Who's Eating What: A Molecular Diet Analysis of Patio kindae and Eidolon helvum in Kasanka National Park

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A Thesis Presented

by

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ABSTRACT
WHO'S EATING WHAT: A MOLECULAR DIET ANALYSIS OF PAPIO KINDAE AND EIDOLON HELVUM IN KASANKA NATIONAL PARK

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Understanding dietary requirements and the ecological roles of species is vital for effective conservation management. This study uses DNA metabarcoding, specifically the *trnL* intron, to examine the plant diet composition of Kinda baboons (*Papio kindae*) and straw-colored fruit bats (*Eidolon helvum*) in Kasanka National Park, Zambia. Our results revealed significant seasonal variation in the baboon diet highlighting their omnivorous nature and dietary flexibility. We also confirmed dietary overlap between the baboons and bats during the roosting period when both species are present in the park. This research highlights the importance of utilizing molecular techniques to better understand the dietary ecology of species and their interactions within ecosystems in order to provide critical insights for conservation strategies aimed at preserving these important species and their habitats.

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CHAPTER 1

INTRODUCTION

Conservation efforts and species management planning requires a comprehensive understanding of each species' biology, habitat use, and dietary needs (Montoya et al. 2012). Diet is especially crucial for understanding an animal's ecological role and habitat requirements (Roslin & Majaneva, 2016). Wildlife ecologists often use non-invasive methods, such as observational data collection and physical fecal analysis, to infer an animal's diet (Ando et al. 2020). Molecular techniques, including DNA metabarcoding, are becoming increasingly important tools to effectively construct animal diets and infer ecosystem interactions (De Barba et al. 2014).

Early research using metabarcoding to analyze diet focused on carnivorous mammals, but its use has been expanded to study a variety of consumers, especially nonhuman primates. Deagle and colleagues designed primers to amplify the DNA of various prey animals in the stool of Steller sea lions (*Eumetopias jubatus*) (2005). This method was applied to folivorous primate species —specifically western lowland gorillas (*Gorilla gorilla*) and black and white colobus monkeys (*Colobus angolensis*) —to extract and amplify plant DNA from their feces (Bradley et al. 2007). To increase the robusticity of plant DNA metabarcoding, Mallott and colleges evaluated the effectiveness of using different plant genetic markers, specifically the *trnL* and *rbcL* introns, in a study on white-faced capuchins (*Cebus capucinus*) (2018). The higher resolution of DNA metabarcoding relative to observational data alone in the inference of diet was confirmed in a study of wild vervet monkeys (Brun et al. 2022). De Barba and colleagues targeted plant, vertebrate, and invertebrate markers in brown bear (*Ursus arctos*) feces, demonstrating the efficiency and resolution of using metabarcoding to assess omnivorous diets (2014).

The application of DNA metabarcoding for the purpose of inferring primate diets is particularly important to global conservation for several reasons: 1) primates are behaviorally flexible, thus, their feeding habits may be more diverse than their morphology or taxonomic grouping suggests, 2) they are keystone species which perform ecological roles such as seed dispersal via consumption and excretion of flora, and 3) they are extremely diverse

taxonomically, geographically, behaviorally, and morphologically (Estrada et al. 2017). Understanding the diets of the world's primates can also shed light on the status of global ecosystems, which can ultimately assist their conservation. In this regard, bats should be a clade of high priority for DNA metabarcoding analysis as they are not only taxonomically, geographically, and morphologically diverse, but are major keystone species which are difficult to observe over long periods due to their aerial, nocturnal nature. DNA metabarcoding has been utilized to assess the diverse diets of neotropical bat assemblages, identifying plants, vertebrates, and arthropods in their diets (which are difficult to discern from observational data alone) (Ingala et al. 2021).

One bat species of high ecological importance in Africa is *Eidolon helvum* (straw colored fruit bat), a large species of fruit bat with dark, black wings and light, blond colored fur (Ossa et al. 2012). These bats are widely distributed across Africa and serve as important pollinators and seed dispersers (Ossa et al. 2012). Despite their ecological role, *E. helvum* are listed as "Near Threatened" by the IUCN as their global population is trending downwards due to habitat loss and degradation (IUCN, 2020). Additionally, they are the most harvested bat species in Africa for bushmeat, which further contributes to their population decline (IUCN, 2020). *E. helvum* is highly social— living in groups of at least 100,000 individuals— and migrates across sub-Saharan Africa, likely following the timing of fruit production in different regions of the continent (Hurme et al. 2022). Starting in October and through December, 10 million bats descend on relatively small forest patches in Kasanka National Park, Zambia to roost (Hurme et al. 2022). The timing of this event coincides with the seasonal peak in the availability of ripe fruit (Hurme et al. 2022). At least two fruiting plants in the park, *Uapaca kirkiana* (masuku) and *Syzygium cordatum* (water berry) are consumed by resident mammals, including fruit bats such as *Epomophorus crypturus* and *Epomops dobsonii,* and the Kinda baboon (*Papio kindae*). However, the degree of dietary overlap among bats and primates during the period of time is largely unknown.

Kinda baboons are unique among baboons: while they resemble the closely related yellow baboon (*Papio cynocephalus*) and are closely related to chacma baboons from southern Africa, they are the smallest baboon species (Sørensen et al. 2023). They are highly social and live in groups of up to 100 individuals with complex mating systems and social organizations that differ from other baboon species (Petersdorf et al. 2019; Weyher et al. 2014). Like all baboon species, Kinda baboons are omnivorous and eat a wide variety of foods including plant material (such as corms, seed pods, fruits, and leaves), invertebrates, small vertebrates, and fungus (Weyher et al.2014).

In this study, we aimed to explore the following questions about these two species:

1) Do the straw-colored fruit bats and Kinda baboons exhibit dietary niche separation during the bat roosting period (the hot/wet season)?
2) How does Kinda baboon plant diet composition change across and throughout the seasons?

Because the bat roosting period coincides with the availability of sought-after fruit species, we predicted diet convergences between the bats and Kinda baboons would be evident. We predicted that known fruit species, such as *Uapaca kirkiana* (masuku) and *Syzygium cordatum* (waterberry), would be abundant in both species' diet. We expected the plant diet composition of the Kinda baboons to show high seasonal variation in both composition and species richness since they exploit different habitat types throughout the seasons, each including different vegetation (Figure 1). We predicted different seasons would have a strong impact on the beta-diversity of the baboon diet, and that the beta-diversity of the baboons during the hot/wet season would be similar to that of the bats.

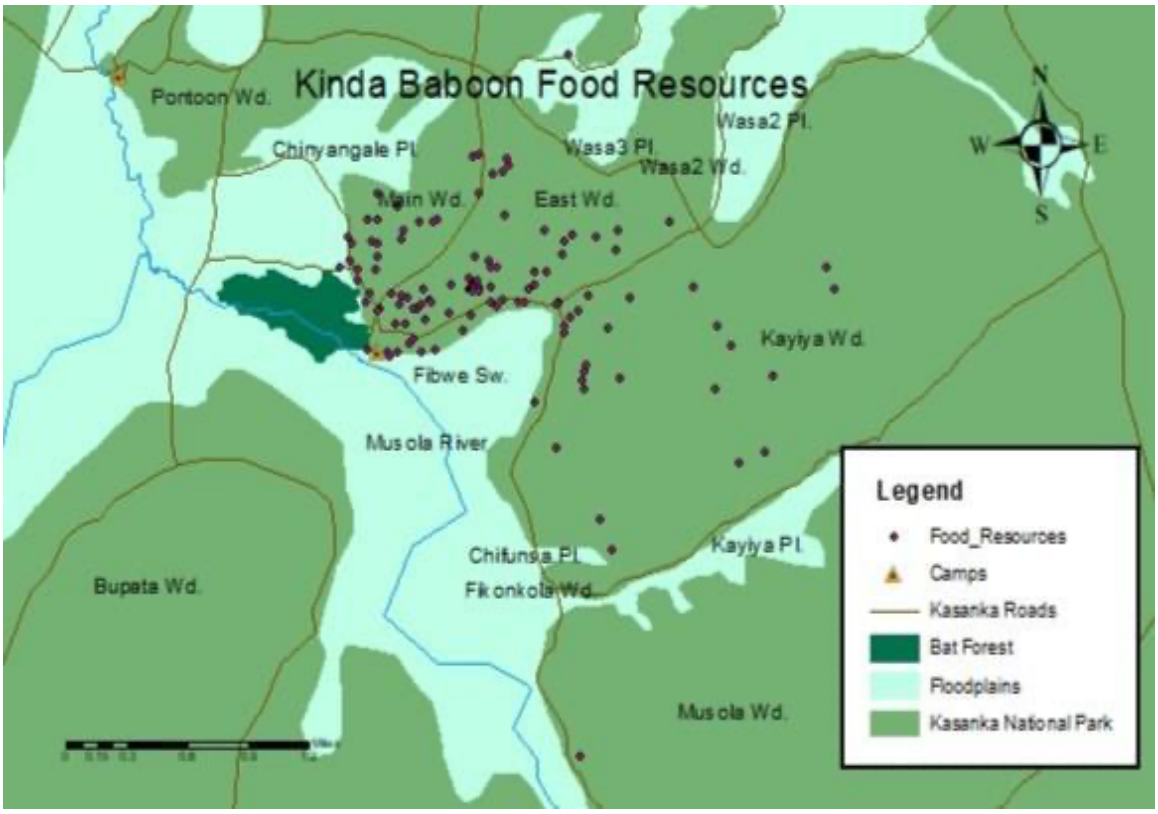

Figure 1: This map illustrates the food resources used by Kinda baboons from March 2022 to January 2023. Resources were recorded on a Garmin GPSMAP 65s Handheld GPS when three baboons used the resource for more than two minutes. Map created by A. J. Fuchs.

METHODS

Sample Collection

We collected fecal samples between April 2022 and January 2023 at Kasanka National Park (KNP), Zambia. This time frame incorporated all three major seasons at KNP: the hot/wet season (November through April), cool/dry (May through August), and hot/dry season (September through October). KNP [coordinates S12.40 and 12.66 latitude and E30.05 and E 30.38 longitude] is a small national park (390 km^2) located in the Central Province of northeastern Zambia (Leonard, 2005). KNP is part of the miombo ecoregion and is predominantly made up of deciduous woodlands, including miombo and dwarf miombo woodlands (Rduch & Jentke, 2021). KNP encompasses other habitat types, including chipya (wooded grassland), mushitu (evergreen forests), and plains/seasonal wetlands (Munishi et al., 2011; Timberlake & Chidumayo, 2011). Our observational data of the Kinda baboon troop indicate they exploit all of these habitat types, and though there may be some overlap of plant species across habitat types, some plant species are only found in certain habitat types that are used differentially throughout the year (Weyher, Fuchs, Kamilar, 2020; Fuchs & Kamilar, 2020; Fuchs unpublished). Puku (*Kobus vardonii*), sitatunga (*Tragelaphus spekii*), hippopotamus (*Hippopotamus amphibius*), vervets (*Cercopithecus pygerythrus*) and blue monkeys (*Cercopithecus mitis*) are among the other mammals who live in the Park (Kasanka Trust).

Bat fecal samples (total n = 42, which includes both guano (n = 15) and swabs (n = 27)) from three fruit bat species: *Eidolon helvum* (straw-colored fruit bat) (n=36), *Epomophorus crypturus* (Peters's epauletted fruit bat) (n = 4), and *Epomops dobsonii* (Dobson's fruit bat) (n=2) were collected during the *E. helvum* roosting period (hot/wet season) over two weeks (from late November to early December). The other two species are year-round residents at KNP. Hereafter all three bat species together are referred to as 'bats'. Bats were captured via mist nets, sampled, and released within 2-3 hours. The bat sampling protocol was approved by the UMass IACUC (approval # 2866). All bat samples were stored in DNA/RNA Shield (Zymo Research

Corporation) at ambient temperature for 3 to 4 weeks while in Zambia and subsequently frozen at -20°C in the lab.

We collected 102 fecal samples from a habituated troop of Kinda baboon (*Papio kindae*) throughout the year, including the times when *E. helvum* was present and absent in the park. One set of baboon fecal samples (set 1, n=42) were stored in DNA/RNA Shield while a second set of samples (set 2, n=60) were stored in RNAlater (Invitrogen).

In addition, we included four fruit samples from two plant species, *U. kirkiana* and *S. cordatum*, that are commonly consumed by the bats and baboons during the time when *E. helvum* is in park. We stored plant samples in DNA/RNA Shield.

All samples were primarily collected for previous projects, but were used in this current research to quantify the plant portion of the bat and baboon diet via genomic analyses.

DNA Extraction

DNA extraction was performed on the bat and plant samples using a Purelink Microbiome kit with the following modifications to the manufacturer's recommended protocol: samples were incubated at 95℃ instead of 65℃; all centrifugation was at 16000 g for 1 minute and 30 seconds. DNA extraction was performed on set 1 of the baboon samples with the Qiagen PowerFecal Pro DNA kit following the manufacturer's recommended protocol with the following modification: all centrifuging was at 16000 g for 1 minute and 30 seconds.

DNA extraction was performed on set 2 of the baboon samples with the Qiagen DNeasy PowerSoil Kit following the manufacturer's recommended protocol. We quantified the concentration of all DNA concentrations using a Qubit 3 Fluorometer (Invitrogen) using the Broad Range buffer for baboon samples and High Sensitivity buffer for the bat and plant samples, which had lower DNA concentrations.

PCR for plant barcoding using the *trnL* intron

We used a two-step PCR protocol to amplify and index the plant DNA from our samples. The first PCR targets a fragment *trnL* chloroplast gene, which has been a commonly used gene for plant barcoding (Taberlet et al. 2007; Boukhdoud et al. 2021). The second PCR adds a unique index to each sample/amplicon which allows us to construct a multiplexed library for Illumina sequencing.

We targeted the P6 loop region of the chloroplast *trnL* intron (10-143 bp) with the following primers and overhang adapters: trnL-g, 5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-GGGCAATCCTGAGCCAA-3' and trnL-h, 5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-CCATTGAGTCTCTGCACCTATC-3' (Taberlet et al. 2007; Boukhdoud et al. 2021). The following protocol was adapted from Boukhdoud et al. (2021). Each PCR reaction was made of 25µl total volume consisting of 8µl MoBIO Superclean H_2O, 1µl BSA, 0.75µl trnL-g primer(10uM), 0.75µl trnL-h primer(10uM), 12.5µl KAPA Hifi HotStart ReadyMix, and 2µl of the extracted DNA template. The PCR cycling conditions (as per KAPA Hifi HotStart Ready Mix Standard Protocol) are as follows: initial denaturing and activation for 3 min at 95 °C, followed by 35 cycles of 3-step cycling (denaturing for 20 seconds at 98 °C, annealing for 15 seconds at 60 °C then an extension for 60 seconds at 72 °C), and a final extension of 1 min @ 72 °C.

We amplified each sample in triplicate, and subsequently pooled them to obtain 75ul of PCR product for each sample. We confirmed the *trnL* gene was amplified by visualizing each amplicon on a 2% agarose gel. Samples that did not amplify successfully were subjected to another PCR using the same protocol except for a higher quantity of template DNA (3-8µl) and less H2O to keep the total volume of each reaction at 25 µl. If samples did not successfully amplify with a higher quantity of template they were not included in the study after this point; 2 *E. helvum* samples and 5 baboon samples did not amplify.

Pooled amplicons were cleaned with a Monarch PCR & DNA Cleanup Kit (NEB) following the kit protocol. We used an initial volume of 50 µl (having used 5µl or more for gel visualization) and a final elution of 15 µl to ensure we had enough to run multiple second-step PCRs but keep the elution concentrated.

The second PCR was performed on each cleaned pooled amplicon to attach a unique combination of iTruSeq dual indices to differentiate between each sample during sequencing (Glenn et al. 2019). Each reaction of 25 µl consisted of 12.75 µl MoBIO Superclean H2O, 5.75 µl KAPA Hifi HotStart ReadyMix, 1.25 µl forward iTru primer (10 uM), 1.25 µl reverse iTru primer (10u M), and 4 µl of the DNA template (i.e., first PCR product). We used the following cycling conditions: initial denaturing and activation for 45 seconds at 98 °C, followed by 13 cycles of 3 step cycling (denaturing for 20 seconds at 98 °C, annealing for 30 seconds at 61 °C, extension for 30 seconds at 72 °C) with a final extension for 7 minutes at 72 °C.

The indexed amplicons were then quantified using a Qubit 3, and subsequently pooled together at equimolar concentrations to make the final multiplexed library. This library was cleaned using the Monarch PCR & DNA Cleanup Kit following the kit protocol (with a sample volume of 100µl and a final elution of 20µl).

Species	Number of Samples
Papio kindae (Kinda baboon)	97
Eidolon helvum (Straw-colored fruit bat)	34
Epomophorus crypturus (Peter's epauletted fruit bat)	4
Epomops dobsonii (Dobson's epauletted fruit bat)	2
Syzygium cordatum (Water berry)	2
Uapaca kirkiana (Sugar plum, Masuku)	2

Table 1: Samples sequenced and used in final analysis grouped by species.

Sequencing

We sent our trnL library to the Genomics Resource Laboratory (GRL) at the University of Massachusetts Amherst. The library quality was examined using an Agilent 2100 Bioanalyzer and sequenced on an Illumina MiSeq with a V3 kit using 201 bp paired-end reads.

Bioinformatics

We uploaded the raw sequences to mBRAVE (multiplex barcode research and visualization environment), a cloud-based platform designed to store, process and analyze high-throughput sequencing data (Ratnasingham, 2019). Our reads are available under project title "MBR-ASTRNLGH". Our bioinformatics pipeline used the default mBrave parameters except for: trim front (changed from 50 bp to 3 bp), min length (pre-trim) (changed from 100 bp to 29 bp), max length (pre-trim) (changed from 1000 bp to 253 b), and the ID min overlap (changed from 100 bp to 20 bp). These changes were to account for the short length of the *trnL* intron to make sure our target sequences were not filtered out. Paired end merging was set to Pool (for Illumina instruments). We used an ID distance threshold of 5% to include a greater number of matched BINS in our identification. Full details about our parameters can be found in Suppl. Table 1.

We searched mBRAVE, BOLD, Genbank, and Google Scholar for reference databases containing the *trnL* gene of African plant species. While mBRAVE is directly linked to BOLD (barcode of life data system), the BOLD Identification System (IDS) uses *rbcL* and *matK* for default plant identification. Researchers who use BOLD or GenBank can make their datasets publicly available and accessible through BOLD, even if they use different genetic markers (such as *trnL*). We selected the databases created by Gill et al. (2019), listed as mBrave project codes: DS-UHURUR1, DS-UHURUR2, and DS-UHURUR3. These databases contain plant species from Mpala Research Center in Laikipia, Kenya (Gill et al. 2019). Their databases were uploaded to BOLD and readily accessible from mBrave. In addition, we aligned and compared the three most abundant OTUs from the plant samples we sequenced and used them to create a custom

database in BOLD to detect *U. kirkiana* and *S. cordatum*; the custom dataset is under project code "DS-TRNLAFRI".

Statistical analysis

The alpha diversity of each sample was calculated in R using the *vegan* package. We calculated the Simpson and Shannon index of each sample. We tested for differences in alpha diversity between seasons and bats vs baboons using Kruskal-Wallis tests with p values less than 0.05 are considered significant. Next, we performed permutational multivariate analysis of variance (PERMANOVA) tests based on both Bray-Curtis and Euclidean distance matrices to compare species and season as predictors of diet composition. We used 9999 permutations to estimate statistical significance. The six samples from *E. crypturus* and *E. dobsonii* were grouped with the 34 *E. helvum* for all analysis under the category 'bats'.

RESULTS

Quantifying baboon and bat plant diet based on the *trnL* gene

The sequencing run produced 28,797,664 total (unfiltered) paired-end reads from all 141 samples sequenced.

Only including the bat and baboon samples, a total of 17,889,512 reads were left after trimming and filtering (via the mBrave filtering parameters). The average number of reads per sample was 130,5809 (ranging from 1,066 to 306,445 reads). Of these, 11,681,659 (65.52%) were identified/matched with a plant species in the reference databases. The average number of reads identified per sample was 85,267 (ranging from 12 to 248,100 reads) and the average proportion of reads identified was 61.24%.

A total of 182 unique plant taxa were identified in the bat and baboon fecal samples, 68 of them were found in bats and 179 were found in baboons. The plant species most prevalent across all sample types were *Neonotonia wightii* (also referred to as *Glycine wightti*), accounting for 13.47% of total reads, *Euclea divinorum* (13.17%), and *S. cordatum* (9.52%). 48 plant species comprised 1% or more of the post-filtered reads from at least one sample and 30 plant species comprised more than 5% of post-filtered reads from at least one sample. A table listing all plants ID'd based on all filtered reads; 1% or greater, and 5% or greater can be found in Supplemental Table 2.

Seasonal variation in baboon plant diet alpha diversity based on the *trnL* gene

Across all seasons, there were 179 plant species detected in the baboon samples with the most abundant species being *N. wightii* (14.97% of baboon sample reads), *E. divinorum* (14.65%), and *Scutia myrtina* (8.13%). During the hot/wet season 96 species were detected in the baboon diet; *N. wightii* (21.77%), *S. myrtina* (14.37%), and *S. cordatum* (10.32%) were the most abundant species detected during this season. *E. divinorum* (37.91%) and *N. wightii* (9.49%) were the most abundant plant species identified during the cool/dry season of the 105 species detected.

A total of 50 plant species were detected during the hot/dry season, with the most prevalent being *E. divinorum* (23.21%) and *Indigofera volkensii* (18.14%).

The hot/dry season had higher median alpha diversity in plant consumption relative to other seasons (Figure 2). Though, both the Simpson and Shannon indices show no significant differences in alpha diversity for plants consumed by the baboons across the three seasons (Simpson index p = 0.199; Shannon index p = 0.357). In contrast, monthly variation in alpha diversity metrics was significant (Simpson index p = 0.0162; Shannon index p = 0.008) (Figure 2).

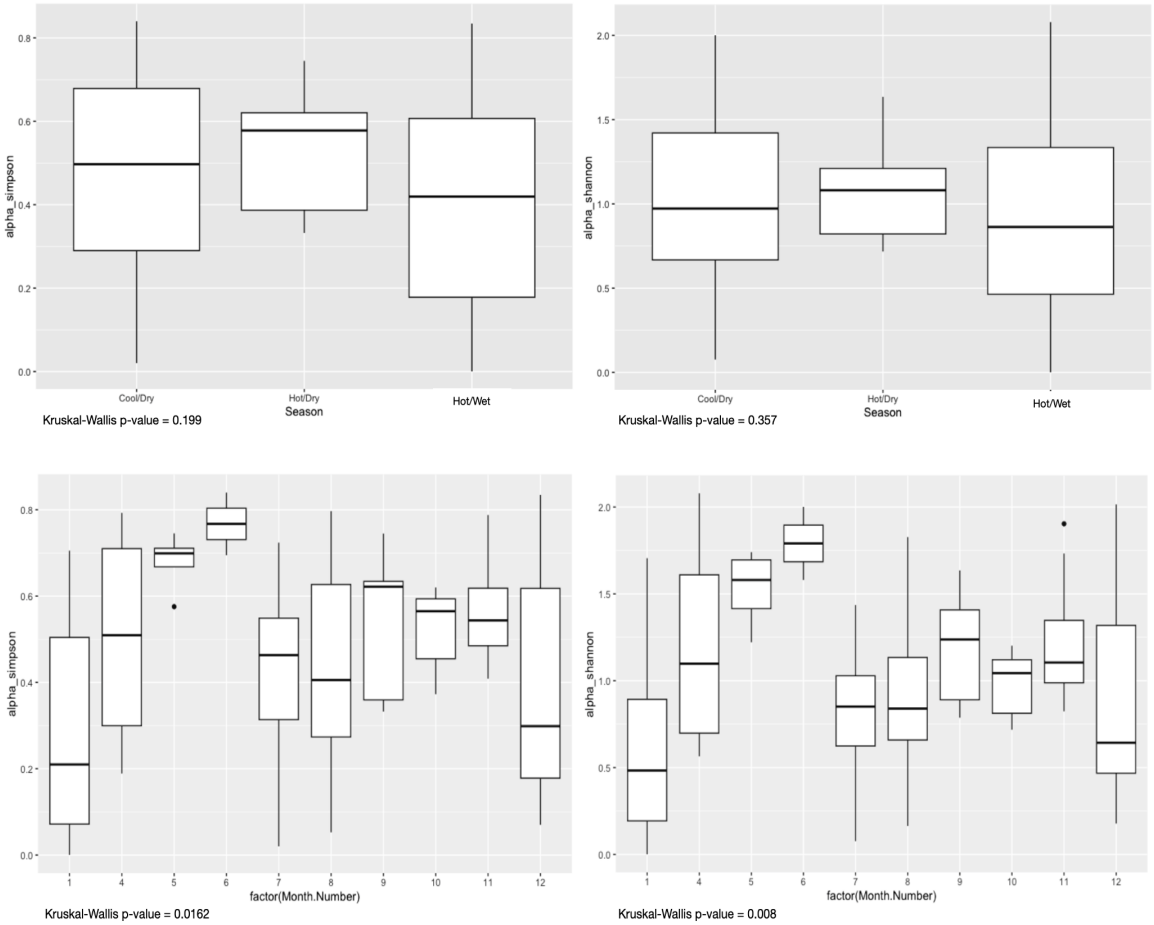

Figure 2: Alpha diversity of the baboon diets by season (top row) and by month (bottom). Simpson index on the right, Shannon index on the left.

Differences in the plant diet alpha diversity between baboons and bats during the hot/wet season

N. wightii, S. myrtina, and *S. cordatum* were the most abundant species detected in the baboon diets in the hot/wet season. *Euphorbia septentrionalis* (19.64%)*, S. cordatum* (42.37%)*,* and *Ficus sycomorus* (10.61%) were the most abundant species detected in the bat samples, all of which were from the hot/wet season.

Both Shannon and Simpson indexes reveal significant differences between bat and baboon diet alpha diversity during the bat roosting period (hot/wet season), when both species are in the park (Simpson index $p < 0.001$; Shannon index $p < 0.001$) (Figure 3). A total of 96 plant species were detected in the baboon diets during this season and 68 plant species were detected in the bat diets.

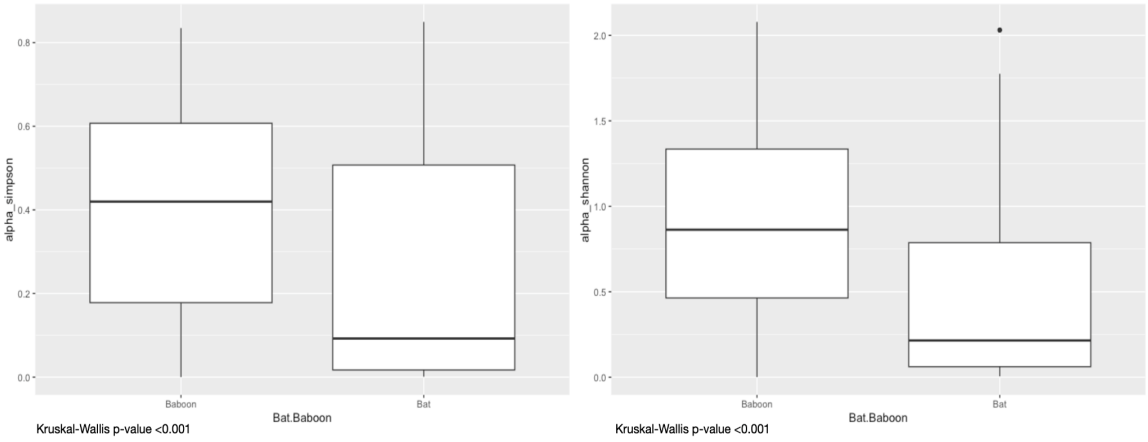

Figure 3: Alpha diversity of bats and baboons during the hot/wet season.

Season and species predicting plant diet composition

The PERMANOVA tests, based on both Bray-Curtis and Euclidean distance matrices yielded p values of 0.0001 for both species (bat or baboon) and season, indicating that both were important and independent predictors of plant diet composition. In addition, we used these distance matrices in nonmetric multidimensional scaling (NMDS) analyzes to visualize the diet composition of

samples in multivariate space (Figure 4). Bat and baboon samples from the hot/wet season occupied the most space and were found primarily on the left side of the plot. The baboon samples from the other two seasons tended to be found on the right side of the graph and occupied much smaller areas of plant composition space. Within the warm/wet samples, the bats were found in the left-most and bottom-most portions of the plot. Additional bat and baboon samples during this season overlapped each other closer to the origin of the plot.

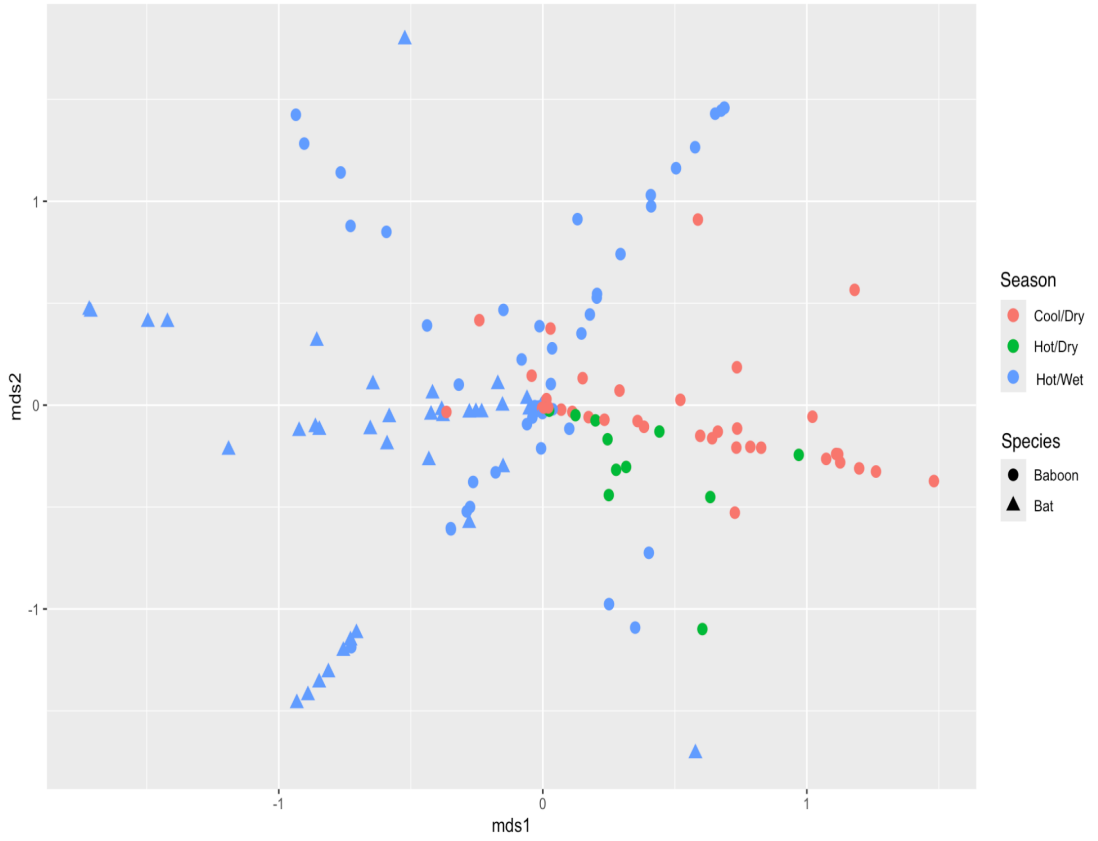

Figure 4: Non-metric multidimensional scaling plot based on Bray Curtis distance matrix. Baboon samples are represented by circles and bat samples are triangles. Colors represent the season during which the samples were collected. Greater proximity between points represents greater dietary similarity.

DISCUSSION

This study explored the change in plant diet composition and diversity in Kinda baboons (*P. kindae*) throughout the seasons and examined diet overlap between the baboons and the migratory straw-colored fruit bat (*E.helvum*) during the hot/wet season in KNP. Using fecal DNA metabarcoding targeting the *trnL* chloroplast marker, we were able to identify plant species consumed by the host species.

We expected the diet composition to change seasonally as the baboons exploit different habitat types due to differential availability of fruits and plant food across seasons. We found that the baboon plant diet varied widely across seasons, which corresponds to the substantial shifts in food availability and diversity at the site which constrain food availability seasonally. We found no significant differences in species richness of plant diversity between the three major seasons at KNP, however, when we divided the year into months, a significant difference was found. This suggests inter-seasonal food availability is extremely high, and species may need to alter their feeding strategy often. This is consistent with our prediction that the baboon diets will shift throughout the year but remain diverse, as they are an omnivorous species. Our results confirmed that season is a strong predictor of diet beta-diversity, which is consistent with our hypothesis that the seasonality will be reflected in the baboon diets throughout the year.

Furthermore, we find that Kinda baboons, *E. helvum*, and local bat species likely overlap in diet profiles during the bat roosting season, as all three groups consume relatively large portions of *N. wightii , S. myrtina,* and *S. cordatum.* However, our data indicate that baboons and bats consume a wide variety of other plants during this period. Driving forces behind this dietary niche separation may include different nutritional requirements or need to reduce food competition. Our data indicates that there is a significant difference in species richness of the bat and baboon diets during the hot/wet season, with the baboons consuming more plant species than the bats. This could be due to the fact that the baboons are omnivorous while the bat species are primarily frugivorous. Fruit bats suck on and squeeze the juice from the fruit then spit out the

fibrous parts of the fruit, including the skin and any seeds (Aziz et al. 2021). Baboons are able to chew and consume the more dense, fibrous parts of the vegetation. These feeding ecology differences between the bats and baboons could further account for the difference in plant species detected using this method.

While the composition of the bat and baboon diets are significantly different, *S. cordatum* is abundant in both species' diets. This suggests *S. cordatum* is an important food source for both residential and migratory mammals in KNP. Our findings are consistent with studies describing the timing of the fruit bat migrations being linked to fruiting and greening events (Hurme et al. 2022). Previous research highlights the abundance of *S.cordatum* and *U. kirkiana* in KNP during the arrival of *E. helvum*, accounting for over 80% of fruiting trees at this time (Richter & Cumming, 2005). Our results show that both species are found in the majority of the bat samples (supple. Table 3), but *S. cordatum* makes up a higher proportion of the bat diet. Understanding the drivers of migration for this gregarious and ecologically important fruit bat is critical to predicting potential threats to the health of the species (Hurme et al. 2022). Results from metabarcoding concur on some points with observational study but differ in others which demonstrates that this method can provide both support for observational methods and greater focus on specific species found to be important in both forms of research.

Several of the plant species identified in the bat and baboon diets are also important resources for the local populations in Zambia and the surrounding areas. *S. cordatum* is an important medicinal resource for people through east and southern Africa (Maroyi, 2018). In Zambia, the leaves, bark, and roots are used to treat diarrhea and malaria (Maroyi, 2018). Another locally important plant is *E. divinorum*, which was detected in abundance in the baboon diets in the cool/dry and hot/dry seasons. While the fruits are edible, the roots are more commonly used medicinally to treat a wide range of ailments including toothaches and digestive issues (PlantZAfrica). The *Eulcea* plant genus has been shown to have bioactive compounds with strong

pharmacological properties including: antimalarial, anticancer, and antimicrobial properties (Taye et al. 2023). Local researchers and park scouts suggest that the baboons might be consuming certain plants for medicinal purposes.

Primatologists have been documenting and researching the possible use of medicinal plants among several wild primate populations. Researchers recently documented a Sumatran orangutan (*Pongo abelii*) applying chewed leaves to a facial wound from a plant with antibacterial, anti-inflammatory, and other properties helpful for healing open wounds (Laumer et al. 2024). Freymann and colleagues conducted a study to evaluate the pharmacological properties of plants consumed by wild chimpanzee groups in Uganda (*Pan troglodytes*), and were able to link the ingestion of certain plant species with consumer infections and injuries (2024).

The *trnL* intron, specifically the P6 loop, is short and may offer lower accuracy when differentiating between closely related species (Taberlet et al. 2007). Our reference database includes the top three OTUs sequenced from our local plant samples, *S. cordatum* and *U. kirkiana*. We observed greater variation between the top *U. kirkiana* sequences, which could be due to intraspecies variation in the *trnL* intron, or could represent closely related species of *Uapaca* consumed by the hosts (Silva et al. 2016). *E. helvum* has been recorded consuming three different species of *Uapaca* in KNP (Richter & Cumming 2005). In previous studies, the *trnL* intron (and other plant chloroplast markers) has identified plant taxa that were not present in the geographic area, likely due to limitations in the reference database (Mallott et al. 2018). The *trnL* intron is, however, a robust choice when working with highly fragmented and degraded DNA due to its short length (Taberlet et al. 2007).

This method exclusively examines the plant content in the diet of the host species. Kinda baboons are omnivorous, therefore this approach does not tell us what proportion of their diets are made up of plants vs. non-plant sources (fungus, invertebrates, etc.).

Chloroplast density varies between plant species and between plant materials (leaves, stems, fruits, roots, etc.) (Garrido et al. 2023). Fruits do contain chloroplast and photosynthesize, however the chloroplast levels vary greatly throughout fruit development, between species, and can be environmentally impacted (Garrido et al. 2023). On average, there is a higher density of chloroplast in leaves than in fruit or any other part of the plant (Valentini et al. 2008). Using the *trnL* intron to identify plant species cannot differentiate between plant tissue types and cannot currently help determine the difference in relative abundance when comparing different plant materials consumed.

Several previous studies using the *trnL* intron to determine diet composition used custom reference databases generated from collecting common plant species from the geographical area of interest (Boukhdoud et al. 2021). The resolution of this study could be improved by collecting and sequencing more local plants from KNP to create a local database.

This study highlights the diverse nature of Kinda baboon diets in Kasanka National Park, which shifts seasonally and reflects changes in food availability. While the baboons and migratory straw-colored fruit bats exhibit some dietary overlap, the differences in their feeding strategies, dietary needs, and preferences are evident in the differences detected in their diets. This research contributes to our understanding of both species' feeding ecology and habitat needs and underscores the important role of crucial plant species for both wildlife and local communities.

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Supplementary Materials

Supplementary Table 1. mBrave project parameters used in our bioinformatics pipeline:

Trimming	
Trim Front	3bp
Trim End	0bp
Primer Masking	Off
Filtering	
Min QV	10qv
Min Length (Pre-Trim)	29bp
Max Length (Pre-Trim)	253bp
Max Bases with Low QV (<20)	4.0%
Max Bases with Ultra Low QV (<10)	1.0%
Other Parameters	
Dereplication Min Rep	1
Pre-Clustering Threshold	None
ID Distance Threshold	5.0%
ID Min Overlap	20bp
Exclude From OTU Threshold	5.0%
Minimum OTU Size	1
OTU Threshold	2.0%
Paired End (Illumina instruments only)	
Paired End Merging	Off
Assembler Min Overlap	20bp
Assembler Max Substitution	5bp

Supplementary Table 2. Plant species identified. The 2nd column has plant species making up 1% or more of at least one sample's filtered reads. The 3rd column has species making up 5% or more of at least one sample's filtered reads.

All Plants Identified	Plants >1%	Plants >5%
Abutilon mauritianum	*Acalypha lanceolata*	*Bothriochloa insculpta*
Acalypha lanceolata	*Blepharis maderaspatensis*	*Carissa spinarum*
Acanthospermum hispidum	*Bothriochloa insculpta*	*Chrysopogon plumosus*
Adenia volkensii	*Carissa spinarum*	*Commiphora habessinica*
Ageratum conyzoides	*Chamaecrista grantii*	*Cordia sinensis*
Aristida adoensis	*Chrysopogon plumosus*	*Eragrostis rigidior*
Aristida adscensionis	*Commelina erecta*	*Euclea divinorum*
Aristida congesta	*Commiphora habessinica*	*Euphorbia septentrionalis*
Asparagus falcatus	*Cordia sinensis*	*Ficus ingens*
Aspilia mossambicensis	*Crinum macowanii*	*Ficus sycomorus*
Aspilia pluriseta	*Digitaria velutina*	*Gladiolus dalenii*
Balanites aegyptiaca	*Dodonaea viscosa*	*Glycine wightii*
Blepharis edulis	*Eragrostis rigidior*	*Helichrysum glumaceum*
Blepharis maderaspatensis	*Euclea divinorum*	*Heliotropium steudneri*
Bothriochloa insculpta	*Euphorbia septentrionalis*	*Hibiscus calyphyllus*
Brachiaria eruciformis	*Ficus ingens*	*Indigofera volkensii*
Brachiaria leersoides	*Ficus sycomorus*	*Ipomoea cairica*
Brachiaria semiundulata	*Gladiolus dalenii*	*Ipomoea hildebrandtii*
Brachiaria xantholeuca	*Glycine wightii*	*Malva parviflora*
Bulbine abyssinica	*Grewia similis*	*Melinis repens*
Carissa spinarum	*Helichrysum glumaceum*	*Mystroxylon aethiopicum*
Chamaecrista grantii	*Heliotropium steudneri*	*Ornithogalum sp.*
Chloris amethystea	*Hibiscus aponeurus*	*Pennisetum mezianum*
Chloris gayana	*Hibiscus calyphyllus*	*Phoenix reclinata*
Chloris nutans	*Indigofera volkensii*	*Plicosepalus sagittifolius*
Chloris pycnothrix	*Ipomoea cairica*	*Rhynchosia malacophylla*
Chlorophytum gallabatense	*Ipomoea hildebrandtii*	*Scutia myrtina*
Chlorophytum subpetiolatum	*Ischaemum afrum*	*Syzygium cordatum*
Chrysopogon plumosus	*Kanahia laniflora*	*Uapaca kirkiana*
Commelina africana	*Malva parviflora*	*Vachellia gerrardii*

All Plants Identified	Plants >1%	Plants >5%
Commelina benghalensis	*Melinis repens*	
Commelina erecta	*Mystroxylon aethiopicum*	
Commelina reptans	*Ocimum filamentosum*	
Commiphora habessinica	*Ornithogalum sp.*	
Coptosperma graveolens	*Oxalis corniculata*	
Corallocarpus epigaeus	*Pennisetum mezianum*	
Cordia monoica	*Phoenix reclinata*	
Cordia sinensis	*Phyllanthus sepialis*	
Crabbea velutina	*Plectranthus barbatus*	
Craterostigma plantagineum	*Plicosepalus sagittifolius*	
Crinum macowanii	*Polygala sphenoptera*	
Crossandra massaica	*Rhynchosia malacophylla*	
Crotalaria incana	*Scutia myrtina*	
Crotalaria pycnostachya	*Syzygium cordatum*	
Cynanchum viminale	*Uapaca kirkiana*	
Cynodon nlemfuensis	*Urochloa brachyura*	
Cyperus cyperoides	*Vachellia gerrardii*	
Cyphostemma serpens	*Ziziphus mucronata*	
Dactyloctenium aegyptium		
Digitaria ternata		
Digitaria thouaresiana		
Digitaria velutina		
Dodonaea viscosa		
Echinochloa colona		
Echinochloa pyramidalis		
Echiochilon lithospermoides		
Enneapogon cenchroides		
Eragrostis papposa		
Eragrostis racemosa		
Eragrostis rigidior		

All Plants Identified	Plants >1%	Plants >5%
Eragrostis sp.		
Eriochloa fatmensis		
Erythrococca fischeri		
Euclea divinorum		
Euphorbia inaequilatera		
Euphorbia septentrionalis		
Eustachys paspaloides		
Evolvulus alsinoides		
Ficus glumosa		
Ficus ingens		
Ficus sycomorus		
Gladiolus dalenii		
Gloriosa superba		
Glycine wightii		
Grewia bicolor		
Grewia similis		
Harpachne schimperi		
Helichrysum glumaceum		
Helinus mystacinus		
Heliotropium steudneri		
Heliotropium strigosum		
Heteropogon contortus		
Hibiscus aponeurus		
Hibiscus calyphyllus		
Hibiscus flavifolius		
Hibiscus trionum		
Hyparrhenia anamesa		
Hyparrhenia papillipes		
Hyparrhenia sp. aff. Nubica		
Indigofera ambelacensis		

All Plants Identified	Plants >1%	Plants >5%
Indigofera arrecta		
Indigofera bogdanii		
Indigofera circinella		
Indigofera hochstetteri		
Indigofera schimperi		
Indigofera sp.		
Indigofera volkensii		
Ipomoea cairica		
Ipomoea hildebrandtii		
Ipomoea mombassana		
Ipomoea obscura		
Ipomoea oenotherae		
Ischaemum afrum		
Justicia diclipteroides		
Kanahia laniflora		
Kedrostis leloja		
Leptothrium senegalense		
Lotononis platycarpos		
Ludwigia stolonifera		
Malva parviflora		
Melanthera scandens		
Melinis repens		
Mentha longifolia		
Microchloa kunthii		
Mystroxylon aethiopicum		
Ocimum americanum		
Ocimum filamentosum		
Ocimum gratissimum		
Ormocarpum kirkii		
Ornithogalum sp.		

All Plants Identified	Plants >1%	Plants >5%
Orthosiphon parviflorus		
Oxalis corniculata		
Panicum deustum		
Panicum maximum		
Pennisetum hohenackeri		
Pennisetum mezianum		
Pennisetum squamulatum		
Pennisetum stramineum		
Persicaria pulchra		
Phoenix reclinata		
Phyllanthus maderaspatensis		
Phyllanthus sepialis		
Plectranthus barbatus		
Plectranthus prostratus		
Plectranthus sp.		
Plicosepalus sagittifolius		
Polygala sphenoptera		
Psydrax schimperiana		
Rhamnus staddo		
Rhynchosia malacophylla		
Ricinus communis		
Ruellia patula		
Sansevieria robusta		
Scutia myrtina		
Senegalia brevispica		
Senegalia mellifera		
Senna didymobotrya		
Setaria sphacelata		
Setaria verticillata		
Sida ovata		

All Plants Identified	Plants >1%	Plants >5%
Solanum hastifolium		
Sonchus asper		
Sphaeranthus suaveolens		
Sporobolus africanus		
Sporobolus agrostoides		
Sporobolus confinis		
Sporobolus discosporus		
Sporobolus stapfianus		
Syzygium cordatum		
Tagetes minuta		
Tetradenia riparia		
Themeda triandra		
Thunbergia tsavoensis		
Trachyandra saltii		
Tragus berteronianus		
Tribulus cistoides		
Tribulus terrestris		
Trimeria grandifolia		
Turraea mombassana		
Uapaca kirkiana		
Urochloa brachyura		
Vachellia gerrardii		
Vachellia seyal		
Vangueria madagascariensis		
Vepris nobilis		
Verbena bonariensis		
Vernonia galamensis		
Vigna membranacea		
Vigna oblongifolia		
Withania somnifera		

All Plants Identified	Plants >1%	Plants >5%
Ziziphus mucronata		

Supplementary Table 3: Plant species detected in the majority of samples, by category.

All Samples	%	Baboons (All Season)	%	Cool/Dry	%
Bothriochloa insculpta	56.2	*Bothriochloa insculpta*	76.29	*Bothriochloa insculpta*	83.33
Chamaecrista grantii	52.55	*Chamaecrista grantii*	70.1	*Chamaecrista grantii*	75
Commiphora habessinica	59.58	*Commiphora habessinica*	68.04	*Commiphora habessinica*	58.33
Euclea divinorum	94.16	*Digitaria velutina*	53.61	*Cordia sinensis*	52.78
Euphorbia septentrionalis	55.47	*Euclea divinorum*	95.88	*Eragrostis rigidior*	66.67
Glycine wightii	89.78	*Euphorbia septentrionalis*	51.55	*Eriochloa fatmensis*	55.56
Helichrysum glumaceum	62.77	*Gladiolus dalenii*	52.58	*Euclea divinorum*	100
Heliotropium steudneri	66.42	*Glycine wightii*	97.94	*Glycine wightii*	100
Indigofera volkensii	70.07	*Helichrysum glumaceum*	86.6	*Helichrysum glumaceum*	83.33
Melinis repens	69.34	*Heliotropium steudneri*	81.44	*Heliotropium steudneri*	77.78
Rhynchosia malacophylla	69.34	*Indigofera volkensii*	82.47	*Indigofera volkensii*	86.11
Scutia myrtina	54.74	*Melinis repens*	83.51	*Melinis repens*	88.89
Syzygium cordatum	85.4	*Panicum deustum*	59.79	*Panicum deustum*	83.33
Vachellia gerrardii	52.55	*Rhamnus staddo*	55.67	*Pennisetum mezianum*	69.44
		Rhynchosia malacophylla	85.57	*Pennisetum stramineum*	58.33
		Scutia myrtina	54.64	*Rhynchosia malacophylla*	80.56
		Syzygium cordatum	81.44	*Scutia myrtina*	55.56
		Vachellia gerrardii	65.98	*Setaria verticillata*	52.78
		Vachellia seyal	56.7	*Syzygium cordatum*	75
		Ziziphus mucronata	54.64	*Themeda triandra*	61.11
				Urochloa brachyura	52.78
				Vachellia gerrardii	97.22
				Vachellia seyal	61.11
				Ziziphus mucronata	69.44

Hot/Dry	%	Hot/Wet	%	Bats	%
Bothriochloa insculpta	75	*Bothriochloa insculpta*	71.43	*Euclea divinorum*	90
Carissa spinarum	66.67	*Chamaecrista grantii*	59.18	*Euphorbia septentrionalis*	65
Chamaecrista grantii	100	*Commiphora habessinica*	73.47	*Glycine wightii*	70
Commiphora habessinica	75	*Digitaria velutina*	65.31	*Scutia myrtina*	55
Crotalaria pycnostachya	66.67	*Euclea divinorum*	91.84	*Syzygium cordatum*	95
Euclea divinorum	100	*Euphorbia septentrionalis*	75.51	*Uapaca kirkiana*	62.5
Ficus ingens	58.33	*Gladiolus dalenii*	83.67		
Ficus sycomorus	66.67	*Glycine wightii*	95.92		
Glycine wightii	100	*Helichrysum glumaceum*	85.71		
Helichrysum glumaceum	100	*Heliotropium steudneri*	81.63		
Heliotropium steudneri	91.67	*Heliotropium strigosum*	53.06		
Indigofera arrecta	58.33	*Indigofera volkensii*	75.51		
Indigofera circinella	91.67	*Melinis repens*	77.55		
Indigofera schimperi	58.33	*Phoenix reclinata*	67.35		
Indigofera volkensii	100	*Rhamnus staddo*	67.35		
Melanthera scandens	58.33	*Rhynchosia malacophylla*	85.71		
Melinis repens	91.67	*Scutia myrtina*	61.22		
Panicum deustum	58.33	*Syzygium cordatum*	89.8		
Rhynchosia malacophylla	100				
Syzygium cordatum	66.67				
Vachellia gerrardii	66.67				
Vachellia seyal	100				
Ziziphus mucronata	75				