrated in long-term storage buffer prior to transport to the Field Museum of Natural History. Sample locations (Figure 1) and number of individuals used for sequence analysis per population are given in Table 1. Blood was also obtained from an Amur tiger at Brookfield Zoo.

DNA extraction, amplification and sequencing

Following overnight digestion of the blood/buffer or tissue sample with 5–10 μ Proteinase K, DNA was extracted using standard phenol, phenol-chloroform/isoamyl alcohol, and chloroform/isoamyl alcohol washes followed by precipitation with 3 M sodium acetate and 100% ethanol (Sambrook et al. 1989). Genomic DNA was used as a template for PCR amplification of the mitochondrial genes, cytochrome *b* (CYTB), NADH dehydrogenase subunits 5 and 6 (ND5/6) and the Rs3 portion of the control region. The following PCR parameters were used for CYTB: 94 °C for 40 s, 50 °C for 45 s, 72 °C for 45 s for 35 cycles, followed by a 10 min extension at 72 °C. For ND5/6 and Rs3 control region, the annealing temperature was increased to 52 °C. Primer sequences can be found in Table 2.

All PCR products were cleaned for sequencing using QIAquick Spin Columns (Qiagen) and submitted to Iowa State Nucleic Acid Facility for Automated sequencing on either an ABI 373 or an ABI 377 system or sequenced on a Beckman/Coulter CEQ2000XL. To verify sequence accuracy, several haplotypes were sequenced on both instruments. Sequences have been deposited in Genebank under the accession numbers AF384809-AF384818 for CYTB, AF385613 and AF385614 for the complete ND5/6, and AF385615-AF385623 for ND5 partial sequences.

Table 1. Sampling localities within Africa by country and region, population name, population ID and sequence sample sizes for cytochrome *b*/NADH dehydrogenase 5 (CYTB/ND5) for the lion, *Panthera leo*

Country/region	Population	ID	Sample size CYTB/ND5
Southwestern Africa			
Namibia	Bushmanland	BML	2
	Etosha National Park	ETO	4
	Caprivi Strip	CAP	2
Botswana	Moremi Game Reserve[a]	BOT	2
Southeastern Africa			
KwaZulu-Natal	Hluhluwe-Umfolozi Park	UMF	3
	Fannie Roberts Reserve	FRR	2
Transvaal	Kapama Game Reserve	KPR	1
	Sabi Sands Region - Southern Kruger National Park[b]	SSR	3
East Africa			
Uganda[c]	Baltimore Zoo (ID BLT80-144)	UGD	1
Kenya			
Coast	Tsavo East National Park	TSV	5
Central	Aberdare National Park	ANP	1
Amur tiger	Brookfield Zoo, ISIS# 970248	*P. tigris*	1

[a]Southern border of the game reserve in the Gomoti region of Botswana.
[b]Samples from lions translocated from Sabi Sands Region to Phinda Reserve.
[c]Offspring (F1) from wild parents, identified as *P. leo massaicus*, brought from Uganda in 1968.

Validation of sequence fidelity and independence

The CYTB and ND5/6 genes were initially selected because Lopez et al. (1994, 1996) concluded they were not part of the transposed mtDNA in the felid nuclear genome (Numt). However, our CYTB sequences differed from those reported by Janczewski et al. (1995) in nucleotide composition. Cracraft et al.(1998) has subsequently identified both nuclear and mitochondrial copies of CYTB. Therefore, to determine if the variation noted between populations was due to nuclear pseudogene contamination or PCR artifact, validation of the fidelity of two CYTB sequences reported here was undertaken.

The CYTB gene was PCR amplified using genomic DNA from the Uganda and the Transvaal lions. PCR products were cleaned using the Quaquick PCR kit (Qiagen), ligated into pGEM-T (pGEM®-T Easy Vector System, Promega), and transformed into JM109 High Efficiency Competent cells (Promega) following the Promega protocol. Plasmid DNA was isolated from positive transformants using Wizard® Plus SV Miniprep Purification Kit (Promega). Inserts were amplified using CYTB primers (Table 2) and sequenced on both strands using a Beckman/Coulter CEQ2000XL sequencer. A total of 10 clones from the Uganda lion and 8 clones from the Transvaal lion were sequenced. All sequences were repeated from the same PCR product and from a second PCR reaction to verify authentic substitutions from those due to PCR artifact.

To ensure that individuals within populations, included in this study, were not related along matrilines, the highly variable repetitive Rs3 portion of the control region, found only in the mitochondria (Hoelzel et al. 1994; Cracraft et al. 1998) was amplified using TaKaRa Ex Taq™ and sequenced for each lion (Table 2). A pair of female siblings and three offspring, spanning three generations, were included in the data set to examine potential haplotype variability among known first-order relatives.

Sequence analysis

Sequences were aligned using the sequence editor and multiple alignment operations of MacDNASIS v3.2 (Hitachi Software Engineering America,

Ltd.). Since these genes have reduced intraspecific variation, and they yielded similar results when analyzed separately, they were combined to enhance the phylogenetic signal. The sequences were analyzed using Phylogenetic Analysis Using Parsimony (PAUP) Beta Version 4.0B1 for Macintosh (Swofford 1998). An Amur tiger, *Panthera tigris*, was included in the analyses for outgroup rooting. Trees were constructed using either maximum parsimony or distance methods and branch reliability was evaluated with 1000 bootstrap replications. A standard error test of neighbor-joining interior-branch lengths (Felsenstein 1988; Nei 1996) was obtained using MEGA (v 1.01) (Kumar et al. 1993). The standard-error test calculates a confidence probability (CP) for each interior branch length, where $CP = 1-\alpha$, α being the significance level of type I error (Rzhetskky & Nei 1992; Takezaki et al. 1995).

In order to evaluate substitution rate constancy among haplotypes, Maximum Likelihood analyses were performed using PAUP. The transition-to-transversion ratio was used to compute log-likelihood values with and without a molecular clock. Substitution rate constancy was tested using the log-likelihood ratio (Felsenstein 1988; Nei 1996) and evaluated as a $\chi^2_{[0.05,df=n-2]}$ distribution.

The significance of the genetic variation was first evaluated among all lion populations using an Analysis of Molecular variation (AMOVA) (Excoffier et al. 1992) implemented in Arlequin Version 2001 (Schneider et al. 2000). Populations were then partitioned into three geographic groups for a two-factor AMOVA to test for significant subdivision among regions and populations within regions. The significance of the fixation indices, F_{ST}, F_{SC}, and F_{CT}, was determined with 1023 haplotype permutations at each level.

Results

DNA sequence

Twenty-six lions were compared for DNA sequence variation at the mitochondrial genes for CYTB (1140 bp) and ND5 (1222 bp) (Table 1). The complete ND5/6 sequence (2338 bp) was obtained for the Uganda and the Transvaal lions, and internal primers were designed from these sequences to amplify a portion of ND5 beginning at position 561 and ending at position 1782 for all other lions (Table 2).

Table 2. Primer pairs used to amplify and sequence each gene region. All Internal primers were developed for this study

Region	Site	Sequence	Source
CYTB	L-glu L14724	5' CGAAGCTTGATATGAAAAAC-CATCGTTG	Irwin et al. (1991)
	H-Thr H15915[a]	5' AACTGCAGTCATCTCCGGTTTA-CAAGAC	Irwin et al. (1991)
	CB538L	5' GCCTTCCACTTCATCCTTCC	Internal
	CB553H	5' GGATGAACTGGAAGGCAAAG	Internal
ND5	ND5L-Leu 763	5' AATAGTTTATCCATTGGTCT-TAGG	Georgiadis et al. (1994)
	ND5L-560	5' GTAGGATTTATCACGGCTATAG	Internal
	ND5H-560c	5' CTATAGCCGTGATAAATCCTAC	Internal complement
	ND5L-1180	5' TAATCATCGAGACAGCCAATAC	Internal
ND6	ND6H-530	5' CTAAGTAGGAGTAGGCTAAG	Internal
	ND6H-Glu 764	5' TTACAACGATGGTTTTTCATAT-CA	Georgiadis et al. (1994)
DLOOP	HCAT-Phe	5' ATTTTCAGTGTCTTGCTTT	Shankaranarayanan & Singh (1998)
	LDL	5' CATCTGGTTCCTACTTCAGG	Internal
	HCAT-221	5' GGCAAGACAGAAATAGACACG	Sequencing, internal

[a]For sequencing H-Thr H15915-s 5' TCATCTCCGGTTTACAAGAC was substituted.

Analysis of 16 cloned sequences, including sequence repeated from the same clone and the same PCR template, verified the initial CYTB haplotypes observed for the Uganda and Transvaal lions. The mitochondrial origin of these sequences are supported by complete translation (including ND5 and ND5/6), comparisons with multiple mammalian CYTB sequences reported by Irwin et al. (1991), and by close agreement with data reported by Arnason et al. (1995) and Cracraft et al. (1998). While the haplotype reported by Cracraft et al. (1998) was very similar to the haplotype found in the Transvaal region, the haplotype from Arnason et al. (1995) was not observed in any of the samples examined here. Two additional clones shared many substitutions with the nuclear pseudogene for tigers, reported by Cracraft et al. (1998) and were substantially different from our mitochondrial sequences. Both CYTB and ND5/6 sequences from these two lions are also concordant with sequence reported for the domestic cat (Lopez et al. 1996).

The control region Rs3 sequence was similar to that reported by Shankaranarayanan and Singh (1998) and Cracraft et al. (1998), and was identical among the two sibling females and their offspring, indicating that this region can detect maternally related individuals. Sequences differed in repetitive pattern and length among 25 of 26 individuals included in this study (unpublished data) suggesting these individuals represent unrelated matrilines. Two lions from the Umfolozi population shared the same haplotype, a finding that was not surprising since this population was founded by two Transvaal females and one Mozambique male (Anderson 1980; Maddock et al. 1996).

Overall, sequence variation for the combined genes (2362 bp) were suggestive of geographic differentiation (Table 3). Among the geographic samples there were six haplotypes that contained 26 nucleotide substitutions, one of these a transversion. The only haplotype polymorphism within a population occurred in Etosha National Park. Five nucleotide substitutions were non-synonymous. For CYTB, the Namibian/Botswana populations and the Sabi Sands lions each had one amino acid substitution, and a third was shared by both. For ND5, one amino acid substitution was found in the Transvaal group (KPR, FRR, TSV) but not in Umfolozi and another was observed in the Sabi Sands lions.

The rate of substitution is most likely constant over these lion lineages as indicated by a non-significant log-likelihood ratio test (Takezaki et al.

Table 3. Nucleotide substitutions within the mitochondrial genes, NADH dehydrogenase subunit 5 and cytochrome *b*, among 12 populations of lion, *Panthera leo*, located throughout eastern and southern Africa

			ND5	CYTB
		Nucleotide Position:	1111	111111
			334560011	347778000111
			258190861512	34470169258123
Haplotype	(N)	Population	520679998194	15219924302621
1	(2)	Uganda,Aberdare(ANP)	GTTTTTGCGGGT	GTCGTTCCGCTTTC
2	(5)	Tsavo (TSV)	ACC.CCATAA..	.......TAT.CC.
2	(3)	Transvaal(KPR,FRR)	ACC.CCATAA..	.......TAT.CC.
3	(3)	Umfolozi (UMF)	ACC.CCA.AA..	.......TAT.CC.
4	(3)	Sabi Sands(SSR)	ACCCC.A..AAC	.CT.CCT.A.C.C**G**
5	(2)	Botswana (BOT)	ACCCC.A..A..	A.TA..T.A.C.C.
5	(2)	Caprivi (CAP)	ACCCC.A..A..	A.TA..T.A.C.C.
5	(2)	Bushmanland(BML)	ACCCC.A..A..	A.TA..T.A.C.C.
5	(2)	Etosha (ETO)	ACCCC.A..A..	A.TA..T.A.C.C.
6	(2)	Etosha (ETO)	ACCCC.A..A..	A.T...T.A.C.C.
	(1)	*P. tigris*	A.CC.C.TCAA.	A.T.C.T.A...CT

The Amur tiger, *P. tigris*, nucleotide is included for those positions that vary within lions. Total sequence length is 1222 bp for ND5 and 1140 bp for cytb. "." denotes a base that is identical to the first row nucleotide; boldface identified the single transversion ($Ti/Tv = 25/1$ among lion populations). N = sample size.

Table 4. Average genetic distances among geographic populations of African lion, denoted by haplotype number 1 through 6, and an Amur tiger outgroup, based on uncorrected "*p*", the total number of pairwise differences/total number of nucleotide sites

1-Uganda[a]		2-Tsavo[b]	3-Umfolozi	4-SabiSands	5-Etosha[c]	6-Etosha
Tiger	0.101	0.101	0.101	0.100	0.099	0.100
1	–	0.006	0.006	0.008	0.006	0.006
2		–	0.0004	0.007	0.005	0.005
3			–	0.006	0.004	0.004
4				–	0.003	0.003
5					–	0.0004
6						

Kimura-two-parameter values were identical
[a]Includes Uganda (UGD), Aberdare (ANP)
[b]Includes Tsavo (TSV), Transvaal (KPR), Fannie Roberts (FRR)
[c]Includes Etosha (ETO), Caprivi (CAP), Bushmanland (BML), Botswana (BOT)

1995; Nei 1996). Genetic distances (Table 4), averaged 10% between *P. leo* and *P. tigris* and less than 1% among all African lion populations. Comparisons of CYTB with the *P. leo* reported by Arnason et al. (1995) yielded an average distance value of $D = 1.2\%$ (range 1.0% with FRR to 1.4% with SSR).

Phylogeographic analysis

Parsimony analysis of the combined sequences (PAUP), including the tiger for outgroup rooting, produced two trees of 259 steps, with a consistency index of 0.973 (Figure 2a). There were 17 parsimony-informative characters. In the two most parsimonious trees, the position of Etosha haplotype #6 was unstable, grouping with the Etosha lions or remaining unresolved. The Sabi Sands haplotype remained in an unresolved polytomy.

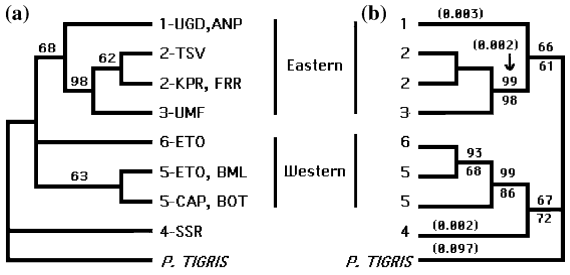

Figure 2. (a) The 50% majority-rule consensus parsimony tree. Bootstrap values (above each branch) were obtained using the branch-and-bound algorithm and sequence addition "as is". (b) Distance-based Neighbor Joining phylogram obtained using minimum evolution and either uncorrected '*P*' or Kimura's-two-parameter method. Bootstrap values are above the line before the corresponding node and confidence probabilities (interior branch test, from MEGA) are below the line. Branch lengths in parentheses are above the four longest branches (all remaining branches are $\leqslant 0.0008$).

Distance-based analysis (Figure 2b) produced a slightly different topology showing two clades. The southwestern and Sabi Sands haplotypes are united in one clade and all eastern haplotypes were placed together in a second clade with high bootstrap values. Only one confidence probability was significant for the eastern cluster.

Maximum Likelihood analysis, without the molecular clock, produced a topology similar to the parsimony tree (Figure 2a), except the Sabi Sands haplotype was united with the eastern clade at a position basal to the Uganda haplotype. When the molecular clock was enforced, the resulting tree was identical to the neighbor-joining distance tree (Figure 2b).

The single-factor AMOVA showed that 99.5% of the variation is due to differences among populations, the remaining due to the polymorphism observed in the Etosha lions. When this variation was partitioned geographically into Uganda–western Kenya, eastern savannas, and western savannas, the majority of the variance, 62.3%, was among groups ($F_{CT} = 0.623$, $P < 0.005$), while 36.6% was among populations ($F_{ST} = 0.988$, $P < 0.001$). These results indicate a high level of genetic structuring among populations and even greater divergence across geographic regions.

Discussion

Systematic relationships

Mitochondrial sequence variation among African lions is modest and supports the current view that all extant African lions belong to a single subspecies (Ellerman et al. 1953; Meester & Setzer 1971 (as revised 1977); O'Brien et al. 1987). Without clear evidence to indicate that Barbary lions differed genetically from other African lions, this taxon should be called *Panthera leo leo*. Although the name *Panthera leo leo* is based on the now extinct Barbary lion (Allen 1939), applying other subspecific names from the extensive synonymy of *Panthera leo* should await a comprehensive analysis of this species across its extensive geographic and temporal range and include both genetic and morphological diagnoses.

Geographic variation

Lions exhibit sex-biased dispersal, in which male offspring leave the natal group at puberty, whereas females may remain in their natal area for life (Schaller 1972; Pusey & Packer 1987; Spong et al. 2002). Observed regional sequence differentiation among lion populations for these two conserved mitochondrial genes supports female fidelity to natal regions.

Distance-based analyses identified two major geographic clades of lions: those in southwestern Africa and those to the east, extending from eastern Kenya south to KwaZulu-Natal. A similar east-southwest dichotomy among genetic haplotypes was observed in seven African bovids (Arctander et al. 1999; Nersting & Arctander 2001; Pitra et al. 2002; Van Hooft et al. 2002); cheetah (*Acinonyx jubatus*)(Freeman et al. 2001); black rhinoceros (*Diceros bicornis*)(Brown & Houlden 2000); and African elephant (*Loxodonta africana*)(Georgiadis et al. 1994). Many additional wide-ranging mammals show range disjunctions that coincide with this haplotype distribution (see maps in Kingdon 1997).

The eastern lions can be further subdivided along each side of the Great African Rift that stretches into South Africa. The lion haplotype from Tsavo, Kenya was identical to the haplotype from the Transvaal region, yet differed considerably from the haplotype found at Aberdares National Park in western Kenya. A similar pattern of variation across the Rift Valley was also reported for sable (*Hippotragus niger*) (Pitra et al. 2002); wildebeest (*Connochaetes taurinus*) and impala (*Aepyceros melampus*) (Templeton & Georgiadis 1996; Arctander et al. 1999); and African wild dogs (*Lycaon pictus*) (Girman et al. 2001). The distinctness and basal position of the Ugandan haplotype suggest isolation and independent evolution by lion populations north of the Congo Basin and west of the Rift, or reflect an ancestral polymorphism alternately fixed by lineage sorting (Lieberman & Vrba 1995; Tosi et al. 2000). AMOVA analysis suggest that the significant portion of genetic variation is found among geographic regions while populations within regions are less divergent most likely due to a shared evolutionary history.

The Sabi Sands haplotype was unresolved by parsimony. Maximum Likelihood placed it basal to the Ugandan haplotype and distance-based analysis grouped it with the southwestern lions at a basal node. Several ancestral and derived substitutions are shared with the southwestern group and the Ugandan haplotype suggesting some type of historical connection. Finding a divergent haplotype in the Sabi Sands area, bounded to the north and south by the haplotype shared by Tsavo and Transvaal lions was surprising. Collection of additional samples from Kruger National Park would be a future priority.

Additional surveys are needed to expand the geographic coverage into West Africa, Tanzania, Angola, Zambia, and Zimbabwe. Collection of samples between KwaZulu-Natal and Botswana in South Africa will be especially important. A geographic contact zone was observed between *Diceros bicornis* subspecies in Namibia and KwaZulu-Natal (Swart & Ferguson 1997) and between distinct east and southwest wild dog populations thought to be isolated (Girman et al. 2001). Until these central regions of Africa are more thoroughly studied, conclusions about the distinctness of the eastern and southwestern clades cannot be made.

Conservation implications

While free ranging lion populations appear to be prevalent in some areas of their distribution, the African Lion Working Group recently estimated

that as few as 22,600 lions remain in Africa, perhaps 10% of the number living there only 25 years ago (Loveridge et al. 2002). Consequently, there are an increasing number of lions in peripherally isolated populations or confined in reserves and zoological parks that are closed to gene flow. Management of these populations must balance the need to maintain stable densities at or below the carrying capacity of the reserve and, at the same time, minimize loss of genetic variability through drift or inbreeding. The management history of the Hluhluwe-Umfolozi Park lions exemplifies how difficult this task is (Anderson 1980; Maddock et al. 1996).

Ideally, translocations to increase genetic diversity would mimic natural gene flow by moving only individuals from the nearest areas with similar haplotypes. However, lions have already been translocated from Namibia into southeast Africa, and appear to have successfully reproduced there. For long-term management, however, a geographic determination of conservation units should be made. The unit for conservation management has been the subject of much discussion (see Cracraft et al. 1998; Bininda-Emonds et al. 2000; Crandall et al. 2000 and references contained in these papers) and depends on taxonomic, ecological and genetic factors. At this time, ecological and genetic exchangeability have not been studied in translocated lions in a controlled fashion.

Phylogenetic data from this study suggest there are at least four lion groups – the southwestern populations, the populations to the east and west of the rift valley and the Sabi Sands population. While taxonomic distinctions await further sampling for resolution, these regions might define evolutionary significant units (ESU) as defined by Crandall et al. (2000).

Finally, conservation management plans should consider disease status as an overriding factor for locating a source population for genetically depressed populations. Lion populations are being exposed to various diseases, such as canine distemper (Alexander et al. 1996; Roelke-Parker et al. 1996), rabies (Kat et al. 1995), and tuberculosis (Moore 1999), with catastrophic results. The effects of the feline lentivirus or feline immunodeficiency virus (FIV) are still poorly understood, but may also play a significant role in their ultimate survivability (Briggs, personal communication). Translocating diseased lions or lions carrying a latent virus could potentially harm the resident population particularly if they have become inbred with reduced immune (MHC) variability (Gilbert et al. 1991). Plans for translocations should include an assessment of disease exposure. Future genetic studies should include additional individuals, locations, and nuclear genes, such as microsatellites, that will provide more detailed information regarding population diversity to guide veterinary and management efforts for health and conservation purposes.

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