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# Carnivore Diet Identification Through Scat and Genetic Analysis in Namibia, Africa

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# Carnivore Diet Identification Through Scat and Genetic Analysis in Namibia, Africa

## **Abstract**

Worldwide the cheetah population is declining making them Africa's most endangered large cat. Namibia, Africa currently has the largest population of cheetahs in the world. During the summer of 2014, I did scat analysis of carnivores on the property of the Cheetah Conservation Fund (CCF) in Namibia in order to better understand the predators that compete with the cheetah and how the ecosystem works as a whole. I worked at CCF for nine weeks to analyze the diets of carnivores in the area through genetic and scat analysis. Analyzing carnivore feces would ultimately identify the diet of various carnivores in the area. One hundred and eight carnivore scat samples were analyzed including jackal, hyena, genet, serval, leopard, african wildcat, caracal, civet, aardwolf and cheetah samples. This information would help CCF understand how the entire ecosystem interacts and aid them in their efforts to manage and protect the wild cheetah. DNA was extracted from each scat sample to determine the species each sample came from. The DNA was then amplified using polymerase chain reaction. Ultimately, the sequences were compared to a genome reference database and the species were determined by sequence similarity. The scat samples were then washed and the contents were analyzed microscopically and macroscopically. Microscopic analysis involved burning hairs to create imprints and looking at the patterns underneath a microscope. My results revealed that the cheetahs are primarily competing with leopards for their prey. When CCF releases cheetahs back into the wild they will now be able to take my findings into consideration to locate the best release site.

## **Keywords**

wildlife, endangered species, population, hunting, cheetah, conservancy, PCR, DNA sequencing, bone analysis, jackal, leopard, african wildcat, serval, brown hyena, genet

## **Subject Categories**

Animal Sciences | Behavior and Ethology | Biodiversity | Genetics | Laboratory and Basic Science Research | Other Animal Sciences | Population Biology

## **Comments**

Please contact professor Conroy with any questions at [drew.conroy@unh.edu](mailto:drew.conroy@unh.edu)

**Carnivore Diet Identification Through Scat and  
Genetic Analysis in Namibia, Africa**

Undergraduate Senior Thesis

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## **Abstract**

Namibia, Africa has the largest population of cheetahs in the world. However, the cheetah population worldwide is declining. During the summer of 2014, I worked at the Cheetah Conservation Fund (CCF, 2015) in Namibia for nine weeks to analyze the diets of carnivores in the area through genetic and scat analysis. CCF has used scat analysis in the past to determine the diet of cheetahs, but the diet of other carnivores in the area has never been examined. Including this additional information will give researchers insight as to how the ecosystem functions as a whole, which is crucial when managing a population. One hundred and eight various carnivore scat samples were analyzed including jackal, hyena, genet, Serval, leopard, African wildcat, caracal, civet, aardwolf and cheetah samples. This work will help CCF in their efforts to manage and protect the wild cheetah. The first step was determining the species each scat sample came from. To determine this DNA was extracted from each scat sample. The DNA was then amplified using polymerase chain reaction and then sequenced. Ultimately, the sequences were compared to a genome reference database and the species were determined by sequence similarity. After determining the species the scat belonged to, the scat samples were washed and the contents were analyzed. Microscopic analysis involved burning hairs to create imprints and looking at the patterns underneath a microscope. Results revealed that the cheetahs have the greatest degree of dietary overlap and are primarily competing for their prey with leopards. Now when CCF releases cheetahs back into the wild they will be able to take these findings into consideration to locate a release site that will ensure cheetah survival.

## **Introduction**

I initially found out about the Cheetah Conservation Fund through my professor Dr. Andrew Conroy at the University of New Hampshire. I soon came in contact with CCF's Assistant Director for Animal Health and Research, Anne Schmidt-Küntzel. With the assistance from both of these mentors I was able to put together a proposal for a grant through the International Research Opportunities Program at the University of New Hampshire. After receiving the grant I spent months preparing for the cultural change I would experience as well as the laboratory techniques I would need to understand. I learned about Namibia and how it manages wildlife as a country and met with multiple professors to get a basic understanding of PCR, DNA sequencing and bone analysis.

Through my preparation beforehand and during my time spent abroad I learned that the loss of a single species could have a large multifaceted affect on an ecosystem. If a large carnivore is lost, smaller predator populations can grow too large and cause many problems (Winterbach et al., 2013). Therefore, protecting endangered large carnivore species is key to maintaining a functional ecosystem.

Understanding the ecology of an area is necessary when attempting to manage an endangered species population. In order to evaluate a particular carnivores' role in the ecosystem it is necessary to have knowledge of the carnivores' diet (Klare, Kamler & Macdonald, 2011). This information can provide insight as to which predators are competing for prey. Knowing which predators are competing for the same prey and understanding how the different species in the area are interacting can influence future management strategies.

## **Review of Literature**

### ***Global Animal Extinction:***

It is critical to review the history of extinction as a whole to fully understand the degree of severity and significance of problems the cheetah population faces today. Humans have impacted the environment in many ways including using natural resources, changing the global climate, introducing nonnative species, increasing the spread of pathogens, fragmenting species habitats and directly killing species. These effects combined make humans responsible for this sixth mass extinction (Barnosky, 2011).

Biodiversity is the variation of species on a genetic level as well as variation within an ecosystem or population, including the varying amounts and distribution of species over an area (National Research Council (US) Committee on Noneconomic and Economic Value of Biodiversity, 1999). Human impact has caused destruction of earth's biodiversity at a startling rate (Purvis, 2000).

Namibia is generally a very dry country making it especially vulnerable to becoming significantly more dry and hot due to human induced climate change. This is anticipated to have a negative effect on the biodiversity of the country (Barnard, 2002). Namibia was one of the first countries in Africa to create a National Biodiversity Strategy to protect the natural resources (Barnard, 2002). Namibia is also unique in the way that it manages and protects its wildlife to prevent future species from becoming extinct.



### ***Wildlife in Africa:***

With human populations increasing across Africa, human-wildlife conflict is increasing and will continue to become a greater problem if not addressed (Lamarque et. al, 2009). However, it has also been found that threatened large animals can be conserved with the assistance of proper wildlife management technique. Studies on wolf and cougar populations in North America and lynx, bear, and wolf populations in Europe revealed that using correct management is the number one factor contributing to the success of saving endangered species and has no correlation to human density (Linnell, Swenson & Anderson, 2001). This points to the urgency to educate people and create successful wildlife management strategies.

Local people in Africa kill wildlife for various reasons. This includes herbivores foraging on their crops, large animals migrating through their crop farms causing extensive damage, and carnivores preying on their livestock (Treves et al., 2006). The killing of carnivores by humans as a way of reducing depredation or to prevent them from preying on livestock, is occurring worldwide and is causing severe population reductions and range shrinkages in numerous carnivore species (Rust, 2013). Wildlife is also being killed due to cultural beliefs and practices. Traditional Chinese medicine falsely believes that many animal parts have healing powers and they are hunted for this reason. These misconceptions are seriously threatening rhino, elephant, leopard and pangolin populations in Africa (Ellis,

2013). In Kenya, Maasai men kill lions during the ritual in which warriors enter into manhood (Hazzah, Mulder & Frank, 2009).

For these reasons it has been shown that it is necessary to get the local peoples' support in conserving the wildlife (Brockington, 2003). Attitude surveys and other social science research tools have been used to learn mutually beneficial methods in which the wildlife can be conserved and managed cooperatively with the local people and their cultures (Browne-Nunez, 2008).

Wildlife is protected many different ways throughout Africa including national parks, Southern African private parks, and community based natural resource management. Each of these different conservation techniques has various advantages and disadvantages when trying to protect wildlife.

National parks for example are beneficial to certain predators, like lions. However, other predators such as cheetahs do not do well in these protected areas. This is because there are high numbers of stronger larger predators such as lions, leopards and hyenas and these predators often force the cheetahs away from their kills. National parks and other protected areas also don't sufficiently accommodate the needs of wide-ranging species, such as cheetahs, making long-term conservation in these protected areas difficult (Muntifering et al, 2006; Linnell, Swenson & Anderson, 2001).

The Southern African private parks are often fenced in and the farmers often have control of the animals within their fenced land. These farms can be beneficial for species conservation and provide economic incentives to protect wildlife. Yet, the fences can disrupt wildlife movement of species in the area (Marker, 2001).

Another method used to manage wildlife in Africa is community based natural resource management (CBNRM). This system allows the community members to work together to protect the wildlife and create a tourism market. The downfall is that this system, while it has been found to be extremely successful in some countries, is not as beneficial in others, in part due to legal constraints of people being able to directly manage their resources (Armitage, 2005). This system has been a great success in Namibia. The factors that made CBNRM successful in Namibia have been closely studied and can be used in other areas to create further success in protecting wildlife (Jones & Weaver, 2008).

### ***Namibia:***

Namibia is located on the southwestern coast of Africa and is often referred to as the “Jewel of Africa”. Namibia became an independent country in 1990. Namibia is impressively organized and well run making it one of the few consistently economically and politically stable countries in Africa (Namibian Sun, 2013). People all around the world come to Namibia to see its wildlife, amazing landscapes and cultural diversity. In 1995 it was shown that tourists coming to see wildlife in Namibia contributed an estimated N\$250.3 million to the economy proving that tourism has substantial national benefits (Barnes, 1999). Trophy hunting, which makes up fourteen percent of the total tourism, was estimated to have produced N\$134 million in direct expenditures in 2000 (Humavindu, 2003). In 2010 there were 984,000 international visitors to Namibia (World Bank: Trading Economics, 2015). Based on the 2012-2013 Namibian tourist exit survey, 67% of the

people who visited said they came to Namibia to see its wildlife. Other popular reasons for visiting included scenery, culture, and sense of space. For U.S. citizens in particular, curiosity was mentioned as a reason for visiting by 43% of the tourists. (Millennium Challenge Account for the Directorate of Tourism 2013). Due to Kenya's political and security issues, Namibia has recently gained popularity as a safari destination and seen a significant increase in tourism.

Namibia is one of the only countries to include conservation and the protection of wildlife in its constitution. Article 95(1) in the Namibian constitution states:

*“ maintenance of ecosystems, essential ecological processes and biological diversity of Namibia and utilization of living natural resources on a sustainable basis for the benefit of all Namibians, both present and future; in particular, the Government shall provide measures against the dumping or recycling of foreign nuclear and toxic waste on Namibian territory”*

The techniques and systems used to manage the land and wildlife in Namibia are some of the most innovative conservation programs in Africa and act as a model for other African countries (Weaver & Petersen, 2008). Namibia is admired for its great efforts in conserving its wildlife and land through the conservancies (Rust, 2013). A conservancy is a legally recognized, protected area co-managed by community land occupiers, who aim to collaboratively use and benefit from natural resources and wildlife in a sustainable manner (CANAM, 2010; Weaver & Petersen, 2008).

It was shown in 2009 that conservancies contributed about N\$487 million to the Namibian economy and N\$64.5 million in direct income (Conniff, 2011). This shows that the system of conservancies has not only wildlife conservation benefits but also has financial benefits for the country as a whole and its members individually.

The Veterinary Cordon Fence (VCF) was created in the 1960s and divides the country into North-South portions. The fence was originally created to prevent the spreading of contagious animal diseases to protect the livestock owned by the white farmers (Tjaronda, 2008). North of the fence is majority communal or native black farmers and south of the fence is primarily commercial or wealthier white farmers, who have titles to their land, often called free-hold land, this affects the type of conservancy formed.

There are two types of conservancies in Namibia, commercial conservancies and communal conservancies. Commercial conservancies are similar to the method used in the United States where a person owns their farm property. Commercial farmers, which are mainly found in the southern portion of Namibia, have primarily been owned by the white population and tend to be wealthier and have traditionally been of Afrikaner and German descent (Rust, 2013; Ashley & Barnes, 1996). Commercial conservancies are formed by landowners who farm livestock and game extensively on a commercial level (Rust, 2013). In commercial conservancies it is legal to hunt the animals that are on the property. Fences are permitted as well as the management of wildlife on the property. These commercial farmers commonly host hunting safaris on their property and legally sell their wildlife's products and

game meat. Since commercial farmers have gained the rights to manage the wildlife on their land and have been motivated to do so for their own financial gain, the wildlife numbers on commercial farms have gone up by at least 80% (Weaver & Skyer, 2003).

In contrast, the majority of poor native Namibians live in communal farms (Mendelsohn et al., 2012; Ashley & Barnes, 1996). Communal farming regions are located in the northern third of the country. The land titles on communal farms are retained by the government (Rust, 2013). This means that the farmers in the communal farms do not have rights over the wildlife on the land as in commercial farms. Communal land has more free roaming wildlife because fences are not allowed, making migration patterns possible.

Commercial farmers are able to recover more easily from any economic loss caused by predators killing their livestock. On the other hand poorer communal farmers suffer greater from the loss of even a single livestock animal. This explains why it is common for communal farmers to kill predators to protect their livestock. Communal farmers have hunted animals such as hippos or elephants almost to the point of extinction because these large species would damage the farmers' crops (NACSO, 2015).

In 1996, Community Based Natural Resource Management (CBNRM) system was created in the communal areas to promote sustainable management of the natural resources while also creating economic growth (NACSO, 2015). In 1998 the Nyae Nyae conservancy was the first communal conservancy to be established in Namibia.

This system allows the conservancy members to manage the land and animals together to create and benefit from a tourism market. This system encourages the locals to protect the wildlife in their conservancy and prevent poaching. They carefully manage the wildlife populations through hunting and have created a tourism market. The locals realize they can't over hunt because then they will lose the wildlife and lose the tourists and income. This system benefits both the local people and the wildlife. The community based natural resource management system provides economic benefits from the presence of these carnivores. These economic benefits include income from trophy hunting, photographic safaris, camping and locally owned lodges for tourists, as well as income from selling items such as game meat. These economic benefits give the communal farmers an incentive to protect the predators on their land and have been proven to be necessary for lasting successful conservation. (Rust, 2013; Muntifering et al, 2006).

Namibia is known for its national parks such as Etosha, Waterberg and the Skeleton Coast. National parks manage wildlife differently than both the communal and commercial conservancies. In contrast to commercial or communal conservancies, there is not a direct individual gain from National parks. However, they do play an important role in conservation and the country's tourism (Ashley & Barnes, 1996). National parks are large fenced in areas that protect wildlife. As mentioned earlier much of Namibia's wildlife resides outside of these parks because of the fences and the competition with other larger predators.

## ***The Cheetah:***

The cheetah is currently Africa's most endangered large cat. Cheetah numbers are declining globally as well as the amount of areas they occur in (Purchase et al., 2007). The wild cheetah population has seen a drastic decline throughout the past century. In 1990, there were 100,000 cheetahs left in the world. Today, there is only an estimated 10,000 remaining and in 23% of the original region that they once lived in (CCF, 2015). Now cheetahs are present in Africa and Asia but Namibia has the highest population of free ranging cheetahs in the world (Marker, Mills & Macdonald, 2003; Marker et al., 2007; Krenzel, 2015; Nowell, K. 1996). It is extremely difficult to get an accurate count but there is an estimated 3,000 cheetahs remaining in Namibia (Marker & Dickman 2004; Schumann et al., 2012). In Namibia cheetahs live mainly below the Veterinary Corodon Fence on commercial farmland outside of protected wildlife areas (Nowell, 1996). The main problems the cheetahs face in Namibia are habitat loss, fragmentation and degradation, human wildlife conflict and the illegal wildlife trade (CCF, 2015; Purchase et al., 2007).

The cheetah has various physical adaptations for quick acceleration and high-speed locomotion, which is necessary for them to catch their prey (Hudson et al, 2011). Because of their small build they are frequently forced away from their kills because larger predators such as leopards, hyenas or lions threaten them (Winterbach et al., 2013). This problem of competition for prey amongst other larger and stronger predators is making it harder for cheetahs to survive by limiting their density and distribution. There are often less of these larger dominant



predators on farmlands but there are still adequate amounts of prey so cheetahs tend to reside in these areas (Marker & Dickman 2004). This creates a conflict between the farmers and cheetahs.

In contrast to other large predators in Namibia, cheetahs hunt during the day. This means the farmers see the cheetahs and not any other predators and assume they are responsible for livestock kills (CCF, 2015). In a desperate attempt to protect their livestock the farmers resort to trapping and killing cheetahs (Marker et al., 2008; Schumann et al., 2008). Therefore it is important to inform farmers and change these misconceptions for cheetah numbers to improve (Schumann et al., 2008).

Cheetahs face habitat loss and fragmentation due to human interference ultimately reducing the areas carrying capacity. This fragmentation has heightened the problem of loss of genetic variation by separating cheetah populations and increasing the rate of inbreeding. A population needs genetic diversity to allow it to survive and overcome environmental alterations or unpredicted disasters.

Lastly, the illegal wildlife trade is yet another issue the cheetah is facing. Cheetahs were once a status symbol for wealth and royalty. Today there is still a market for people trying to keep cheetahs as pets especially in the United Arab Emirates (Ahmed, 2010). To do this cheetah cubs are captured from their mother and sent off to where they are wanted. Only one in six of the cubs captured survive the trip due to inhumane travelling conditions so more cubs are then captured to meet demand (CCF, 2015). A cheetah will always remain a wild animal; it can be

considered abusive and unethical to raise them as a pet and this is assisting cheetah population decline (CCF, 2015).

### ***Importance of Working with the Farmers:***

It is estimated that 90% of the cheetahs in Namibia reside outside protected areas on commercial farms (Marker, Mills & Macdonald, 2003). Human caused mortality has been the leading threat to the cheetah population. Saving the cheetah will not be possible without changing the attitudes of these farmers who come into contact with the cheetahs (Muntifering et al, 2006).

The Cheetah Conservation Fund (CCF, 2015) has worked to determine the cheetahs' diet in order to prove to the farmers that the cheetahs are not responsible for these livestock kills. Surveys with the Namibian farmers have shown that farmers who see cheetahs as a problem kill, on average, 29 per year. After educating and working with the farmers this number dropped to an average of 3.5 cheetahs killed per year (Marker, Mills & Macdonald, 2003).

### ***Techniques to Determine Diet:***

Analysis of scat (Burns et al., 1998), stomach content (Contesse et al., 2004), field observation and stable isotopes (Hilderbrand et al., 1996) are some of the various methods that have been used to study and determine an animals diet.

When working with an endangered species like the cheetah, analyzing the stomach contents of alive animals is not an option. The elusive nature of the cheetah

also makes field observation unreliable and extremely difficult. Scat analysis is a simple and non-invasive method to determine a species diet.

Scat analysis looks at the undigested parts of prey such as the hair, feathers and bones in the predator scat. This method is ideal because the scat is easy to collect. Scat analysis is the most commonly used method when determining the diets of terrestrial carnivores (Klare, Kamler & Macdonald, 2011). This method has been used and shown to be reliable in determining the diets of Weddell seals (Burns et al., 1998) as well cheetahs (Marker et al., 2003).

## **Research Methods and Materials**

This research included the prey preferences of the cheetah (*Acinonyx jubatus*), black-backed jackal (*Canis aureus*), brown hyena (*Hyaena brunnea*), leopard (*Panthera pardus*), caracal (*Caracal caracal*), serval (*Leptailurus Serval*), african wildcat (*Felis silvestris lybica*), aardwolf (*Proteles cristata*), genet (*Genetta genetta*) and other genet-like carnivores.

### ***Predator Species Identification:***

The carnivore predator species was determined using molecular genetic tools (mitochondrial segment ATP6). The prey species was determined through hair analysis and bone identification.

To determine which predator species the scat belonged to, DNA was extracted from the scat using the Qiagen stool extraction kit methods described in Appendix I.

Next the extracted DNA was run through a polymerase chain reaction. A PCR amplifies a specific section of the DNA template indicated by the primers. This makes a complimentary strand of DNA. After PCR there are lots of copies of the specific ATP6 sequence. The PCR recipe for each sample is shown below. The program used on the PCR was Td Igd.

The PCR recipe for each sample was: 7.5  $\mu$ l of Master Mix 3, .6  $\mu$ l of ATP6-Reverse (10  $\mu$ M concentration), .6  $\mu$ l of ATP6-Forward (10  $\mu$ M concentration), .3  $\mu$ l of BSA (20mg/ml), 4.5  $\mu$ l of H<sub>2</sub>O and 1.5  $\mu$ l of the DNA.

After the PCR was completed, to determine if the PCR was successful or not a 1 % agarose gel electrophoresis was performed. To do this recycled gel was heated in the microwave until it became a clear liquid. The solution was cooled to about 55°C. A gel tray was prepared by sealing the ends with tape. Two combs were placed into the tray and positioned vertically. The gel solution was then poured into the tray. This took about 20 minutes to solidify. The combs were then removed slowly leaving wells in the gel. The tray was then placed in the electrophoresis chamber filled with TAE buffer. 2  $\mu$ l of heavy band loading dye and 1  $\mu$ l of gel red dye was added to each 10  $\mu$ l sample. The mixtures were carefully loaded into the wells. The

gel was run for 25 minutes at 75V. The gel was visualized using the UV station and the gel image was printed.

The samples were then prepared for sequencing. This was done by combining 2  $\mu$ l of exosap (10:) with 5  $\mu$ l of PCR product. The exosap removes dNTPs and primers that are still remaining in the PCR product. After the samples were cleaned the sequencing reaction was performed. The recipe for each sample is shown below.

Recipe for each cleaned PCR product: 1  $\mu$ l of ATP6 Reverse primer (2  $\mu$ M concentration), 1  $\mu$ l of Big Dye, 2  $\mu$ l of Big Dye Buffer, 4.5  $\mu$ l of sterile water and 1.5  $\mu$ l of cleaned PCR product.

The mixtures were then put in to be sequenced for two and a half hours using program Big Dye User: "PE". Following sequencing an ethanol precipitation was performed for each sample. This is the final step to prepare the sequences for the 310 analyzer. To do this a 1.5ml tube with 12 $\mu$ l of master mix one is prepared for each tube in the pre-PCR area. After preparing this master mix two was prepared separately. The recipe for master mix one and two is shown below.

Master Mix 1 Recipe: 10 $\mu$ l of DI-H<sub>2</sub>O and 2 $\mu$ l of 125 mM EDTA

Master Mix 2 Recipe: 2 $\mu$ l of 3M NaOAc pH 4.6 and 50 $\mu$ l of "pure" ethanol

The 1.5ml tubes and master mix 2 was then brought over to the post-PCR area. 10µl of the sequencing product was then added to each tube containing master mix 1. Pipetting up and down mixed the solution. 52µl of master mix two was then added to each 1.5ml tube and then quickly vortexed. The tubes were left to incubate at room temperature for 15 minutes.

The samples are then spun at 12,000g for 20 minutes at room temperature. The supernatant was then carefully decanted with out removing the pellet. 250µl of 70% ethanol was added to each sample and then they were spun again at 12,000g for 10 minutes at room temperature. The supernatant was again decanted carefully making sure not to lose the pellet. 250µl of 70% ethanol was added to each sample and they were again spun at 12,000g for 10 minutes for a final time. The supernatant was decanted. After the precipitation was completed the samples are left open in a dark cabinet overnight to fully dry.

The following day 13µl of Hi-Di form amide was added then quickly vortexed. Each sample was then transferred to tubes that will be used in the 310 analyzer. The samples are denatured and finally placed into the 310 analyzer.

After being analyzed the sequences were edited through the genius program and manually checked for mistakes. The final sequences were then compared to reference sequences to determine from which species the sample came.

### ***Prey Species Identification:***

After determining the predator species the scat samples were washed. This was done by putting the frozen samples into a nylon stocking. The stocking must be

rolled down to the very bottom before putting the first sample in. A metal identification number must be put in with each sample and recorded so the sample can be identified after washing. After placing the first sample into the nylon with the specific identification number a knot should be tied right above the sample to prevent samples from mixing with each other. The next sample is placed above this knot and the protocol was repeated.

The stocking was left out for a few hours to allow the samples to completely defrost before washing. Once the samples were defrosted they were placed into a washing machine and run on one 25-minute wash cycle with no detergent. After washing the samples should only have hair, bones, sand and vegetation remaining if there was still scat residual then that specific sample was washed again. After washing, the samples are left to dry over night. While drying a metal bowl was placed over the samples so bugs can't get into the samples. The following day the remains of the samples are removed from their stocking and placed into a plastic bag labeled with their corresponding scat number.

If the sample had hair, an imprint of the hair was burned onto a plastic cover slip. This was not done to samples that clearly only had rodent hair because our hair reference guide does not include rodent hair. These samples normally had bones that could be used to identify prey instead. This was done by separating the bone fragments from the hair and comparing them to a reference sheet. The scapula shape was usually a good indicator if the skeleton was a shrew or rodent. The jaw and teeth were also used to differentiate between rodent and shrew skeletons. Larger bone fragments such as pieces of long bones were harder to identify from the

bone alone. From the size of the bone I immediately knew that it was not part of a rodent or shrew skeleton and instead belonged to something the size of a springbok, dik dik or warthog. For samples that had larger bones from larger prey there was normally enough prey hair to identify the specific prey using the hair burning method.

To burn an imprint of the hair the following methods were used. Five glass slides were laid out for each sample. Two plastic cover slips were placed onto each slide. One hair from the sample is placed onto each coverslip. Another glass slide is then placed over the hairs on the coverslips. Four binder clips are used to hold the two glass slides together (See Figure 1 below).

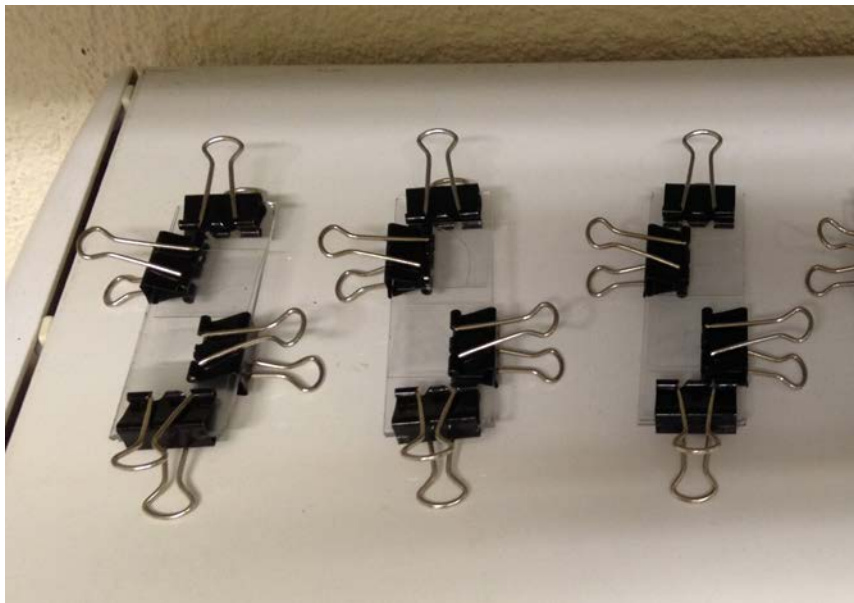


Figure 1: Hair burning set up.

The sandwich of slides with cover slips in between was then placed in a pre-heated toaster oven for five minutes. After five minutes the slides were taken out and left to cool. Once they are cool to the touch the binder clips were removed and the slides were taken apart. The hair was then taken off of the coverslip and taped



onto the results page. The coverslip with the remaining imprint of the hair is taped next to the corresponding hair. The hair was kept for macroscopic examination. A total of ten coverslips with imprints were made for each scat sample.

The coverslips with imprints are examined first at x4 to locate the hair and then further scrutinized under 10x and if necessary 40x objectives. The magnification of the eyepiece is 10x so this was a total magnification of 40, 100 and 400. The imprint was inspected from proximal to distal end. The pattern in each region of the hair was studied and compared to the references of labeled hair imprints. After determining the prey, based on the pattern of the hair, the result was recorded. Later a second person examined the hair and also record their findings. This helped eliminate inaccuracies and confirm the correct prey. If the two readers disagreed on what the pattern indicates as a prey then a third person would review the hair. The color and thickness of the actual hair could be compared to reference hairs to also help indicate what the prey was.

For samples that did not have the hair burned the sample was removed from the plastic bag and put into a rectangle plastic container for macroscopic examination (See Figure 2).



Figure 2: Macroscopic analysis set up.

The contents of the samples were inspected and if bones, berries, seeds or feathers are found they were removed and placed into a small petri dish. The contents found were recorded and then arranged and photographed for future access. A description of each sample was later entered into a word document.

## Results

Table 1: Predator species and amount of samples.

<b>Species</b>	<b>Number of Samples</b>	<b>Species</b>	<b>Number of Samples</b>
Jackal	33	Serval	1
Leopard	28	Civet	1
Cheetah	6	Caracal	1
Brown Hyena	6	Aardwolf	1
African Wildcat	6	Unknown	1
Genet-like	8		

Table 1 show above illustrates the amount of predator samples studied for each species. The overall results showed that jackals showed no preference on prey and would prey on anything that was available. The results of the brown hyenas show a large preference in elands. Hyenas are primarily scavengers. The large size of elands leads me to believe that eland carcasses would often be left unfinished leaving it available for hyenas to eat. This theory may explain the high percentage of

eland in the hyenas' diet. African wildcats and genets prey on rodents and bugs. Leopards preferred springbok and eland but still had quite a variety in their diet. On average leopards range from 30-80 kg, whereas cheetahs range from 35 to 65 kg (National Geographic Website & Big Cat Rescue Website), they are similar in weight, however cheetahs are much longer and slender while leopards are thick and muscular. The heavier build of leopards gives them an advantage when searching for prey because they are capable of killing various sizes of prey. Therefore they have a larger amount of prey available for them to hunt because they are not limited by the size of the prey.

The cheetahs had a much smaller variety in the large prey they ate. They seemed to prefer red hartebeest, springbok and kudu. Cheetahs have a harder time killing larger prey, therefore are more limited in prey species. Cheetahs will have an easier time finding kills in regions with fewer leopards because there will be less competition. The data below is a further explanation of the findings of each species dietary preference.

## Jackal

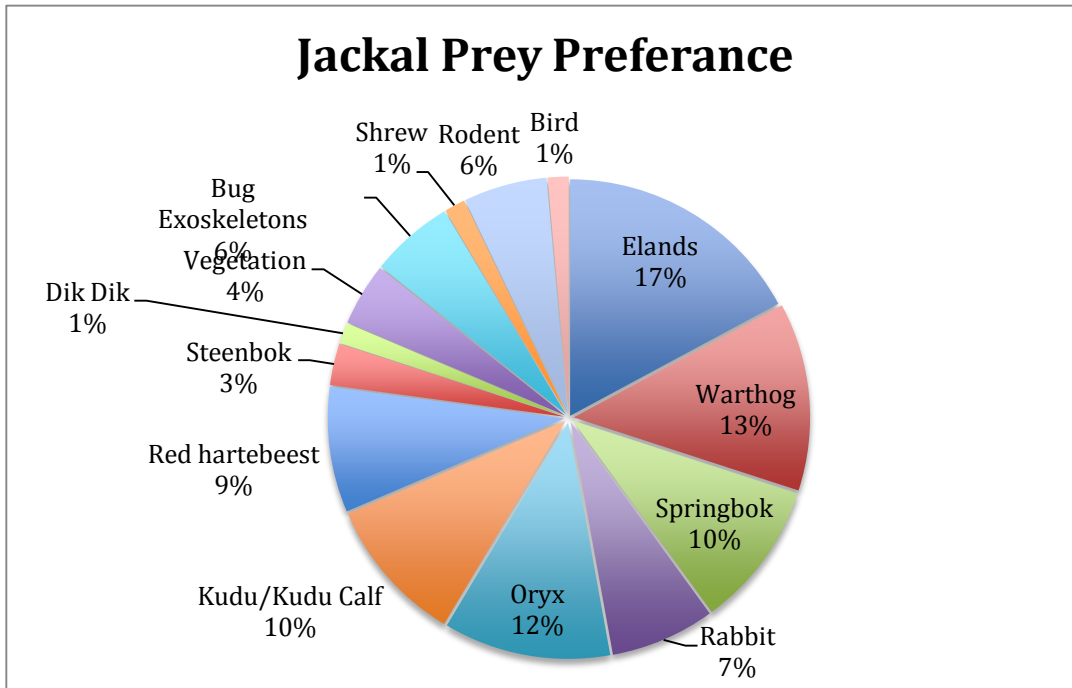


Figure 3: Jackal prey results: percent of each prey species found in jackal samples.

Out of the thirty-three jackal samples in this project twenty-three of these had hair that could be burned. One of the samples that was thought to have prey hair that could be burned turned out to be pieces of plants that were mistaken as hairs. Out of the remaining twenty-two samples that had the hair burned twelve of the jackals preyed on elands, nine of the jackals preyed on warthog, seven preyed on springbok, five preyed on rabbit, eight preyed on oryx, seven preyed on kudu and or kudu calf, six preyed on red hartebeest, two preyed on steenbok and one preyed on dik dik. There were ten samples that did not have hair that could be burned so these samples were analyzed macroscopically. Out of these samples only two included

vegetation, one included vegetation and unidentifiable bug exoskeletons. Four included rodent bones. One included beetle exoskeletons. One included a grasshopper exoskeleton. One showed remains of a shrew as well as a snake or some sort of reptile skin. One revealed that the prey was a bird as well as seeds and some bugs. In conclusion Jackals are opportunistic hunters and do not necessarily show a preference for prey.

### **Leopard**

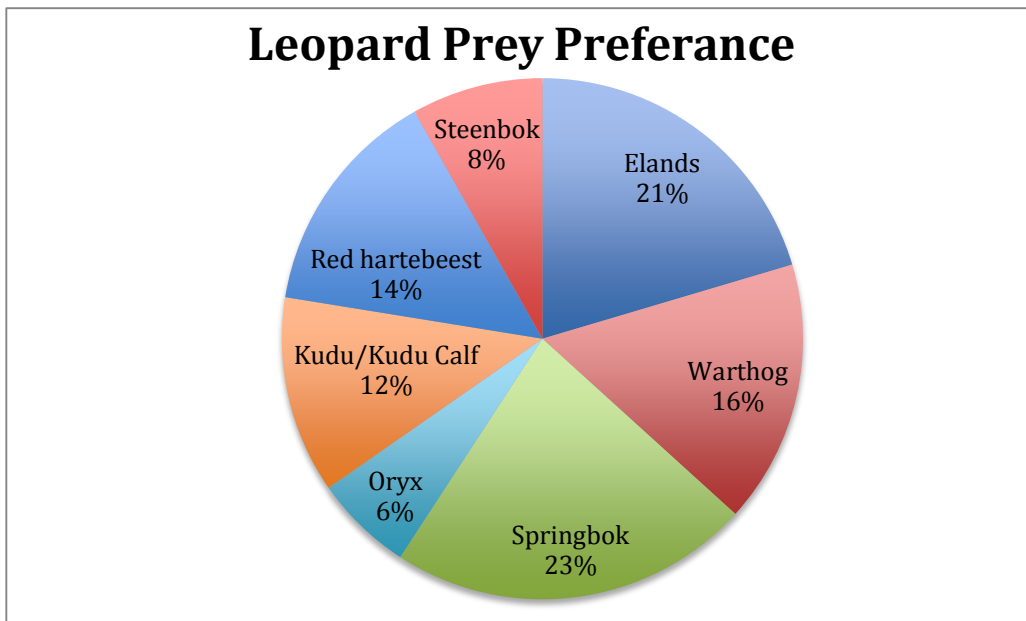


Figure 4: Leopard prey results: percent of each prey species found in leopard samples.

Out of the twenty-eight leopard samples, twenty-six had prey hair that could be burned. Four of these samples that had hair burned also had bone fragments. Three of the leopard samples did not have any hair or bones. The hair burning revealed that eight of the leopards preyed on warthogs, six preyed on kudu and or

kudu calf's, seven preyed on red hartebeest, ten preyed on elands, eleven preyed on springbok, four preyed on steenbok and three preyed on oryx.

### Cheetah

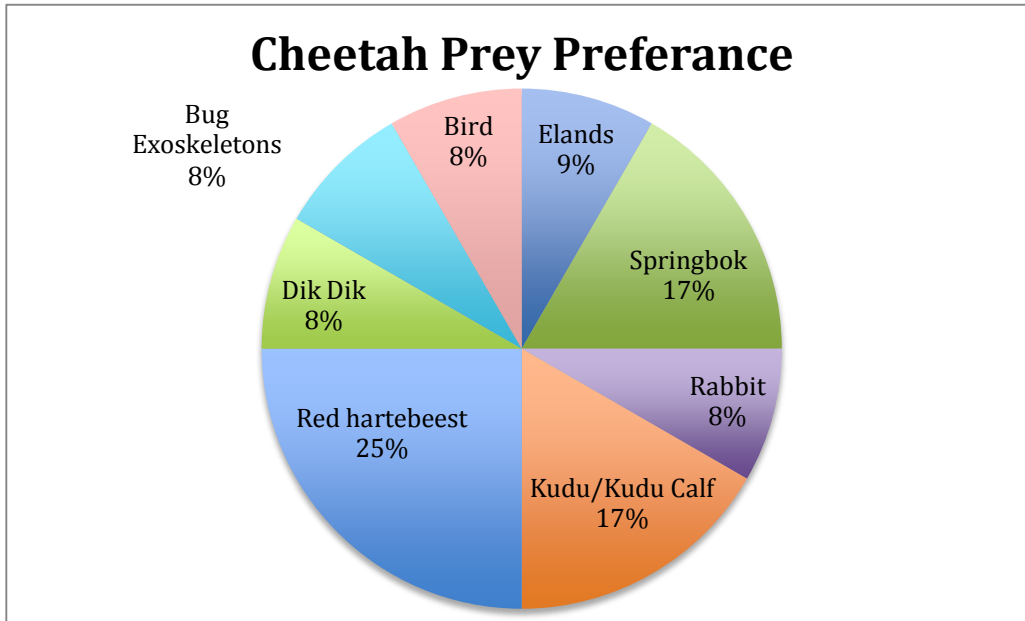


Figure 6: Cheetah prey results: percent of each prey species found in cheetah samples.

Out of the six cheetah samples in this project five of them had hair that could be burned to determine the prey. The one remaining sample contained bird feathers and bug exoskeletons. One cheetah preyed on an eland, two preyed on springbok, one preyed on a rabbit, two preyed on kudu and or kudu calf, three preyed on red hartebeest and one preyed on a dik dik. If you compare the leopard and cheetah preferred prey pie charts, the overlap of prey between these two predators is evident.

## Brown Hyena

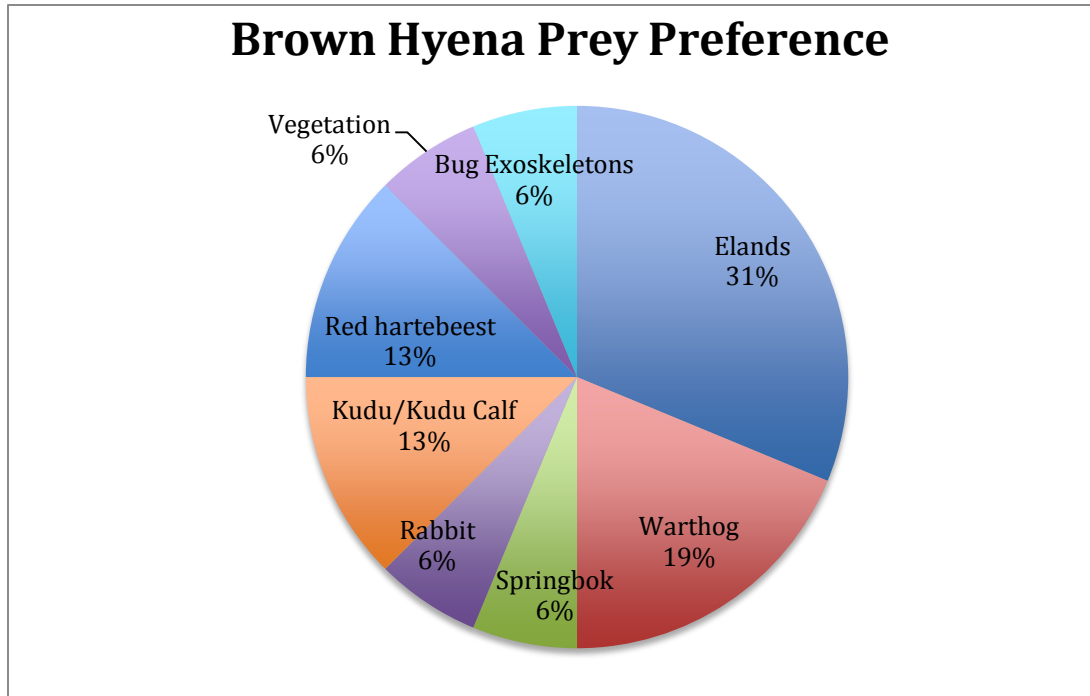


Figure 5: Brown Hyena prey results: percent of each prey species found in brown hyena samples.

Out of the six brown hyena samples, all six had enough prey hair to be burned and two of these samples also had a few bone fragments. One of the samples that had bone fragments also had some leaves and other vegetation however this could have been from collecting the sample post digestion. Five of the hyenas preyed on elands, three preyed on warthogs, one preyed on a springbok, one preyed on a rabbit, two preyed on kudu and or kudu calf and two preyed on red hartebeest.

## Genet "Like" Samples

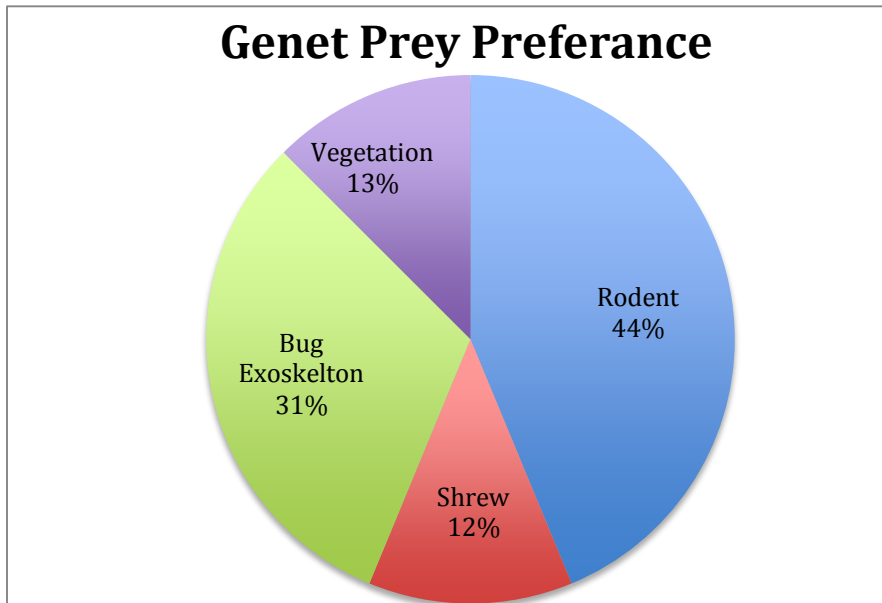


Figure 7: Genet prey results: percent of each prey species found in genet samples.

None of the eight genet-like samples had prey hair that could be burned. The bones were separated from the samples and compared to the bone reference guide to determine the prey. Seven of the genets preyed on rodents, two preyed on shrews, five preyed on various bugs and two ate vegetation.



## African Wildcat

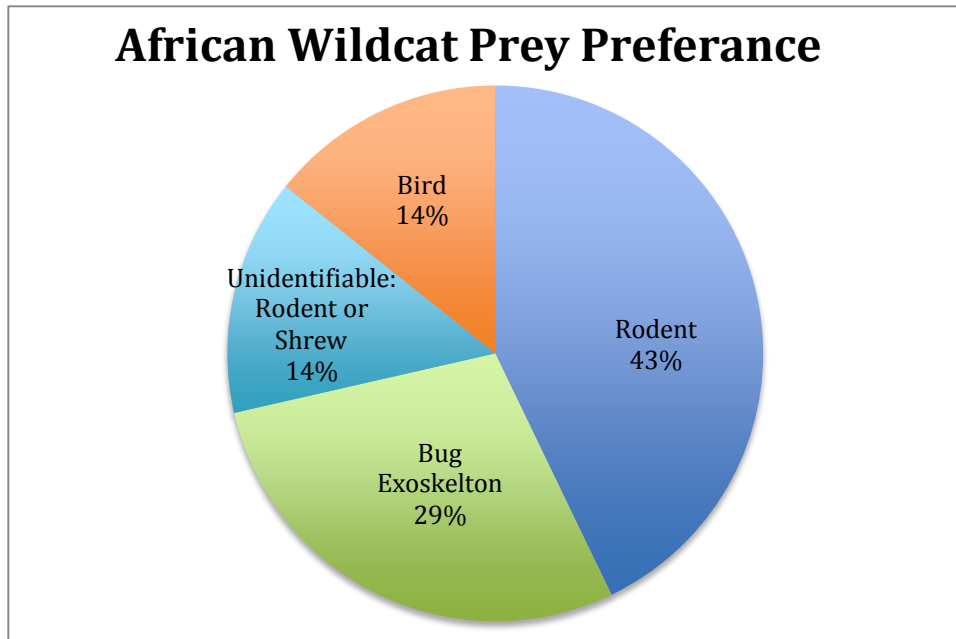


Figure 8: African Wildcat prey results: percent of each prey species found in the African wildcat samples.

None of the six African wildcat samples had prey hair that could be burned. The bone fragments were analyzed and compared to the bone reference guide to determine the prey. In one sample the prey could not be completely determined but was either a shrew or rodent. Three African wildcats preyed on rodents, two ate bugs and one preyed on a bird.

## **Serval**

There was only one Serval sample in this project. The bones in the sample revealed that it preyed on a bird and a rodent. In the center of Figure 10 a bird long bone with a digit attached is visible. Other digits were also found as well as fragments of other bird bones. A bird foot with black scaly skin and bones still attached was also found. Black and white spotted feathers were also found. Three small teeth, most likely rodent teeth, were also found so the bird couldn't have been the only prey.



Figure 9: Components of Serval sample.

## **Caracal**

This sample included a tick exoskeleton and a few bone fragments. The prey could not be identified from these fragments.

## Civet

The genetic analysis of this sample was similar to a genet and was most likely a civet. This sample included remains of a bird and rodent as well as some small spherical objects that could be seeds or berries (See Figure 11). In the top left corner of the photo you can see a bird foot that was found. Lots of black and white feathers and feather roots were also found. Small spherical black berries or seeds are shown in right side of the picture. In the bottom right of the photo you can see a few bug exoskeletons specifically beetle exoskeletons that were discovered. Underneath the bird foot there is a small rodent jaw with teeth. There was a lot of light brown rodent hair in the sample.



Figure 11: Components of Civet sample.

## Aardwolf

This sample included lots of grass and other vegetation. There were many small black bead like objects that are most likely pieces of termites (possibly the heads). No hair or bones were found.

## **Unknown**

This predator could not be identified genetically because it did not match anything in the reference library. This sample included lots of bug exoskeletons that were most likely pieces of grasshopper exoskeleton.

## **Discussion**

CCF has done studies in the past to determine what the cheetahs in central Namibia were preying on. This information could be used to educate farmers on the truth about what cheetahs were eating and help farmers realize that the cheetahs in most cases were not predominately preying on livestock and therefore not causing them an economic loss (Marker et al., 2003).

In this project I took CCF's research a step further than what had been done in the past. Instead of just focusing on the cheetah, we also considered other carnivores in the ecosystem and determined what they were preying on. This information gave us a better picture of how the ecosystem was functioning. This research showed how the carnivores in the ecosystem were linked and how they were affecting each other. We tried to answer questions such as: which species are competing for prey? Which prey populations directly affect the cheetah? This could prove helpful in the future when determining the ideal area to release cheetahs. This information was also needed to help devise a reliable population management plan for the cheetah in the future.

Past research has shown that leopards can coexist easily with large predators because they are flexible to eating different prey (Karanth & Sunquist 2000, Marker & Dickman 2005). These results did agree with these previous findings, however I am led to believe that even though leopards can coexist easily with large predators this does not mean that large predators can coexist easily with them. The results showed the cheetah prey overlapped greatly with the leopards prey revealing competition for prey between these two predators. As mentioned earlier the leopards will have the advantage over cheetahs when competing for prey because of their large size. The small size of cheetahs imposes limitations on which prey they can catch (Hayward et al., 2006). Cheetahs are often forced away from their kills because they are threatened by dominant competitors for prey (Winterbach et al., 2013).

In a previous study it was found that red hartebeest followed by springbok were the most common prey for cheetahs (Wachter et al., 2006). The results from our project the cheetah scat samples also showed the red hartebeest was represented most followed by springbok and kudu. The results from this research illustrate that cheetahs primarily capture medium sized prey. Previous studies agree with these findings and suggest that this is the case because these prey can be eaten relatively quickly before larger predators steal the cheetahs kill (Hayward et al., 2006).

If this research was to be repeated it could be improved in various ways. First there could have been more scat samples collected over a larger area. In general, doing this project on a larger scale would make the results more significant

and reliable. In this project I had far more jackal and leopard scat samples in comparison to other predator species. If this were done again I would attempt to include a similar number of each of the different predator scat samples. I would also use a genome reference library that contained more references. The specific species of some of the samples could not be determined because we did not have them in the genome reference library available to us. Instead I determined the genome that matched the closest to these samples. This was evident with the genet-like samples, the genomes matched closest to the common genet genome that we had in the reference library however it could not be determined exactly which species of genet the scat samples belonged to. Finally, I would also look at all of the hair samples under a greater magnification and use a more in depth reference book for greater accuracy when determining the prey.

In conclusion, to aid in successful transition of cheetahs being released back into the wild I would suggest that researchers look into the populations densities of leopard around possible cheetah release sites. It would also be helpful to look at population densities of the cheetahs preferred prey in these areas. This information will aid in assuring a successful transition back into the wild for these cheetahs. Further studies on whether the abundance of the prey species has an effect on what carnivores prey on would be useful.

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## Appendix I: Predator Species Identification Methods

1. Set heat block to 70°C
2. Add 1.6 ml of Buffer ASL to the first 2ml tube for each sample.
  - a. Buffer ASL is a stool lysis buffer that is provided by the Qiagen Kit
3. Prepare freezer drawer with three icepacks, clean with bleach and water.
4. Take samples that are to be extracted and place them into the freezer drawer.
5. Place approximately 100-200 mg of the scat sample into the tube with the 1.6 ml of buffer ASL.
6. Return the scat samples to the freezer.
7. Vortex each tube from 1-10 minutes or until the scat is dissolved into the buffer.
8. Centrifuge tubes for 1 minute at full speed (14,000rpm).
  - a. This creates a pellet of stool particles on the bottom of the tube.
9. Remove 1.4ml of the supernatant and place it into a new 2 ml tube, discard the pellet.
10. Add InhibitEX tablet to each of the three four tubes.
  - a. The InhibitEX tablet binds the PCR inhibitors.
11. Vortex the tubes for 1 minute and then leave them at room temperature for an additional minute.
12. Centrifuge the tubes for 6 minutes at full speed creating a pellet of stool particles and an InhibitEX matrix.
13. Transfer as much supernatant as possible to a new 1.5 ml tube and discard the pellet.
14. Centrifuge the new tubes for 3 minutes at full speed.
15. Prepare new 2ml tubes with 25 µl of proteinase K.
  - a. Proteinase K is an enzyme that is able to digest keratin and is often used in DNA extractions.

16. Transfer 600  $\mu$ l of the supernatant from each tube to a new tube that contains proteinase K.
17. Add 600  $\mu$ l of Buffer AL to each tube.
18. Vortex the tubes for 15 seconds and then incubate them in the heat block for 10 minutes at 70°C.
19. Add 600  $\mu$ l of ethanol and vortex the tubes.
20. Transfer 600  $\mu$ l of the lysate to a QIAamp spin column. Do not discard the tube with left over lysate.
21. Centrifuge the spin columns for 1 minute at full speed and place them into two new 2ml collection tubes. Discard the old tubes. Repeat two more times until all the lysate is spun.
22. Add 500  $\mu$ l of Buffer AW1 and centrifuge for 1 minute at full speed.
23. Place the spin columns into a new 2ml tube and discard the filtrate.
24. Add 500  $\mu$ l of Buffer AW2 and centrifuge for 3 minutes at full speed.
25. Place the column into a new 1.5 ml tube (with no lid) and discard filtrate.
26. Centrifuge for one minute at full speed.
27. Transfer the spin column to a new 1.5 ml tube (that is labeled) and discard the previous tube.
28. Add 100  $\mu$ l of Buffer AE.
  - a. Buffer AE is an elution buffer that is provided by the Qiagen kit.
29. Allow the tubes to sit for 1 minute at room temperature and then centrifuge for 1 minute at full speed to elute.
30. The eluate contains the purified DNA.