Received: 25 April 2022 | Accepted: 2 February 2023

DOI: 10.1111/2041-210X.14078

Methods in Ecology and Evolution

RESEARCH ARTICLE

Contemporary Methods for Studying Animal Sociality in the Wild

Molecular assessment of dietary variation in neighbouring primate groups

Judith Schneider[1] | Loïc Brun[1,2] | Pierre Taberlet[3,4] | Luca Fumagalli[1,5] | Erica van de Waal[2,6,7]

[1]Laboratory for Conservation Biology, Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland; [2]Inkawu Vervet Project, Mawana Game Reserve, Swart Mfolozi, South Africa; [3]Laboratoire d'Ecologie Alpine, Université Grenoble Alpes, CNRS, Grenoble, France; [4]UiT – The Arctic University of Norway, Tromsø Museum, Tromsø, Norway; [5]Swiss Human Institute of Forensic Taphonomy, University Centre of Legal Medicine Lausanne-Geneva, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland; [6]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland and [7]Centre for Functional Biodiversity, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa

Correspondence
Judith Schneider
Email: judith.schneider@unil.ch

Funding information
Branco Weiss Fellowship - Society in Science; H2020 European Research Council, Grant/Award Number: 949379; Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 310030_192512 and PP00P3_170624; Université de Lausanne

Handling Editor: Thibaud Gruber

Abstract

1. Facing rapid environmental changes and anthropogenic habitat destruction, animal behavioural plasticity becomes an adaptive potential that needs to be considered in conservation strategies along with, for example, genetic diversity. Here, we evaluate to what extent non-invasive environmental DNA (eDNA) methods may contribute to the assessment of intraspecies behavioural plasticity in terms of foraging behaviour.
2. We analysed DNA metabarcoding data for plant components in the diet of four neighbouring groups of wild vervet monkeys *Chlorocebus pygerythrus* to identify intergroup variation (IGV). The faecal samples considered for the analyses were limited to the summer season to minimise the impact of seasonality. Each sample was attributed by observation to individuals with known life history data. A plant survey was conducted in each group home range during the study period to account for environmental variation.
3. We observed mixed results when testing whether IGV in plant consumption was greater than intragroup variation, indicating that the influence of social dynamics must be considered. Intragroup variation was positively correlated with group size. We observed IGV in diet composition among all groups as well as in some pairwise comparisons. We found significant dietary differences between two group pairs when considering only adult females. Lastly, we observed IGV in foraging of specific plants that were not explained by their distribution, suggesting behavioural differences in selectivity between groups.

Judith Schneider and Loïc Brun joint first authors.

Luca Fumagalli and Erica van de Waal joint last authors.

4. Our study system and organism, being a highly social and non-threatened primate species, with constant gene flow and overlapping territories between groups, provides an ideal model to evaluate the usage of eDNA-based methods to better understand the impact of social factors on IGV. Our results highlight the need to consider social and demographic factors, the impact of which remains complicated to disentangle from environmental factors. However, we emphasise the great potential for studying social groups using eDNA and that such studies are needed to better understand intraspecific behavioural plasticity in wild populations.

KEYWORDS
DNA metabarcoding, environmental DNA, foraging behaviour, intergroup variation, primate population, vervet monkeys

1 | INTRODUCTION

The analysis of DNA extracted from environmental samples (i.e. environmental DNA; eDNA) has seen a rapid implementation in various research fields (Bohmann et al., 2014; Ruppert et al., 2019; Taberlet et al., 2018; Thomsen & Willerslev, 2015). In particular, the development of DNA metabarcoding (PCR amplification of short but informative metabarcodes with universal primers and next generation sequencing [NGS] of DNA mixtures, Taberlet et al., 2012) enables comprehensive taxonomic identification of complex environmental samples.

DNA metabarcoding often provides higher taxonomic resolution and coverage than traditional methods (Nørgaard et al., 2021; Ruppert et al., 2019). For terrestrial species, studies commonly rely on faecal sampling for diet characterisation (De Barba et al., 2014; Shehzad et al., 2012), parallel prey and predator identification (Galan et al., 2018; Gillet et al., 2015) and biodiversity assessment (Nørgaard et al., 2021; Shao et al., 2021). In the field of primatology, the most commonly used methods for dietary analyses are direct observation of feeding events and microhistology of faecal samples as discussed in (Matthews et al., 2020). Both are time- and labour-intensive, rely heavily on taxonomic expertise and results are often constrained in taxonomic resolution and coverage (Nielsen et al., 2018), as the identification of consumed items or the observation of feeding events themselves are often challenging (Matthews et al., 2020; Pickett et al., 2012). Depending on the studied organism and field conditions, observations on broad temporal and spatial scales are complicated. Recently, the use of DNA metabarcoding in the field of primatology has enabled new insights, in particular regarding the consumption of arthropods (Lyke et al., 2018; Mallott et al., 2015, 2017; Rowe et al., 2021) but also plants (Brun, Schneider, Mas Carrió, Dongre, van de Waal, et al., 2022; Mallott et al., 2018; Quéméré et al., 2013). The sampling procedure of eDNA promises new opportunities to investigate behavioural plasticity through the study of foraging behaviour of species that are challenging to observe but for which faecal samples can be obtained.

DNA metabarcoding is constrained, however, in that it cannot, for example, identify different life stages or states of detected organisms, and it is more complicated to estimate abundances than with observations due to several potential biases that need to be considered (Piñol et al., 2019). Nevertheless, eDNA approaches can add valuable information for ecological network analysis (Clare, 2014) and several studies, for example in the context of niche partitioning, have used relative read abundance (RRA; the number of a specific sequence divided by the total number of reads within a sample) to use DNA metabarcoding semi-quantitatively (Deagle et al., 2019; Kartzinel et al., 2015; Pansu et al., 2019; Vesterinen et al., 2018). However, there are few studies that assess differences beyond the population level (see the work of Voelker et al., 2020). DNA metabarcoding can hence be useful to assess feeding patterns between different organisms, groups or populations.

Considering the intergroup-level, the unit of analysis avoids species-wide generalisations based on behavioural studies of single populations (Kaufhold & van Leeuwen, 2019; van de Waal, 2018). To date, most studies assessing intergroup variation (IGV) in primates have set the focus on tool-use (Luncz et al., 2012; Tan et al., 2015), social behaviour (Borgeaud et al., 2016; DeTroy et al., 2021; van Leeuwen et al., 2018, 2021) or both (Santorelli et al., 2011; Whiten et al., 1999). There are few studies on IGV in foraging behaviour (Quéméré et al., 2013; Samuni et al., 2020; Tournier et al., 2014). The majority of studies on IGV assess differences qualitatively rather than quantitatively (see, e.g. the studies of Luncz et al., 2012; Samuni et al., 2020; van Leeuwen et al., 2021). This study investigates to what extent DNA metabarcoding data allows assessment of IGV quantitatively in foraging behaviour, that is, intraspecies behavioural variation at the group level. The aim is to assess whether the method works effectively in our study system, and to evaluate its potential as a means of capturing cultural diversity in a wider context for consideration in conservation measures.

Research at the Inkawu Vervet Project (IVP), Mawana game reserve (28°00.327S, 031°12.348E), South Africa, focuses on the behavioural ecology of wild vervet monkeys *Chlorocebus pygerythrus*.

This species lives in social groups with female philopatry and male dispersal. The constant gene flow between groups reduces genetic differences within the population (Cheney & Seyfarth, 1983). The high social learning capacity of vervet monkeys has been demonstrated in manifold experiments (Mertz et al., 2019; Whiten & van de Waal, 2018). Indeed, in philopatric species, the social structure promotes the development of distinct group cultures (van de Waal, 2018) and females, the philopatric sex in vervets, have been shown to be preferred role models under experimental conditions (van de Waal et al., 2010, 2014). Therefore, we predict that the diet of adult females best represents that of a group, and hence IGV (measured by RRA) will be accentuated when focussing on adult females only.

We have shown previously a strong seasonal effect on vervet monkeys' diet analysing 823 faecal samples collected over a year, and a strong correlation between plant RRA and observational data, validating the use of RRA as a semi-quantitative measure of consumption in this system. The results indicate that selective feeding behaviours are more likely to occur in summer when resources are more abundant than in scarcer seasons (Brun, Schneider, Mas Carrió, Dongre, van de Waal, et al., 2022). The current study therefore focuses on assessing IGV between four neighbouring groups of vervets during the summer season. We used the RRA of dietary items detected in faecal samples to investigate intrapopulation behavioural plasticity, possibly learnt and transmitted through social learning. According to the 'exclusion principle' variation in a behaviour that is not induced by genetic or environmental differences is likely to result from social learning (van de Waal et al., 2015). Therefore, we assessed (a) whether IGV in the diet was greater than intragroup variation; (b) whether IGV was greater when considering all individuals in the group or only adult females, the philopatric sex; and (c) we investigated the relation between the availability of single food items per home range and their consumption. We use these data to illustrate the potential of the method, in particular the use of an eDNA approach as a promising tool to go beyond classical observational analyses of diet composition. Finally, we discuss challenges arising from the method broadening the perspectives on how to assess intraspecies foraging behavioural variation in wild animals using eDNA approaches.

2 | MATERIALS AND METHODS

2.1 | Studied vervet monkey groups and sampling

Our data were collected from four IVP long-term studied groups with overlapping territories (Figure S1): Ankhase (AK), Baie Dankie (BD), Kubu (KB) and Noha (NH). All individuals were identified using specific bodily and facial features (e.g. scars, colours, shape) and each sample was assigned by experienced field assistants to a specific vervet monkey and consequently linked to available life history data. We defined adulthood for males and females separately, as their life cycles follow different patterns: 3 years for females, and 4 years for males if they dispersed, otherwise 5 years. To avoid redundancy, the social factors sex and age were combined into one category with three levels (female adults, male adults, juveniles) during analyses. Infants were not sampled because they are born at the start of the summer and only feed by nursing for the first 3 months. Infants of the previous year were already 1 year old, and thus in the juvenile class. Approximately 0.5 cm^3 from inside a scat were collected with gloves and a disposable plastic spoon into 20 mL HDPE scintillation vials (Carl Roth GmbH, Karlsruhe, Germany) and covered with 10 mL absolute ethanol, immediately after an individual was observed defecating. After 24–36 h, the ethanol was replaced by silica gel beads and samples stored until DNA extraction. For this study we use the sequence data of 372 faecal samples collected in summer, from mid-November 2018 to mid-March 2019 (Table S1).

Fifty and 95% core areas of each group's home range were calculated based on GPS positions (639 scat sampling and 4669 locations of observations, Table S2) using the *Brownian bridge movement model* (Horne et al., 2007). A full year's data was included, as the use of more data provided better estimates of the model parameters for the different home ranges and their respective core areas. Furthermore, these remain stable throughout the year in this species (Cheney, 1981; Struhsaker, 1967). Subsequently, to account for variable plant distributions across groups' home ranges, ten square vegetation plots (each 1600 m^2) were randomly allocated per 50% core area of each group using QGIS software, and the vegetation cover of 52 presumed forage plants was recorded to estimate local abundance (Figure S1). The final dataset comprised coverage data of 27 plants that the monkeys consumed, and which were also detected in faecal samples. Species accumulation curves (SACs) made with the VEGAN package (Oksanen et al., 2014) showed the adequacy of this survey for representing the distribution of plants in the study area (Figure S2). We controlled for homogeneity of group dispersions with the *betadisper* function (*vegan*) before investigating potential variation in plant coverage between groups' territories with a permutational multivariate analysis of variance (*PERMANOVA* with Bonferroni correction, pseudo $F_{40} = 1.44$, $R^2 = 0.11$, $p = 0.12$) on Bray–Curtis dissimilarity matrices, and pairwise tests also revealed no significant difference (Figure S3).

2.2 | DNA metabarcoding

DNA extraction of scat samples was performed using a phosphate buffer-based approach (Taberlet et al., 2018) following a modified protocol of the NucleoSpin Soil Kit (Macherey-Nagel, Düren, Germany), as described in (Brun, Schneider, Mas Carrió, Dongre, van de Waal, et al., 2022). Extractions were performed in a pre-PCR laboratory exclusively dedicated to low DNA-content analyses (Laboratory for Conservation Biology, University of Lausanne, Switzerland). To assess the plant part of the diet, DNA extracts were amplified in triplicates with a primer pair (Sper01) targeting the P6 loop of the *trn*L intron (UAA) of chloroplast DNA (Taberlet et al., 2018). Library preparation was performed using the TruSeq

DNA PCR-Free Library Prep Kit (Illumina) and libraries were 150 paired-end sequenced on the Illumina Miniseq Sequencing System (Illumina). Bioinformatic processing of raw sequences was conducted with the OBITOOLS package (Boyer et al., 2016) and in R (version 4.0.2). All details of experimental conditions and sequence alignment, filtering, clustering, data cleaning based on controls and taxonomic assignments are described in (Brun, Schneider, Mas Carrió, Dongre, van de Waal, et al., 2022).

2.3 | Data analyses

In order to test the assumption that intergroup variation was greater than intragroup variation, we used the weighted means of Bray–Curtis dissimilarities ranging from 0 (complete overlap) to 1 (complete nonoverlap). For all analyses, data was aggregated as the mean of RRA per plant item and per monkey to account for pseudoreplication. Dietary patterns of the four groups were visualised with non-metrical dissimilarity scaling (NMDS). The assumption of homogeneity of group dispersions was tested with the *betadisper* function in VEGAN package (Oksanen et al., 2014). If these were homogenous, we performed *PERMANOVA* with 9999 permutations and Bonferroni correction on Bray–Curtis dissimilarity matrices to test the effect of the group and sex/age variables on diet composition. If dispersions were heterogenous, we used beta regression models taking into account these dispersions to assess differences in proportions of dietary plant species per group with the package BETAREG (Cribari-Neto & Zeileis, 2010) and the *joint-tests* function in the EMMEANS package (Lenth, 2019) to assess the main effects of the models.

For certain plant species ($n = 9$), we investigated the extent to which consumption was related to environmental factors, that is, plant coverage in the groups' home ranges. In a first step, a feature selection analysis based on a random forest algorithm with 2000 permutations was conducted in the R BORUTA package (Kursa & Rudnicki, 2010) to determine, which species were significantly impacting on IGV in diet. Of the 61 plant species consumed, the random forest algorithm identified 16 species that differed significantly between groups and for nine of these distribution data was available (Figure S4, Table S3). Subsequently, for these nine species RRA data was corrected for the coverage in a group's territory to account for environmental differences (RRA*(1-(percentage of coverage in the group's territory/100))). The Jacob's D index was calculated for these species ranging from −1 (avoidance) to +1 (preference) to visualise differences in selectivity between the groups.

3 | RESULTS

Regarding our hypothesis that intragroup variation was lower than intergroup variation, we found inconsistent results when all individuals were included, since it holds for the smaller groups (AK, KB) but not for the larger ones (BD, NH), as shown in Table 1. A Pearson's product–moment correlation confirmed this positive relationship between group size and increased intragroup variation (0.97, $p = 0.03$). The intragroup dispersions of the four groups were heterogenous ($F = 27.15$, $p = 0.001$). However, we observed group clustering in the NMDS (Figure 1a) and the boxplots of centroids (Figure S5) also indicated location effects; the heterogenous dispersions might have been caused by the unbalanced sample size. Using beta regression, the variable group was significant for all groups ($F_{ratio} = 8.49$, $p < 0.0001$). Testing the groups pairwise, we observed significant effects of the factor group for AK/BD ($F_{ratio} = 4.43$, $p = 0.0353$), AK/KB ($F_{ratio} = 4.62$, $p = 0.0316$), BD/KB ($F_{ratio} = 22.95$, $p < 0.0001$) and KB/NH ($F_{ratio} = 13.17$, $p = 0.0003$). PERMANOVA showed no significant effect of the variable sex/age on dietary variations between groups ($F = 1$, $R^2 = 0.02$, $p = 0.4021$).

TABLE 1 Weighted means of Bray–Curtis dissimilarities of intragroup and intergroup dietary variation using plant RRA data. Green colour indicates that our results were in line with the hypothesis that intergroup variation was greater than intragroup variation (compared to intragroup RRA per row) and red colour that they were not in line with our hypothesis meaning that intragroup variation was greater than intergroup variation. Stars indicate significance level for the group variable in pairwise regression models (*** < 0.001, ** < 0.01, * < 0.05).

Group	AK	BD	KB	NH	Intragroup
AK		0.59*	0.36*	0.48	0.37
BD	0.59*		0.57***	0.58	0.60
KB	0.36*	0.57***		0.46***	0.28
NH	0.48	0.58	0.46***		0.50

The results above point out that group demographics and social dynamics are important factors influencing the foraging behaviour of the studied groups, highlighting the need of further analyses for certain classes of individuals or on the intragroup level. Our hypothesis that intergroup variation was higher than intragroup variation was more accurate when including only adults of the philopatric sex (Table 2), illustrated also by the NMDS (Figure 1b). While for adult females of all groups, dispersions were also heterogenous ($F = 5.46$, $p = 0.005$), the results differed for pairwise tests as these were homogenous for KB/AK and BD/NH. PERMANOVA showed that group explained part of the variance in diet composition for KB/AK ($F = 3.69$, $R^2 = 0.22$, $p = 0.0024$) and BD/NH ($F = 3.76$, $R^2 = 0.13$, $p = 0.0056$). With the beta regression model, the variable group was neither significant for all groups ($F_{ratio} = 1.5$, $p = 0.21$) nor for the other group combinations. We found no significant effect of the variable sex/age at the intragroup level. The small sample size for adult males biases comparisons but there is nevertheless some structuring in the NMDS plots per group (Figure S6).

Figure 2 shows the group-specific selection patterns between resource availability and consumption (measured by Jacob's D index), for those plant species indicated by random forest analysis as being variably consumed between groups and for which distribution data were available. We observed that *Berchemia zeyheri*, a tree whose fruits are a main resource in summer, is highly consumed by all groups, but the least by BD. The resource distribution does not in

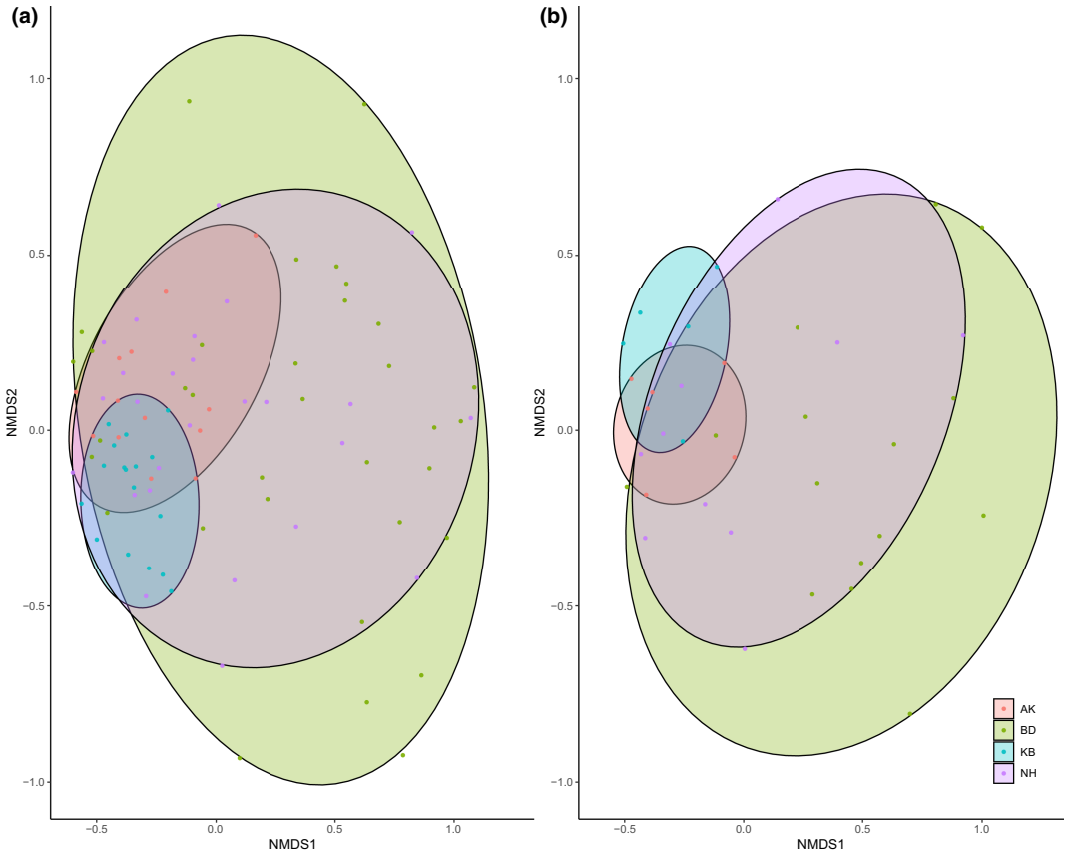

FIGURE 1 Nonmetric multidimensional scaling (NMDS), based on relative read abundances (RRA) of consumed plants detected in faecal samples aggregated per monkey per group in summer for (a) all individuals ($n = 103$) and (b) adult females ($n = 42$). The colours represent the four groups (Ankhase, AK; Baie Dankie, BD; Noha, NH and Kubu, KB).

all cases explain consumption patterns, as it is the case for example for *Cereus jamacaru*, *Hippobromus pauciflorus* and *Premna mooeinsis*. *Ziziphus mucronata* is a main food source in winter, explaining here the pattern of low consumption in summer.

4 | DISCUSSION

Behavioural diversity can be a product of both ecological factors and of cultural traits and it is very difficult to distinguish the effect of even small-scale variation (Brakes et al., 2021; Samuni et al., 2020). We have shown previously that there are strong seasonal patterns in the diet of our population, thus being strongly correlated with resource availability (Brun, Schneider, Mas Carrió, Dongre, van de Waal, et al., 2022). Therefore, to assess IGV, we focused here on one season to reduce environmental variation, selecting summer when food resources are more abundant as it increases individuals' opportunities to select food according to their preferences. The results did not fully match our assumption (IGV was not consistently higher than intragroup variation). Intragroup variation was correlated with group size and may reflect higher inter-individual competition for resources; higher in larger groups (BD and NH) and lower in smaller ones (AK and KB). Both smaller groups in our study had only one adult male at that time and it was therefore not possible to disentangle the relative impact of group size and sex on the observed reduction of intragroup variation. We found an effect of group by assessing the differences in proportions of consumed plants. We suppose that the heterogenous dispersions of group variances were a consequence of the unbalanced design and that both the NMDS (Figure 1a) and boxplots of centroids (Figure S5) indicated true differences in centroids. Future individual-level analyses with more balanced sample numbers will be beneficial to investigate the relationship between group

TABLE 2 Weighted means of Bray–Curtis dissimilarities of intragroup and intergroup dietary variation using plant RRA data for adult females only. Green colour indicates that our results were in line with the hypothesis that intergroup variation was greater than intragroup variation and red colour that they were not in line with our hypothesis meaning that intragroup variation was greater than intergroup variation. Triangles indicate significance level for differences between groups in pairwise *PERMANOVA* with 9999 permutations for the group variable, where model assumption where fulfilled (▲▲ <0.01).

Group	AK	BD	KB	NH	Intragroup
AK		0.56	0.37▲▲	0.46	0.34
BD	0.56		0.57	0.56▲▲	0.55
KB	0.37▲▲	0.57		0.47	0.33
NH	0.46	0.56▲▲	0.47		0.49

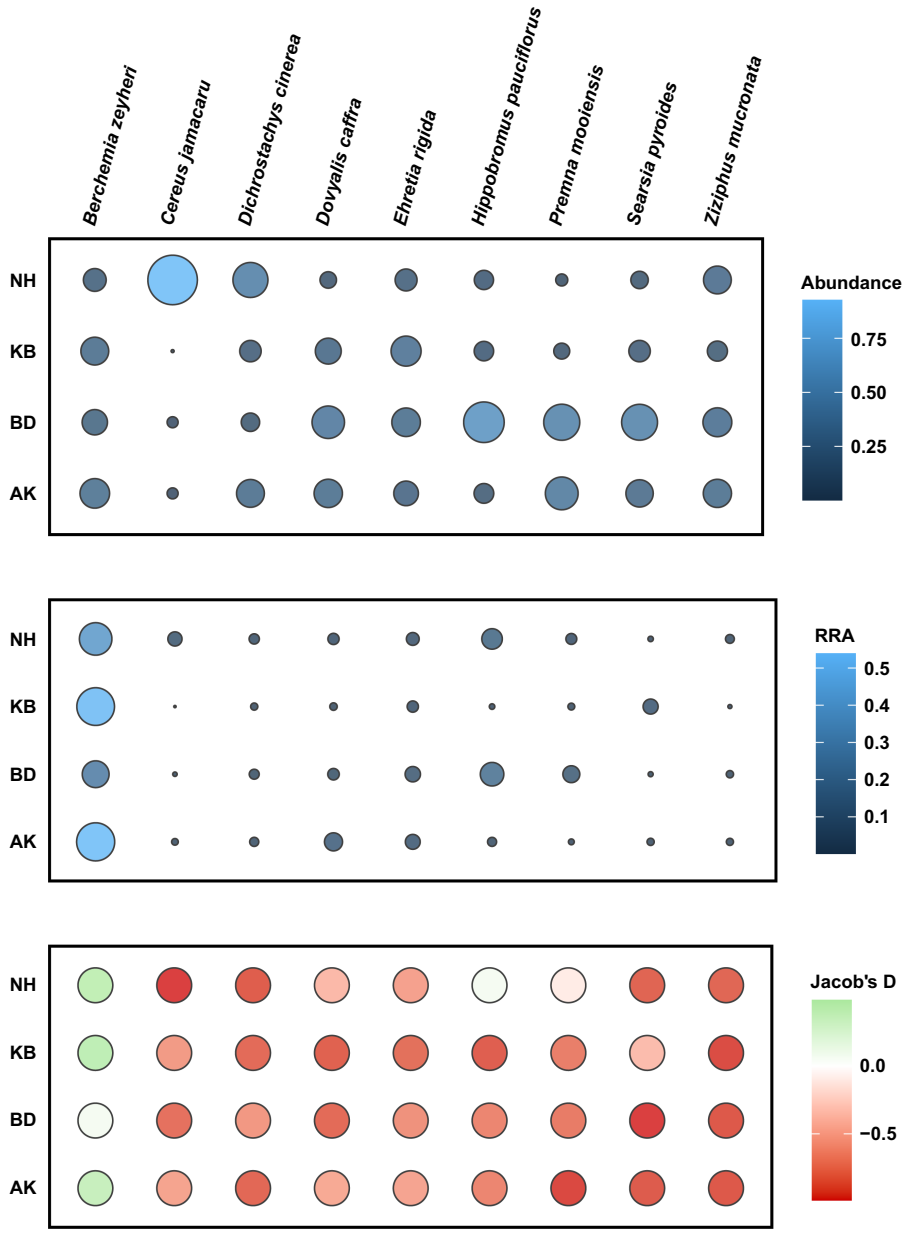

FIGURE 2 Resource availability, consumption, and selectivity of plant species indicated by random forest analysis and present in both plant cover assessments and sequence data. On the y-axis of each plot are the four groups: Noha (NH), Kubu (KB), Baie Dankie (BD), Ankhase (AK). (a) Proportional coverage per group home range; circle size and colour reflect relative abundance. (b) Proportional contribution of each plant to the diet of each group; circle size and colour reflect the mean of relative read abundances. (c) Jacob's D selectivity index for each plant taxon, ranging from −1 (low consumption compared to abundance, red) to 1 (strongest selection or high consumption compared to abundance, green).

size, composition, and diet. Demographic parameters may have an impact on an individual's diet, particularly when social interactions are strongly involved, as in the case of strong competition or when food acquisition is strongly mediated by social learning (Kaufhold & van Leeuwen, 2019; van Boekholt et al., 2021). Obtaining this kind of information in the wild is challenging, yet it is helpful to better understand the driving forces of IGV (DeTroy et al., 2021). While our results show that DNA metabarcoding is a useful approach when studying IGV, and that it may also bring insights into the study of social learning in non-experimental settings, it also emphasises the importance and difficulty of having balanced sampling when demographic parameters must be accounted for.

In this context, the focus on the philopatric sex also produced interesting results. Previous studies on social learning in our population demonstrated in the context of foraging experiments a bias to copy dominant females (van de Waal et al., 2010) and vertical transmission happening primarily in mother-infant dyads where naive infants first rely on their mothers' experience regarding foraging choices (van de Waal et al., 2013, 2014). In the context of a female philopatric system, these premises lay the necessary foundation for

the evolution of cultural variants of foraging behaviours that would essentially be maintained and transmitted by adult females. The heterogenous dispersions prevented unequivocal conclusions about all groups at once, but we observed the factor group to significantly affect the diet of two group pairs. It should be noted that these were on the one hand the smaller groups (AK/KB) and on the other hand the larger ones (BD/NH). A more balanced design may thus allow us to overcome the statistical issue of heterogenous dispersions and to also trace IGV between the remaining groups. However, the observed homogeneity could either be an effect of sample size reduction or of the exclusion of individuals foraging more diversely. When we looked at the results for all individuals, we saw the greatest variation between the smallest group with only one adult male (KB) and the largest group with the most adult males (BD), while we did not see any effect when only looking at adult females.

There is a range of interesting research questions regarding the effect of social factors that could be studied using eDNA data. Future analyses at the intragroup level could assess whether individual foraging is consistent over time and if matrilines (mother–offspring) show foraging behaviour distinct from other matrilines or if different energetic needs due to the reproductive state of females influence the diet. Albeit, sex was not a significant predictor for the present data, it could be worthwhile to study possibly greater behavioural flexibility of the dispersing group members, adult males, as shown previously in behavioural experiments and discussed above (Bono et al., 2018; van de Waal et al., 2013). Furthermore, it has been found that the sex of an individual can already lead to dietary variations in juveniles as described for orangutans (Ehmann et al., 2021). Here, this effect was not significant but the structure within the NMDS still suggests differences between male and female juveniles (Figure S7).

An observational study reported variable feeding on tree species between neighbouring groups of vervet monkeys with multiple mismatches between the dietary importance of a species and its local abundance (Tournier et al., 2014). After taking into account the differences in plant species distribution in the groups' home ranges, we observed variation in consumption of certain plant species that is not entirely explained by a difference in abundance in the territories (Figure 2). For instance, our data showed that monkeys in NH are more selective towards *P. mooiensis* and *H. pauciflorus* relative to its abundance in their home range than the other groups, and the same pattern can be observed for KB with *C. jamacaru*. A possible explanation for variation in selectivity could be that different plant species provide different nutrients, and when species are less abundant in a group's home range, the monkeys must compensate by consuming other species providing similar nutrient intakes. Alternatively, monkeys might have developed distinct food preferences not constrained by physiological needs but rather by social learning. Whenever possible, sampling of environmental factors should also respect seasonal variations, for example the phenology of plants. Here, the plant census was done solely in terms of coverage in the same season as the analysed samples, assuming equal intraspecies-phenology for the presented data. One option is to sample across seasons and to control for this factor in data analyses, but as we have seen, limited sample numbers and unbalanced sampling may be an issue and thus within season designs are a good alternative despite the risk of overlooking important patterns (Matthews et al., 2020).

Reliable and robust estimations of biomass or abundances are crucial to many research questions in ecology and conservation biology (Pimm et al., 2014). Often these revolve not around simple detection or non-detection but need abundance measures to lead to meaningful results. While abundances can be measured by observational methods, the quantification potential of eDNA-approaches is an unresolved debate (Calderón-Sanou et al., 2019; Cuff et al., 2022; Zinger et al., 2019). For example, PCR primer-induced biases, that is, the preferential amplification of certain taxa and the under- or non-representation of others, are considered a main source of biases in PCR-based target enrichment approaches such as DNA metabarcoding (Jusino et al., 2019; Piñol et al., 2015, 2019), and multiplexing of primers or the use of degenerated primers has been proposed as alternative (Dowle et al., 2016; Krehenwinkel et al., 2017). Diet studies are faced with the additional challenge of possible digestion-related biases (Clare, 2014). Macroscopic studies in primates provided evidence for different digestibility of different items (e.g. Matthews et al., 2020). A feeding trial with little penguins *Eudyptula minor* in captivity indicated that the initially fed proportions were not directly correlated to sequence counts (Deagle et al., 2010). Nonetheless, DNA metabarcoding offers the potential to semi-quantitatively study IGV provided that the same experimental conditions are maintained for all groups. Vervet monkeys are omnivorous, however, plants represent the main food source which makes them the first choice for assessment but also implies certain challenges (Cuff et al., 2022). We acknowledge that while we relied on a promising study system, the limited sample numbers and probably the targeted diet components did not allow us to draw the conclusions we had hoped for. An alternative is to study food items that are less frequently consumed or that are more difficult to prey on to inquire about IGV using occurrence data; for example, vertebrates in vervet monkeys or crabs in chimpanzees *Pan troglodytes verus* (Koops et al., 2019). In a study on bonobos *Pan paniscus*, Samuni et al. (2020) observed variation in hunting behaviour of mammalian prey of two groups sharing the same habitat.

The main advantages of an eDNA-based approach are that no experimental setup is needed, it is non-invasive, studied animals are hence not disturbed or influenced in their natural behaviour, and it can provide a thorough picture including both wide temporal and spatial scales (reviewed in Bohmann et al., 2014; Taberlet et al., 2012, 2018; Thomsen & Willerslev, 2015). DNA metabarcoding can also serve to study population structure (Bohmann et al., 2018) and in principle, using SNPs or microsatellite genotyping of faeces DNA would allow assessment of relatedness and genetic structure. A wealth of information can be extracted from samples particularly when conservation status and ethical issues prevent invasive tissue sampling. eDNA samples can *ideally* provide data on genetic *and* behavioural diversity.

To conclude, eDNA-based approaches offer new research opportunities to assess the influence of social factors on dietary variation, in particular for species that are not prone to observation, such as rare and endangered, nocturnal, elusive or dangerous ones. Obtaining information at the individual level might not always be feasible but investigating whether there are dietary variations between males and females, whether diets differ between groups that share a similar environment or, in contrast, that live in very different ones, would greatly contribute to our current knowledge of dietary IGV which has been little studied so far. When different foraging behaviours are detected, possible social transmission, and sometimes even cultural traits, can be studied later. Understanding the driving force and the circumstances that regulate IGV in different populations or species could provide significant insights for various fields of research, including behavioural ecology and cultural evolution but also for applied conservation. In light of climate change and increased anthropogenic habitat destruction, behavioural plasticity might be an important means of responding to rapid disturbances (Brakes et al., 2019, 2021; Gruber et al., 2019). Similarly to the identification of hotspots of genetic diversity to prioritise conservation efforts, cultural traits should be taken into consideration to define populations with the greatest potential for survival (Keith & Bull, 2017; Kühl et al., 2019; Sih, 2013). Cultural transmission of different behaviours through social learning may establish distinct traditions that define a culture, differentiating populations or subpopulations, leading implicitly to varying adaptive potentials. However, the identification of socially transmitted variants and the subsequent potential for cultural differentiation remains challenging to observe in the wild and in this context eDNA techniques might prove valuable.

AUTHOR CONTRIBUTIONS

Erica van de Waal and Luca Fumagalli conceived the ideas and designed methodology; Loïc Brun collected the data; Loïc Brun, Judith Schneider and Pierre Taberlet performed lab work; Judith Schneider and Loïc Brun analysed the data; Judith Schneider led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

The authors thank the van der Walt family for permission to conduct the study on their land. The authors are very grateful to Pooja Dongre for coordinating the start of sample collection and to the whole IVP team who assisted in the field work. The authors thank Eduard Mas Carrió for his precious help during data curation. The authors also thank the Editor and three anonymous reviewers for their critical and valuable comments on earlier versions of the manuscript. The observational research was approved by the relevant local authority "Ezemvelo KZN Wildlife" in South Africa. The field costs during data and sample collection were funded by grants to EW from the Branco Weiss Fellowship-Society in Science and the Swiss National Science Foundation (grant number PP00P3_170624), and the grant 'ProFemmes' of the Faculty of Biology and Medicine, University of Lausanne. At the time of writing, Erica van de Waal and Loïc Brun were supported by the European Research Council under the European Union's Horizon 2020 research and innovation programme for the ERC 'KNOWLEDGE MOVES' starting grant (grant agreement No. 949379). This project was supported by a Swiss National Science Foundation grant to Luca Fumagalli (nr. 310030_192512).

CONFLICT OF INTEREST STATEMENT

The authors note that Pierre Taberlet is co-inventor of a patent related to the Sper01 primers and the use of the P6 loop of the chloroplast *trnL* (UAA) intron for plant identification using degraded template DNA. This patent only restricts commercial applications and have no impact on the use of this loci by academic researchers.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/2041-210X.14078.

DATA AVAILABILITY STATEMENT

Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.6q573n621 (Brun, Schneider, Mas Carrió, Dongre, Taberlet, et al., 2022). Sanger sequences for the local database have been deposited in GenBank under accession numbers OL898555-OL898608.

ORCID

Judith Schneider https://orcid.org/0000-0003-2663-6130
Loïc Brun https://orcid.org/0000-0003-3009-9091
Pierre Taberlet https://orcid.org/0000-0002-3554-5954
Luca Fumagalli https://orcid.org/0000-0002-6648-2570
Erica van de Waal https://orcid.org/0000-0001-7778-418X

REFERENCES

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W., & de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution*, *29*(6), 358–367. https://doi.org/10.1016/j.tree.2014.04.003

Bohmann, K., Gopalakrishnan, S., Nielsen, M., Nielsen, L. D. S. B., Jones, G., Streicker, D. G., & Gilbert, M. T. P. (2018). Using DNA metabarcoding for simultaneous inference of common vampire bat diet and population structure. *Molecular Ecology Resources*, *18*, 1050–1063. https://doi.org/10.1111/1755-0998.12891

Bono, A. E. J., Whiten, A., van Schaik, C., Krützen, M., Eichenberger, F., Schnider, A., & van de Waal, E. (2018). Payoff- and sex-biased social learning interact in a wild primate population. *Current Biology*, *28*, 2800–2805. https://doi.org/10.1016/j.cub.2018.06.015

Borgeaud, C., Sosa, S., Bshary, R., Sueur, C., & van de Waal, E. (2016). Intergroup variation of social relationships in wild vervet monkeys: A dynamic network approach. *Frontiers in Psychology*, *7*, 915. https://doi.org/10.3389/fpsyg.2016.00915

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2016). OBITOOLS: A UNIX-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, *16*, 176–182. https://doi.org/10.1111/1755-0998.12428

Brakes, P., Carroll, E. L., Dall, S. R. X., Keith, S. A., McGregor, P. K., Mesnick, S. L., Noad, M. J., Rendell, L., Robbins, M. M., Rutz, C., Thornton, A., Whiten, A., Whiting, M. J., Aplin, L. M., Bearhop, S., Ciucci, P., Fishlock, V., Ford, J. K. B., Notarbartolo di Sciara, G., & Garland, E. C. (2021). A deepening understanding of animal culture suggests lessons for conservation. *Proceedings of the Royal Society B: Biological Sciences*, *288*, 20202718. https://doi.org/10.1098/rspb.2020.2718

Brakes, P., Dall, S. R. X., Aplin, L. M., Bearhop, S., Carroll, E. L., Ciucci, P., Fishlock, V., Ford, J. K. B., Garland, E. C., Keith, S. A., McGregor, P. K., Mesnick, S. L., Noad, M. J., Notarbartolo di Sciara, G., Robbins, M. M., Simmonds, M. P., Spina, F., Thornton, A., Wade, P. R., & Rutz, C. (2019). Animal cultures matter for conservation. *Science*, *363*(6431), 1032–1034. https://doi.org/10.1126/science.aaw3557

Brun, L., Schneider, J., Mas Carrió, E., Dongre, P., Taberlet, P., van de Waal, E., & Fumagalli, L. (2022). Focal vs. faecal: Seasonal variation in the diet of wild vervet monkeys from observational and DNA metabarcoding data. *Dryad Digital Repository*, *12*, 9358. https://doi.org/10.5061/dryad.6q573n621

Brun, L., Schneider, J., Mas Carrió, E., Dongre, P., van de Waal, E., & Fumagalli, L. (2022). Focal vs. faecal: Seasonal variation in the diet of wild vervet monkeys from observational and DNA metabarcoding data. *Ecology and Evolution*, *12*, e9358. https://doi.org/10.1002/ece3.9358

Calderón-Sanou, I., Münkemüller, T., Boyer, F., Zinger, L., & Thuiller, W. (2019). From environmental DNA sequences to ecological conclusions: How strong is the influence of methodological choices? *Journal of Biogeography*, *47*, 193–206. https://doi.org/10.1111/jbi.13681

Cheney, D. L. (1981). Intergroup encounters among free-ranging vervet monkeys. *Folia Primatologica*, *35*, 124–146. https://doi.org/10.1159/000155970

Cheney, D. L., & Seyfarth, R. M. (1983). Nonrandom dispersal in free-ranging vervet monkeys: Social and genetic consequences. *The American Naturalist*, *122*(3), 392–412.

Clare, E. L. (2014). Molecular detection of trophic interactions: Emerging trends, distinct advantages, significant considerations and conservation applications. *Evolutionary Applications*, *7*, 1144–1157. https://doi.org/10.1111/eva.12225

Cribari-Neto, F., & Zeileis, A. (2010). Beta Regression in R. *Journal of Statistical Software*, *34*(2), 1–24. https://doi.org/10.18637/jss.v034.i02

Cuff, J. P., Windsor, F. M., Tercel, M. P. T. G., Kitson, J. J. N., & Evans, D. M. (2022). Overcoming the pitfalls of merging dietary metabarcoding into ecological networks. *Methods in Ecology and Evolution*, *13*, 545–559. https://doi.org/10.1111/2041-210X.13796

De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., & Taberlet, P. (2014). DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: Application to omnivorous diet. *Molecular Ecology Resources*, *14*, 306–323. https://doi.org/10.1111/1755-0998.12188

Deagle, B. E., Chiaradia, A., McInnes, J., & Jarman, S. N. (2010). Pyrosequencing faecal DNA to determine diet of little penguins: Is what goes in what comes out? *Conservation Genetics*, *11*(5), 2039–2048. https://doi.org/10.1007/s10592-010-0096-6

Deagle, B. E., Thomas, A. C., McInnes, J. C., Clarke, L. J., Vesterinen, E. J., Clare, E. L., Kartzinel, T. R., & Eveson, J. P. (2019). Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology*, *28*, 391–406. https://doi.org/10.1111/mec.14734

DeTroy, S. E., Ross, C. T., Cronin, K. A., van Leeuwen, E. J. C., & Haun, D. B. M. (2021). Cofeeding tolerance in chimpanzees depends on group composition: A longitudinal study across four communities. *IScience*, *24*(3), 102175. https://doi.org/10.1016/j.isci.2021.102175

Dowle, E. J., Pochon, X., Banks, J. C., Shearer, K., & Wood, S. A. (2016). Targeted gene enrichment and high-throughput sequencing for environmental biomonitoring: A case study using freshwater macroinvertebrates. *Molecular Ecology Resources*,*16*(5), 1240–1254. https://doi.org/10.1111/1755-0998.12488

Ehmann, B., van Schaik, C. P., Ashbury, A. M., Mörchen, J., Musdarlia, H., Atmoko, S. U., van Noordwijk, M. A., & Schuppli, C. (2021). Immature wild orangutans acquire relevant ecological knowledge through sex-specific attentional biases during social learning. *PLoS Biology*, *19*(5), 1–21. https://doi.org/10.1371/journal.pbio.3001173

Galan, M., Pons, J.-B., Tournayre, O., Pierre, É., Leuchtmann, M., Pontier, D., & Charbonnel, N. (2018). Metabarcoding for the parallel identification of several hundred predators and their prey: Application to bat species diet analysis. *Molecular Ecology Resources*, *18*, 474–489. https://doi.org/10.1111/1755-0998.12749

Gillet, F., Tiouchichine, M.-L., Galan, M., Blanc, F., Némoz, M., Aulagnier, S., & Michaux, J. R. (2015). A new method to identify the endangered Pyrenean desman (*Galemys pyrenaicus*) and to study its diet, using next generation sequencing from faeces. *Mammalian Biology*, *80*, 505–509. https://doi.org/10.1016/j.mambio.2015.08.002

Gruber, T., Luncz, L., Mörchen, J., Schuppli, C., Kendal, R. L., & Hockings, K. (2019). Cultural change in animals: A flexible behavioural adaptation to human disturbance. *Palgrave Communications*, *5*(64), 1–9. https://doi.org/10.1057/s41599-019-0271-4

Horne, J. S., Garton, E. O., Krone, S. M., & Lewis, J. S. (2007). Analyzing animal movements using Brownian bridges. *Ecology*, *88*(9), 2354–2363. https://doi.org/10.1890/06-0957.1

Jusino, M. A., Banik, M. T., Palmer, J. M., Wray, A. K., Xiao, L., Pelton, E., Barber, J. R., Kawahara, A. Y., Gratton, C., Peery, M. Z., & Lindner, D. L. (2019). An improved method for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous animals. *Molecular Ecology Resources*, *19*, 176–190. https://doi.org/10.1111/1755-0998.12951

Kartzinel, T. R., Chen, P. A., Coverdale, T. C., Erickson, D. L., Kress, W. J., Kuzmina, M. L., Rubenstein, D. I., Wang, W., & Pringle, R. M. (2015). DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 8019–8024. https://doi.org/10.1073/pnas.1503283112

Kaufhold, S. P., & van Leeuwen, E. J. C. (2019). Why intergroup variation matters for understanding behaviour. *Biology Letters*,*15*(11), 20190695. https://doi.org/10.1098/rsbl.2019.0695

Keith, S. A., & Bull, J. W. (2017). Animal culture impacts species' capacity to realise climate-driven range shifts. *Ecography*,*40*(2), 296–304. https://doi.org/10.1111/ecog.02481

Koops, K., Wrangham, R. W., Cumberlidge, N., Fitzgerald, M. A., van Leeuwen, K. L., Rothman, J. M., & Matsuzawa, T. (2019). Crabfishing by chimpanzees in the Nimba Mountains, Guinea. *Journal of Human Evolution*, *133*, 230–241. https://doi.org/10.1016/j.jhevol.2019.05.002

Krehenwinkel, H., Kennedy, S., Pekár, S., & Gillespie, R. G. (2017). A cost-efficient and simple protocol to enrich prey DNA from extractions of predatory arthropods for large-scale gut content analysis by Illumina sequencing. *Methods in Ecology and Evolution*, *8*(1), 126–134. https://doi.org/10.1111/2041-210X.12647

Kühl, H. S., Boesch, C., Kulik, L., Haas, F., Arandjelovic, M., Dieguez, P., Bocksberger, G., McElreath, M. B., Agbor, A., Angedakin, S., Ayimisin, E. A., Bailey, E., Barubiyo, D., Bessone, M., Brazzola, G., Chancellor, R., Cohen, H., Coupland, C., Danquah, E., & Kalan, A. K. (2019). Human impact erodes chimpanzee behavioral diversity. *Science*, *363*(6434), 1453–1455. https://doi.org/10.1126/science.aau4532

Kursa, M. B., & Rudnicki, W. R. (2010). Feature selection with the Boruta package. *Journal of Statistical Software*, *36*(11), 1–13. https://doi.org/10.18637/jss.v036.i11

Lenth, R. (2019). Emmeans: Estimated marginal means, aka least-squares mean. R package version 1.4.1.

Luncz, L. V., Mundry, R., & Boesch, C. (2012). Evidence for cultural differences between neighboring chimpanzee communities. *Current Biology*, *22*, 922–926. https://doi.org/10.1016/j.cub.2012.03.031

Lyke, M. M., Di Fiore, A., Fierer, N., Madden, A. A., & Lambert, J. E. (2018). Metagenomic analyses reveal previously unrecognized variation in the diets of sympatric Old World monkey species. *PLoS ONE*, *14*, e0218245. https://doi.org/10.1371/journal.pone.0218245

Mallott, E. K., Garber, P. A., & Malhi, R. S. (2017). Integrating feeding behavior, ecological data, and DNA barcoding to identify developmental differences in invertebrate foraging strategies in wild white-faced capuchins (*Cebus capucinus*). *American Journal of Physical Anthropology*, *162*, 241–254. https://doi.org/10.1002/ajpa.23113

Mallott, E. K., Garber, P. A., & Malhi, R. S. (2018). trnL outperforms rbcL as a DNA metabarcoding marker when compared with the observed plant component of the diet of wild white-faced capuchins (*Cebus capucinus*, Primates). *PLoS ONE*, *13*, e0199556. https://doi.org/10.1371/journal.pone.0199556

Mallott, E. K., Malhi, R. S., & Garber, P. A. (2015). Brief communication: High-throughput sequencing of fecal DNA to identify insects consumed by wild Weddell's saddleback tamarins (*Saguinus weddelli*, Cebidae, Primates) in Bolivia. *American Journal of Physical Anthropology*, *156*, 474–481. https://doi.org/10.1002/ajpa.22654

Matthews, J. K., Ridley, A., Kaplin, B. A., & Grueter, C. C. (2020). A comparison of fecal sampling and direct feeding observations for quantifying the diet of a frugivorous primate. *Current Zoology*, *66*, 333–343. https://doi.org/10.1093/CZ/ZOZ058

Mertz, J., Surreault, A., van de Waal, E., & Botting, J. (2019). Primates are living links to our past: The contribution of comparative studies with wild vervet monkeys to the field of social cognition. *Cortex*, *118*, 65–81. https://doi.org/10.1016/j.cortex.2019.03.007

Nielsen, J. M., Clare, E. L., Hayden, B., Brett, M. T., & Kratina, P. (2018). Diet tracing in ecology: Method comparison and selection. *Methods in Ecology and Evolution*, *9*, 278–291. https://doi.org/10.1111/2041-210X.12869

Nørgaard, L., Olesen, C. R., Trøjelsgaard, K., Pertoldi, C., Nielsen, J. L., Taberlet, P., Ruiz-Gonzáles, A., De Barba, M., & Iacolina, L. (2021). eDNA metabarcoding for biodiversity assessment, generalist predators as sampling assistants. *Scientific Reports*, *11*, 6820. https://doi.org/10.1038/s41598-021-85488-9

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2014). Vegan: Community ecology package. R package version 2.5–7.

Pansu, J., Guyton, J. A., Potter, A. B., Atkins, J. L., Daskin, J. H., Wursten, B., Kartzinel, T. R., & Pringle, R. M. (2019). Trophic ecology of large herbivores in a reassembling African ecosystem. *Journal of Ecology*, *107*, 1355–1376. https://doi.org/10.1111/1365-2745.13113

Pickett, S. B., Bergey, C. M., & Di Fiore, A. (2012). A metagenomic study of primate insect diet diversity. *American Journal of Primatology*, *74*, 622–631. https://doi.org/10.1002/ajp.22014

Pimm, S. L., Jenkins, C. N., Abell, R., Brooks, T., Gittleman, J. L., Joppa, L. N., Raven, P. H., Roberts, C. M., & Sexton, J. O. (2014). The biodiversity of species and their rates of extinction, distribution, and protection. *Science*, *344*, 1246752. https://doi.org/10.1126/science.1246752

Piñol, J., Mir, G., Gomez-Polo, P., & Agustí, N. (2015). Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. *Molecular Ecology Resources*, *15*, 819–830. https://doi.org/10.1111/1755-0998.12355

Piñol, J., Senar, M. A., & Symondson, W. O. C. (2019). The choice of universal primers and the characteristics of the species mixture determine when DNA metabarcoding can be quantitative. *Molecular Ecology*, *28*, 407–419. https://doi.org/10.1111/mec.14776

Quéméré, E., Hibert, F., Miquel, C., Lhuillier, E., Rasolondraibe, E., Champeau, J., Rabarivola, C., Nusbaumer, L., Chatelain, C., Gautier, L., Ranirison, P., Crouau-Roy, B., Taberlet, P., & Chikhi, L. (2013). A DNA metabarcoding study of a primate dietary diversity and plasticity across its entire fragmented range. *PLoS ONE*, *8*, e58971. https://doi.org/10.1371/journal.pone.0058971

Rowe, A. K., Donohue, M. E., Clare, E. L., Drinkwater, R., Koenig, A., Ridgway, Z. M., Martin, L. D., Nomenjanahary, E. S., Zakamanana, F., Randriamanandaza, L. J., Rakotonirina, T. E., & Wright, P. C. (2021). Exploratory analysis reveals arthropod consumption in 10 lemur species using DNA metabarcoding. *American Journal of Primatology*, *83*, e23256. https://doi.org/10.1002/ajp.23256

Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, *17*, e00547. https://doi.org/10.1016/j.gecco.2019.e00547

Samuni, L., Wegdell, F., & Surbeck, M. (2020). Behavioural diversity of bonobo prey preference as a potential cultural trait. *eLife*,*9*, 1–10. https://doi.org/10.7554/ELIFE.59191

Santorelli, C. J., Schaffner, C. M., Campbell, C. J., Notman, H., Pavelka, M. S., Weghorst, J. A., & Aureli, F. (2011). Traditions in spider monkeys are biased towards the social domain. *PLoS ONE*, *6*(2), e16863. https://doi.org/10.1371/journal.pone.0016863

Shao, X., Lu, Q., Liu, M., Xiong, M., Bu, H., Wang, D., Liu, S., Zhao, J., Li, S., & Yao, M. (2021). Generalist carnivores can be effective biodiversity samplers of terrestrial vertebrates. *Frontiers in Ecology and the Environment*, *19*, 557–563. https://doi.org/10.1002/fee.2407

Shehzad, W., Riaz, T., Nawaz, M. A., Miquel, C., Poillot, C., Shah, S. A., Pompanon, F., Coissac, E., & Taberlet, P. (2012). Carnivore diet analysis based on next-generation sequencing: Application to the leopard cat (*Prionailurus bengalensis*) in Pakistan. *Molecular Ecology*, *21*, 1951–1965. https://doi.org/10.1111/j.1365-294X.2011.05424.x

Sih, A. (2013). Understanding variation in behavioural responses to human-induced rapid environmental change: A conceptual overview. *Animal Behaviour*, *85*(5), 1077–1088. https://doi.org/10.1016/j.anbehav.2013.02.017

Struhsaker, T. T. (1967). Ecology of vervet monkeys (*Cercopithecus aethiops*) in the Masai-Amboseli game reserve, Kenya. *Ecology*, *48*, 891–904. https://doi.org/10.2307/1934531

Taberlet, P., Bonin, A., Zinger, L., & Coissac, É. (2018). *Environmental DNA: For biodiversity research and monitoring*. Oxford University Press. https://doi.org/10.1093/oso/9780198767220.001.0001

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, *21*, 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Tan, A., Tan, S. H., Vyas, D., Malaivijitnond, S., & Gumert, M. D. (2015). There is more than one way to crack an oyster: Identifying variation in burmese long-tailed macaque (*Macaca fascicularis aurea*) stone-tool use. *PLoS ONE*, *10*(5), 1–25. https://doi.org/10.1371/journal.pone.0124733

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA—An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, *183*, 4–18. https://doi.org/10.1016/j.biocon.2014.11.019

Tournier, E., Tournier, V., van de Waal, E., Barrett, A., Brown, L., & Bshary, R. (2014). Differences in diet between six neighbouring groups of vervet monkeys. *Ethology*, *120*, 471–482. https://doi.org/10.1111/eth.12218

van Boekholt, B., van de Waal, E., & Sterck, E. H. M. (2021). Organized to learn: The influence of social structure on social learning opportunities in a group. *iScience*, *24*(2), 102117. https://doi.org/10.1016/j.isci.2021.102117

van de Waal, E. (2018). On the neglected behavioural variation among neighbouring primate groups. *Ethology*, *124*, 845–854. https://doi.org/10.1111/eth.12815

van de Waal, E., Borgeaud, C., & Whiten, A. (2013). Potent social learning and conformity shape a wild primate's foraging decisions. *Science*, *340*(6131), 483–485. https://doi.org/10.1126/science.1232769

van de Waal, E., Bshary, R., & Whiten, A. (2014). Wild vervet monkey infants acquire the food-processing variants of their mothers. *Animal Behaviour*, *90*, 41–45. https://doi.org/10.1016/j.anbehav.2014.01.015

van de Waal, E., Claidière, N., & Whiten, A. (2015). Wild vervet monkeys copy alternative methods for opening an artificial fruit. *Animal Cognition*, *18*, 617–627. https://doi.org/10.1007/s10071-014-0830-4

van de Waal, E., Renevey, N., Favre, C. M., & Bshary, R. (2010). Selective attention to philopatric models causes directed social learning in wild vervet monkeys. *Proceedings of the Royal Society B: Biological Sciences*,*277*, 2105–2111. https://doi.org/10.1098/rspb.2009.2260

van Leeuwen, E. J. C., Cronin, K. A., & Haun, D. B. M. (2018). Population-specific social dynamics in chimpanzees. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(45), 11393–11400. https://doi.org/10.1073/pnas.1722614115

van Leeuwen, E. J. C., Van Donink, S., Eens, M., & Stevens, J. M. G. (2021). Group-level variation in co-feeding tolerance between two sanctuary-housed communities of chimpanzees (*Pan troglodytes*). *Ethology*, *127*(7), 517–526. https://doi.org/10.1111/eth.13154

Vesterinen, E. J., Puisto, A. I. E., Blomberg, A. S., & Lilley, T. M. (2018). Table for five, please: Dietary partitioning in boreal bats. *Ecology and Evolution*, *8*, 10914–10937. https://doi.org/10.1002/ece3.4559

Voelker, M. R., Schwarz, D., Thomas, A., Nelson, B. W., & Acevedo-Gutiérrez, A. (2020). Large-scale molecular barcoding of prey DNA reveals predictors of intrapopulation feeding diversity in a marine predator. *Ecology and Evolution*,*10*, 9867–9885. https://doi.org/10.1002/ece3.6638

Whiten, A., Goodall, J., Mcgrew, W. C., Nishida, T., Reynolds, V., Sugiyama, Y., Tutin, C. F. G., Wrangham, R. W., & Boesch, C. (1999). Cultures in chimpanzees. *Nature*, *399*, 15–18. https://doi.org/10.1038/21415

Whiten, A., & van de Waal, E. (2018). The pervasive role of social learning in primate lifetime development. *Behavioral Ecology and Sociobiology*, *72*, 80. https://doi.org/10.1007/s00265-018-2489-3

Zinger, L., Bonin, A., Alsos, I. G., Bálint, M., Bik, H., Boyer, F., Chariton, A. A., Creer, S., Coissac, E., Deagle, B. E., De Barba, M., Dickie, I. A., Dumbrell, A. J., Ficetola, G. F., Fierer, N., Fumagalli, L., Gilbert, T. P., Jarman, S., Jumpponen, A., & Taberlet, P. (2019). DNA metabarcoding–Need for robust experimental designs to draw sound ecological conclusions. *Molecular Ecology*, *28*, 1857–1862. https://doi.org/10.1111/mec.15060

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Sample counts per group in summer: number of samples/number of individuals sampled (number of individuals present in group at the start of summer; 15th November 2018). Discrepancies between samples/individuals and individuals/group possible when age categories changed between 15th of November and the time of sampling (e.g. AK adult females and BD one individual sampled as juvenile F and adult F). Pearson's Chi-square tests confirmed the goodness of fit per age/sex category of sampled individuals per group for AK, KB and NH. For BD the null hypothesis of good fit was only confirmed when excluding the infant category. Age categories were defined as follows: infant <1 year, juvenile 1-2 years for females and 1-4 years for males (if not dispersed), adult 3 years for females and 4 years for males if they dispersed, otherwise 5 years.

Table S2. Number of GPS locations sampled per group from the faecal sample spots and through observations, used to calculate the 50 % and 95 % isopleths of the groups' home ranges (*total* rows). The *faecal samples* and *observations* rows indicate the number of these found within their respective isopleths.

Table S3. Comparison of the predicted group (from the random forest model) and the observed group from which the faecal samples were originating for the test dataset (25 % of the observations). Observed samples are in columns and predicted samples in rows. Values on the diagonals represent the true positive of the models. Sensitivity (True positives/(True positives + False negatives)) and Specificity (True negatives/(True negatives + False positives)) are indicated for each group.

Figure S1. Home ranges (50 % and 95 % isopleths) of the four groups based on GPS data of sampling locations of faecal samples and observations. Points indicate the location of the 40 vegetation plots.

Figure S2. Species accumulation curves (SACs) of plant abundance in terms of coverage for the 40 vegetation plots. On the x-axis are the ten vegetation plots per groups and on the y-axis the cumulative number of plant species, grey shading indicates 95 % confidence intervals.

Figure S3. Nonmetric multidimensional scaling (NMDS) with *envfit* function of plant abundance in terms of coverage for the 40 vegetation plots taken in the respective 50 % home ranges of the four groups. Vectors of plants are shown for those with $p<0.005$. *PERMANOVA* indicates no significant difference in plant coverage between groups' territories (pseudo$F40 = 1.44$, $R^2 = 0.11$, $P = 0.12$).

Figure S4. The plot shows the plant species that were selected by the random forest algorithms implemented in the Boruta R package as relevant features to explain dietary variation between groups. The higher the importance, the higher the group specificity of the corresponding species. Blue shows the minimum, average and maximum importance scores obtained by chance after 2000 random row permutations. Species in red were below the maximum threshold and considered not specific to any of the groups. *E. undulata* in yellow was very close to the maximum threshold and also not kept for further analyses. For species in green the group specificity was higher than that obtained by chance. Species above the threshold were corrected according to their respective abundance in the different groups' home ranges when available.

Figure S5. Boxplots displaying the dispersion from the centroids for each group, for (a) all individuals (average distance to median: AK = 0.25, BD = 0.43, KB = 0.19, NH = 0.35; $p<0.001$) and (b) only adult females (average distance to median: AK = 0.23, BD = 0.38, KB = 0.21, NH = 0.33; $p<0.005$).

Figure S6. Nonmetric multidimensional scaling (NMDS), based on relative read abundances (RRA) of consumed plants detected in faecal samples aggregated per monkey in summer for all groups (Ankhase, AK; Baie Dankie, BD; Noha, NH and Kubu, KB) with variable sex/age with three factor levels. The colours represent female adults (red), male adults (green) and juveniles (blue).

Figure S7. Nonmetric multidimensional scaling (NMDS), based on relative read abundances (RRA) of consumed plants detected in faecal samples aggregated per monkey in summer for all groups (Ankhase, AK; Baie Dankie, BD; Noha, NH and Kubu, KB) with variable sex/age with four factor levels. The colours represent female adults (red), male adults (green), female juveniles (blue) and male juveniles (violet).

How to cite this article: Schneider, J., Brun, L., Taberlet, P., Fumagalli, L., & van de Waal, E. (2023). Molecular assessment of dietary variation in neighbouring primate groups. *Methods in Ecology and Evolution*, *14*, 1925–1936. https://doi.org/10.1111/2041-210X.14078