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CHAPTER

6

Conservation Genetics of the Cheetah: Genetic History and Implications for Conservation

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INTRODUCTION

The cheetah (*Acinonyx jubatus*) is one of the most recognized examples of the important links between evolutionary history, genetic variation, and conservation. Its value to the biodiversity of the world is not only warranted by its unique physical characteristics, such as being the fastest land mammal (Chapter 7), but also its unique evolutionary lineage as the only extant representative of its genus, *Acinonyx*. Concerns over levels of genetic variation among cheetahs were first raised as captive programs grappled with difficulties in breeding cheetahs (Chapter 27). These observations led to research investigating the biological basis of the low rates of captive breeding success (10%–15%) and the concurrent high rate of infant mortality (29%) (O'Brien et al., 1985). This research led to the discovery of low levels of genetic diversity in the cheetah, which were attributed to one or several severe population bottlenecks. As a consequence, debates arose regarding the impact of low genetic diversity on the survival of the species, and the cheetah has been featured in genetic textbooks since the 1980s. Early research on cheetah represented one of the first studies in the new field of conservation genetics.

In the last century, cheetah numbers have declined drastically due to loss of habitat and prey, persecution due to real or perceived livestock depredation, and removal from the wild to supply captive facilities and private individuals (Chapters 10, 11, 13, and 14). The reduction in numbers and fragmented distribution add to the urgency to preserve the genetic diversity left today.

In this chapter, we review the current status of cheetah genetics and its impact on the species' conservation. While publications on cheetah genetics may appear contradictory at times, the fundamental conclusions have been consistent for over 30 years, with various measures confirming low genetic diversity (section "Genetic Diversity"), which was shown to have originated thousands of years ago (section "Historic Demography"), and with a relatively recent divergence of extant published subspecies (section "Subspecies Definition and Divergence"). The differences debated among geneticists only affect the interpretation of genetic results regarding precise timing of events and extent of reduced genetic diversity. This chapter also covers the cheetah's phylogenetic (evolutionary relation based on genetic data) position among other felids (section "Species-Level Taxonomy"), the genetic structure of the subspecies and within geographical regions (section "Phylogeography"), an overview of additional genetic studies including kinship (section "Additional Insights Into Cheetah Genetics"), and implications of genetic findings for cheetah conservation (section "Discussion").

SPECIES-LEVEL TAXONOMY

The cheetah is a member of the family Felidae (Fig. 6.1), which comprises 41 living species that are distributed throughout the world, with the exception of Australasia and the polar regions (Kitchener et al., 2017). One of the most striking aspects of molecular genetic studies in the Felidae was how rapidly felids evolved into eight different lineages (over a 6-million-year period), each with unique biogeographical histories. Earlier groupings of felid lineages were largely based on morphological features and life-history patterns. The cheetah was generally considered to be an early divergence from the felid radiation due to some of its unique adaptations, including its incompletely retractile claws (Chapter 7). However, the advent of genetic approaches has provided clarity to more confidently reconstruct felid evolutionary history, and today the cheetah is included in the Puma lineage, which was the sixth of eight lineages to branch off during felid evolution [7 million years ago (MYA); Johnson et al., 2006; Li et al., 2016; Werdelin et al., 2010; Fig. 6.1]. The cheetah's closest living relatives are known to be the puma (*Puma concolor*) and the jaguarundi (*Herpailurus yagouaroundi*)

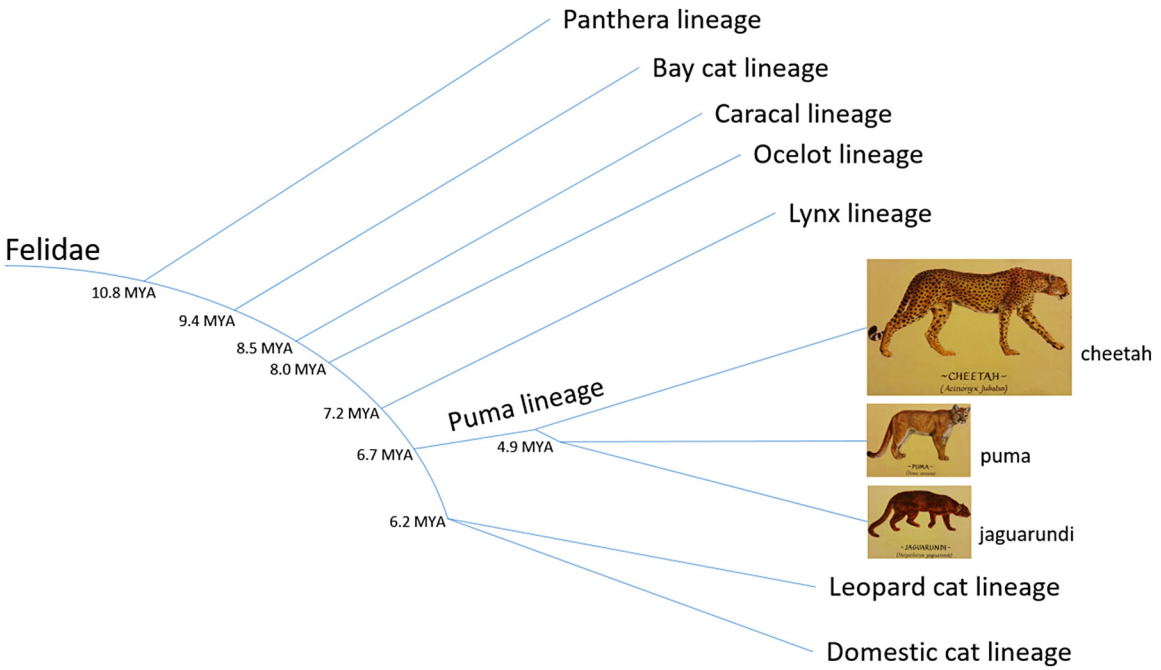

FIGURE 6.1 **Phylogenetic tree depicting the cheetah (*Acinonyx jubatus*) within the *Puma* lineage, relative to the other lineages of extant felid species.** Time of divergence for each lineage is indicated at the base of the branch in million years ago (MYA). The figure is based on the molecular data presented in Johnson et al. (2006).

(Johnson et al., 2006; Li et al., 2016; Werdelin et al., 2010; Fig. 6.1), with whom the cheetah likely shared a common ancestor and evolutionary history prior to their divergence. Today the cheetah is found in the Old World, and the puma and jaguarundi in the New World (Chapter 3). The cheetah has 19 chromosomal pairs (i.e., 38 chromosomes) like most felid species (O'Brien et al., 2006). The cheetah is the only extant representative of its genus.

GENETIC DIVERSITY

Genetic variation (polymorphism) forms the raw material of evolution. Novel variation rises from DNA mutations. DNA mutation rates that alter the amino acid sequence and may be detectable in allozyme migration rates (section "Allozymes") are several orders of magnitude slower than those observed in the mitochondrial DNA (section "Mitochondrial DNA") or in repetitive elements in nuclear DNA, such as microsatellites (section "Microsatellites"). While significant gain of genetic variation is limited by mutation rates and takes numerous generations, variation can be lost within a single generation if only a subset of the population reproduces due to either high death rates or a high number of nonreproducing individuals.

Allozymes

The first study to indicate reduced genetic diversity of the cheetah documented low variation at protein-based markers compared to other felids and mammals (O'Brien et al., 1983; O'Brien et al., 1985; Table 6.1). Allozymes are

TABLE 6.1 Overview of Studies on Genetic Diversity in the Cheetah

Marker detail[a]	*N* (region)[b]	Polymorphism[c]	Diversity[d]	Study	Comparison
ALLOZYMES/SOLUBLE PROTEINS					
47 allozyme loci	55 (S)	0 (0%) pol loci		O'Brien et al. (1983)	8%–12%[e]
155 sol. prot. loci	55 (S)		0.013 Ho	O'Brien et al. (1983)	0.03–0.07 Ho[e]
52 allozyme loci	55 (S)	0 (0%) pol loci		O'Brien et al. (1985)	
49 allozyme loci	30 (E)	2 (4%) pol loci	0.014 Ho	O'Brien et al. (1987)	
	43 (S)	1 (2%) pol locus	0.0004 Ho		
MITOCHONDRIAL MARKERS (mtDNA)					
505 nt (28 RFLP)	39 (E), 35 (S)	6 variants, 7 hapl	0.182% div	Menotti-Raymond and O'Brien (1993)	
525 nt (all CR)	1 (E), 17 (S), 2 (NE)	15 variants	1.31% div	Freeman et al. (2001)	4.16–7.45%[f]
915 nt (221 CR)	29 (S), 26 (NE), 11 (E), 1 (N), 11 (Asia)	29 pol sites, 18 hapl	0.66% div	Charruau et al. (2011)	N/A[g]
Whole genome	4 (S)		0.071% div	Dobrynin et al. (2015)	
	3 (E)		0.008%div		
MICROSATELLITE MARKERS					
Whole genome (2 probes; RFLP 3 ez)	15–17 (E and S)	167 pol fragments	0.435 Ho	Menotti-Raymond and O'Brien (1993)	
10 loci (random)	5 (S), 5 (E)		0.39 Ho	Menotti-Raymond and O'Brien (1995)	0.61–0.77 Ho[h]
82 loci (random)	10 (NE)		0.44 Ho	Driscoll et al. (2002)	0.08–0.63 Ho[i]
	20 (S)		0.44–0.46 Ho		
38 loci	98 unrel (S)		0.64–0.70 He	Marker et al. (2008)	
13 loci	147 (E)		0.65 He	Gottelli et al. (2007)	
18 loci	27 (S)		0.70 He	Charruau et al. (2011)	
	25 (NE)		0.67 He		
14 loci	32 (S)		0.62 He	Dalton et al. (2013)	
MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)					
MHC I (RFLP 4 ez)	9 (S)		0.05 Ho	Yuhki and O'Brien (1990)	0–0.51 Ho[j]
	13 (E)		0.07 Ho		
MHC I (~1100 nt seq)	2 (S?)	2 alleles		Yuhki and O'Brien (1994)	
MHC I (SSCP)	108 (S)	10 alleles		Castro-Prieto et al. (2011)	
MHC I (seq)	4 (S), 3 (E)	11 alleles		Dobrynin et al. (2015)	136 alleles[f]
MHC II-DRB (RSCA)	25	5 alleles		Drake et al. (2004)	

TABLE 6.1 Overview of Studies on Genetic Diversity in the Cheetah (*cont.*)

Marker detail[a]	*N* (region)[b]	Polymorphism[c]	Diversity[d]	Study	Comparison
MHC II-DRB (SSCP)	139 (S)	4 alleles		Castro-Prieto et al. (2011)	14/52 alleles[k]
MHC II-DRB (seq)	4 (S), 3 (E)	7 alleles		Dobrynin et al. (2015)	54 alleles[f]
WHOLE GENOME SEQUENCING					
Whole genome	4 (S), 3 (E)		0.02% div	Dobrynin et al. (2015)	

Studies are organized by marker type. Details about the marker and the number of animals tested from each region (Columns 1, 2) as well as the measured outcome (Columns 3, 4) are shown. Values from other species are indicated for comparison (Column 6).

[a] *CR, control region; ez, restriction enzyme; nt, nucleotide; RFLP, restriction fragment length polymorphism; RSCA, reference strand mediated conformational analysis; seq, sequencing; sol. prot., soluble proteins; SSCP, single strand conformation polymorphism.*

[b] *E, eastern Africa; NE, northeastern Africa; S, southern Africa; unrel, unrelated.*

[c] *hapl, Haplotypes; pol, polymorphic.*

[d] *He, expected heterozygosity; Ho, observed heterozygosity; % div, % nucleotide diversity.*

[e] *25 loci in caracal, Caracal caracal; leopard, Panthera pardus; lion, Panthera leo; ocelot, Leopardus pardalus; tiger, Panthera tigris; Newman et al. (1985).*

[f] *In domestic cat, Felis catus; same study.*

[g] *Not comparable to other studies due to other studies covering different mitochondrial regions.*

[h] *In domestic cat, lion, puma, Puma concolor; same study.*

[i] *0.08–0.16 Ho in lions from the Gir forest, pumas from Florida; 0.33–0.47 in lions from Serengeti and Ngorongoro, pumas from Idaho; 0.63 in domestic cat; same study.*

[j] *0–0.08 Ho in lions from the Gir forest and Ngorongoro; 0.17–0.51 in domestic cat, lions from the Serengeti, mole rat, human; same study*

[k] *14 alleles in 14 Bengal tigers; Pokorny et al. (2010); 52 in 25 Gir; Sachdev et al. (2005).*

variant forms of enzymes and can be detected with electrophoretic analyses. The observed differences of protein migration reflect differences at the amino acid level, which correspond to alleles (alternative genetic variants) of the corresponding gene(s) (locus/loci) at the DNA level. The percentage of polymorphic allozyme loci within several mammal species was estimated to range from 15% to 60% (O'Brien et al., 1987). Thus, it was surprising that analyses of 55 southern African cheetahs failed to identify genetic polymorphisms across a total of 52 allozyme loci (O'Brien et al., 1983; O'Brien et al., 1985; Table 6.1), and that additional analyses only identified 3 polymorphic loci in 73 eastern and southern African cheetahs [observed heterozygosity (differing alleles at a given locus/set of loci; measure of diversity) = 0.014 and 0.0004, respectively; O'Brien et al., 1987; Table 6.1]. Presence of multiple protein forms at 155 abundant soluble protein loci within individual cheetahs was also low (observed heterozygosity = 0.013) compared to 7 other felid species (O'Brien et al., 1983; Table 6.1).

However, insights from these studies were limited, because protein-based markers only assess amino acid changes that alter the electrophoretic mobility of the protein. DNA changes, such as silent/synonymous substitutions are masked in proteins, resulting in an underestimation of the amount of genetic variation in all species. In addition, protein-based markers are more likely to be linked to functional differences and as such allele frequency differences may be susceptible to selective pressure (environmental conditions affecting survival of organisms with a particular characteristic). The subsequent development of DNA-based markers provided more detailed information about the degree of genetic diversity in cheetahs.

Skin-Graft Acceptance

Concurrently with allozyme studies, functional studies demonstrated that reciprocal skin allografts between 12 unrelated cheetahs and 2 siblings showed no signs of acute graft rejection, whereas xenografts (from domestic

cat, *Felis catus*) were rapidly rejected (O'Brien et al., 1985). These results were attributed to reduced *functional* allelic variation at the cheetah's major histocompatibility complex (MHC), an important immune gene family, which encodes cell surface proteins responsible for distinguishing foreign from self molecules. The inferred reduced functional variation was ultimately supported by molecular studies (section "Major Histocompatibility Complex").

Mitochondrial DNA

As sequencing techniques became available, low levels of genetic variation were also observed in mitochondrial DNA (mtDNA). mtDNA is independent from the nuclear genome, and represents the maternal demographic history. The mitochondrial genome evolves faster than the nuclear coding genome, with the control region (CR) being the most rapidly evolving region of the mitochondrial genome. mtDNA, in particular the CR, has been informative for investigations of diversity patterns, population structure, and phylogeography (phylogenetic structure in relation to location). The complete cheetah mtDNA genome has 17,047 bp (Burger et al., 2004) and has 91% similarity with the mtDNA genome of the domestic cat (Lopez et al., 1996).

In the 1990s, a study based on restriction fragment length polymorphism (RFLP) inferred low levels of nucleotide variation (0.18% diversity) in cheetah mtDNA relative to comparable studies in other species (Menotti-Raymond and O'Brien, 1993; Table 6.1). In 2001, nucleotide variation in the mtDNA-CR in 20 cheetahs was observed to be relatively low (1.31%; Freeman et al., 2001; Table 6.1). Nucleotide variation was even lower when a short sequence of the mtDNA coding region was included with the mtDNA-CR (Charruau et al., 2011), or when the entire mtDNA genome was evaluated (Dobrynin et al., 2015; Table 6.1). Dobrynin et al. (2015) found a 90% reduction in nucleotide variation across 7 cheetahs (4 Namibian, 3 Tanzanian) relative to other mammals (Dobrynin et al., 2015; Table 6.1).

Microsatellites

Microsatellites (nuclear DNA markers consisting of variable numbers of tandem repetitions of 2–6 nucleotides; also called STR or short tandem repeats) accumulate new variation quickly as they have high mutation rates (several orders of magnitude higher than DNA coding for proteins), and are usually not associated with any function (i.e., they do not code for a protein) and thus are not subjected to selection pressure. These markers have been used widely in conservation genetics, population genetics, and wildlife forensics. Most non-domestic felid studies have selected a subset from 583 polymorphic microsatellite loci, which were mapped and characterized in the domestic cat (Menotti-Raymond et al., 2003).

Initial studies, with 10 microsatellite markers in 10 cheetahs, demonstrated low observed heterozygosity (0.39; Menotti-Raymond and O'Brien, 1995; Table 6.1). Expected heterozygosity was slightly higher (0.46–0.48) when measured in 82 randomly selected microsatellite markers in 30 cheetahs (Driscoll et al., 2002; Table 6.1). These estimates were comparable to the heterozygosity levels of other felids in small isolated populations (e.g., pumas from Idaho, lions from Serengeti and Ngorongoro crater), but lower than in domestic cats (Driscoll et al., 2002; Table 6.1). Subsequent studies of cheetahs only included a subset of these microsatellite markers, which were not selected randomly, but instead for their known relatively high level of polymorphism, leading to higher heterozygosity estimates. Hence, these increased heterozygosity estimates do not reflect the genetic diversity of the cheetah species *per se*, but are summarized in Table 6.1 for informational purposes.

Terrell et al. (2016) suggested a reduction in genetic diversity in the wild population from a dataset spanning 30 years. The study was based

on 46 individuals born between 1976 and 2007 from South Africa and Namibia, which were genotyped (characterized on a genetic level) with 12 microsatellite markers.

While microsatellite markers demonstrated levels of heterozygosity in the cheetah that were not always significantly lower than for other species (Table 6.1), this does not contradict findings of low genetic diversity in cheetahs at other, more slowly evolving markers. It merely indicates that the variation at microsatellite loci is more recently evolved in origin. The length of time needed to accumulate this new microsatellite variation can inform estimates of the timing of events that led to the loss of genetic variation (section "Historic Demography").

Major Histocompatibility Complex

The MHC is one of the most polymorphic loci known in vertebrates and has important immune functions. An increasing number of studies have documented an association between the diversity of MHC genotypes or individual alleles with disease susceptibility in wildlife, thus confirming the importance of the selection pressure from pathogens on the MHC (reviewed in Sommer, 2005).

Most comprehensive studies of the MHC in felids (also known as the feline leucocyte antigen), have been conducted in the domestic cat (Winkler et al., 1989). The cat is a good model as the general architecture of the MHC appears relatively conserved within each class of vertebrates; the number of MHC class I or II genes, however, can vary substantially among species. The domestic cat MHC region is located on chromosome B2 and includes 19 MHC I and 8 MHC II genes (Yuhki et al., 2008). The cheetah MHC sequence resolved 278 genes with complete homology to the domestic cat for all MHC II and most MHC I genes. Its structural organization was also found to be highly similar to that of the domestic cat (Dobrynin et al., 2015).

An early study of cheetah MHC I based on RFLP markers showed reduced genetic diversity in cheetah (observed heterozygosity = 0.05–0.07) compared to other species, which was only comparable to that of lions from isolated populations (Gir Forest and Ngorongoro Crater; Yuhki and O'Brien, 1990; Table 6.1). Only 2 MHC I alleles were identified in 2 individual cheetahs through sequencing (Yuhki and O'Brien, 1994; Table 6.1) and 5 MHC II-DRB alleles were identified in 25 individuals through Reference Strand-Mediated Conformational Analysis (Drake et al., 2004; Table 6.1). Castro-Prieto et al. (2011) identified 10 unique MHC I and 4 MHC II-DRB alleles in 108 and 139 Namibian cheetahs, respectively (Table 6.1). While a 5-fold increase in the number of MHC I alleles identified in 2011 may appear like an increase in genetic diversity, the identification of only 8 additional alleles despite a 54-fold increase in the number of study animals is in fact further confirmation of low levels of allelic diversity in the cheetah. The low level of allelic diversity was further confirmed by comparison to other species, which harbor more alleles in fewer individuals (Table 6.1). More recently, a 95%–98% reduction in single nucleotide variants (SNVs) was observed when the complete MHC sequence of 7 cheetahs (4 Namibian, 3 Tanzanian) was compared to that of human (*Homo sapiens*), dog (*Canis familiaris*), and an outbred domestic cat; only 11 variants affecting the amino acid sequence were detected in the MHC I coding region of cheetahs (Dobrynin et al., 2015; Table 6.1).

Whole Genome Sequence Variants

Dobrynin et al. (2015) described patterns of diversity across the entire genome of the cheetah. Five commonly employed metrics confirmed the genic and genomic lack of diversity of the species: SNV incidence was 90% less than that observed in a feral domestic cat; SNV density was 8–15× less than in the domestic cat,

European wildcat (*Felis silvestris silvestris*), or human (however it was higher than in lions from the Gir Forest); regions of continuous homozygosity (identical alleles at a given locus/set of loci) were 10–15× longer than in the domestic cat; heterozygosity levels were 15%–61% of the levels observed in the domestic cat, tiger (*Panthera tigris*), and human; SNVs in coding genes were 98% reduced compared to the domestic cat or European wildcat (Dobrynin et al., 2015; Table 6.1; Fig. 6.2).

Since the initial discovery of reduced genetic diversity in the cheetah in the 1980s, conservation, and scientific interest have turned toward identifying its cause (section "Historic Demography") and assessing the impact of low genetic diversity on the cheetah's chances of long-term survival (section "Importance of Low Genetic Diversity on Cheetah Survival").

HISTORIC DEMOGRAPHY

The cumulative results, indicative of reduced genetic diversity in the cheetah, were consistent with a genetic bottleneck or a series of demographic reductions over time and space. Menotti-Raymond and O'Brien (1993) proposed a scenario of distant past, rather than recent, reduction in the global population size. The demographic event causing this drastic loss of diversity was estimated to have occurred during the end of the Pleistocene (10,000–12,000 years ago; Table 6.2). This proposal was based on the time estimated for the near-reconstitution of genetic variation at rapidly evolving minisatellite (nuclear DNA marker consisting of variable numbers of tandem repetitions of 5–50 nucleotides) loci. The authors obtained similar estimates with mtDNA RFLP data calibrated on estimates of divergence of the species from the *Panthera* genus. This estimated timeframe was later corroborated with 82 microsatellite markers (Driscoll et al., 2002), with the time needed for nuclear alleles to reach fixation in the MHC (Castro-Prieto et al., 2011), and with whole genome data (Dobrynin et al., 2015) (Table 6.2). In the Asiatic cheetah population a more recent, independent, bottleneck was inferred based on a significant heterozygosity excess observed in the 18 microsatellite loci tested (Charruau et al., 2011).

Several alternative hypotheses to the single bottleneck scenario have been proposed to explain the severe loss of genetic variation. First, that the uniformity resulted from a persistent low effective population size (*Ne*; theoretical number which roughly reflects the number of animals genetically contributing to the population), possibly resulting from the high reproductive variance linked with the cheetah's presumed polygynous mating system (Pimm et al., 1989). Second, that low effective population sizes were maintained by a continuous cycle of extinction of subpopulations followed by recolonization, that is, metapopulation dynamics (Gilpin, 1991; Hedrick, 1996; Pimm et al., 1989).

The availability of SNVs derived from whole genome data of individuals from both eastern and southern Africa permitted more robust analyses of historical demographic patterns (Dobrynin et al., 2015). These analyses support the premise that cheetah populations expanded uniformly following a founder event 100,000 years ago. This was followed by a more recent split into an eastern and southern population, which were subjected to a bottleneck around 10,000–12,000 years ago. An alternative scenario of a gradual decline in the effective population size was supported by analyses of diploid whole genome sequence data to estimate past population sizes (Table 6.2).

Fabiano et al. (in preparation) suggested a gradual decline in population numbers, commencing at least 20,000 years ago, based on different coalescent-based approaches applied to published microsatellite profiles in the Namibian cheetah. While there was evidence

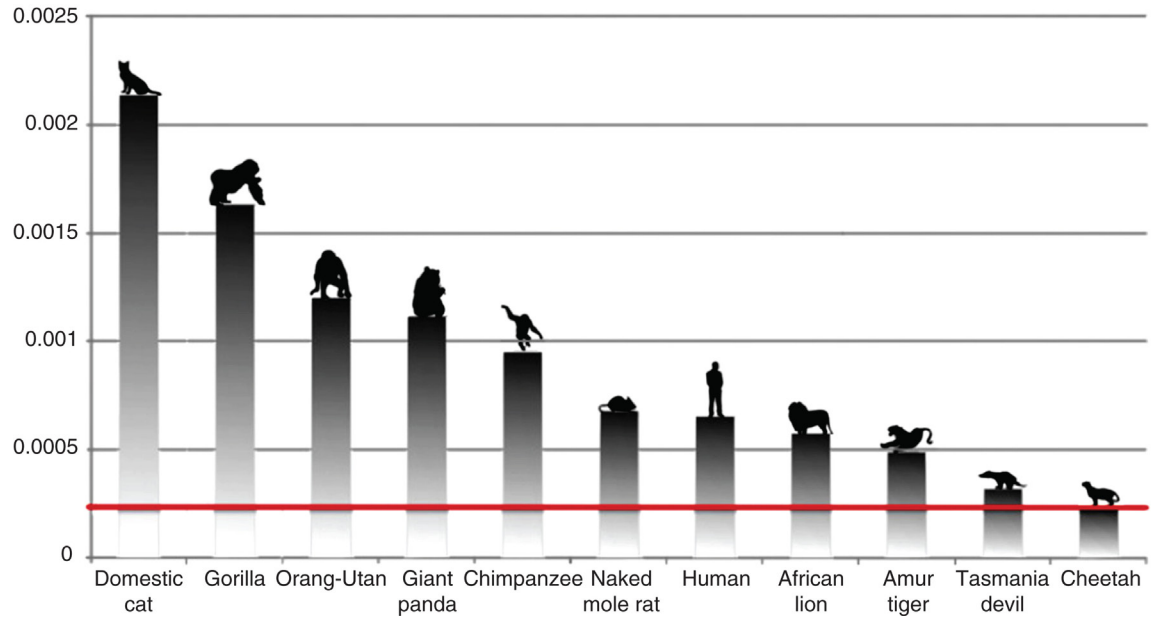

FIGURE 6.2 **Rates of single nucleotide variants (SNV) representing the diversity of the cheetah (*Acinonyx jubatus*) genome relative to other mammal genomes.** SNV rates were estimated for one individual per represented species using all variant positions, without filtering for repetitive regions. *Source: Reprinted from Dobrynin et al., 2015.*

of a continuous decline during this time period, some methods suggest an accelerated decline around 10,000 and 13,000 years ago (Table 6.2).

SUBSPECIES DEFINITION AND DIVERGENCE

Prior to the availability of genetic data, the species' taxonomy was based on morphological and geographical information. According to these taxonomic criteria, the extant cheetah populations were classified into four African and one Asiatic subspecies (Smithers, 1975), namely: *A. jubatus hecki* Hilzheimer, 1913 in northwest Africa; *A. j. raineyi* Heller, 1913 (for which the name *ngorongorensis* Hilzheimer, 1913 has priority) in east Africa; *A. j. jubatus* Schreber, 1775 in southern Africa; *A. j. soemmeringii* in northeast Africa; and *A. j. venaticus* Griffith, 1821 from north Africa to central India.

A. j. jubatus and *A. j. raineyi* were the first two subspecies to be assessed with molecular tools. Initial allozyme analyses in 1987 detected some minor differences (O'Brien et al., 1987; Table 6.3). The separation of the two sub-Saharan populations was further supported with mtDNA-CR (Freeman et al., 2001), microsatellites (Driscoll et al., 2002), and whole genome variation (Dobrynin et al., 2015; Table 6.3). The time of divergence was estimated to be a minimum of 4500 years ago by both Driscoll et al. (2002) (Table 6.3) and O'Brien et al. (2017). In 2017, based on their interpretation of the published evidence, the International Union for Conservation of Nature (IUCN) Cat Specialist Group's Cat Classification Task Force has suggested that *A. j. raineyi* and *A. j. jubatus* be synonymized into a

TABLE 6.2 Studies Investigating the Historic Demography of the Cheetah With Molecular Genetic Methods

Marker[a]	N	Generation time (years)[b]	Mutation rate/ calibration[c]	Model[d]	Time range (years ago)	Conclusion
Menotti-Raymond and O'Brien (1993)						
mtDNA RFLP (505 nt; 28 ez)	74	6	Cal: Panthera ancestor: 1.6–2.0 MYA	Molecular clock (1 initial hapl)	28,000–36,000	
Minisat fing prt (2 probes, 3 ez)	7–16	6	μ: 4.7×10^{-4}–1.7×10^{-3}	$1/\mu \times G$ (1 initial allele)	3,529–12,766	10,000–12,000
Driscoll et al. (2002)						
Microsatellite (82 loci)	20	6	μ: 5.6×10^{-4}–2.05×10^{-3}	$1/\mu \times G$	2,928–10,716	Min 12,000
				SMM	4,631–16,950	
Castro-Prieto et al. (2011)						
MHC I/II-DRB	108/139	2.4		Fixation $4N_e$	2,976–14,880	
Dobrynin et al. (2015)						
Whole-genome (1,8 M var. sites)	7	3	μ: 0.3×10^{-8}	DaDi	11,084–12,589	12,000
		3		PSMC	Since 100,000	Gradual decline
Fabiano et al. (in preparation)						
Microsatellite (31 loci)	89	2.4 and 6		Coal (DIYABC-FDA, MSVAR1.3, VarEff)	Since >20,000	Gradual decline[e]

Marker information, number of animals, main parameters (Columns 1–5), as well as outcomes (Columns 6 and 7) are shown for each study.

[a] *ez, Restriction enzyme; M var. sites, Million variable sites; Minisat fing prt, minisatellite fingerprint; mtDNA, mitochondrial DNA; nt, nucleotide; RFLP, restriction fragment length polymorphism.*

[b] *2.4 years (Kelly et al.,1998); 3 years (modified from Kelly et al., 1998); 6 years (Marker and O'Brien, 1989).*

[c] *cal: Reference time point used as calibration; μ: mutation rate given per generation; MYA, million years ago.*

[d] *$1/\mu \times G$ (Nei, 1987); coal, coalescent methods, MSVAR1.3 (Storz and Beaumont, 2002), VarEff (Nikolic and Chevalet, 2013), DIYABC-FDA (Cornuet et al., 2010); DaDi, Diffusion approximation to the allele frequency spectrum (Gutenkunst et al., 2009); fixation $4N_e$, fixation of a neutral nuclear marker is expected after $4N_e$ generations (Nichols, 2001); PSMC, Pairwise Sequential Markovian Coalescent (Li and Durbin, 2011); SMM, Stepwise Mutation Model (Valdes et al., 1993).*

[e] *An accelerated decline may be present 10,000 and 13,000 years ago.*

single subspecies (Kitchener et al., 2017). Additionally, Kitchener et al. (2017) put forward that as additional data become available the four subspecies that the IUCN Cat Specialist Group currently recognizes may be further merged in the future. However, this does not affect the amount of diversity found between the populations identified to date, which we will present here.

A. j. soemmeringii was first compared to *A. j. jubatus* in 2001 based on mtDNA (Freeman et al., 2001), with additional support provided in 2011 (Charruau et al., 2011). Time of divergence was estimated to be between 1,600 and 72,296 years ago by Charruau et al. (2011) (Table 6.3) and approximately 5,000 years by O'Brien et al. (2017).

A. j. venaticus was only genetically assessed in 2011, when Charruau et al. (2011) analyzed the first samples from the subspecies in a range-wide study. This finding refuted the possibility

that the current Asiatic cheetah population may have originated from individuals imported from eastern Africa for hunting purposes in the past century. The time of divergence between *A. j. venaticus* and *A. j. jubatus* was estimated at 4,700–67,400 years ago. The extent of the separation of *A. j. venaticus* from the African subspecies was not clear-cut. mtDNA data placed the split between *A. j. jubatus* and *A. j. venaticus* slightly more recently than that of *A. j. jubatus* with *A. j. soemmeringii*, while microsatellite data suggested that the divergence with *A. j. soemmeringii* was the more recent event (Charruau et al. 2011; Table 6.3). The pairwise genetic distance (F_{ST}: measure of difference between populations) of *A. j. venaticus* to the African subspecies appeared to be possibly slightly larger than distances within African populations (mtDNA F_{ST} values: 0.818–0.958 compared to 0.724–0.930; Charruau et al., 2011). However, it is important to keep in mind that divergence values between *A. j. venaticus* and the other subspecies could have been stochastically increased due to a postulated recent bottleneck in *A. j. venaticus* (section "Historic Demography"). O'Brien et al. (2017) estimates a time of divergence between *A. j. venaticus* and *A. j. jubatus* of approximately 6500 years ago.

A. j. hecki was not specifically assessed, as none of the studies was able to include confirmed *A. j. hecki* samples from west Africa.

PHYLOGEOGRAPHY

Range Wide

Historically, the distributions of each subspecies were defined based on morphological differences and presumed connectivity between populations. Once the subspecies were confirmed genetically, the distribution of each subspecies and their genetic structure could be verified (Fig. 6.3A). Charruau et al. (2011) obtained a sample collection of 94 cheetahs from 18 countries from the extant and historical cheetah range. These samples were expected to represent four of the five cheetah subspecies recognized at that time.

A. j. jubatus was confined to individuals from southern African countries, which included Botswana, South Africa, and Namibia. These samples consistently clustered (grouped) together, with both nuclear (microsatellite) and mtDNA data. Depending on the type of analyses, a single cheetah sample from the Democratic Republic of Congo grouped with *A. j. jubatus*, or slightly outside. The mtDNA haplotype (linked group of variants that is inherited together) group of *A. j. jubatus* was the most diverse (8 haplotypes) of the investigated sample collection and was centrally positioned in the mtDNA haplotype networks, with the haplotypes of the other subspecies radiating from it.

Haplotypes assigned to *A. j. raineyi* were confined to east African countries, which included Kenya and Tanzania. However, the maternal lineages (mtDNA) fell into 2 separate haplotype groups, one of which clustered with *A. j. jubatus*, separately from other *A. j. raineyi* haplotypes. As a consequence, *A. j. raineyi* has been included in *A. j. jubatus* by Kitchener et al. (2017)

A. j. venaticus was confined to extant samples from Iran. Historical samples of Asiatic cheetahs (Oman, Jordan, India, Iraq, medieval Iran) clustered with the extant Iranian cheetah samples, as observed with both nuclear and mtDNA data. Additionally, one sample from the extinct population in northeastern Egypt also clustered with the Asiatic cheetah samples of *A. j. venaticus*.

Historical samples from Libya appeared distinct based on mtDNA data. With microsatellite data the Libyan sample clustered more closely to, but separately from, the Asiatic cheetah (bootstrap support of 72% for the divergence between the branch of the Asiatic samples and the Libyan sample). Cheetahs from southern Egypt, western Sahara, and Algeria shared the mtDNA haplotype with the Libyan samples, and were separate from the *A. j. venaticus* haplotypes. Thus the mtDNA data supported the Asiatic subspecies

TABLE 6.3 Divergence Between Putative Cheetah Subspecies Estimated by Molecular Genetic Methods

Marker[a]	N_1/N_2[b]	Parameter[c]	Model[d]	Outcome	Conclusion
A. j. jubatus/A. j. raineyi (classified as consubspecific in 2017)					
Allozymes (49 loci)	43/30		*D*	0.004 distance (8× less than humans)	
mtDNA (505 nt; 28 ez)	35/39			100% unique hapl.	
mtDNA (525 nt)	17/1		K2P	15 substitutions	
Minisat (2 probes; 3 ez)	15–17		APD	Mean APD: 48.2 29% unique frag.	
Microsat (82 loci)	10/10	μ: 5.6×10^{-4}–2.05×10^{-3}	$(\delta\mu)^2$	4,253 ya	≥4,500 ya
		Cal: bottleneck 12,000 ya	Prop unique	4,514 ya	
A. j. jubatus/A. j. soemmeringii					
mtDNA (525 nt)	17/2		K2P	9 substitutions	
mtDNA (915 nt)	29/26	Clo: Puma-cheetah 4.92 MYA	Coal	32,200–244,000 ya	66,500 ya
			D_A	26,660–202,100 ya	55,085 ya
			IMa	43,928-379,317 ya	72,296 ya
			0 Migr	24,067–117,615 ya	66,698 ya
Microsat (18 loci)	27/25	μ: 2.05×10^{-4}–2.05×10^{-3}	$(\delta\mu)^2$	3,200–32,400 ya	
			D_{SW}	1,600–15,600 ya	
A. j. jubatus/A. j. venaticus					
mtDNA (915 nt)	29/11	Clo: Puma-cheetah 4.92 MYA	D_A	20,300–153,800 ya	41,900 ya
			Coal	15,570–118,020 ya	32,170 ya
			IMa	27,420–379,222 ya	44,403 ya
			0 migr	16,295–83,677 ya	42,120 ya
Microsat (18 loci)	27/8	μ: 2.05×10^{-4}–2.05×10^{-3}	$(\delta\mu)^2$	6,700–67,400 ya	
			D_{SW}	4,700–47,200 ya	

Studies are organized by subspecies pair. Marker information, animal numbers per subspecies and parameters (Columns 1–4) as well as outcomes (Columns 5 and 6) are indicated for each study. ya, Years ago.
Studies: *A. j. jubatus/A. j. raineyi*: Allozymes, O'Brien et al., 1987; mtDNA (505 nt), Menotti-Raymond and O'Brien, 1993; mtDNA (525 nt), Freeman et al., 2001; Minisat, Menotti-Raymond and O'Brien, 1993; Microsat, Driscoll et al., 2002.
A. j. jubatus/A. j. soemmeringii: mtDNA (525 nt), Freeman et al., 2001; mtDNA (915 nt), Charruau et al., 2011; Microsat, Charruau et al., 2011.
A. j. jubatus/A. j. venaticus: mtDNA, Charruau et al., 2011; Microsat, Charruau et al., 2011.
[a] *ez, Restriction site enzyme; Microsat, microsatellite; Minisat, minisatellite fingerprint; mtDNA, mitochondrial DNA; nt, nucleotide.*
[b] N_1/N_2 *is the no. of individuals* N_1 *of subspecies 1 followed by the no.* N_2 *of subspecies 2; subspecies 1 and 2 correspond to the two subspecies indicated for each section.*
[c] *Generation time used for all studies is 6 years (Marker and O'Brien, 1989); cal, reference time point used as calibration; clo, reference time point used for molecular clock; μ, mutation rate given per generation; MYA, million years ago.*
[d] *0 migr, Isolation with migration rate = 0, conservation method (Wakeley and Hey, 1997);* $(\delta\mu)^2$*, microsatellite genetic distance (Goldstein and Pollock, 1997; Zhivotovsky and Feldman, 1995); APD, average percent difference; Coal, coalescent method (Gaggiotti and Excoffier, 2000);* D*, Nei's raw number of nucleotide differences between populations (Nei and Li, 1979);* D_A*, net number of nucleotide differences between populations (Nei and Li, 1979);* D_{SW}*, stepwise weighted genetic distance (Shriver et al., 1995); IMa, isolation with migration, demographic method (Hey and Nielsen, 2007); K2P, Kimura's 2-parameter model (Kimura, 1980); Prop unique, proportion of unique alleles.*

boundaries suggested by Nowell and Jackson (1986) for *A. j. venaticus*, with cheetahs from the northern Sahara being of a different subspecies (Belbachir, 2007; Krausman and Morales, 2005). The cheetahs from the northern Sahara may be of the same subspecies as the west African cheetah (*A. j. hecki*), but this could not be confirmed, as no west African samples were available.

A. j. soemmeringii was confined to northeast African countries, which included Sudan, Djibouti, Ethiopia, and Somalia. Although these samples clustered together, some substructuring was identified. In Freeman et al. (2001), 1 of the *A. j. soemmeringii* mtDNA haplotypes was more similar to the *A. j. raineyi* haplotype (1 substitution apart) than to the other *A. j. soemmeringii* mtDNA haplotypes (9 substitutions apart). This likely reflects a more complex migration/colonization history, imperfect lineage sorting, or weakly defined boundaries of subspecies. All the *A. j. soemmeringii* mtDNA haplotypes shared a 1-amino acid deletion in the mtDNA-ND5 protein, which, if confirmed, could be used as diagnostic site to trace illegally traded specimens.

Globally, there was no evidence of gene flow between the subspecies in recent generations, as no admixture was detected with Bayesian Analysis of Population Structure. Genetic differentiation was further supported by the Neighbor-Joining (NJ) phylogenetic tree based on 18 microsatellite markers.

Southern Africa

Patterns of genetic variation identified 3 broad regional populations in southern Africa: Namibia, the Kalahari, and South Africa, when analyzing 13 microsatellite loci in 51 cheetahs from Botswana, Namibia, and the northwestern parts of South Africa (Fig. 6.3B; Kotze et al., 2008). F_{ST} values between Namibia and South Africa (0.115) and Namibia and the Kalahari (0.108) were higher than those between South Africa and the Kalahari (0.059). However, these values remain below the F_{ST} values recommended for high statistical certainty of population assignment (0.15–0.20; Manel et al., 2002). Also, while the significance of the differentiation between populations ($P < 0.001$) suggests that accurate population assignment may be possible, attempts to assign 6 cheetahs of unknown origin to a specific region (from a Bayesian exclusion test method and a frequency-based method) were inconclusive. The efficacy of assignment testing in cheetahs will most likely be improved through more comprehensive sampling (more subpopulations and more individuals per region; Manel et al., 2002). mtDNA data may also help to resolve questions of origin within southern Africa, given that 8 haplotypes were identified in a 915 bp sequence of mtDNA in Botswana, Namibia, and South Africa (Charruau et al., 2011).

Namibia

The Namibian cheetah population was the first national population to be assessed genetically. Marker et al. (2008) analyzed 35 microsatellite markers in 89 unrelated individuals whose distribution covered most (over 90%) of the cheetah's natural distribution in the country (Fig. 6.3B). No regional structure was detected when analyzing samples individually. When individuals were grouped by region, very modest support was given to a tentative grouping of the north-western regions (Outjo, Grootfontein; NJ tree, bootstrap support of 98%) and of the southern regions (Windhoek and Gobabis; bootstrap support of 79%). Multiple component analysis distributed the regions somewhat according to their geographical location, with the most northern region (Outjo) appearing to be most distinct, but F_{ST} values for Okahanja and Outjo ($F_{ST} = 0.086$) were below the recommended threshold for separate population assignment (0.15–0.20; Manel et al., 2002). Overall it was concluded that the Namibian cheetah population is panmictic (without barrier to breeding and without population structure), with gene

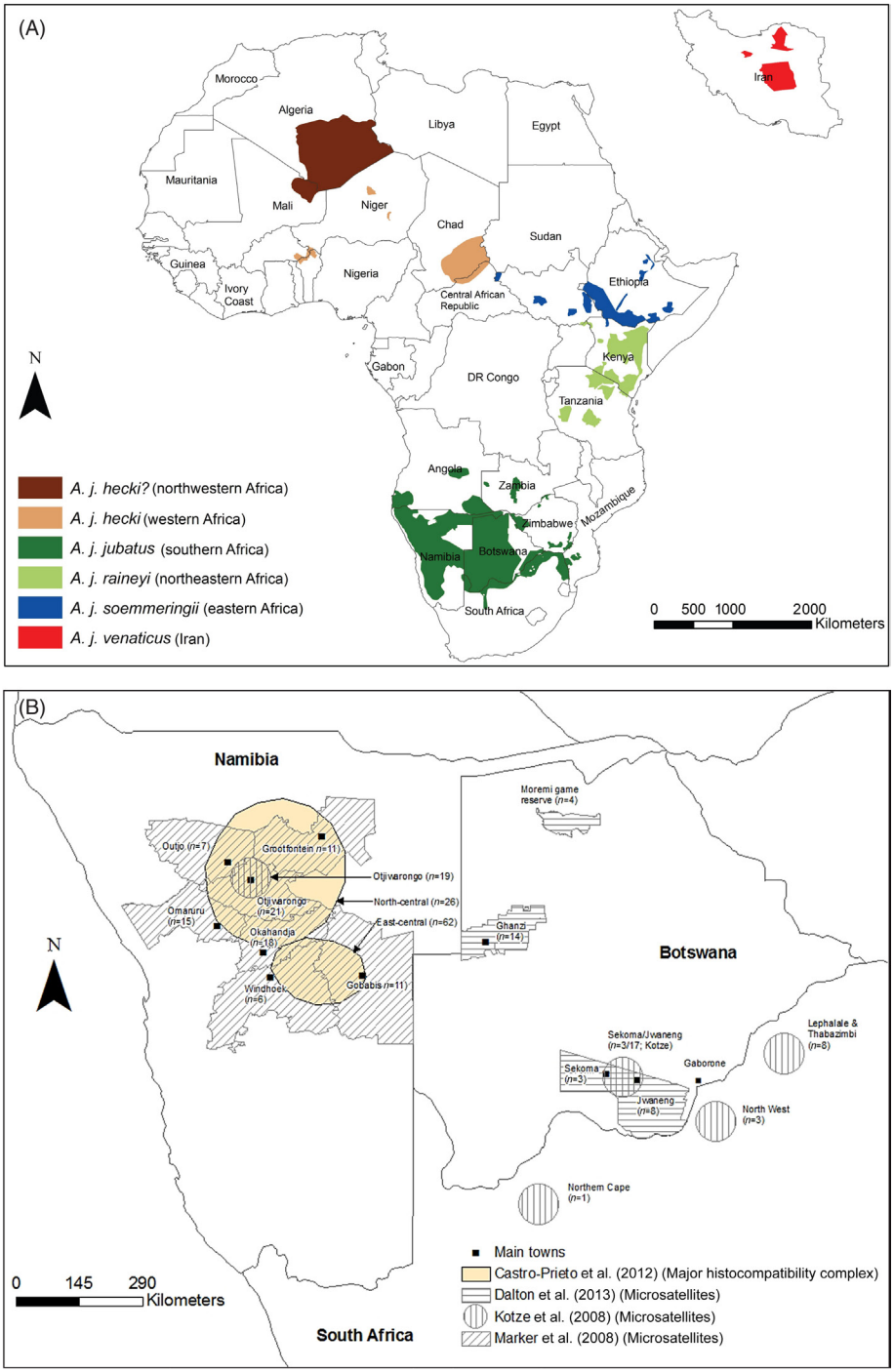

FIGURE 6.3 (A) Current confirmed resident range-wide distribution map (according to Durant et al., 2017), representing the putative subspecies according to genetic verification in Charruau et al. (2011). Subspecies genetically confirmed to date (*A. jubatus jubatus*; *A. j. soemmeringii*; *A. j. venaticus*) are represented with intense colors (*green*, *blue*, *red*, respectively); the northern African subspecies (which was confirmed genetically, and may be *A. j. hecki*) is in *brown*, and the expected *A. j. hecki* range (which has not yet been genetically tested) in *light brown*. *A. j. raineyi* was classified as consubspecific of *A. j. jubatus* in Kitchener et al. (2017) and is represented in lighter shade of *green*. (B) Geographical representation of the regions covered by the southern African phylogenetic studies.

flow maintained between the studied regions and precluding the emergence of any major structure. Connectivity was further supported by identification of two migrants to and from the Outjo region.

More recently, Castro-Prieto et al. (2012) reached similar conclusions through analyses of exon 2 of MHC class I, and class II-DRB genes that were genotyped with the single strand conformational polymorphism method in 26 individuals from the north-central region and 62 individuals from the east-central region (Fig. 6.3B). All identified alleles and resulting haplotypes were present in both regions. No regional differentiation was detected for MHC II-DRB, although haplotype frequency for MHC class I varied between north-central and east-central Namibia with a moderate F_{ST} support ($F_{ST} = 0.07$; $P < 0.01$). The difference in allele frequency at MHC class I (which targets intracellular pathogens) was attributed to different viral selective pressures in the two regions.

Botswana

Dalton et al. (2013) conducted an analysis of the Botswanan population (Fig. 6.3B) in 32 unrelated animals with 14 microsatellite loci. Although this study included a small sampling number, it still provided essential insights into the lack of population structure in Botswana. All animals were assigned to one unique group. Absence of substructure was further supported from the analysis of molecular variance (93% of variation shared among localities) and low genetic distance between the two largest sampled populations (Ghanzi vs. Jwaneng $F_{ST} = 0.035$; $P < 0.05$). Weak subdivision among the geographical populations suggests that gene flow occurs, which can be attributed to natural cheetah movements. The Moremi population appeared slightly "distinct" from the neighboring Ghanzi population ($F_{ST} = 0.079$), which was tentatively attributed to the presence of the Okavango delta as potential natural barrier between these two populations.

ADDITIONAL INSIGHTS INTO CHEETAH GENETICS

Kinship

Genetic analyses of fecal samples in the Serengeti identified multiple paternities for subsequent litters (8 females), as well as within litters (10/23 litters); 3 observed adoption events could also be confirmed genetically (Gottelli et al., 2007). In Namibia 21 of 23 females with cubs were confirmed as biological mothers, and 17 of 21 presumed sibling groups without a dam were confirmed to be related (Marker et al., 2008); in all exceptions (2 mothers and 4 sibling groups) individuals had been sampled from captive facilities with suspected human-induced animal grouping.

Coalition males appeared to be related in Namibia based on 23 of 26 male coalitions (Marker et al., 2008); while in Botswana, 3 of 4 wild-caught coalitions appeared to include at least one unrelated individual (Dalton et al., 2013). All three unrelated Namibian groups and one of the unrelated Botswanan groups dispersed at the time of release, suggesting that the grouping may have been an artifact of capture.

It was also shown that females with higher levels of genetic relatedness had greater home-range overlap (Marker et al., 2008), suggesting a matriarchal society.

Genetic Investigations of Infectious Diseases Affecting Cheetahs

The identification of the infectious agent responsible for one of the better documented viral outbreaks in the captive cheetah population (Chapter 25) was made possible due to samples properly stored since the outbreak in the early 1980s (Pearks Wilkerson et al., 2004). Using a phylogenetic approach, the virus was identified as a coronavirus, very similar to domestic cat coronavirus responsible for feline infectious peritonitis. However, in cheetahs, morbidity

was 100% (symptoms included diarrhea, jaundice, and seizures) and overall mortality was 60% within 3 years (85% in cubs). This was significantly higher compared to the 5%–10% mortality in domestic cat or the corresponding coronavirus (SARS) outbreak in humans in 2002, making this the deadliest documented death toll of a coronavirus. The authors attributed the high death toll to the lack of genetic diversity and the naïveté of the cheetah population to this virus, which appeared to have jumped from the domestic cat into the cheetah species.

A new species of infectious blood-borne parasite, *Babesia lengau*, was characterized genetically in cheetah (Bosman et al., 2010). While, as opposed to its effect in domestic cats, *Babesia* is not currently known to cause any pathology in the cheetah, additional research is continuing to investigate the effect of *Babesia* on specific health parameters (Schmidt-Küntzel et al., in preparation).

Investigations of Potential Genetic Predisposition to Disease

A single nucleotide polymorphism (SNP), described in the *Serum Amyloid A* (*SAA*) gene, was genotyped in captive cheetahs to assess whether there was a correlation between amyloidosis disease status (Chapter 25) and the SNP genotype (Franklin et al., 2015). It was found that the SNP had a semidominant effect on the associated protein level within each study population (N = 58), but that the institution at which the animals were housed had an even larger effect. In addition, there was no significant association between genotype and disease status (N = 48). Thus, the genetic impact of *SAA* on amyloid levels in cheetahs is minimal and outcompeted by other factors.

No correlation could be detected between variants in the mtDNA genome and myelopathic pathology (Burger et al., 2004). And to date no correlation could be identified between oxalate nephrosis and the coding regions of published candidate genes (Cheetah Conservation Fund and National Zoological Gardens of South Africa, unpublished data).

Investigations of the Molecular Basis for Heritable Traits

The cheetah has long been known for its poor sperm quality, with less than 20% viable sperm observed in reproductive studies (Chapter 27). In a whole genome study comparing the cheetah sequence to that of other species, several mutations affecting gene function were found in *A-kinase anchor protein 4* (*AKAP4*), a gene involved in spermatogenesis, and were shown to be likely fixed (only 1 allele present in the population) in the cheetah (Dobrynin et al., 2015). Those mutations may be in part responsible for the documented poor sperm quality.

Other phenotypes (heritable traits that can be seen/measured) of interest observed in the cheetah are kinked tails, crowded incisors, palatal depression, and coat variations (Chapter 7). While no molecular work has been performed on the morphological traits to date, insight was gained on several coat related phenotypes. Genes from the keratin-associated protein family were found to be expressed at higher levels in the yellow background of the cheetah fur (Kaelin et al., 2012), which is consistent with observations that fur of the black spots is softer relative to the coarser textured yellow background. Conversely, genes responsible for pigmentation were expressed at higher levels in the black spots relative to the less pigmented yellow background of cheetah fur (Hong et al., 2011). Another gene whose expression was increased in the black spots was a paracrine hormone, which was hypothesized to be involved in coordination of the spot pattern (Kaelin et al., 2012). The genetic basis for the king cheetah coat variant was determined to be a mutation in the *transmembrane aminopeptidase Q g*ene (Kaelin et al., 2012). Rare cases of gross morphological deformities have occurred

in the past, but no literature is available to substantiate whether they were based on low genetic diversity, inbreeding in a captive setting, or teratogenic influences.

Signatures of Selection, Copy Number Variation, and Changes in Gene Families

In a recent genome-wide analysis of the cheetah (Dobrynin et al., 2015) signatures of positive selection (by comparison with lion, tiger, cat, human, and mouse) were identified in close to a thousand genes. Ten of the genes were involved in muscle contraction (both cardiac and striated muscles), specifically the mitogen-activated protein kinase pathway (which is linked to stress), and in the regulation of catabolic processes, indicating that the cheetah underwent some degree of specialization in these pathways. In the same study over 10 million nucleotides of segmental duplications were identified, and affect genes that are believed to be involved in energy balance, nutrition, and sensory adaptation. In addition, gene expansion was observed in the MHC extended class I region, which includes vomeronasal receptors, as well as olfactory and G-coupled receptor genes; these gene expansions were tentatively linked to behavior (pheromones) and physiology (e.g., LDH-A and LDH-B are linked to a carnivorous diet). Both segmental duplications and gene expansions lead to temporary redundancy, allowing new gene functions to arise.

Evidence of historical positive selection on antigen binding sites that interact directly with pathogen-derived proteins was detected for both MHC classes, particularly MHC I (Castro-Prieto et al., 2011). Signatures of selection in the MHC were also identified in the whole genome study (Dobrynin et al., 2015). A study of the cytochrome P450 gene (CYP2D6), involved in drug metabolism, showed considerable genetic diversity and signs of relaxed selection pressure in felids, including cheetahs (Schenekar et al., 2011).

DISCUSSION

Importance of Low Genetic Diversity on Cheetah Survival

The most notable and still poorly understood feature of cheetah evolutionary history is how the cheetah has persisted in spite of remarkably low levels of genetic variation. Genomic variation is generally considered to be crucial for long-term survival of species as it provides potential for adaptive responses (natural selection of advantageous genetic variation) to environmental changes, such as climate change (Chapter 12), and adaptability of immunity to disease outbreaks (O'Brien and Evermann, 1988). Therefore, the initial discovery of genetic uniformity of the cheetah was quickly followed by concerns about the species' chances of long-term survival. However, the discovery that the event or events leading to the loss of diversity could be placed over 10,000 years ago and that cheetah numbers had recovered by the 19th century, indicate the cheetah's ability to survive and thrive, despite reduced levels of genetic diversity, over extended periods of time. However, this does not guarantee the cheetah's survival in the future, as lack of genetic diversity limits the ability to adapt and evolve, in particular in the light of major changes in environmental conditions or pathogenic pressure.

Reduced genetic variation, particularly at adaptively important MHC loci, has been associated with high susceptibility to infectious diseases in captive cheetahs (O'Brien et al., 1985; O'Brien et al., 1986; O'Brien and Evermann, 1988). A prime example is the high death toll caused by a coronavirus outbreak in a North American zoo (section "Genetic Investigations of Infectious Diseases Affecting Cheetahs," Chapter 25). Despite this, free-ranging cheetahs from eastern and southern Africa show robust health (Caro 1994; Munson et al., 2004; Munson et al., 2005; Thalwitzer et al., 2010) and do not seem to have compromised immunocompetence (Castro-Prieto et al., 2011). In addition, in the wild the cheetah's

large home ranges (Chapter 8) reduce the risk of infectious disease transmission. However, this may not be sufficient to protect the species in the event of an emerging disease, especially given the low levels of MHC diversity.

At an individual level manifestation of deleterious traits caused by excessive levels of homozygosity (inbreeding depression) appear to be limited compared with the puma population in Florida (Florida panther), which suffered from atrial defects, poor sperm quality, cryptorchidism, and high disease load (Roelke et al., 1993). The cheetah is only known to suffer from poor sperm quality; none of the other traits observed in the cheetah (e.g., kinked tails, crowded incisors; section "Investigations of the Molecular Basis for Heritable Traits") are detrimental to individual health, or the capacity to survive and reproduce. This, and evidence of positive signatures of selection on genes involved in muscle contraction and stress metabolism (section "Signatures of Selection, Copy Number Variation, and Changes in Gene Families"), suggests that the low levels of genetic diversity were caused, or followed by, strong selective pressures, which perhaps purged deleterious alleles from the species (e.g., Hedrick and Garcia-Dorado, 2016).

Despite limited sperm quality, cheetah matings produce sufficient viable cubs (up to 6 cubs per litter every 2 years; Chapter 9) to maintain and even increase the population. However, further loss of genetic diversity could impair reproductive success, which is the ultimate requirement for species survival. Indeed, increased infant mortality was observed in captive inbred individuals (O'Brien et al., 1985).

Genetic Diversity and *In Situ* Cheetah Conservation

With the reduction of its natural range, cheetah numbers are declining (Durant et al., 2017; Chapter 4), and most cheetah populations today are fragmented with loss of connectivity between them (Chapter 10). Small populations, such as the critically endangered Iranian cheetah (*A. j. venaticus*; Chapter 5), which has the lowest amount of genetic diversity of all the currently recognized cheetah subspecies (Charruau et al., 2011), are particularly at risk of losing further genetic diversity. Therefore, it is crucial to maintain or regain sufficiently large population sizes and connectivity, while preserving existing variation through viable long-term storage of sperm and oocytes (Chapter 27).

Whenever possible, animals should remain in, or if captured, be returned to the wild (Chapter 20). Captured animals that are not suitable for release back into the wild should be considered for breeding programs (section "Genetic Diversity and *Ex Situ* Cheetah Conservation"). Wild cheetah populations of the same subspecies and geographical region were generally panmictic; minor population structure was only observed in allele frequencies of the rapidly evolving immune response genes. As such, translocations of wild caught individuals performed as part of conservation actions within these populations, only mimic natural connectivity (which may be restricted by anthropogenic barriers to gene flow).

An additional level of complexity arises when populations are from different subspecies. While in principle exchange between populations of different subspecies should be avoided as they may be considered evolutionary significant units (Moritz, 1994), a compromise between preserving the existing structure and the urgency to rescue a small population at risk of disappearing, may have to be reached. Relatively recent times of subspecies divergence and the merging of two subspecies in 2017, are additional considerations during such decision making processes (section "Subspecies Definition and Divergence"). This dilemma may have to be addressed for the Iranian population at some point if the numbers remain below 100 individuals (Chapter 5). Additional information on the genetic health of the existing population is critically and urgently needed to determine if this population is likely to survive without management actions.

Genetic Diversity and *Ex Situ* Cheetah Conservation

Captive cheetah populations have been established in part to serve as a reservoir for the wild population (Chapter 23). Breeding decisions are guided by the genealogical data managed through the regional and international cheetah studbooks (Chapter 23). Reputable captive programs (Chapter 22) aim to retain 90% of genetic diversity over 100 years (Lacy, 2012). However, this may not be sufficient in the long term as the small number of founders only represents a subset of the genetic diversity found in the wild (founder effect) and the 10% genetic diversity lost is irreversible. This loss of diversity can only be compensated for by the recovery of lost breeding lines through "reinjection" of viably preserved reproductive material (e.g., gametes) of founders into the captive population, addition of new founder individuals (or gametes) obtained from the wild, or inclusion of unrelated captive individuals to the breeding pool. As with wild populations, interconnectivity of captive populations should be maintained as much as possible, through regional and interregional exchange of animals or reproductive material. As the cost of genetic research continues to decrease, it will provide the possibility to assess the genetic makeup of all captive cheetahs, and thus the integration of animals of unknown origin into the cheetah breeding pool, if genetic evaluation determines that they represent a new breeding lineage. Of note, inbred individuals (high homozygosity levels) can also be used for further breeding if they represent a unique breeding lineage, as homozygosity is not heritable.

CONCLUSIONS

The field of conservation genetics will see more advanced analyses emerging, including the evaluation of heritable traits, landscape genetics, and increased precision in assessing the extent and timing of the events that cause the loss of genetic diversity. Non invasive samples are increasingly employed to provide answers regarding populations that have not yet been intensely studied (Chapter 31). Additional research involving contemporary and museum samples is currently under way to fill existing knowledge gaps regarding the published subspecies (Léna Godsall Bottriell, personal communication). However, it is crucial to remember that data obtained from genetic studies published to date agree sufficiently in confirming the low genetic diversity of the species at nonrepetitive loci (section "Genetic Diversity"), dating the origin of the low diversity to more than 10,000 years ago (section "Historic Demography"), providing support for genetic differences, although short divergence times, between the populations corresponding to most published subspecies (section "Subspecies Definition and Divergence"), and showing only minimal population structure within geographical regions (section "Phylogeography"). This in turn enables a joint message in terms of recommendations for cheetah conservation, as well as *in situ* and *ex situ* management (sections "Genetic Diversity and *In Situ* Cheetah Conservation" and "Genetic Diversity and *Ex Situ* Cheetah Conservation"). We hope that by presenting all available data on cheetah genetics, this chapter provides clarity to the results and conclusions arising from the field of conservation genetics, and contribute to the global efforts for the cheetah's long-term survival.

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