The importance of protein in leaf selection of folivorous primates

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Abstract

Protein limitation has been considered a key factor in hypotheses on the evolution of life history and animal communities, suggesting that animals should prioritize protein in their food choice. This contrasts with the limited support that food selection studies have provided for such a priority in nonhuman primates, particularly for folivores. Here, we suggest that this discrepancy can be reconciled if folivores only need to select for high protein leaves when average protein concentration in the habitat is low. To test the prediction, we analyzed published and unpublished results of food selection and protein concentrations from 47 studies of folivorous primates. To counter potential methodological flaws, we differentiated between methods analyzing nitrogen and soluble protein concentrations. We found that leaves containing either high concentrations of total nitrogen or high soluble protein were selected more in low protein forests. There was no relationship (either negative or positive) between the concentration of protein and fiber in the food. Overall our study suggests that protein is limiting only in protein-poor environments, explaining the sometimes contradictory results in previous studies on protein selection.

Key words: primates, food chemistry, food selection, leaf-eating, nutrient requirements, protein availability

INTRODUCTION

Protein has been considered a major limiting factor involved in the evolution of animal communities and life history traits [e.g., White, 1993]. The need to satisfy protein requirements plays a central role in hypotheses on the evolution of morphological, physiological and behavioral life history traits (such as gut specialization, reduced metabolism in folivores, social systems linked to the distribution of different types of food, and community composition; e.g., White, 1993). The essentials of this idea have been developed for primates by Kay [1984] and illustrated by Terborgh [1992]. Specifically, while most primates eat fruit to satisfy their energy requirements, fruits typically do not provide enough available protein for survival and reproduction, though this may not always be the case [reviewed by Klaasen and Nolet 2008; Ganzhorn et al., 2009; Schwitzer et al 2009]. Therefore, smaller-bodied species feed on insects and fruit to support their protein needs. Larger species are unable to obtain enough protein from insects because the capture rate of insects is independent of body mass [Hladik, 1978; Rothman et al., 2014]. Consequently they eat leaves, which usually contain more protein than fruit and can be found in sufficient quantities to satisfy the protein needs of a larger species. According to this scenario, within the broad constraints of body mass, protein represents the ultimate factor that determines whether a species is insectivorous or folivorous. The idea that protein is limiting has received support from the studies of Milton [1979], Oates et al*.,* [1990] and Davies and Oates [1994 and their contributors]. Milton [1979] postulated that the densities and biomass of folivorous howler monkeys are closely related to the average leaf quality of a forest expressed as the ratio of protein to fiber (most commonly measured as acid detergent fiber – ADF) concentrations. Oates and collaborators (1990) tested and found support for this idea through a wide comparison of colobine monkeys. ADF concentrations were included because ADF should represent the refractory fraction of the cell wall (cellulose + lignin) and increasing ADF concentrations are also likely to reflect greater amounts of indigestible protein [Rothman et al., 2008]. The concept of protein to fiber ratios was extended to additional populations of colobines [e.g., Chapman et al*.,* 2002, 2004; Wasserman & Chapman, 2003; Fashing et al., 2007] and supported with independent datasets on lemurs [Ganzhorn, 1992; Simmen et al., 2012] and howler monkeys [Peres, 1997]. The biological relevance of this ratio has been questioned based on biochemical considerations, statistical issues around the use of ratios [Wallis et al., 2012], and empirical grounds [Gogarten et al., 2012; Chapman et al., 2014] but it seems to retain some predictive capacity.

Restricting the considerations to protein alone, several studies have shown that protein can be limiting with lasting effects on development and lifetime fitness [e.g., Fleagle et al., 1975; Elias & Samonds, 1977; Altmann, 1991, 1998; Degabriel et al., 2009]. However, the evidence that folivorous primates actually select leaves with high protein content is ambiguous. Considering protein alone, some studies found positive selection by primates for high protein leaves [e.g., *New World howler monkeys*: Milton, 1979, 1998; Glander, 1981; *Old World non-colobine monkeys*: Beeson, 1989; Barton & Whiten, 1994; *Old World colobines*: Davies et al., 1988; Waterman et al., 1988; Mowry et al., 1996; Koenig et al., 1998; Yeager et al*.,* 1997; *Apes*: Calvert, 1985; *Lemurs*: Ganzhorn, 1988, 1992, 2002; Mutschler, 1999] but others failed to do so [e.g., *New World howler monkeys*: Gaulin & Gaulin, 1982; Estrada & Coates-Estrada, 1986; *Old World colobines*: Oates et al*.,* 1980; McKey et al*.,* 1981; Waterman et al*.,* 1988; Kool, 1992; Dasilva, 1994; Chapman et al*.,* 2002; *Apes*: Conklin-Brittain et al*.,* 1998; Rothman et al., 2011; *Lemurs*: Ganzhorn, 1988; Ganzhorn et al., 2004; Simmen et al., 2014]. Thus, we are left with the conundrum that protein is hypothesized to be an important component in primate food selection while only about half of the studies on food selection criteria demonstrate that primates actively select high protein leaves. This discrepancy can be due to methodological, ecological, or species-specific reasons, or the hypothesis may simply be wrong.

On the methodological side, different studies have applied different methods to measure “protein”. While the conventional method of measuring crude protein uses total nitrogen concentrations multiplied by 6.25 (or a species specific factor [Milton & Dintzis, 1981]) as a surrogate for protein, this measure does not actually distinguish between protein and non-protein nitrogen [e.g. N in cyanogenic glycosides, non-protein amino acids, nitrates or alkaloids], or between available protein and protein bound to other components and thus unavailable for digestion [DeGabriel et al., 2008; Rothman et al., 2008]. To overcome this shortcoming, some studies have analyzed total amino acids [e.g., Glander, 1981; Simmen & Sabatier, 1996; Mutschler, 1999; Curtis, 2004] or soluble protein [e.g., Ganzhorn, 1988; Koenig et al., 1998; Conklin-Brittain et al*.,* 1999; for methodological considerations see Ortmann et al*.,* 2006; Rothman et al., 2012]. Although the selection for high protein items was more consistent in studies that analyzed soluble protein than in studies based on crude protein, none of these methods accounts for differences in protein quality (defined by essential amino acids), or digestibility [Robbins, 1983; NRC, 2003; Wallis et al., 2012; DeGabriel et al., 2014].

From an ecological perspective, the lack of positive selection for high protein items could also be explained by the assumption that primates are able to satisfy their protein requirements with a diet containing about 6.4 – 8% crude protein [NRC, 2003]. The crude protein concentration of leaves and the average concentration of protein in primate foods are around or well above these requirements [e.g., Hladik, 1977; Oftedal, 1991; Conklin-Brittain et al., 1998; Ganzhorn et al., 2009]. Thus, primates might not need to select high protein items but could simply feed according to the average availability of protein in the environment provided that the digestibility of protein from the food was not hindered by other components such as fiber or tannins [Mowry et al., 1996; Yeager et al., 1997; Simmen et al., 2014].

Deviations from selecting high protein leaves may also be caused by species-specific adaptation of gut morphology and digestive physiology [Chivers et al., 1984;

Cork & Foley 1991; Hughes, 1993; Langer & Chivers, 1994; Van Soest, 1994; Lambert, 1998; Milton, 1998, 1999; Campbell et al., 1999, 2004; Edwards & Ullrey, 1999a,b; Godfrey et al., 2004]. The effect of gut physiology may be more important than the effect of body mass on dietary characteristics in primates as hindgut-fermenters process food differently than foregut fermenters and both deviate from species with unspecialized digestive tracts, regardless of size. For example, Campbell et al. [2004] found that different adaptations of the digestive tract result in food passage times largely independent of body mass [see also Clauss et al., 2008], such as larger primate species with foregut fermentation (colobines) or hindgut fermentation (gorillas), and small primates with hindgut fermentation and caecotropy (e.g., *Lepilemur* spp.) [Charles-Dominique & Hladik 1971], or enlargement of the small intestine (Indriidae). This supports the conclusion that body mass is not a useful surrogate to understand primate feeding and digestion, including protein requirements [Lambert, 1998].

Thus, in order to investigate protein selection in folivorous primates, we consider the availability of protein in the environment and test the hypothesis that protein is a limiting component and therefore primates should search for high protein and/or low fiber leaves. According to this hypothesis, selection for high protein items would not be necessary if animals could obtain enough protein from their overall diet. However, if protein concentrations in the environment are low, folivorous primates should seek high protein leaves. Therefore, we predict that selectivity for high protein leaves declines with increasing average protein content in leaves encountered by the animals in their home range. We could expect there to be an inverse relationship between concentrations of protein and fiber in foliage reflecting a maturation of the leaf ontogenetically and temporally. We also tested for this relationship and separately tested whether fiber in the food selected differed from that of a general sample.

METHODS

Database

The analyses presented here are based on published data from all primate radiations (except for apes; see below), supplemented by new data of folivorous primates from Madagascar, the New World and Nepal (Table 1). Analyses were restricted to forest dwelling species that have been classified as “folivores” because the majority of their food items were from photosynthetic material [Kappeler & Heymann, 1996]. As more studies are conducted, it appears that the classification of species into specific feeding guilds does not reflect the species-specific variability of diet [Hemingway & Bynum, 2005; Garber et al., 2015]. Thus, we call those species “folivores” that are supposed to derive their protein from leaves and not insects according to Kay’s [1984] hypothesis.

Species that feed primarily on the leaves of grasses, bamboo (*Hapalemur* spp., *Prolemur simus*) and herbs (*Gorilla* spp.) were not included, as grass and herbs have different physico-chemical properties than leaves from trees, such as different lignin, a general lack of tannins and incorporation of silica in grasses [Robbins, 1983]. However, *Hapalemur meridionalis* from Mandena (south-eastern Madagascar) was included as these animals live in an area without bamboo and feed on grass and other leaves [Eppley et al., 2011]. For the current analysis we removed all grasses that were used as food and restricted the analysis to the proportion of their diet that consists of leaves from trees. We also included body mass in the database provided in Table I. Data for primate body mass were taken from Smith and Jungers [1997] and Mittermeier et al. [2010] and averaged between sexes.

Food Types and Nutritional Analyses

Foods included in the present analysis were leaves or flower buds from trees, shrubs or vines. We further restricted the analysis to concentrations of nitrogen (measured by the Kjeldahl method), or by a combustion procedure with subsequent analysis of elementary nitrogen (the Dumas method), or based on near infrared reflectance spectroscopy (NIRS) (calibrated against the Kjeldahl or Dumas method), soluble protein and acid detergent fiber (ADF). Data presented as “crude protein” (i.e. total nitrogen multiplied by 6.25) was re-transformed to total nitrogen concentration as the biological significance of the conversion factor is presently debated and its biological meaning is unclear (Milton & Dintzis, 1981; NRC, 2003; for methodological reviews see Ortmann et al*.,* 2006; Rothman et al., 2012). The Kjeldahl and Dumas methods yield almost identical results (regression between nitrogen measured by Kjeldahl [y] and by the Dumas method [x] forced through the origin: $y = 0.94x$; $R^2 = 0.99$; $n = 90$; Supplementary Material [Terboven, 2014]). Near infrared reflectance spectroscopy also provides accurate estimates for nitrogen concentrations when models were tested with truly independent data (Kjeldahl: $y = 1.06x$, $R^2 = 0.97$, $N = 18$; combustion: $y = 0.97x$, $R^2 = 0.97$; $N = 18$; Supplementary Material).

Studies that published soluble protein concentrations (measured by the method outlined by Bradford, [1976]) but without estimates of crude protein were included in the analysis, when available. However, these two datasets were analyzed separately. “Available protein” would be a more biologically appropriate measure of protein than crude protein [DeGabriel et al., 2008, 2014; Wallis et al., 2012] and probably also than soluble protein as soluble protein concentrations are correlated with available protein in some studies but not in others [Ganzhorn, unpubl.]. To date, too few data exist for available protein to allow for comparative analyses.

In primate studies, fiber concentrations are most commonly reported as acid detergent fiber (ADF). However, not all studies report exact details of the procedures (e.g. whether ADF is analyzed sequentially following isolation of neutral detergent fiber (NDF)). In addition, most studies do not specify whether ADF is reported on an ash-free basis or corrections are made for residual dry matter. Furthermore, there is little appreciation in primate literature that fiber residues can be contaminated with tannin-protein complexes [Wallis et al., 2012]. All these factors can contribute to unknown errors in the reported ADF concentrations, but how significant they are in different studies is hard to gauge and it is not possible to apply a consistent correction factor to compensate for methodological differences. We emphasize the need for rigorous analysis to avoid these uncertainties [Rothman et al., 2012]. As a result, the accuracy of the “ADF” data is likely to be low and conclusions derived from fiber concentrations should be considered with these limitations in mind

All as yet unpublished chemical analyses were carried out in the laboratory of the University of Hamburg [Donati et al., 2007] (Table I). All results are expressed as % of dry matter.

Insert Table I here

Quality of Leaves Available in Different Forests (“representative samples”)

Most measures of the availability of protein and leaf quality in different forests (here termed “representative samples”) are based on mature tree leaves. Leaves were collected opportunistically or from the most abundant tree species and were assumed to represent a proxy for year-round leaf quality [e.g., Oates et al., 1990; Ganzhorn, 1992; Chapman et al., 2002, 2004; Wasserman & Chapman, 2003; Simmen et al., 2014]. The representative samples for *Semnopithecus schistaceus* in

Ramnagar (Nepal) are based on mature leaves of the 25 most abundant tree species [Chalise 1995; Chalise & Koenig, unpubl.] and for *Propithecus edwardsi* in Ranomafana (Madagascar) on 14 tree species sampled haphazardly [Wright & Daniels, unpubl.].

Some studies collected separate representative samples for young and mature leaves [Mowry et al., 1996; Liu et al., 2013] or separate samples for the wet and the dry season [Ganzhorn, 2002]. These samples were considered as independent data points and were entered in the analyses as independent units. Our rationale is that we wanted to have some measure of leaf nutritional quality in samples of leaves that we could use for the analyses of selection of leaves consumed as food against this representative sample (see “Selection Criteria for Consumed Leaves” below).

Selection Criteria for Consumed Leaves

Determination of the significance of selection for specific chemical components was restricted to photosynthetic parts (leaves, sometimes differentiated in different parts of leaves). Analyses of selection were always restricted to the same types of plant parts because we wanted to know when selection occurs with respect to the representative sample. For example; if the representative sample consisted of mature leaves, then only food items consisting of mature leaves were considered. If the representative sample consisted of young leaves, then only young leaf food items were considered. If the representative sample consisted of mature leaves and the animals were feeding only on young leaves, no comparison was calculated.

The data for *Propithecus coronatus* are based on the early dry season. During this time of the year, the diet consisted of 85-90% leaves. The chemical analyses were based on a reconstructed diet, made by mixing aliquot proportions of each food species consumed according to its dietary proportion [Pichon, 2012].

Selection criteria were taken from the original paper, or leaves that were consumed were compared with representative samples from the forest, or concentrations of chemical components were correlated with the frequency of consumption (assumed to represent the amount of leaf material ingested). Thus, p-values listed in Table I and Figure 1 are based on t-tests between samples of material consumed versus the representative sample or on correlations between the frequency of consumption and the concentration of the chemical component in question.

Statistical Analyses

Published data are based on the analysis of a single individual per plant species or averages based on several different individuals of the same plant species or on averages weighted by the frequency of abundance or the frequency of consumption. When possible, we base our analyses on unweighted means of plant species. Surprisingly, and despite the known temporal and inter-individual variation within plant species [Ganzhorn & Wright 1994; Chapman et al., 2003], the variation between weighted and unweighted samples seems to average out in large samples (Table II). Statistical tests were made with SPSS 21.0.

Insert Table II

RESULTS

Selection of Leaves in Relation to the Average Concentrations of Nitrogen, Soluble Protein or ADF in a Given Forest

Measures of nitrogen, soluble protein and ADF in representative samples of plant leaves were found for 19, 18 and 33 studies, respectively (Table I). Concentrations of the same components in food plants were found for 35, 22 and 41 studies. The data for soluble protein were unevenly distributed in the dataset, and were mainly available for foods of lemurs. Studies of the same species in different areas or during different times of the year were treated as independent units since the concentrations of chemical components vary significantly between sites and seasons.

Selectivity for leaves containing high concentrations of nitrogen increased significantly with declining nitrogen concentrations in forests ($r_s = 0.62$, $P = 0.008$, $N = 17$; Figure 1; Table I). Restricting the correlation to the Colobinae does not alter the principal result but removes significance ($r_s = 0.51$, $P = 0.075$, $N = 13$).

For soluble protein data, selection of high protein leaves was stronger in forests with low concentrations of soluble protein in representative samples of leaves than in forests with high concentrations ($r_s = 0.66$, $P = 0.004$, $N = 17$). Removing *Semnopithecus schistaceus* from the correlation (the only species for which soluble protein data are available for representative samples of leaves outside Madagascar; thus restricting it to lemurs) does not change the result ($r_s = 0.66$, $P = 0.005$, $N = 16$).

Combining the data for the two measures of protein and including the type of protein analysis as a random categorical variable in a GLMM results in a highly significant effect of the concentrations of protein in representative samples of leaves on the strength (significance) of selection ($F = 21.58$; $P < 0.001$).

There was no relationship between concentrations of nitrogen or soluble protein and fiber in the data set. There were no significant correlations between the selection (or rather discrimination) against ADF and the ADF in representative samples, either over all the data ($r_s = 0.12$, $P = 0.534$, $N = 31$), or when considering various primate radiations separately.

Insert Figure 1

DISCUSSION

The present analysis sought to better understand the discrepancy between the findings of some studies that identify protein as a limiting resource, including those that focus on non-human primates [Kay 1984] and others that find no evidence for this phenomenon. Primates (and animals in general) need to satisfy their protein needs by selecting protein-rich food, but we found that many primatological studies failed to demonstrate such a selection for high protein food (Table I). A number of studies have pointed out that selection of high protein food would only be required if the food items in the environment have average protein concentrations below the required needs [e.g., Mowry et al., 1996; Yeager et al., 1997; Ganzhorn et al., 2009; Simmen et al., 2014] and that, once protein concentrations are above requirements, selection could be based on other components and criteria, such as the availability within the environment [e.g., Oftedal, 1991; Fashing et al., 2007] or secondary plant chemicals [Moore & Foley 2005] or minerals such as sodium [Rothman et al 2006]. While this idea has been around for some time, it has rarely been tested [Marsh et al., 2014; Jensen et al., 2015]. Studies started to focus instead on long-term nutrient budgets and nutrient balancing using the conceptual approach of geometric frameworks [e.g., Felton et al., 2009; Rothman et al., 2011; Johnson et al., 2013; DeGabriel et al., 2014; Irwin et al., 2014], on new methods on how to measure protein that is actually available [DeGabriel et al., 2008], or on an understanding of other confounding variables [Wallis et al., 2012], Our results illustrate that primates select for high protein leaves especially in situations where the average protein content of leaves in a forest is low. No such correlation was found with respect to fiber concentrations. Thus, it appears that protein is limiting for folivorous primates under certain conditions, but clearly not in the majority of tropical forests studied. In contrast, we found no evidence of either an expected inverse relationship between protein and fiber concentrations in food or indeed any evidence that animals were selecting against fiber. We cannot judge whether there is a significant effect of methodology on this result but it is clear that fiber is analyzed inconsistently in primatological studies with little regard to the effects of ash, tannins or other interfering substances [Makkar & Singh 1995; Wallis et al., 2012]

Our comparative study also indicates a fundamental problem of field studies on food selection. Animals are most frequently studied where they occur in high densities. These are probably the best areas for survival and reproduction with high quality food availability. Under these conditions, it is probably hard, if not impossible, to identify factors that are actually limiting. Having enjoyed considerable time in forests with plentiful animals, it may be an unfortunate conclusion, but in order to find out what limits primates, researchers will likely need to turn their attention to regions where animals are naturally scarce (e.g. Stalenberg 2015).

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We confirm that the research adhered to the legal requirements of the country in which the research was conducted and that this research adhered to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates.

References

Altmann SA. 1991. Diets of yearling female primates (*Papio cynocephalus*) predict lifetime fitness. Proceedings of the National Academy of Sciences USA 88:420-423.

Altmann SM. 1998. Foraging for Survival. Chicago: The University of Chicago Press.

Arrigo-Nelson S. 2006. The impact of habitat disturbance on the feeding ecology of the Milne-Edwards' Sifaka (*Propithecus edwardsi*) in Ranomafana National Park, Madagascar. New York: Stony Brook.

Barton RA, Whiten A. 1994. Reducing complex diets to simple rules: food selection by olive baboons. Behavioral Ecology and Sociobiology 35:283-293.

Beeson M. 1989. Seasonal dietary stress in a forest monkey (*Cercopithecus mitis*). Oecologia (Berlin) 78:565-570.

Bradford M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye-binding. Analytical Biochemistry 72:248-254.

Calvert JJ. 1985. Food selection by western gorillas (*G.g.gorilla*) in relation to food chemistry. Oecologia (Berlin) 65:236-246.

Campbell JL, Eisemann JH, Glander KE, Crissey SD. 1999. Intake, digestibility, and passage of a commercially designed diet by two *Propithecus* species. American Journal of Primatology 48:237-246.

Campbell JL, Williams CV, Eisemann JH. 2004. Use of total dietary fiber across four lemur species (*Propithecus verreauxi coquereli, Hapalemur griseus griseus, Varecia variegata*, and *Eulemur fulvus*): Does fiber type affect digestive efficiency? American Journal of Primatology 64:323-335.

Chalise MK. 1995. Comparative study of feeding ecology and behavior of male and female langurs (*Presbytis entellus*) (PhD Thesis, Kathmandu: TU Nepal.

Chapman CA, Bonnell TR, Schoof VAM, Calme S. 2015. Competing pressures on populations: how disease may interact with food availability and stress to influence animal abundance. Philosophical Transactions of the Royal Society B. 370: DOI: 10.1098/rstb.2014.0112

Chapman CA, Chapman LJ, Bjorndal KA, Onderdonk DA. 2002. Application of protein-to fiber ratios to predict colobine abundance on different spatial scales. International Journal of Primatology 23:283-310.

Chapman CA, Chapman LJ, Naughton-Treves L, Lawes MJ, McDowell LR. 2004. Predicting folivorous primate abundance: validation of a nutritional model. American Journal of Primatology 65:55-69.

Chapman CA, Chapman LJ, Rode KD, Hauck EM, McDowell LR. 2003. Variation in nutritional value of primate foods: among trees, time periods and areas. International Journal of Primatology 24:317-333.

Charles-Dominique P, Hladik CM. 1971. Le Lepilemur du sud de Madagascar: écologie, alimentation et vie sociale. La Terre et la Vie 25:3-66.

Chivers DJ, Wood BA, Bilsborough A. 1984. Food acquisition and processing in primates. New York: Plenum Press.

Clauss M, Streich WJ, L. Nunn CL, Ortmann S, Hohmann G, Schwarm A, Hummel J. 2008. The influence of natural diet composition, food intake level, and body size on ingesta passage in primates. Comparative Biochemistry and Physiology, Part A 150:274-281.

Conklin-Brittain NL, Dierenfeld ES, Wrangham RW, Norconk M, Silver SC. 1999. Chemical protein analysis: A comparison of Kjeldahl crude protein and total ninhydrin protein from wild, tropical vegetation. Journal of Chemical Ecology 25:2601-2622.

Conklin-Brittain NL, Wrangham RW, Hunt KD. 1998. Dietary response of chimpanzees and cercopithecines to seasonal variation in fruit abundance. II. Macronutrients. International Journal of Primatology 19:971-998.

Cork SJ, Foley WJ. 1991. Digestive and metabolic strategies of arboreal mammalian folivores in relation to chemical defenses in temperate and tropical forests. In: Palo RT, Robbins CT, editors. Plant Defenses Against Mammalian Herbivory. Boca Raton, Florida: CRC Press. p 133-166.

Curtis DJ. 2004. Diet and nutrition in wild Mongoose Lemurs (*Eulemur mongoz*) and their implications for the evolution of female dominance and small group size in lemurs. American Journal of Physical Anthropology 124:234-247.

Dasilva GL. 1994. Diet of *Colobus polykomos* on Tiwai Island: selection of food in relation to its seasonal abundance and nutritional quality. International Journal of Primatology 15:655-680.

Davies AG, Bennet EL, Waterman PG. 1988. Food selection by two South-east Asian colobine monkeys (*Presbytis rubicunda* and *Presbytis melalophos*) in relation to plant chemistry. Biological Journal Linnean Society 34:33-56.

Davies AG, Oates JF. 1994. Colobine Monkeys: Their Ecology, Behaviour and Evolution. Cambridge: Cambridge University Press. 415 p.

Degabriel JL, Moore BD, Foley WJ, Johnson CN 2009. The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. *Ecology* 90**:** 711-719.

DeGabriel JL, Moore BD, Felton AM, Ganzhorn JU, Stolter C, Wallis IR, Johnson CN, Foley WJ. 2014. Translating nutritional ecology from the laboratory to the field: Milestones in linking plant chemistry to population regulation in mammalian browsers. Oikos 123:298-308.

DeGabriel JL, Wallis IR, Moore BD, Foley WJ. 2008. A simple, integrative assay to quantify nutritional quality of browses for herbivores. Oecologia (Berlin) 156:107-116.

Donati G, Bollen A, Borgognini-Tarli SM, Ganzhorn JU. 2007. Feeding over the 24-hour cycle: dietary flexibility of cathemeral collared lemurs (*Eulemur collaris*). Behavioral Ecology and Sociobiology 61:1237-1251.

Edwards MS, Ullrey DE. 1999a. Effect of dietary fiber concentration on apparent digestibility and digesta passage in non-human primates I: Ruffed lemurs (*Varecia variegata variegata* and *V. v. rubra*). Zoo Biology 18:529-536.

Edwards MS, Ullrey DE. 1999b. Effect of dietary fiber concentration on apparent digestibility and digesta passage in non-human primates II. Hindgut and foregut fermenting folivores. Zoo Biology 18:537-549.

Elias MF, Samonds KW. 1977. Protein and calorie malnutrition in infant *Cebu* monkeys - Growth and behavioral development during deprivation and rehabilitation. American Journal of Clinical Nutrition 30:355-366.

Eppley TM, Verjans E, Donati G. 2011. Coping with low-quality diets: a first account of the feeding ecology of the southern gentle lemur, *Hapalemur meridionalis*, in the Mandena littoral forest, southeast Madagascar. Primates 52:7-13.

Estrada A, Coates-Estrada R. 1986. Use of leaf resources by howling monkeys (*Alouatta palliata*) and leaf-cutting ants (*Atta cephalotes*) in the tropical rain forest of Los Tuxtlas, Mexico. American Journal of Primatology 10:51-66.

Fashing PJ, Dierenfeld ES, Mowry CB. 2007. Influence of plant and soil chemistry on food selection, ranging patterns, and biomass of *Colobus guereza* in Kakamega Forest, Kenya. International Journal of Primatology 28:673-703.

Felton AM, Felton A, Raubenheimer D, Simpson SJ, Foley WJ, Wood JT, Lindenmayer DB. 2009. Protein content of diets dictates the daily energy intake of a free-ranging primate. Behavioral Ecology 20:685-690.

Fleagle JG, Samonds KW, Hegsted DM. 1975. Physical growth of *Cebus* monkeys, *Cebus albifrons*, during protein or calorie deficiency. American Journal of Clinical Nutrition 28(3):246-253.

Ganzhorn JU. 1988. Food partitioning among Malagasy primates. Oecologia (Berlin) 75:436-450.

Ganzhorn JU. 1992. Leaf chemistry and the biomass of folivorous primates in tropical forests. Oecologia (Berlin) 91:540-547.

Ganzhorn JU. 2002. Distribution of a folivorous lemur in relation to seasonally varying food resources: integrating quantitative and qualitative aspects of food characteristics. Oecologia (Berlin) 131:427-435.

Ganzhorn JU, Abraham J-P. 1991. Possible role of plantations for lemur conservation in Madagascar: food for folivorous species. Folia Primatologica 56:171-176.

Ganzhorn JU, Arrigo-Nelson S, Boinski S, Bollen A, Carrai V, Derby A, Donati G, Koenig A, Kowalewski M, Lahann P et al. 2009. Possible fruit protein effects on primate communities in Madagascar and the Neotropics. PLoS ONE 4(12).

Ganzhorn JU, Pietsch T, Fietz J, Gross S, Schmid J, Steiner N. 2004. Selection of food and ranging behaviour in a sexually monomorphic folivorous lemur: *Lepilemur ruficaudatus*. Journal of Zoology, London 263:393-399.

Ganzhorn JU, Wright PC. 1994. Temporal pattern in primate leaf eating: the possible role of leaf chemistry. Folia Primatologica 63:203-208.

Garber PA, Righini N, Kowalewski MM. 2015. Evidence of alternative dietary syndromes and nutritional goals in the genus *Alouatta*. In: Kowalewski MM, editor. Howler Monkeys. New York: Springer. p 85-109.

Gaulin SJ, Gaulin CK. 1982. Behavioral ecology of *Alouatta seniculus* in Andean cloud forest. International Journal of Primatology 3:1-32.

Glander KE. 1981. Feeding patterns in Mantled howling monkeys. In: Kamil AC, Sargent TD, editors. Foraging behavior: ecological, ethological, and psychological approaches. New York: Garland Press. p 231-257.

Godfrey LR, Samonds KE, Jungers WL, Sutherland MR, Irwin MT. 2004. Ontogenetic correlates of diet in Malagasy lemurs. American Journal of Physical Anthropology 123:250-276.

Gogarten JF, Guzman M, Chapman CA, Jacob AL, Omeja PA, Rothman JM. 2012. What is the predictive power of the colobine protein-to-fiber model and its conservation value. Tropical Conservation Science 5:381-393.

Hemingway CA, Bynum N. 2005. The influence of seasonality on primate diet and ranging. In: Brockman DK, van Schaik CP, editors. Seasonality in Primates: Studies of Living and Extinct Human and Non-human Primates. Cambridge: Cambridge University Press. p 57-104.

Hladik CM. 1977. A comparative study of the feeding strategies of two sympatric species of leaf monkeys: *Presbytis senex* and *P.entellus*. In: Clutton-Brock TH, editor. Primate ecology: studies of feeding and ranging behaviour in lemurs monkeys and apes. London New York: Academic Press. p 324-353.

Hladik CM. 1978. Adaptive strategies of primates in relation to leaf-eating. In: Montgomery GG, editor. The Ecology of Arboreal Folivores. Washington D.C.: Smithsonian Institution Press. p 373-395.

Huang ZP, Huo S, Yang SG, Cui LW, Xiao W. 2010. Leaf choice in black-and-white snub-nosed monkeys *Rhinopithecus bieti* is related to the physical and chemical properties of leaves. Current Zoology 56:643-649.

Hughes RN. 1993. Diet Selection. Oxford: Blackwell Scientific.

Irwin MT, Raharison J-L, Raubenheimer D, Chapman CA, Rothman JM. 2014. Nutritional correlates of the “lean season”: effects of seasonality and frugivory on the nutritional ecology of diademed sifakas. American Journal of Physical Anthropology 153:78-91.

Jensen LM, Wallis IR, Foley WJ. 2015. The relative concentrations of nutrients and toxins dictate feeding by a vertebrate browser, the greater glider *Petauroides volans* PLoS One. 10:e0121584

Johnson CA, Raubenheimer D, Rothman JM, Clarke D, Swedell L. 2013. 30 Days in the life: Daily nutrient balancing in a wild chacma baboon. PLoS One 8:e70383

Kappeler PM, Heymann EW. 1996. Non-convergence in the evolution of primate life history and socio-ecology. Biological Journal Linnean Society 59:297-326.

Kar-Gupta K, Kumar A. 1994. Leaf chemistry and food selection by common langurs (*Presbytis entellus*) in Rajaji National Park, Uttar Pradesh, India. International Journal of Primatology 15:75-93.

Kay RF. 1984. On the use of anatomical features to infer foraging behavior in extinct primates. In: Rodman RS, Cant JGH, editors. Adaptation for Foraging in Non-human Primates. New York: Columbia University Press. p 21-53.

Klaassen M, Nolet BA. 2008. Stoichiometry of endothermy: shifting the quest from nitrogen to carbon. Ecology Letters 11:785-792.

Koenig A, Beise J, Chalise MK, Ganzhorn JU. 1998. When females should contest for food - testing hypotheses about resource density, distribution, and quality with Hanuman langurs (*Presbytis entellus*). Behavioral Ecology and Sociobiology 42:225-237.

Kool KM. 1992. Food selection by the silver leaf monkey, *Trachypithecus auratus sondaicus*, in relation to plant chemistry. Oecologia (Berlin) 90:527-533.

Lambert JE. 1998. Primate digestion: interactions among anatomy, physiology, and feeding ecology. Evolutionary Anthropology 7:8-20.

Langer P, Chivers DJ. 1994. Classification of foods for comparative analysis of the gastro-intestinal tract. In: Chivers DJ, Langer P, editors. The Digestive System in Mammals: Food, Form and Function. Cambridge: Cambridge University Press. p 74-86.

Liu XC, Stanford CB, Yang JY, Yao H, Li YM. 2013. Foods eaten by the Sichuan Snub-Nosed Monkey (*Rhinopithecus roxellana*) in Shennongjia National Nature Reserve, China, in relation to nutritional chemistry. American Journal of Primatology 75:860-871.

Makkar HPS, Singh B. 1995. Determination of condensed tannins in complexes with fiber and proteins. Journal of the Science of Food and Agriculture 69:129-132.

Marsh KJ, Moore B, Wallis I, Foley W (2014) Feeding rates of a mammalian browser confirm the predictions of a “foodscape” model of its habitat. *Oecologia (Berlin)* 174: 873-882 .

Matsuda I, Tuuga A, Bernard H, Sugau J, Hanya G. 2013. Leaf selection by two Bornean colobine monkeys in relation to plant chemistry and abundance. Scientific Reports 3: 1873.

McKey DB, Gartlan JS, Waterman PG, Choo GM. 1981. Food selection by black colobus monkeys (*Colobus satanas*) in relation to plant chemistry. Biological Journal Linnean Society 16:115-146.

McKey DB, Waterman PG, Gartlan JS, Struhsaker TT. 1978. Phenolic content of vegetation in two African rain forests: ecological implications. Science 202:61-64.

Meyers DM. 1993. The effects of resource seasonality on behavior and reproduction in the Golden-Crowned Sifaka (*Propithecus tattersalli*, Simons, 1988) in three Malagasy forests (Dissertation). Durham: Duke University.

Milton K. 1979. Factors influencing leaf choice by howler monkeys: A test of some hypotheses of food selection by generalist herbivores. American Naturalist 114:362-378.

Milton K. 1998. Physiological ecology of howlers (*Alouatta*): energetic and digestive considerations and comparisons with the Colobinae. International Journal of Primatology 19:513-548.

Milton K. 1999. Nutritional characteristics of wild primate foods: do the diets of our closest living relatives have lessons for us? Nutrition Abstracts and Reviews, Series B 15:488-498.

Milton K, Dintzis FR. 1981. Nitrogen-to-protein conversion factors for tropical plant samples. Biotropica 13:177-181.

Mittermeier RA, Louis Jr. EE, Richardson M, Schwitzer C, Langrand O, Rylands AB, Hawkins F, Rajaobelina S, Ratsimbazafy J, Rasoloarison MR et al. 2010. Lemurs of Madagascar. Bogota: Conservation International.

Moore BD, Foley WJ. 2005. Tree use by koalas in a chemically complex landscape. Nature 435: 488-490.

Mowry CB, Decker BS, Shure DJ. 1996. The role of phytochemistry in dietary choices of Tana River Red Colobus Monkeys (*Procolobus badius rufomitratus*). International Journal of Primatology 17:63-84.

Mutschler T. 1999. Folivory in a small-bodied lemur: the nutrition of the Alaotran gentle lemur (*Hapalemur griseus alaotrensis*). In: Rakotosamimanana B, Rasamimanana H, Ganzhorn JU, Goodman SM, editors. New Directions in Lemur Studies. New York: Kluwer Academic / Plenum Press. p 221-239.

Norscia I, Ramanamanjato J-B, Ganzhorn JU. 2012. Feeding patterns and dietary profile of the nocturnal southern woolly lemur, *Avahi meridionalis*, in south-east Madagascar. International Journal of Primatology 33:150-167.

NRC. 2003. Nutrient Requirements of Non-human Primates. Washington, D.C.: National Research Council. The National Academies Press. 286 p.

Oates JF, Waterman PG, Choo GM. 1980. Food selection by the South Indian leaf monkey, *Presbytis johnii*, in relation to plant chemistry. Oecologia (Berlin) 45:45-56.

Oates JF, Whitesides GH, Davies AG, Waterman PG, Green SM, Dasilva GL, Mole S. 1990. Determinants of variation in tropical forest primate biomass: new evidence from West Africa. Ecology 71:328-343.

Occhibove F, Ferro C, Liponi GB, Borgognini-Tarli SM, Ganzhorn JU, Donati G. 2015. Living in islands of forests: nutritional ecology of the howler monkey (*Alouatta palliata*) at La Suerte Biological Field Station, North-eastern Costa Rica. In: Huettmann F, editor. Central American Biodiversity. New York, Springer.

Oftedal OT. 1991. The nutritional consequences of foraging in primates: the relationship of nutrient intake to nutrient requirements. Philosophical Transaction of the Royal Society London B 334:161-170.

Ortmann S, Bradley BJ, Stolter C, Ganzhorn JU. 2006. Estimating the quality and composition of wild animal diets - a critical survey of methods. In: Hohmann G, Robbins MM, Boesch C, editors. Feeding ecology in Apes and other Primates

Ecological, Physical and Behavioural Aspects. Cambridge: Cambridge University Press. p 397-420.

Patel ER. 2012. Acoustic and olfactory communication in Eastern sifakas (*Propithecus* sp.) and rhesus macaques (*Macaca mulata*). Ann Arbor: Cornell University. 160 p.

Peres CA. 1997. Effects of habitat quality and hunting pressure on arboreal folivore densities in Neotropical forests: a case study of Howler Monkeys (*Alouatta* spp.). Folia Primatologica 68:199-222.

Pichon C. 2012. Contraintes écologiques et sociales sur l'acquisition alimentaire du propithèque couronné (*Propithecus coronatus*) dans une forêt sèche semi-caducifoliée du nord-ouest de Madagascar. Paris. 167 p.

Robbins CT. 1983. Wildlife Feeding and Nutrition. New York: Academic Press.

Rothman JM, PJ, Pell AN (2006) Decaying wood is a sodium source for mountain gorillas. Biology Letters 2:321–324

Rothman JM, Chapman CA, Pell AN. 2008. Fiber-bound nitrogen in gorilla diets: Implications for estimating dietary protein intake of primates. American Journal of Primatology 70:690-694.

Rothman JM, Chapman CA, Van Soest PJ. 2012. Methods in primate nutritional ecology: A user's guide. International Journal of Primatology 33:542-566.

Rothman JM, Raubenheimer D, Bryer MAH, Takahashi M, Gilbert CC. 2014. Nutritional contributions of insects to primate diets: Implications for primate evolution. Journal of Human Evolution 71:59-69.

Rothman JM, Raubenheimer D, Chapman CA. 2011. Nutritional geometry: gorillas prioritize non-protein energy while consuming surplus protein. Biology Letters 7:847-849.

Schwitzer C, Polowinsky SY, Solman C. (2009). Fruits as foods. Common misconceptions about frugivory. In: *Zoo Animal Nutrition IV*. Clauss M, Fidgett A, Janssens G, Hatt J-M, Huisman T, Hummel J, Nijboer J, Plowman A. (eds). Fürth: Filander Verlag, 131–168.

Silver SC, Ostro LET, Yeager CP, Dierenfeld ES. 2000. Phytochemical and mineral components of food consumed by black howler monkeys (*Alouatta pigra*) at two sites in Belize. Zoo Biology 19:95-109.

Simmen B, Sabatier D. 1996. Diets of some French Guianan primates: food composition and food choices. International Journal of Primatology 17:661-693.

Simmen B, Tamaud L, Hladik A. 2012. Leaf nutritional quality as a predictor of primate biomass: further evidence of an ecological anomaly within prosimian communities in Madagascar. Journal of Tropical Ecology 28:141-151.

Simmen B, Tarnaud L, Marez A, Hladik A. 2014. Leaf chemistry as a predictor of primate biomass and the mediating role of food selection: A case study in a folivorous lemur (*Propithecus verreauxi*). American Journal of Primatology 76:563-575.

Smith RJ, Jungers WL. 1997. Body mass in comparative primatology. Journal of Human Evolution 32:523-559.

Stalenberg E, Wallis IR, Cunningham RB, Allen C, Foley WJ, 2014. Nutritional correlates of koala persistence in a low-density population. PLoS One 10: e113930.

Terborgh J. 1992. Diversity and the Tropical Rain Forest. New York: Scientific American Library.

Terboven J. 2014. Evaluierung verschiedener Methoden zur Analyse von Stickstoff in Pflanzen. Hamburg: Hamburg University. 26 p.

Van Soest PJ. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Ithaca, Cornell University Press.

Wallis IR, Edwards MJ, Windley H, Krockenberger AK, Felton A, Quenzer M, Ganzhorn JU, Foley WJ. 2012. Food for folivores: nutritional explanations linking diets to population density. Oecologia (Berlin) 169:281-291.

Wasserman MD, Chapman CA. 2003. Determinants of colobus monkey abundance: the importance of food energy, protein, and fibre content. Journal of Animal Ecology 72:650-659.

Waterman PG, Ross JAM, Bennett EL, Davies AG. 1988. A comparison of the floristics and leaf chemistry of the tree flora in two Malaysian rain forests and the influence of leaf chemistry on populations of colobine monkeys in the Old World. Biological Journal Linnean Society 34:1-32.

White TCR. 1993. The Inadequate Environment: Nitrogen and the Abundance of Animals. Berlin: Springer-Verlag.

Yeager CP, Silver SC, Dierenfeld ES. 1997. Mineral and phytochemical influences on foliage selection by proboscis monkey (*Nasalis larvatus*). American Journal of Primatology 41:117-128.

Tables

TABLE I. Nitrogen, soluble protein and acid detergent fiber (ADF) concentrations in leaves eaten by folivorous primates and in “representative samples” of leaves (RS) in a given forest. “P” indicates significance of selection for high protein or low ADF concentrations.

TABLE II. Comparison of the concentration of chemical components in leaves based on measures of several individuals of the same plant species and on the mean per plant species. Values are means ± standard deviations; N = sample size. Data on *Propithecus edwardsi* from Arrigo-Nelson (2006; unpubl.) based on mature leaves; data on *P. candidus* from Patel [2012; unpubl.], restricted to leaves of species identified unambiguously.

Figure captions

Fig. 1. Significance values for the selection of leaves in relation to the average concentrations of nitrogen (upper graph), soluble protein (middle graph) and ADF (lower graph) in leaves available in different forests. Dots are lemurs, squares are Old World Monkeys (Colobinae) and triangles are New World monkeys (*Alouatta* spp.).