

**Evaluating the Genetic & Demographic Integrity of South
Africa's Cheetah Metapopulation**

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Abstract

In 2011, the Endangered Wildlife Trust launched the Cheetah Metapopulation Project to provide coordinated relocation management to a group of 300+ cheetahs that were removed from commercial farmland in Namibia & South Africa. These cheetah were subsequently introduced onto smaller, fenced, privately held reserves in South Africa. This paper addresses 2 challenges associated with the cheetah metapopulation's long-term viability: the ecological capacity of the fenced reserves and the genetic diversity of the reserve cheetahs.

Density prediction models based on prey biomass were used to project carrying capacity estimates for 21 reserves. Three equations were used in order to capture differences in prey characteristics. When compared with actual densities, the model projections did not provide adequate estimates of carrying capacity. The finding implies that prey biomass alone cannot explain cheetah density. Heterozygosity, or gene diversity, was used to evaluate the population's genetics so that future analyses can capture a loss or gain in diversity. As a reference population, 33 cheetah individuals from South Africa's free roaming population were genotyped using 16 microsatellite loci. Forty three of the reserve cheetahs were genotyped at the same loci. This data were used to calculate and compare heterozygosity for the two populations, enabling a comparison of genetic diversity between the free roaming cheetahs and the metapopulation cheetahs which are actively managed in fenced reserves.

Overall, the reserve population has higher levels of heterozygosity than the free roaming population, suggesting that genetic diversity has been maintained on the reserves. This is possibly due to a temporary outbreeding effect from 'artificial migration' (translocation between reserves). This suggests that the reserve cheetahs would not benefit from crossbreeding with the free-roamers, however the free roamers would benefit. As the reserve population approaches its overall capacity, mating suppression will be required to avoid selling these wild cheetah into captivity. Genetic information should continue to be utilized for management, in order to further increase the population's biological fitness over the long term.

Introduction

Acinonyx jubatus, commonly known as the cheetah or hunting leopard, is thought to have disappeared from 76% of its historic range in Africa (Ray et al 2005). Many population estimates have been proposed, although there is no clear consensus in the literature on which ones are accurate. In 1975, the global population of cheetah was thought to be 15,000 (Meyers 1975). At the Global Cheetah Conservation Plan Meeting in South Africa in 2002, Dr. Laurie Marker cited the global cheetah population at just under 15,000 individuals (GCCP 2002), while IUCN estimates the current (2014) cheetah population at 7-10,000 individuals (Durant 2014). Furthermore, the IUCN's cheetah distribution map shows stark voids over areas where cheetah are known to exist in reality (e.g., the entire southern half of Botswana).

Of the 7-10,000 cheetah reported by IUCN, an estimated 4,500 reside in southern Africa (Durant 2014). However, researchers local to southern Africa believe the true number to be significantly higher, based on the amount of individual cheetahs taken off ranches without a visible decline in the free-ranging population. This is especially so in Namibia and Botswana, where many areas are devoid of lions and are even more likely to contain cheetahs. Regardless, southern Africa contains the species stronghold and is an important region for conservation of the species.

Conservation Status

There are 5 subspecies of cheetah; three of them exist outside of southern/eastern Africa and two of those are critically endangered. The Asiatic cheetah (*A.j. venaticus*) exists in Iran and possibly Pakistan and Afghanistan. According to Hunter et al 2007, this group consists of 60-100 individuals. This cheetah subspecies is categorized by the IUCN as critically endangered. In northwest Africa, one or more small population(s) of Northwest African cheetah- (*A. j. heckii*) totaling 250 individuals- is also categorized as critically endangered according to the IUCN. The remaining three cheetah subspecies are: *A.j. raineyii* of east Africa, *A.j. jubatus* of southern Africa, *A.j. soemmerringi* of northeast Africa.

IUCN has listed the cheetah on the Red List as 'Vulnerable', because of the approximate 30% population decline over 3 generations (18 years) (Durant 2014). Habitat loss and fragmentation is the main driver of this decline, although the capturing and killing of cheetahs who are thought to prey on livestock is also significant (IUCN Cats Red List Workshop 2007). In southern Africa, conflicts with farmers and ranchers are largely responsible for cheetah population declines (Purchase et al 2007). In Namibia specifically, exorbitant numbers of cheetah have been live-trapped and removed or killed by ranchers who seek to protect their livestock (Nowell 1996). In actuality, cheetah were only shown to be responsible for 3% of livestock losses to predators (Marker et al 2003). However, what moves the farmers and ranchers to action is not so much the actual threat of the cheetah but rather it is the perceived threat. A study by Nowell in 1996 using Namibian government permit records calculated upwards of 9,500 cheetahs were removed between 1978-1995. Beginning as early as 1965, 'problem' cheetahs assumed responsible for livestock losses were removed from commercial farmland in Namibia, South Africa, and Botswana and reintroduced to a number of small, fenced reserves in South Africa.

Table 1-Reserves of the Cheetah Metapopulation

Cluster	Reserve	Biome	Size (km ²)	Tenure	Reasons for Cheetah Reintroduction	Lions Present	Population September 2014
Waterberg	Mokolo & Ka'ingo PGRs	Savannah	157	Private	Ecological	Yes	0
	Entabeni PGR	Savannah	64	Private	Ecotourism	Yes	3
	Shambala PGR	Savannah	115	Private	Ecotourism	Yes	1
	Welgevonden PGR	Savannah	375	Private	Ecotourism	Yes	22
	Monate Game Lodge	Savannah	18	Private	Rewilding	No	0
	Witwater PGR	Savannah	65	Private	Ecotourism	No	7
	Ukulima Farm	Savannah	9	Private	Private leisure and research	No	3
	Thaba Tholo Game Farm	Savannah	300	Private	Free roamers always present	Yes	12
	Mabula PGR	Savannah	120	Private	Ecotourism	No	3
	Marakele NP & Pty	Savannah	900	State	Free roamers always present	Yes	0
	Pilansberg NP	Savannah	572	State	Ecotourism	Yes	3
	Madikwe Nature Reserve	Savannah	750	State	Ecotourism	Yes	5
	Dinokeng Game Reserve	Savannah	85	Private	Ecotourism	Yes	4
	Rietvlei Nature Reserve	Grassland	36	State	Ecological	No	1
	Meletse PGR	Savannah	130	Private	Ecological	No	2
Lowveld	Blue Canyon Conservancy	Savannah	160	Private	Ecotourism	Yes	5
	Hoedspruit AFB	Savannah	20	State	Promote flight safety	No	0
	Kapama PGR	Savannah	170	Private	Free roamers always present	Yes	3
	Karongwe PGR	Savannah	80	Private	Ecotourism	Yes	2
	Louis Trichart AFB	Savannah	26	State	Promote flight safety	No	1
	Makutsi PGR	Savannah	40	Private	Ecotourism	No	2
	Nkomazi PGR	Grasslands	120	Private	Ecotourism	Yes	3
	Pidwa Wilderness and Makalali PGR	Savannah	250	Private	Free roamers always present	Yes	9
	SanWild Wildlife Sanctuary	Savannah	60	Private	Rewilding	No	2
KwaZulu-Natal	AmaKhosi PGR	Savannah	125	Private	Ecotourism	Yes	2
	Mkuze Falls PGR	Savannah	125	Private	Ecotourism	Yes	5
	Zimanga PGR	Savannah	79	Private	Ecological	No	4
	uMhkuze Game Reserve	Savannah	400	State	Ecological & Ecotourism	No	5
	Nambiti PGR	Savannah	100	Private	Ecotourism	Yes	1
	Phinda PGR	Savannah	235	Private	Ecotourism	Yes	21
	Thanda PGR	Savannah	155	Private	Ecotourism	Yes	5

	Zulu Nyala PGR	Savannah	20	Private	Ecotourism	No	2
	Zululand Rhino PGR	Savannah	220	Private	Ecological & Ecotourism	Yes	15
	Hluhluwe-Imfolozi Game Reserve	Savannah	960	State	Ecological & Ecotourism	Yes	40
Eastern Cape	Amakhala PGR	Thicket	57	Private	Ecotourism	No	2
	Gondwana PGR	Fynbos	103	Private	Ecotourism	Yes	2
	Hopewell PGR	Thicket	55	Private	Ecotourism	No	2
	Kwandwe PGR	Thicket	176	Private	Ecotourism	Yes	8
	Mount Camdeboo PGR	Nama Karoo	45	Private	Ecotourism	No	6
	Samara PGR	Nama Karoo	120	Private	Ecotourism	No	8
	Sanbona PGR	Succulent Karoo	540	Private	Ecotourism	Yes	12
	Shamwari PGR	Thicket	47	Private	Ecotourism	Yes	3
	Lalibela PGR	Thicket	75	Private	Ecotourism	Yes	6
	Phumba PGR	Thicket	20	Private	Ecotourism	No	3
	Mountain Zebra NP	Grasslands	240	State	Ecological & Ecotourism	Yes	12
	Addo NP	Nama Karoo	160	State	Ecotourism	Yes	5
	Garden Route Game Lodge	Fynbos	20	Private	Ecotourism	No	1
Kalahari	Glen Lyon PGR	Savannah	660	Private	Private leisure	No	4
	Khamab PGR	Savannah	940	Private	Free roamers always present	Yes	30
	Tswalu PGR	Savannah	1000	Private	Conservation	Yes	19
	Loahu Valley Reserve	Grassland	86	Private	Conservation	No	2
Total Cheetahs in Metapopulation							318

Table 2- Area of Reserves

	Total Area (km ²)	% area South Africa	% area Kruger NP
Total Reserve Area	11384	0.9	58
ZA total area = 1219000 km ² ; Kruger total area = 19633 km ²			

Reintroductions in South Africa and the Endangered Wildlife Trust

Approximately 500 cheetah individuals to date have been reintroduced to these reserves from farmland since 1965. Namibian cheetahs were relocated from 1965-1998, and South African cheetahs followed suit between 1999 and 2009. The current population is 318 individuals on 49 fenced reserves. All the reserves are in South Africa and most of them are privately held fenced reserves in South Africa (**Table 1**). The total combined area of the reserves is approximately 11384 km² (**Table 2**) although the reserves are very widely dispersed across South Africa (**Figure 2**). The reserve population today represents a mixture of founders and offspring from those cheetahs originally reintroduced over the specified timespan.

The Endangered Wildlife Trust (EWT) is a non-profit “dedicated to conserving threatened species and ecosystems in southern Africa to the benefit of all people” (Endangered Wildlife Trust 2014) and recognized the need for a more coordinated relocation strategy for these reintroduced cheetah. In 2011, EWT launched the Cheetah Metapopulation Project to:

1. Ensure the long-term viability of cheetahs in small fenced reserves.
2. Ensure the long term genetic and demographic integrity of the metapopulation.
3. Increase the resident range of Cheetahs in South Africa
4. Maximize the conservation benefits of Cheetahs in small fenced reserves.

Today, EWT is the central contact point for reserve managers and owners, veterinarians, government and permitting officials, etc. As such, the organization coordinates and executes management interventions such as bringing individuals from disparate reserves together to mate, or conversely, transferring littermates to other reserves to prevent inbreeding.

Cheetah are no longer relocated from farmland (ie from free-roaming populations) to reserves. The practice has ceased for a variety of reasons. First, as previously mentioned there was uncertainty around the impact this practice had and/or will have on free-roaming cheetah populations. Second, the relocations did not solve human-wildlife conflict. The problems of livestock depredation continued regardless of the ‘problem’ animals being removed and relocated to as far away as another country in some cases. The last reason is simply that over time, regional and national action plans were formed which did not support relocation as a

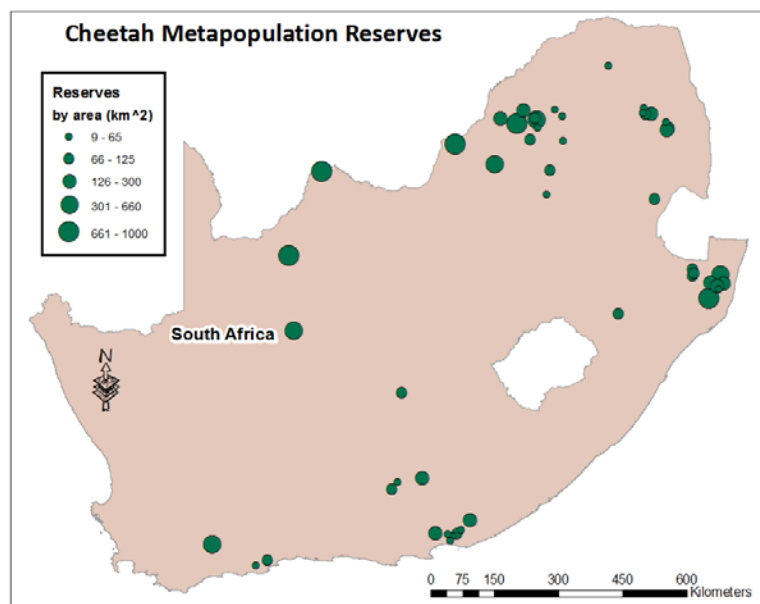


Figure 1-Endangered Wildlife Trust is the central contact point for 49 fenced reserves which hold a total of 318 cheetahs across South Africa.

conservation action: Regional Conservation Strategy for the Cheetah and African Wild Dog in Southern Africa (IUCN/SSC (2007).

Unique Attributes of the Cheetah Metapopulation

Metapopulation, defined as “a set of spatially disjunct populations, among which there is some immigration” according to Wells and Richmond (1995). By the time EWT launched the National Cheetah Metapopulation Program (CMP) in 2011, there were already hundreds of cheetahs on fenced reserves. For simplicity, I will refer to the metapopulation as the reserve population throughout this report.

The reserve population size (cheetahs) is comparable to the combined populations of the two subspecies outside of southern Africa (100 in Iran and 250 in western Africa). The estimated number of cheetahs present in southern Africa is 4,500, which is the stronghold for African cheetah. A quarter of these cheetahs are in South Africa, and approximates 1,185 cheetahs (Figure 2). The reserve population constitutes 27% of South Africa’s cheetahs and 7% of the stronghold (if the estimation of 4,500 is correct).

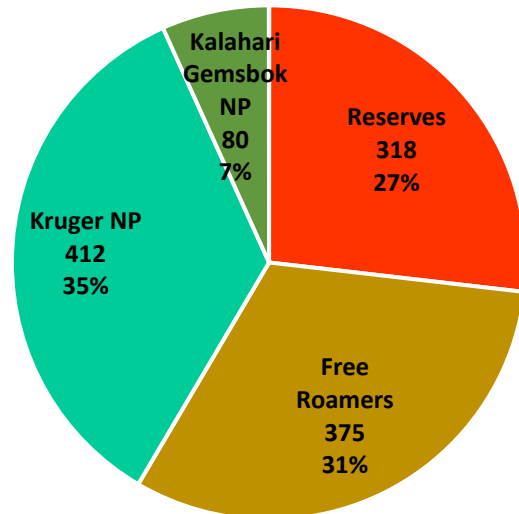
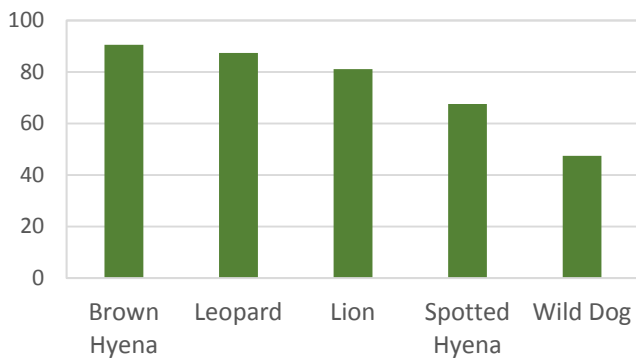


Figure 2- Distribution of South Africa’s cheetahs

The reserve population is neither free-roaming nor captive. Though they are constrained by the fences, these cheetah behave as wild, free-roaming cheetah do. They they hunt their own prey and are subject to prey availability. The cheetahs are not encaged, enclosed, or in any way obfuscated from the view of fellow guild members. They must be predator adapted as in the wild, since a large number of the metapopulation cohabitate with other large predators (Figure 3). Reserve owners do not feed the cheetah, however ~6% contracepts them, and 42% of reserves contain a single sex of cheetah. In essence this means that 48% of the metapopulation cannot conceive without an intervention.



There have been 352 cheetah mortalities to date, and the cause for 234 of those is documented in Figure 4. Lions are responsible for over 30% of total cheetah mortalities in the reserve population, which is consistent with some studies who have found that lions are an important role in maintaining cheetah density levels (Laurenson et al 1995; Eaton 1982). Anthropogenic reasons are the

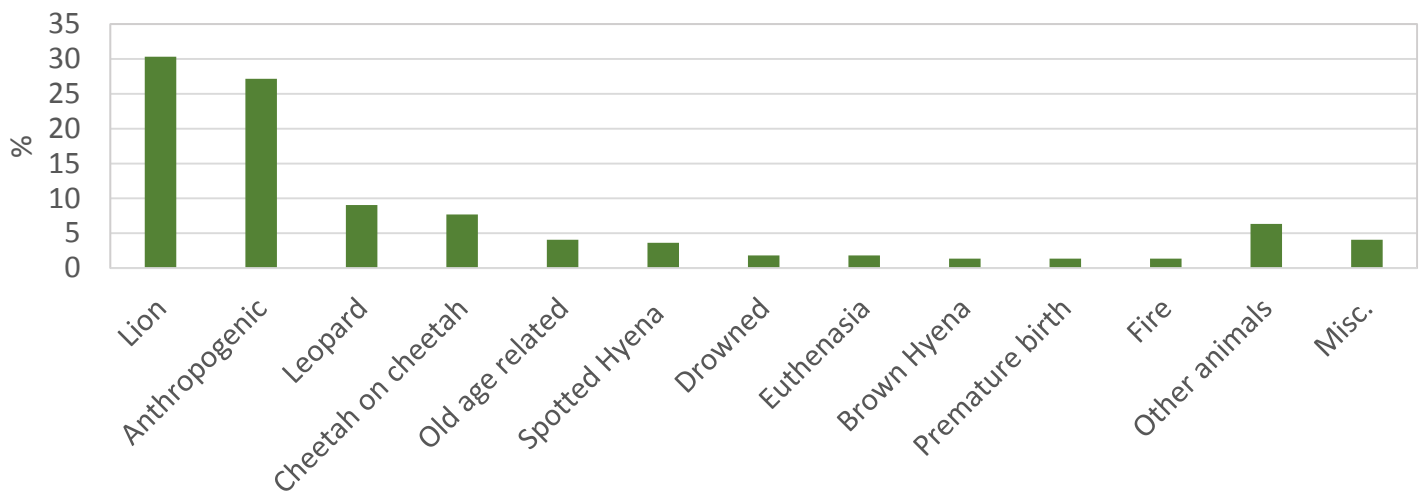
Figure 3- Percentage of reserve cheetahs coexisting with other large predators

second largest cause of death, and mainly happen when cheetahs are being transferred between locations. This is most

certainly human error, and in the wild would probably not contribute to as many mortalities as is seen with this population.

Each reserve is essentially its own small population. A small number of cheetahs get reintroduced onto the reserve as the founder population. Usually the founders are assumed to be unrelated, however that can only be confirmed via direct observations recorded or DNA analysis performed. Therefore, founder relatedness is often unknown on these reserves. Over time, unless some of the cheetahs are translocated between reserves, the cheetahs on the given reserve will be fenced in with their relatives. Small founder populations and high possibility of inbreeding cause major concerns regarding the genetic integrity of the reserve population.

Figure 4-Causes of known cheetah mortalities



Importance of Fenced Reserves

Naturally occurring populations of large African predators are vanishing from small and medium size (36-3,900 km²) habitat patches in Africa (Woodroffe & Ginsberg 1998), especially in countries where natural habitats are becoming increasingly fragmented (Lindsey et al 2011). When habitat patches are broken up, what results is “edge effects” –increased species mortalities from reduced protection and resource availability (Ogada et al 2003). Predators have large area requirements and are therefore even more susceptible to these edge effects (Lindsey et al 2011). Boundary fencing can reduce edge effects in two main ways. First, by reducing human incursion into protected areas and second, by preventing wildlife from moving into adjacent land that is unprotected (Lindsey et al 2011). Fencing has allowed for the effective conservation of wildlife in highly modified habitats, and notably on smaller areas than would otherwise be possible without fencing. Many conservation areas in South Africa are <1,000 km² (EWT 2014), and therefore small, fenced reserves of similar area may be a resource for conservation in South Africa.

Fenced reserves may also prove to be more financially advantageous. A recent study by Packer et al 2013 shows that lion conservation is more effective in fenced reserves. According to the study, “Lion populations in fenced reserves are significantly closer to their estimated carrying capacities than unfenced populations. Whereas fenced reserves can maintain lions at 80% of their potential densities on annual management budgets of \$500/km², unfenced

populations require budgets in excess of \$2000/km² to attain half their potential densities” (Packer et al 2013).

Other animal species are successfully managed in fenced reserves. After the first poaching crisis, white rhinoceroses were bred in a fenced reserve in Kwa-Zulu Natal province and formed a population for reintroductions into other areas, including the Kruger National Park. WWF is currently running a Rhino Range Expansion Project in small fenced reserves, many of which are also taking part in the Cheetah Metapopulation Project. African wild dogs have been reintroduced into nine fenced reserves in South Africa to form a viable population outside of the Kruger National Park. Currently, a national management plan is being developed for lions in small fenced reserves in South Africa.

Cheetahs have been extirpated from large areas of South Africa, and these reintroductions are the only way to develop a viable cheetah population outside of fragmented wild habitats and captivity. Conservation education and research opportunities are inherent attributes of the CMP, as EWT works closely with reserve owners and there is mutual necessity to work together.

A few other species were mentioned as having been managed in fenced reserves. The cheetah possesses traits which prove beneficial for successful reintroductions, making them a worthy prototype for similar attempts at species management on fenced reserves. They have an expansive diet, are responsive to behavioral manipulation, are well-adapted to a wide range of habitats, and commonly exploit new areas (Stanley Price 1989). Furthermore, cheetahs play an important role on these reserves. They are essential members of the trophic guild. They provide economic opportunities for locals which honors the natural heritage of the land.

There is every reason to believe that cheetahs can survive and be sustained on smaller, fenced reserves. And if this is so, fenced management holds great potential to contribution to the preservation of the species: the populations remain wild yet protected, are maintained in an environment conducive to conservation research and education, and provide livelihood for and engagement of the local communities.

A Brief History of cheetah genetics

Captive breeding of endangered species has become essential to the survival or increased abundance of many species (Henkel et al 2011). Managers of captive populations work to minimize inbreeding and equalize founder lineages. Normally, managers of captive populations rely on pedigree information to achieve these objectives. Pedigree information for each individual in a given population is usually kept in a Studbook. Using the studbook information, pedigree analyses can be conducted to determine relationships among individuals and this information can be used to make breeding decisions for the captive population (Henkel et al 2011).

However, estimates of diversity cannot be adequately constructed based on pedigree information alone for a population lacking information on parental lineage or one that has experienced a severe bottleneck prior to establishment of captive population, (Jones 2002). The cheetah metapopulation has both of these limitations: information is lacking or altogether absent on pedigree and parental lineage, and its species is believed to have experienced a genetic bottleneck (Menotti-Raymond and O’Brien 1995).

Reduced genetic variation can have reproductive consequences in large mammals (Frankham et al 2002) and cause increased vulnerability to infectious diseases and inbreeding depression (Dubach et al 2013). While increased susceptibility to infectious diseases has been observed in species (O'Brien 1993), evidence describing inbreeding depression in cheetahs is lacking (Caro & Laurenson 1994).

There is some disagreement in the literature regarding the historical population bottleneck. If it occurred, it was approximately 10,000 years ago (O'Brien 1993). Evidence in support of the bottleneck theory has been given in some studies (Menotti-Raymond and O'Brien 1995), and has been refuted in others (Charruau et al 2011). A 2011 study on phylogeography of cheetah did not find evidence for a bottleneck in the southern and northern-east African cheetah populations (Charruau et al 2011).

An alternative explanation of genetic uniformity to the bottleneck theory is that of metapopulation dynamics. Metapopulation dynamics assumes recurrent colonization events of a limited number of individuals, which results in a continually small effective population (Hedrik 1996). When there are few founders, when extinction and recolonization occurs frequently, and when gene flow is lacking at times excluding recolonization, effective population size can be particularly small (Hedrik & Gilpin 1996). Charruau's 2011 study suggests that a population of cheetah in east Africa is derived from a relatively recent recolonization, though further research is needed to determine how often this may have happened in the past. Local extinction or near extinction would not be unexpected under a metapopulation scenario (Hedrik 1996), and cheetah have gone extinct in 13 countries over the last 30 years (Marker 1998). Whether due to bottleneck theory or metapopulation dynamics is unclear, but it is alarming nonetheless that the effective population of cheetah is estimated to be below 50% of the total population (Marker 1998).

The divergence time of the two sub-Saharan subspecies (*A.j. jubatus of southern Africa* and *A.j. raineyii of eastern Africa*) are estimated to predate the supposed bottleneck (Menotti-Raymond and O'Brien 1993). The Charruau study found that the two sub-Saharan subspecies could not be completely separated in an analysis of mitochondrial population structure (though there is enough support to separation into two distinct subspecies), and concludes that the genetic diversity is lacking among these two subspecies. The remaining three cheetah subspecies (Asiatic, western African, and northeast African) were found to be long-term isolates with independent evolutionary histories. This research shows evidence of greater genetic variation in the worldwide cheetah population than was previously believed, but shows that it is specific to the Asiatic, western African, and northeast African subspecies (Charruau et al 2011). This is important for this study because if the metapopulation is comprised solely or mainly of the two sub-Saharan subspecies, it may not contain the genetic diversity necessary to sustain itself. Since the other three subspecies display a higher level of diversity, they might serve as supplemental additions to the sub-Saharan gene pool should that be necessary. Adding the historical context to the present constraints mentioned above (small founder populations per reserve, fenced in with relatives), it is no surprise that the genetic and demographic integrity of this reserve population is in question. Therefore it is of utmost importance to understand the corridors to gene dispersal and flow and to identify instances where genetic continuity has been interrupted (Dubach et al 2013).

Objectives

This project evaluates the genetic and demographic integrity of South Africa's cheetah metapopulation. The two main objectives of the Cheetah Metapopulation Project are to: 1) ensure the long-term viability of cheetahs on smaller, fenced reserves, and 2) ensure the long-term genetic and demographic integrity of the metapopulation. In short, this metapopulation should be managed such that it remains self-sustaining in ecological and genetic capacities.

This project addresses the former by asking the question: what is the ecological carrying capacity of these reserves? The reserves are privately owned and management interventions are the standard. Though they work with EWT to determine how many cheetahs should reside on their reserves, ultimately they specify the decision. Currently the total demand across reserves is 40, which would be additional to the 318 resident cheetahs. This is important because any cheetahs that cannot be accommodated on a reserve will be sold into captivity. EWT intends to avoid this situation from occurring. Cheetah demand is seldom decided using ecological carrying capacity, which is the number of individuals of a particular species that can be supported by a habitat without depleting available resources (Odum 1993). Predicting the ecological carrying capacity of carnivores is vital in small, enclosed reserves (Hayward et al 2007) so that neither overpopulation of prey species nor cheetah death by starvation occurs. Carrying capacity will be estimated using carnivore density prediction models. The predictions will be tested in comparison to actual density values. Given that reserve owners decide cheetah demand, the carrying capacity models are expected to vary from the actual densities. If predictions are accurate, EWT can work with the reserve owners to plan for the correct number of cheetahs on their reserves to minimize the effects of boom and bust cycles and prey depletion.

Since the reserves no longer receive relocated free-roaming cheetahs (ie what might be fresh DNA), and because cheetahs are notoriously difficult to breed in captivity, the genetic integrity of the population will be of vital importance in determining the management actions that should be taken. Considering that cheetahs are already genetically disadvantaged, the levels genetic diversity of the reserve population is

Therefore, second objective will be addressed by asking the question: how does the genetic diversity of the reserve cheetahs compare to the free roaming population? This project will use genetic diversity and relatedness as parameters to define the biological fitness of the reserve population.

The reserve cheetahs have constraints that the free roamers do not; they are fenced in with their relatives and each reserve originates from a very small founder population (2-4 individuals). Many of the individuals sampled on reserves were specifically chosen because they are thought to be siblings or a direct result of inbreeding. Inbreeding is known to be associated with low genetic diversity. For these reasons, it is expected that the reserve population will have lower levels of heterozygosity (or genetic diversity) than the free roamers. The reserves are expected to be biased towards higher levels of relatedness, for the same reasons as is stated above.

The ultimate goal of this study is to determine whether or not 40 more cheetahs can be generated from the current reserve gene pool and ecologically supported under the current reserve structure.

Methods

Carrying Capacity

Carrying capacity estimates were calculated using three model equations for 21 reserves (42% of total). These models are based solely on prey biomass. Prey data was only available for 21 reserves, otherwise the calculations would have been performed for all reserves. Of these 21 reserves, 8 have demand for additional cheetah and are among the high priority reserves (ranked by EWT based on a series of characteristics measured for each reserve holding metapopulation cheetah).

Using a density prediction equation from Carbone & Gittleman 2002 and two density prediction equations derived in Hayward et al 2007, carrying capacity was estimated at each reserve. All three model equations were based on prey biomass, and were calculated on a log-log scale. Therefore, prey biomass estimates were log-transformed and then back transformed to yield estimates of cheetah density. After the model predictions were calculated, the results were compared to actual density on the reserves.

Cheetah's prey preference is thought to be species specific, with blesbok, impala, springbok, Grant's gazelle, Thomson's gazelle being the top 5 most significantly preferred species (Hayward et al 2006b). Cheetahs also commonly prey on species which fall within the weight range of 23-56 kg (Laurenson 1995b). The preferred species fall within this weight range, as do others such as bushbuck, nyala, reedbuck.

Each equation accounts for different prey characteristics (**Table 3**). Carbone & Gittleman developed a model which controls for resource availability by calculating the relationship between average number of carnivores and units of prey biomass (2002). The Carbone & Gittleman model shows that 10,000 kilograms of prey supports approximately 90 kilograms of a given carnivore. Therefore, the first equation estimates predator density as a scalar relationship between predator & prey (Carbone & Gittleman 2002). Hayward et al 2007 proposes a model which accounts for cheetah behavioral characteristics. The first equation from Hayward uses prey biomass for any species that is significantly preferred by cheetah (blesbok, Impala, Thomson's gazelle, Grant's gazelle, and springbok). The second equation estimates predator density by utilizing the sum of prey biomass for all prey that are within the preferred weight range of cheetah (23-56 kg).

Predictions based on scalar relationship between cheetah and prey:

$$(a) y = (94.54 * (50^{-1.03})) * (z/10000); \text{ (Carbone \& Gittleman 2002)}$$

Predictions based on cheetah preferred prey species:

$$(b) y = -2.543 + (0.369 * (x)); \text{ (Hayward et al 2007)}$$

Predictions based on cheetah preferred prey weight range (23-56 kg):

$$(c) y = -2.641 + (0.411 * (x)); \text{ (Hayward et al 2007)}$$

The value of x represents the sum of the biomass of prey that is either the sum of prey biomass (a), the sum of preferred species (b) or the sum of species within cheetah's preferred weight range (c). The latter is calculated for each prey species by multiplying the prey density (abundance/reserve area) by .75 female body mass, then taking the sum total of biomass for the species of interest.

Table 3- Summary of predictive density models

	Definition	Equation	Source
Model A	$\frac{90 \text{ kg carnivore}}{10,000 \text{ kg prey}}$	$y = (94.54 * (50^{-1.03})) * (z/10000)$	Carbone & Gittleman 2002
Model B	preferred <i>spp</i>	$y = -2.543 + (0.369 * (x))$	Hayward et al 2007
Model C	23-56 kg	$y = -2.641 + (0.411 * (x))$	Hayward et al 2007

**Microsatellite
DNA Analysis**

DNA samples were taken from 110 cheetahs overall. The free roaming cheetah population (n=39) was used as a reference population for which to compare the results of the reserve cheetahs (n= 71) (see **Figure 5**). All samples were humanely collected and sent to Onderstepoort Veterinary Genetics Laboratory in South Africa. Samples included one or more of the following for each cheetah individual: blood, serum, heparin, hair, buccal swabs, and tissue. At OPVGL, each sample was given an identification code, a population code, and was then genotyped at 26

microsatellite loci developed in *Felis catus* and tested on cheetahs: FCA001, FCA008, FCA026, FCA031, FCA057, FCA069, FCA075, FCA085, FCA096, FCA097, FCA105, FCA113, FCA126, FCA193, FCA224, FCA230, FCA240, FCA272, FCA275, FCA310, FCA391, FCA441, FCA453, FCA506, F115, F37, F41, F42 (primer sequences in Menotti-Raymond et al. 1999). Of the 26 loci tested, 10 had too much missing data across loci. Therefore, only 16 loci were used for further analysis.

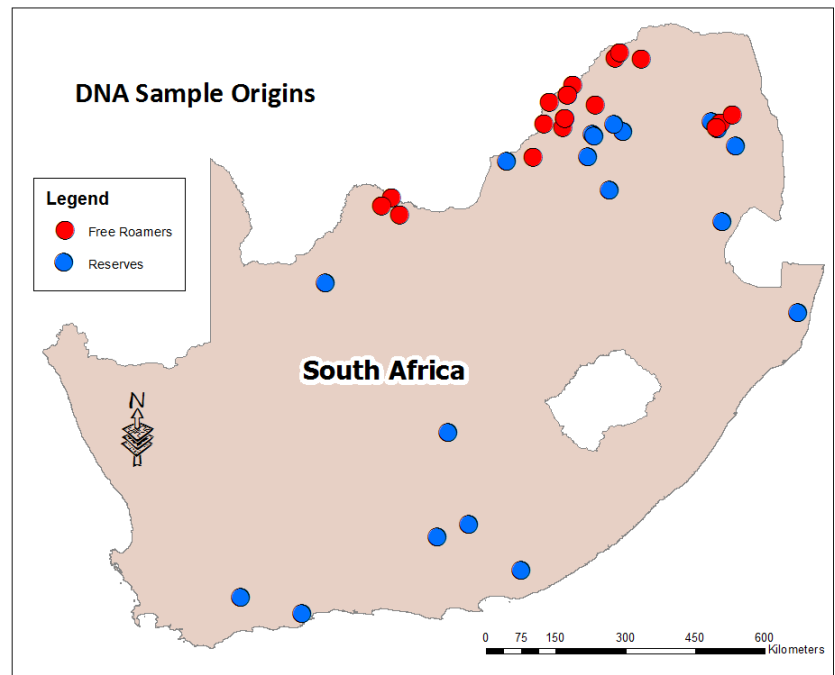
Heterozygosity

Using the The GenAlEx

macro for Excel, individual heterozygosity was calculated for

each of the 110 individuals. Heterozygosity is a measure of gene diversity, calculates the variance of allele frequencies across loci within the individual (Hildebrand et al 1992).

Heterozygosity is a good baseline parameter and can be used in subsequent analysis to determine how much gene diversity is gained or lost over the number of generations between analyses (Fienieg and Galbusera 2013). One sample t-tests were performed in Stata to compare the H-indiv values for the reserve population to the reference population (free roamers). The null hypothesis is that the free roamer population mean is equal to the reserve population mean. The alternate hypothesis is that the free roamer population mean is not equal to the reserve population mean.

**Figure 5-Sample Origins for DNA Analysis**

Then, t-tests were performed to compare the H-indiv value for the free roamer population to each reserve cheetah individual to determine how each reserve individual compares to the mean of the reference population. For each of these t-tests, the null hypothesis is that the free roamer population mean is equal to the given H-indiv. The alternate hypothesis is that the free roamer population mean is not equal to the given H-indiv. All tests used a p-value of 0.05 to decide whether or not to reject the null.

Relatedness

Pairwise relatedness was estimated for all samples in the data using The Queller & Goodnight moment estimator (Queller & Goodnight 1989). QGM is the most commonly used method to evaluate how closely two individuals are related to one another. Pairwise relatedness was also calculated for each population separately so that overall levels of relatedness per population could be determined.

Heterozygosity values were compared to relatedness values for reserve pairs and separately for free roamer pairs to determine whether there is a correlation between genetic diversity and relatedness. Pairs that comprised of individuals from both populations were omitted from these comparisons.

Results

Carrying Capacity

The predicted density values from all 3 models can be seen in **Table 4**. The models did not explain the variance in the data for each reserve. The model based on scalar relationship between predator and prey had the best fit ($R^2 = 0.0025$) compared to the model based on preferred prey ($R^2 = 0.001$) and weight range ($R^2 = 0.0005$). However, the best fit in this case was a poor one.

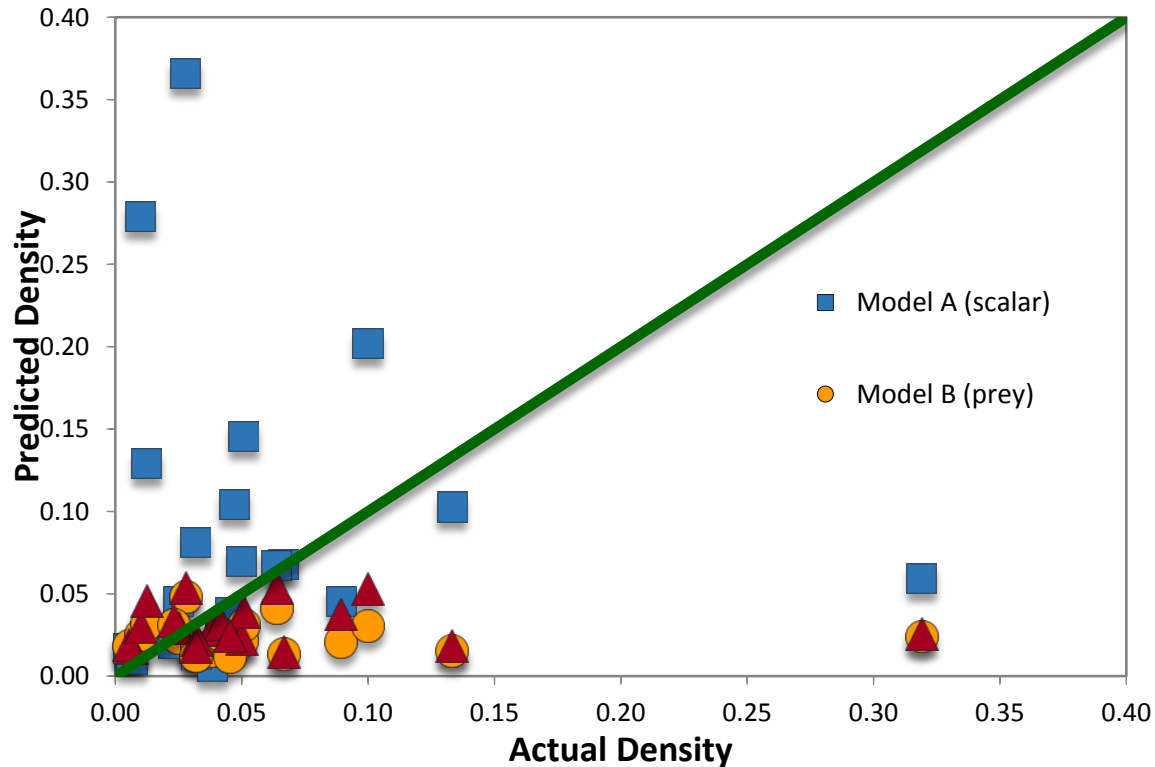
Model A resulted in 48% of predicted densities that were greater than actual values, 19% that approximately equal to actual values, and 33% that were greater than actual values. Model B resulted in 29% of predicted densities that were greater than actual values, 5% that approximately equal to actual values, and 67% that were greater than actual values. Model C resulted in 33% of predicted densities that were greater than actual values, 5% that approximately equal to actual values, and 62% that were greater than actual values. (Refer to **Figure 6**).

Table 4-Predicted & Actual Cheetah Densities

Predicted Cheetah Density for Select Reserves							
Cluster	Reserve	Census	Area (km ²)	Density (scalar) ^a	Density (prey) ^b	Density (weight) ^c	Actual Density
Waterberg	Entabeni	2011-12	63.5	0.104	0.025	0.027	0.047
	Madikwe	2011-12	750	0.010	0.017	0.017	0.007
	Rietvlei	2011-12	36	0.366	0.048	0.054	0.028
	Pilansberg	2011-12	572	0.017	0.018	0.019	0.005
	Ukulima	2011-12	9.4	0.059	0.024	0.026	0.319
	Mabula	2012	120	0.045	0.023	0.029	0.025
	Louis Trichardt	2011-12	26	0.006	0.015	0.028	0.038
Lowveld	Makutsi	2011-12	40	0.070	0.021	0.022	0.050
	Nambiti	2011-12	100	0.279	0.025	0.030	0.010
	Zimanga	2011-12	78.6	0.145	0.031	0.039	0.051
KwaZulu-Natal	Phinda	2010	235	0.046	0.021	0.037	0.089
	Thanda	2011	155	0.013	0.017	0.022	0.032
	uMkhuze	2011-12	400	0.129	0.032	0.045	0.013
	Zulu Nyala	2011-12	20	0.202	0.030	0.053	0.100
	Hluhluwe - Umfolozi	2011-12	960	0.024	0.025	0.031	0.042
	Kwandwe	2011-12	176	0.039	0.012	0.024	0.045
	Mt Cambedoo	2011-12	45	0.103	0.015	0.018	0.133
Eastern Cape	Samara	2011-12	120	0.067	0.013	0.015	0.067
	Shamwari	2011-12	47	0.067	0.041	0.054	0.064
Kalahari	Khamab	2011-12	940	0.081	0.012	0.018	0.032
	Laohu Valley	2011-12	86	0.020	0.031	0.034	0.023
<p>a Biomass refers to predictions based on prey biomass scaled to the reciprocal of carnivore mass $y = (94.54 * (50^{-1.03})) * (z/10000)$ (Carbone & Gittleman 2002)</p>							
<p>b Prey refers to predictions based on the biomass of significantly preferred prey species $y = -2.543 + (0.369 * (x))$ (Hayward et al 2007)</p>							
<p>c Weight refers to predictions based on the biomass of prey within the preferred weight range of a predator $y = -2.641 + (0.411 * (x))$ (Hayward et al 2007)</p>							

All methods predict cheetah density on a log-log scale. Therefore, prey biomass estimates were log-transformed and then back transformed to yield estimates of cheetah density.

Figure 6- Actual vs Predicted Cheetah Density ($\#/km^2$)



Heterozygosity

Heterozygosity values range from 0 to 1, with 0 being the least amount of genetic diversity (homozygous) and 1 representing the most genetic diversity (totally heterozygous). **Table 5** shows the descriptive statistics for the heterozygosity (H-indiv) for the two populations; see **Figure 7** and **Figure 8** for distributions. The reference population (FR) had mean H-indiv value of 0.605 and range of [0.188, 0.813], whereas the reserve population had a mean H-indiv value of 0.711 and a range of [0.438, 1].

The t-test to compare the population means had a p-value of 0.0003. This is strong evidence to reject the null hypothesis that the free roamer population mean is equal to the given H-indiv. The one-tailed p-value of 0.0001 is strong enough evidence to state that the free roamer population mean is smaller than the reserve mean. Furthermore, approximately 63% (45/71) of the reserve cheetah have greater individual genetic diversity than the mean of the free roaming population, 11% have less (8/71), and 23% are not significantly different

Table 5- Descriptive statistics for Heterozygosity by population

	FREE ROAMERS	RESERVES
N	39	71
Sum	23.59	50.89
Mean	0.605	0.717
Median	0.625	0.688
SD	0.156	0.130
SE	0.025	0.015
Min	0.188	0.438
Max	0.813	1.000

(16/71) (Figure 9).

Figure 7-Heterozygosity Distribution of Free Roamer Population

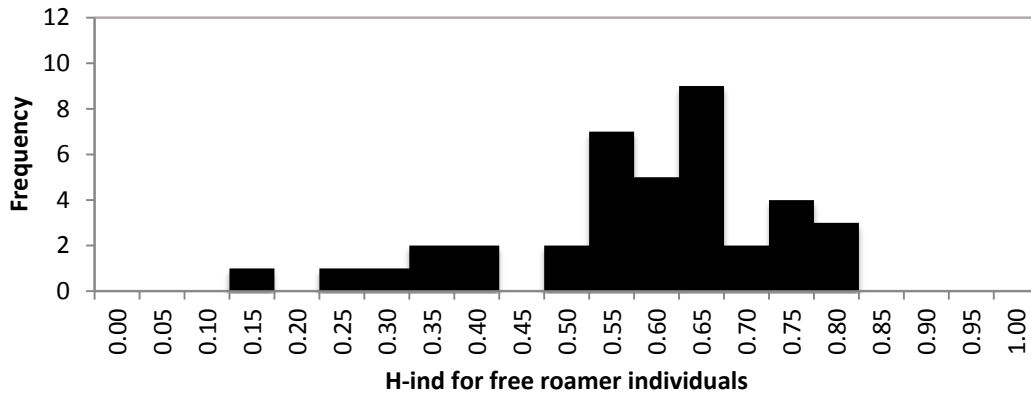


Figure 8- Heterozygosity Distribution of Reserve Population

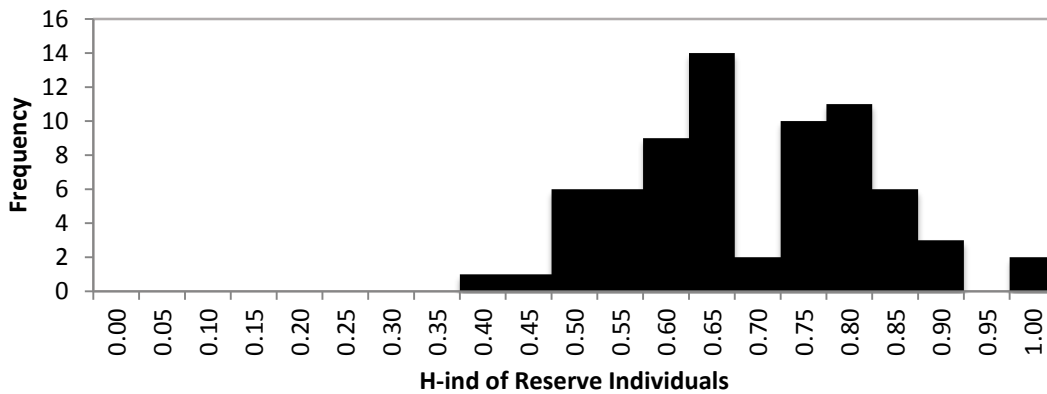
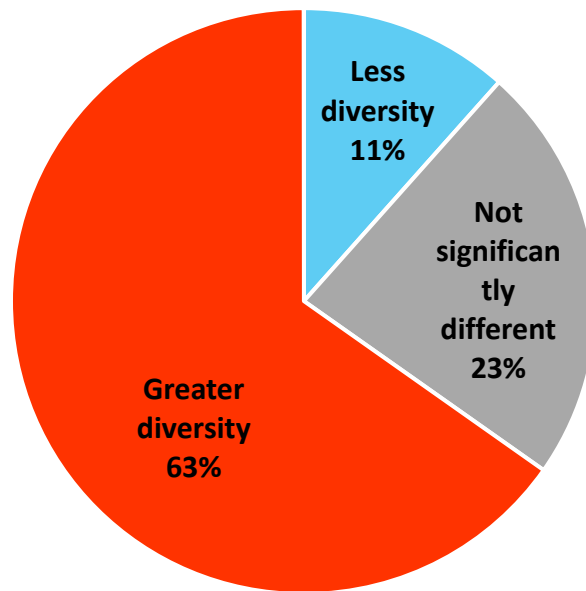


Figure 9-Gene diversity of reserve individuals compared to free roaming population



Relatedness

The summary statistics for pairwise relatedness (QGM) estimator values can be found in **Table 6**. **Table 7** shows the corresponding order and interpretation of estimator values. A simplified version of the relatedness composition is also found in **Table 7**. Roughly, this is the proportion of pairs that would be classified as full-sibling (parent-offspring), half-sibling, and unrelated. There were four cases with a QGM value of 1 in this data. One of these was caused by a duplicate sample being mislabeled as a different individual. The others are due to too much missing data for the given individuals.

Table 6-Summary statistics for pairwise relatedness

Summary statistics; pairwise relatedness for all individuals sampled	
	QGM
N	5995
Sum	-62.531
Mean	-0.010
Median	-0.028
SD	0.214
SE	0.003
Min	-0.625
Max	1.000

Table 7- QGM relatedness estimator values, definitions, and relatedness composition across all sampled individuals

QGM Value	Explanation	% of pairs with given relatedness
1	identical profiles (error, duplicate, or identical twin)	0.1
0.5	related to the level of full-sibling or parent/offspring	2
0.25	related to the half-sibling level	9
<0.25	distantly related	89

Discussion

Carrying Capacity

There are a number of possible explanations for the discrepancies between predicted and actual densities. First, there is a mismatch in timing between the predator data and prey data. The prey population surveys used in here are from 2010-2012, while the actual cheetah densities are from 2014. Since species populations go through boom and bust cycles, this mismatch could account for some of the discrepancies seen between predicted and actual values. Should data become available for the same year, the results may change accordingly. Next, management interference could be affecting the results. For this population of cheetahs, the managers or owners on each reserve decide how many cheetahs they would like on their reserve. This implies a reserve could demand many more cheetahs than their land is ecologically suited for or visa versa. EWT also translocates cheetahs where possible to prevent inbreeding, and this could contribute to the discrepancies seen in the results.

There are many reserves whose predictions are below the actual densities, in particular in Model A. This suggests that the reserves could hold more cheetahs than they currently do. However, cheetahs are competitively inferior to most other predators (Hayward et al 2006b; Frame 1999). **Figure 3** above shows that the majority of reserve cheetahs coexist with more than 1 other large predator, which could indicate that these other predator outcompete the cheetahs for prey. Additionally, **Figure 4** above shows cheetah mortalities on the reserves, we see that 3 of the top 6 causes of mortalities are from other large predators. Lions alone account for over 30% of known mortalities. Data in the right resolution was not available to allow quantitative exploration of the relationship between lions and cheetah density, however Endangered Wildlife Trust should request that lion predation on cheetahs be better documented. Information that would be useful to gather includes: cheetah age at time of mortality, the conditions in which lion predation on cheetah is attempted (eg exposed vs enclosed habitat, scarce vs abundant preferred prey of choice). Mortalities are depressing cheetah densities, though further information is necessary to determine the scale at which it is happening. The second highest cause of known mortalities is anthropogenic, which suggests that they are non-natural and not accounted for in the models. Overall, competitive inferiority and mortality are likely contributing to those reserves which have less actual density than the models predict.

There are also many prediction values which are greater than the actual density values, indicating that these reserves are over capacity. These conservative estimates could be due to the fact that the models are based solely on specific prey preferences. The predictions in the above equations B and C account for situations where at least one type of prey preference is met (species or weight). However, when neither of these conditions are met, cheetah will likely feed on what is present and attainable in order to avoid starvation. This is not accounted for in Models B and C. Finally, it is possible that these models are wrong for reasons not accounted for here. These model predictions, which are based solely on prey biomass do not capture the true density. Therefore, they should not be used to determine the ecological carrying capacity on these reserves.

Heterozygosity

About half of the reserve individuals showed less than the 16 microsatellite loci because of missing data, so associated H-indiv and relatedness values are very coarse. Interpretations are made with caution. Overall, the reserve population has higher levels of heterozygosity than the free roaming population, suggesting that genetic diversity has been maintained on the reserves. This is possibly due to a temporary outbreeding effect from 'artificial migration' (translocation between reserves). So although the genetic diversity appears to be adequately maintained at this time, EWT should continue genetic evaluation to ensure that this is not a temporary effect of translocation. This is especially true for the reserve population since the founders were cheetahs from Namibian and South Africa, which could have been 2 structurally different populations or one contiguous population. The South African founders came from what is now the free roamers, which was the reference population used in this study. Therefore, the outbreeding effect observed in the reserve cheetahs could simply be a result of added diversity from the Namibian population. However, if Charruau's theories (see introduction for a brief history of cheetah genetics) are correct, the Namibian and South African populations are not structurally distinct and so the resultant 'mixed' population would not actually show increased heterozygosity. Since this was the first time genetics were analyzed for the reserve cheetahs, the population structures of the Namibian and South African founders are not known. Therefore it is difficult to know which of these explanations is most likely occurring.

EWT is eager to know about inbreeding at the individual level. With the given data, the only inbreeding estimations possible to make are at the population level. While the heterozygosity and relatedness values do not appear to suggest an inbreeding problem on the reserves, the temporary outbreeding effect could be masking any effects from inbreeding that are occurring in the reserve population. Future analyses of heterozygosity would allow a comparison over generations such that it would be possible to determine how much diversity was gained or lost over the given time span.

Relatedness

Based on the summary statistics of the relatedness analysis, the free-roaming and reserves populations are distantly related overall (QGM <0.2). This implies that for management purposes, the two populations are not distinct enough from one another such that combining the two would promote diversity; nor should there be concern that there is a subspecies within the free-roaming population that should be preserved. In short, the free-roamers and metapopulation cheetah are similar enough to breed with one another without exposing rare alleles or reducing diversity. However, since the reserve population had greater heterozygosity than the free roamers, the reserve population would not necessarily benefit from mating with free roamers unless the outbreeding effect seen now is temporary.

Relatedness values are continuous and not categorical, and they are based solely on the samples included in the analysis. Therefore, relatedness is a sliding scale. A value of 0.5 indicates a pair of cheetahs related at the full-sibling level. However, a value of 0.4 could be full-siblings too, or it could be that inbreeding causes the pair to appear more closely related. For the purposes of this project, interpretations will be limited to approximate relatedness. In

other words, it is not so important to determine that two cheetahs are brother and sister and/or to determine which parent they share. Rather, it is important to know that if the relatedness value of the pair is equal to or greater than 0.2, then they are simply too closely related to mate.

Conclusion

This report evaluated the genetic and demographic integrity of South Africa's cheetah metapopulation by addressing factors associated with the population's long-term viability. The results were intended to be utilized for enhanced management of the reserve population. The carrying capacity estimates should not be used in this case, as the models were poor predictors of cheetah density on reserves. Better data on predation by other large predators, specifically lions, and predator/prey data from the same year could increase the accuracy of the models'.

The few simple genetic analyses that were conducted in this report are already being incorporated into EWT's management of the reserve population. For example, EWT now has clarity on some of the individuals they assumed were relatives and can intervene to ensure that they are either split up or have other non-relatives brought in. Genetic diversity appears to be adequately maintained on reserves, but could be due to a temporary outbreeding effect.

The resolution of the genetics data was quite coarse and the resulting recommendations offered are equally coarse. Mating pairs should be chosen based on genetic information as that is the best way to select for diversity and importantly, to know how closely related individuals are. When choosing individuals to mate, those with higher H-indiv should be more highly ranked. Mating pairs should have relatedness values smaller than 0.2, with lower values being best.

Currently the total number of cheetah openings left across reserves is 40, based on the demands of the reserve owners and managers. As the reserve population approaches its overall capacity, mating suppression will be required to avoid selling these wild cheetah into captivity. Endangered Wildlife Trust should begin to select mating pairs based on the suggestions listed above. Now that there is a baseline genetic profile for the reserve population, further parameters can be estimated or compared such as inbreeding, diversity gained or lost, and rare alleles. Genetic information should continue to be utilized for management, in order to further increase the population's biological fitness over the long term.

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