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Continued decline in genetic diversity among wild cheetahs (*Acinonyx jubatus*) without further loss of semen quality

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ABSTRACT

As a well-studied felid with limited genetic diversity, the cheetah (*Acinonyx jubatus*) has shaped much of the scientific debate surrounding inbreeding depression. The species survived a population bottleneck ~12,000 years ago and was extirpated from >75% of its historical range in the last century. Modern cheetahs produce poor-quality semen, a presumed manifestation of inbreeding depression. Within Felidae, a positive association between genetic diversity and semen quality is well supported by pedigree data and inter-species comparisons. However, this relationship has never been examined among individual cheetahs. Furthermore, whether ongoing population declines are exacerbating inbreeding depression in wild or captive cheetah populations is unknown. Using 12 microsatellite markers, we evaluated the relationship between heterozygosity and reproductive traits among wild ($n = 54$) and captive ($n = 43$) male cheetahs born from 1976–2007. We tested the hypotheses that genetic diversity has declined over the last ~30 years and is positively correlated with semen quality/breeding success in the cheetah. Findings revealed that genetic diversity has decreased in the wild, but not captive, population. Unexpectedly, heterozygosity was *lower* in proven versus unproven breeders and did not correlate with semen quality. A small proportion of all males (<10%) produced relatively high quality ejaculates, with sperm traits similar to those of non-inbred felid species. These data suggest a more complex relationship between inbreeding and male cheetah reproductive traits than previously appreciated. Intensive management of captive cheetahs appears to be minimizing inbreeding, whereas the continued erosion of genetic diversity in wild males is of conservation concern.

1. Introduction

Inbreeding is linked to negative fitness consequences across a diversity of mammal, bird, fish, reptile, amphibian, insect, and plant species in the wild (Allentoft and O'Brien, 2010; Frankham et al., 2002; Keller and Waller, 2002). These negative effects are most profound in traits closely linked to reproductive success, including seminal quality and fecundity (Frankham et al., 2002). Species-level genetic diversity is correlated with semen quality among 20 mammals (Fitzpatrick and Evans, 2009), and analogous correlations have been documented at the individual level (i.e., within species) in the Iberian lynx (*Lynx pardinus*; (Ruiz-Lopez et al., 2012), Mexican gray wolf (*Canis lupus baileyi*; (Asa et al., 2007)), and Mohor gazelle (*Gazella dama mhorr*; (Ruiz-Lopez et al., 2012)). Within Felidae, the link between genetic diversity and male reproductive traits is well established. A single generation of inbreeding reduces semen quality in the domestic cat (*Felis catus*; (Neubauer et al., 2004)) and leopard cat (*Prionailurus bengalensis*; (Wildt, 1994)), while free-ranging inbred lions (*Panthera leo*) produce higher proportions of malformed spermatozoa and have fewer seminiferous tubules compared to non-inbred counterparts (Wildt et al., 1987). Consistent with this relationship, semen quality is relatively high among felid species with greater genetic diversity, including the ocelot (*Leopardus pardalis*), jaguar (*Panthera onca*), and African leopard (*Panthera pardus pardus*; (Pukazhenthi et al., 2006b; Swanson et al., 1995)).

Although some natural populations have persisted over long periods with limited genetic diversity (Reed, 2010), most studies support a general relationship between inbreeding and population decline/

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extirpation (Keller and Waller, 2002). In particular, the Florida panther (*Puma concolor coryi*) provides a compelling example of the consequences of extreme inbreeding. Compared to other puma subspecies, the Florida panther is highly inbred, with a population size of <100 individuals (Johnson et al., 2010; Roelke et al., 1993). Males experience severe reproductive defects, including an increased incidence of cryptorchidism, drastically reduced semen and testicular volumes, impaired sperm motility, and very high percentages (>90%) of structurally-abnormal spermatozoa (Mansfield and Land, 2002; Roelke et al., 1993), which are known to be incapable of fertilization (Howard et al., 1993). Conversely, introgression of DNA from eight Texas pumas (*Puma concolor stanleyana*) increased heterozygosity in the Florida population and resulted in fewer reproductive defects and greater offspring survival (Johnson et al., 2010). Aside from the Florida panther, the cheetah (*Acinonyx jubatus*) is perhaps the most thoroughly-studied wildlife model of inbreeding depression. The cheetah's lack of genetic diversity was originally detected by allozyme analysis and the ability of unrelated conspecifics to accept reciprocal skin grafts (O'Brien et al., 1983). This finding was subsequently confirmed by six additional measures of genomic variation (O'Brien, 1994), a lack of diversity in MHC class II-DRB alleles (Castro-Prieto et al., 2011), and the whole-genome sequencing of Namibian and Tanzanian cheetahs (Dobrynin et al., 2015). The cheetah's lack of genetic diversity is attributed to a severe population bottleneck that occurred ~12,000 years ago (Driscoll et al., 2002; O'Brien et al., 1985), from which the entire extant species is derived (Charruau et al., 2011). Intriguingly, recent genome sequencing suggests that a second ancient bottleneck occurred >100,000 years ago, coincident with the migration of cheetahs into Africa (Dobrynin et al., 2015). Importantly, the cheetah is the only modern felid species that lacks a non-inbred population – a fact that not only limits conservation options, but also complicates understanding the consequences of reduced genetic diversity (O'Brien and Johnson, 2005).

There has been substantial interest in understanding how genetic monomorphism influences health and reproduction in the cheetah, particularly because nearly all individuals studied to date consistently produce poor-quality semen (Crosier et al., 2007; Terrell et al., 2010; Wildt et al., 1983). Cheetahs maintained in zoological collections often fail to reproduce (Marker et al., 2014) and are susceptible to infectious disease (Munson, 1993) and birth defects (O'Brien et al., 1985). Initially, these issues were attributed to the species' lack of genetic diversity (O'Brien et al., 1985), but there is no evidence of impaired reproductive success, increased disease susceptibility, or high incidences of birth defects in wild cheetah populations (Caro and Laurenson, 1994; Castro-Prieto et al., 2011; Laurenson et al., 1995; Munson et al., 2004). Furthermore, although poor semen quality in the cheetah is presumed to have resulted from the ancient bottleneck (O'Brien et al., 1987), there has been no effort to empirically test this relationship. Therefore, while the cheetah is often cited for its extreme lack of genetic diversity, the manifestations of inbreeding are not entirely understood.

Although extensively debated (Caro and Laurenson, 1994; Laurenson et al., 1995; May, 1995; Merola, 1994; O'Brien, 1994), the question of inbreeding depression in the cheetah remains relevant because wild populations continue to decline (Durant et al., 2008). Over the last century, the cheetah was extirpated from >75% of its historical range, resulting in the geographic isolation of the southern African (*Acinonyx jubatus jubatus*) and east African (*Acinonyx jubatus raineyi*) subspecies (O'Brien et al., 1987; Ray et al., 2005). Whether these modern demographic changes have resulted in significant loss of genetic diversity is unknown. Since the early 1980s, captive cheetah populations in North America and Europe have been managed through cooperative breeding programs, with the goals of conserving rare genetic lineages and maintaining 90% of extant genetic diversity for the next 100 years (Association of Zoos and Aquariums, 2014). Given the cheetah's precarious status in the wild, the recent sequencing of its genome (Dobrynin et al., 2015), and the extensive efforts to create captive 'insurance' populations, it is an opportune time to evaluate the relationship between genetic diversity and reproductive traits in this species. We have a unique opportunity to test this relationship because our research group has collected DNA samples and/or reproductive data from >200 southern African cheetahs over the past 30 years. Additionally, these samples and records can provide insight into temporal changes in genetic diversity over several decades of population decline. In this study, our goal was to use archived DNA samples and paired reproductive data to better understand inbreeding depression in modern cheetahs. We predicted that genetic diversity of the southern African cheetah had eroded over the past 30 years, given the species' demographic declines in the wild and poor reproductive success in captivity. We further hypothesized that modern inbreeding (i.e., detected by microsatellite markers) would negatively affect reproductive traits in male cheetahs, specifically testis volume, sperm quantity and quality, and offspring production/survivorship.

2. Methods

2.1. Study populations

Our reproductive dataset included nearly 400 semen collections from wild ($n = 116$) and captive ($n = 99$) southern African cheetahs born from 1976–2007. Wild animals were captured (for reasons other than semen collection) throughout Namibia, excluding regions where the species is rare or absent (i.e., coastal areas, Kalahari Desert, and the Caprivi). The study area is arid to semi-arid, encompassing grassland and savanna, with ~400 mm rainfall per year. We identified 118 individuals in our dataset for which archived DNA samples also were available. These samples had been previously extracted from blood or tissue (using either a commercial kit (Qiagen; Valencia, CA) or standard phenol-chloroform procedure) and subsequently stored at −80 °C. After excluding DNA samples that failed to amplify ($n = 21$, see below), our genetic dataset represented 97 cheetahs, including those that were wild-born ($n = 54$) or captive-born in North America ($n = 27$), Europe ($n = 6$), or South Africa ($n = 10$). The latter group was of South African stock, but all other captive-born cheetahs were descendants of Namibian animals. Wild-born and captive-born populations are subsequently referred to as wild and captive, respectively. Importantly, these designations are based on population of origin (i.e., place of birth) and not whether the animals were subsequently housed in captivity. Wild cheetahs were either released into the wild (after semen collection) or transferred permanently to captive institutions. Captive individuals were born at accredited zoological institutions (North America and Europe) or breeding centers (South Africa). Birth years were obtained from the International Cheetah Studbook (Marker et al., 2014), except for five wild males for which this information was not recorded. Mean age at death for deceased males in our dataset was 12.0 ± 0.4 years, which is typical for a cheetah (Marker et al., 2014). Our dataset included seven suspected sibling groups ($n = 17$ wild cheetahs) and eight known sibling groups ($n = 18$ captive cheetahs). Required permits were obtained from the Namibian Ministry of Environment, and all animal procedures were approved by the Smithsonian Institutional Animal Care and Use Committee.

2.2. Microsatellite genotyping

We amplified 12 previously-described microsatellite markers (FCA8, FCA42, FCA85, FCA96, FCA97, FCA126, FCA214, FCA247, FCA298, FCA310, FCA441, FCA559) (Marker et al., 2008; Menotti-Raymond et al., 1999) using an Applied Biosystems® GeneAmp® 9700 Thermal Cycler and a 'touchdown' protocol (Marker et al., 2008). All loci were unlinked or >20 cm apart in the domestic cat (and therefore assumed to be unlinked in the cheetah), except for one pair (FCA85/FCA96) that was separated by 12 cm (Marker et al., 2008). These markers are unlikely to reflect the cheetah's bottleneck(s) ≥12,000 years ago because the present level of microsatellite diversity has likely accumulated over