

## RESEARCH ARTICLE

### The Importance of Protein in Leaf Selection of Folivorous Primates

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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 resolved if folivores only need to select for high protein leaves when average protein concentration in the habitat is low. To test the prediction, we applied meta-analyses to analyze published and unpublished results of food selection for protein and fiber concentrations from 24 studies (some with multiple species) of folivorous primates. To counter potential methodological flaws, we differentiated between methods analyzing total nitrogen and soluble protein concentrations. We used a meta-analysis to test for the effect of protein on food selection by primates and found a significant effect of soluble protein concentrations, but a non-significant effect for total nitrogen. Furthermore, selection for soluble protein was reinforced in forests where protein was less available. Selection for low fiber content was significant but unrelated to the fiber concentrations in representative leaf samples of a given forest. There was no

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relationship (either negative or positive) between the concentration of protein and fiber in the food or in representative samples of leaves. Overall our study suggests that protein selection is influenced by the protein availability in the environment, explaining the sometimes contradictory results in previous studies on protein selection. *Am. J. Primatol.* © 2016 Wiley Periodicals, Inc.

**Key words:** food chemistry; food selection; leaf-eating; protein availability; meta-analysis

## 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, habitat utilization, and community composition; e.g., Mattson, 1980; Moore & Foley, 2005; 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 & Nolet [2008]; Ganzhorn et al. [2009]; Schwitzer et al. [2009]). Therefore, smaller-bodied species feed on insects to meet 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 their protein needs. 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 et al. [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; Fashing et al., 2007; Wasserman & Chapman, 2003] 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 [Chapman et al., 2004; Gogarten et al., 2012] but it seems to retain some predictive capacity.

Restricting the considerations to protein alone, several studies of folivores have shown that protein can be limiting with lasting effects on development and lifetime fitness [e.g., Altmann, 1991, 1998; DeGabriel et al., 2009; Elias & Samonds, 1977; Fleagle et al., 1975]. 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; Koenig et al., 1998; Mowry et al., 1996; Waterman et al., 1988; 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; Occhibove et al., 2015; *Old World colobines*: Chapman et al., 2002; Dasilva, 1994; Kool, 1992; McKey et al., 1981; Oates et al., 1980; Waterman et al., 1988; *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 differ widely in statistical power of their conclusions and have applied different methods to measure “protein.” The statistical aspect can be reconciled by meta-analyses [e.g., Starrs et al., 2014]. The chemical aspects are more complex. 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., Curtis, 2004; Glander, 1981; Mutschler, 1999; Simmen & Sabatier, 1996] or soluble protein [e.g., Conklin-Brittain et al., 1999; Ganzhorn, 1988; Koenig et al., 1998; 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 [DeGabriel et al., 2014; NRC, 2003; Robbins, 1983; Wallis et al., 2012].

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., Conklin-Brittain et al., 1998; Ganzhorn et al., 2009; Hladik, 1977; Oftedal, 1991]. 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 reduced by other components such as fiber or tannins [Mowry et al., 1996; Simmen et al., 2014; Yeager et al., 1997].

Deviations from selecting high protein leaves may also be caused by species-specific adaptation of gut morphology [Campbell et al., 1999, 2004; Chivers et al., 1984; Cork & Foley 1991; Edwards & Ullrey, 1999a,b; Godfrey et al., 2004; Hughes, 1993; Lambert, 1998; Langer & Chivers, 1994; Milton, 1998, 1999; Van Soest, 1994]. Yet, studies on possible effect of body mass and gut physiology remain inconclusive, supporting the conclusion that body mass is not a useful surrogate to understand primate feeding and digestion, including protein requirements [Lambert, 1998]. 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 (Colobinae) 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 (Indridae). With respect to protein digestion, there is no evidence that there is a difference between the primate digestion types [Schwarm et al., 2009]. Also,

the typical effect of sorting or digesta washing evident in other mammals is not visible in the primate hindgut-foregut dichotomy [Müller et al., 2011], and there is no indication for a functional sorting mechanism in the colobine primate foregut [Matsuda et al., 2015].

In order to investigate protein and fiber selection in folivorous primates, we consider the availability of protein and fiber in the environment and test the hypothesis that these components are limiting primate food selection 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 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 from folivorous primates from Madagascar and the New World (Table I). 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 [Garber et al., 2015; Hemingway & Bynum, 2005]. 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]. We also included body mass in the database provided in Table I to facilitate further comparisons. Data for primate body mass were taken from Smith & Jungers [1997] and Mittermeier et al. [2010] and averaged between sexes.

**TABLE I. Nitrogen, Soluble Protein, and Acid Detergent Fiber (ADF) Concentrations in Leaves (in %) Eaten by Folivorous Primates and in “Representative Samples” of Leaves (RS) in a Given Forest**

Species	Country	Site; Plant part	Body mass (kg)	Nitrogen			Soluble Protein			ADF			References
				Food	Non-food	RS	Food	Non-food	RS	Food	Non-food	RS	
<b>Old World: Africa</b>													
<i>Colobus polykomos</i>	Sierra Leone	Tiwai; ML	9	2.40 ± 0.78 (7)	2.53 ± 1.28 (9)	2.18	0.81	40.30 ± 7.07 (7)	43.09 ± 11.89 (9)	41.6	0.59	Dasilva, 1994; Oates et al., 1990	
<i>Procolobus badius</i>	Uganda	Kibale; ML	8.8	2.74 ± 0.64 (40)	2.79 ± 0.67 (40)	2.64	0.73	34.31 ± 9.14 (40)	35.80 ± 7.30 (14)	34.6	0.59	Chapman et al., 2002; Oates et al., 1990	
<b>Old World: Asia</b>													
<i>Nasalis larvatus</i>	Indonesia	Tanjung Puting National Park	15.1	1.58 ± 0.54 (20)	1.36 ± 0.27 (36)	1.25	0.05	30.5 ± 12.1 (20)	41.0 ± 9.4 (36)	34.7	0.001	Yeager et al., 1997	
<i>Presbytis melalophos</i>	Malaysia	Kuala Lumpur	6.5	2.14 ± 0.95 (5)	1.53 ± 0.59 (13)	2.02	0.11	39.76 ± 4.84 (5)	41.31 ± 12.71 (10)	51.1	0.8	Davies et al., 1988; Oates et al., 1990	
<i>Presbytis rubicunds</i>	Sabah	Sepitok	6.2	2.32 ± 0.12 (3)	1.80 ± 0.68 (9)	1.7	0.23	33.23 ± 4.47 (4)	52.29 ± 18.62 (9)	63.4	0.07	Davies et al., 1988; Oates et al., 1990	
<i>Rhinopithecus roxellana</i>	China	Shemongjia; ML	15	2.05 ± 0.73 (6)	1.82 ± 1.32 (43)	2.68	0.68	12.35 ± 2.85 (5)	16.49* ± 5.65 (27)		0.12	Liu et al., 2013	
<i>Rhinopithecus roxellana</i>	China	Shemongjia; YL	15	2.12 ± 0.77 (9)	2.68 ± 0.86 (10)		0.16	11.29 ± 4.81 (8)	13.28* ± 2.60 (9)		0.3	Liu et al., 2013	
<i>Rhinopithecus bieti</i>	China	Mt Longma	12.5	1.76 ± 0.29 (9)	1.55 ± 0.19 (5)		0.16	21.30 ± 4.51 (9)	33.70 ± 4.71 (5)		0.001	Huang et al., 2010	
<i>Trachypithecus auratus</i>	Java	Pangandaran Nature Reserve	7.1	2.32 ± 0.96 (5)	2.08 ± 0.64 (21)	2.08	0.5	30 ± 7 (5)	37 ± 9 (21)	37	0.11	Kool, 1992	
<b>New World</b>													
<i>Alouatta palliata</i>	Mexico	Los Tuxtlas; ML	5	2.06 (30)	2.29 (20)	2.16	0.22	26.6 (30)	27.1 (20)	23.6	0.4	Estrada & Coates-Estrada, 1986	
<i>Alouatta palliata</i>	Mexico	Los Tuxtlas; YL	5	2.74 (30)	2.94 (20)	2.96	0.25	21.4 (30)	26.4 (20)	25	0.01	Estrada & Coates-Estrada, 1986	
<i>Alouatta pigra</i>	Belize	Community Baboon Sanctuary and Cockscomb Basin Wildlife Sanctuary; ML + YL	8.9	3.51 ± 1.10 (70)	2.22 ± 0.89 (16)		0.001					Silver et al., 2000	
<i>Alouatta seniculus</i>	Ecuador	Yasuni National Park	6	3.11 ± 0.87 (31)	2.41 ± 0.70 (13)		0.01	31.58 ± 9.41 (29)	31.35 ± 13.88 (13)		0.95	Derby and Ganzhorn, unpubl.	
<b>Madagascar</b>													
<i>Avahi laniger</i>	Madagascar	Perinet	1.1	9.03 ± 3.35 (34)	4.70 ± 3.11 (31)			46.26 ± 8.23 (18)	47.91 ± 10.34 (13)	47.91 ± 10.34	0.64	Ganzhorn, 1988, 1992	
<i>Eulemur fulvus</i>	Madagascar	Perinet	1.9	8.46 ± 4.38 (25)	4.70 ± 3.11 (31)			49.00 ± 3.73 (3)	47.91 ± 10.34 (13)	47.91 ± 10.34	0.77	Ganzhorn, 1988, unpubl.	
<i>Indri indri</i>	Madagascar	Perinet	6.34	8.24 ± 3.15 (34)	4.70 ± 3.11 (31)			47.23 ± 11.11 (15)	47.91 ± 10.34 (13)	47.91 ± 10.34	0.87	Ganzhorn, 1988, 1992	
<i>Lepilemur microdon</i>	Madagascar	Perinet	0.97	5.91 ± 4.57 (25)	4.70 ± 3.11 (31)			45.20 ± 9.61 (13)	47.91 ± 10.34 (13)	47.91 ± 10.34	0.49	Ganzhorn, 1988, 1992	

TABLE 1. Continued

Species	Country	Site; Plant part	Body mass (kg)	Nitrogen			Soluble Protein			ADF			References
				Food	Non-food	RS	P	Food	Non-food	RS	P	Food	
<i>Azchi meridionalis</i>	Madagascar	Sainte-Luce; ML	1.1	0.94 ± 0.29 (37)	1.06 ± 0.36 (20)	0.14	4.58 ± 2.24 (37)	3.17 ± 1.73 (20)	0.02	27.36 ± 6.99 (37)	26.75 ± 7.70 (20)	0.74	Norscia et al., 2012
<i>Azchi occidentalis</i>	Madagascar	Ampijoroa	0.8	8.85 ± 2.96 (10)			8.85 ± 2.96 (10)		0.18	45.09 ± 15.26 (9)		0.64	Ganzhorn, 1988, 1992
<i>Lepilemur edwardsi</i>	Madagascar	Ampijoroa	0.9	4.92 ± 3.02 (13)			4.92 ± 3.02 (13)		0.08	41.16 ± 16.57 (14)		0.91	Ganzhorn, 1988, 1992
<i>Propithecus coquereli</i>	Madagascar	Ampijoroa	4	9.77 ± 3.99 (12)			9.77 ± 3.99 (12)		0.05	45.06 ± 13.99 (9)		0.64	Ganzhorn, 1992, unpubl.
<i>Lepilemur ankaranaensis</i>	Madagascar	Ankarana; wet forest	0.75	11.04 ± 4.50 (15)			11.04 ± 4.50 (15)		0.001	28.46 ± 9.17 (13)		0.002	Ganzhorn, 1992, unpubl.
<i>Lepilemur leucopus</i>	Madagascar	Berenty; spiny forest	0.58	2.69 ± 1.02 (156)		1.39 ± 0.18 (340)			0.001	18.20 ± 6.10 (156)		0.001	Dröscher et al., in press; Dröscher & Stalenberg, unpubl.
<i>Lepilemur ruficaudatus</i>	Madagascar	Kirindy N5; dry season	0.78	6.35 ± 6.34 (73)			6.35 ± 6.34 (73)		0.16	27.23 ± 8.95 (75)		0.001	Ganzhorn, 2002
<i>Lepilemur ruficaudatus</i>	Madagascar	Kirindy N5; wet season	0.78	8.66 ± 3.95 (47)			8.66 ± 3.95 (47)		0.001	25.67 ± 10.21 (43)		0.001	Ganzhorn, 2002
<i>Lepilemur ruficaudatus</i>	Madagascar	Kirindy CS7	0.78	9.69 ± 5.05 (9)			9.69 ± 5.05 (9)		0.1	26.13 ± 13.94 (9)		0.19	Ganzhorn, 1992, unpubl.
<i>Propithecus coquereli</i>	Madagascar	Ampijoroa; Plantation	4	6.77 ± 4.10 (8)			6.77 ± 4.10 (8)		0.5	21.05 ± 8.28 (7)		0.26	Ganzhorn & Abraham, 1991
<i>Propithecus edwardsi</i>	Madagascar	Ranomafana	5.8	2.17 ± 0.91 (14)		1.97 ± 1.10 (14)	6.01 ± 2.76 (14)		0.76	30.86 ± 6.96 (14)		0.001	Arrigo-Nelson, Daniels & Wright unpubl.; Ganzhorn 1992
<i>Propithecus tattersalli</i>	Madagascar	Daraina	3.5	7.63 ± 3.75 (16)			7.63 ± 3.75 (16)		0.07	53.79 ± 10.13 (16)		0.16	Meyers, 1993
<i>Propithecus verreauxi</i>	Madagascar	Kirindy CS7	3.1	1.77 ± 0.62 (63)		2.55 ± 1.21 (23)	6.91 ± 3.15 (68)		0.03	25.98 ± 11.64 (54)		0.001	Carral, unpubl.; Ganzhorn, 1992
<i>Propithecus verreauxi</i>	Madagascar	Kirindy CN5	3.1	10.81 ± 3.94 (26)			10.81 ± 3.94 (26)		0.001	29.33 ± 13.26 (24)		0.03	Ganzhorn, 1992, unpubl.
<i>Eulemur rufifrons</i>	Madagascar	Kirindy CN5	2	7.93 ± 3.09 (3)			7.93 ± 3.09 (3)		0.63	32.74 ± 32.74 (3)		0.57	Ganzhorn, unpubl.
<i>Propithecus verreauxi</i>	Madagascar	Berenty Gallery forest	3.25	3.41 ± 1.37 (18)		3.09 ± 1.32 (17)	27.37 ± 9.48 (18)		0.48	30.24 ± 9.74 (17)		0.38	Simmen et al., 2014

\*P\* indicates significance of selection for or against nitrogen, soluble protein, and ADF concentrations. ML, mature leaves; YL, young leaves.

## 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 total 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 ADF. Data presented as “crude protein” (i.e., total nitrogen multiplied by 6.25) were 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 details and reviews see [Foley et al., 1998; Ortmann et al., 2006; Rothman et al., 2012; Stolter et al., 2006]). 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:  $y = 0.94x$ ;  $R^2 = 0.99$ ;  $n = 90$ ; [Terboven, 2014]).

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]. Yet, to date, too few data exist for available protein to allow for comparative analyses.

In primate studies, fiber concentrations are most commonly reported as 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.

## 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 leaf quality available in that forest [e.g., Chapman et al., 2002, 2004; Ganzhorn, 1992; Oates et al., 1990; Simmen et al., 2014; Wasserman & Chapman, 2003]. The representative samples for *Propithecus edwardsi* in Ranomafana (Madagascar) were based on 14 tree species sampled at random [Wright & Daniels, unpubl.]. For *Alouatta palliata* in Los Tuxtlas (Mexico) leaves not consumed by ants (*Atta*) were used as a representative sample [Estrada & Coates-Estrada, 1986].

Some studies collected separate representative samples for young and mature leaves [Estrada & Coates-Estrada, 1986; Liu et al., 2013] or separate samples for the wet and the dry season [Ganzhorn, 2002]. These samples were considered as independent data points since the concentrations of chemical components vary significantly between sites and seasons, 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

Statistical analyses of selection criteria were restricted to photosynthetic parts (leaves, sometimes differentiated in different parts of leaves). If possible, analyses of selection were 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.

Selection criteria were based on comparisons of leaves that were consumed with representative samples from the forest, or leaves consumed were compared with those that were not eaten.  $P$  values listed in Table I are based on  $t$ - tests.

## 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 [Chapman et al., 2003; Ganzhorn & Wright 1994], the variation between weighted and unweighted samples seems to average out in large samples (Table II). Statistical tests were made with SPSS 21.0.

Meta-analysis is a powerful tool to explore the significance and consistency of findings across multiple, independent studies [Borenstein et al., 2009; Starrs et al., 2014]. Effect size meta-analysis was conducted on data extracted from studies that explicitly examined total nitrogen, soluble protein and/or acid detergent fiber content. Data collection was restricted to those studies that provided means, standard deviations and sample sizes of a representative sample of eaten foods, and a “representative sample” of foliage roughly matching that selected by the primate. Where a *t*-statistic, *P*-value and sample size was presented, this was included. Data reporting correlations between nutritional quality and rank order of preference were not included in this analysis, as these data are not directly comparable in the meta-analyses [e.g., Oates et al., 1980; McKey et al., 1981].

Effect sizes (Hedges’ *g*) and variance of Hedges’ *g* were calculated for each independent comparison from all studies. Where a representative sample was shared between multiple comparisons, a single composite effect was calculated, as these cannot be considered totally independent. In addition, the mean value of the representative sample was included to allow for examination between the strength of effect size, and nutritional quality of the habitat examined.

Six separate random effects meta-analyses with inverse-variance weights were performed each on the nitrogen, soluble protein and acid detergent fiber data sets. Firstly, the meta-analytic mean and variance was calculated to determine if there was a

significant effect. Examination for between-study heterogeneity was performed using *Q*-tests. An additional meta-analysis was then conducted on each dataset to determine if the representative sample was a significant moderator variable. Tests for significant residual heterogeneity was undertaken using *QE* tests to determine if additional moderator variables may be required to further explain between-study variation in effects. Sensitivity of analyses was conducted using leave-one-out analyses to explore whether inclusion of additional future studies may alter the conclusions drawn from these analyses. Finally, forest plots were produced to visualize the results. Effect sizes were calculated using the R package “compute.es” (version 0.2.1, Del Re, 2012), and meta-analyses conducted using the package “metafor” [version 1.7–0, Viechtbauer, 2010].

### Ethical Statement

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.

## RESULTS

### Correlations Between Chemical Components

Nitrogen and soluble protein concentrations could not be correlated due to the small number of studies where both components have been measured (Table I). Both of these protein measures were uncorrelated with ADF concentrations in food items (Pearson correlations based on means given in Table I: Nitrogen-ADF:  $r = 0.14$ ,  $N = 16$ ,  $P = 0.595$ ; soluble protein—ADF:  $r = 0.08$ ,  $N = 17$ ,  $P = 0.76$ ) and both components were also uncorrelated with ADF in the representative samples of leaves (nitrogen-ADF:

**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**

	Nitrogen	Soluble protein	ADF
<i>Propithecus edwardsi</i>			
Several measures per plant species	2.16 ± 0.82 N = 100	5.53 ± 2.60 N = 100	26.57 ± 6.95 N = 100
Mean per plant species	2.17 ± 0.91 N = 14	6.01 ± 2.76 N = 14	30.86 ± 6.96 N = 14
<i>Propithecus candidus</i>			
Several measures per plant species	1.38 ± 0.49 N = 309	6.38 ± 3.00 N = 310	33.76 ± 9.87 N = 303
Mean per plant species	1.31 ± 0.42 N = 62	6.27 ± 2.80 N = 62	34.04 ± 8.42 N = 61

Values are means ± standard deviations; N = sample size. Data on *Propithecus edwardsi* from Arrigo-Nelson [2006] based on mature leaves; data on *P. candidus* from Patel [2012], restricted to leaves of species identified unambiguously.

$r = -0.41$ ,  $N = 12$ ,  $P = 0.181$ ; soluble protein—ADF:  $r = -0.12$ ,  $N = 11$ ,  $P = 0.73$ ; Table I).

## Meta-Analyses of Food Selection

### Nitrogen

A meta-analysis of 18 effect sizes revealed a positive but non-significant effect of selection by primates for foliage with higher than average nitrogen content (mean effect size,  $g = 0.34$ ,  $n = 18$ , 95% confidence interval (CI):  $-0.05$  to  $0.73$ ,  $P = 0.09$ ) (Table I, Fig. 1a). Furthermore, there was significant between-study heterogeneity in effect sizes ( $I^2 = 87.3\%$ ) ( $Q_{17} = 326.3$ ,  $P < 0.0001$ ). Inclusion of the nitrogen concentration of a representative sample as a moderator variable was marginally nonsignificant (coefficient =  $-0.60$ ,  $n = 18$ , 95%CI:  $-1.21$  to  $0.00$ ,  $P = 0.051$ ), yet indicated an effect of reduced selectivity when the nitrogen concentration of a representative sample was higher. There was significant residual heterogeneity remaining ( $QE_{16} = 172.2$ ,  $P < 0.0001$ ), indicating that additional factors may explain the variance between studies. A leave-one-out analysis produced a range of mean  $g$  of  $0.19$ – $0.39$ . Removal of one particular effect size resulted in a positive, significant mean  $g$ , indicating that inclusion of additional studies could considerably alter the observed effect.

### Soluble protein

A meta-analysis of 12 effect sizes revealed significant, positive effect of selection for foods of higher than average soluble protein content (mean  $g = 0.55$ ,  $n = 12$ , 95%CI:  $0.26$ – $0.84$ ,  $P < 0.0001$ ) (Table I, Fig. 1b). There was considerable and significant between-study heterogeneity ( $I^2 = 67.7\%$ ,  $Q_{11} = 36.6$ ,  $P = 0.0001$ ). Including the soluble protein concentration of a representative sample as a moderator variable produced a significant negative effect of protein content on selectivity by primates (coefficient =  $-0.22$ ,  $n = 12$ , 95%CI:  $-0.40$  to  $0.04$ ,  $P = 0.013$ ). Significant residual heterogeneity remained ( $QE_{10} = 19.5$ ,  $P = 0.03$ ), suggesting that additional factors may yet further explain the between-study heterogeneity. Finally, a leave-one-out analysis produced a range of mean  $g$  of  $0.47$ – $0.65$ , however, the significance of this meta-analysis was robust to removal of individual effects, suggesting that inclusion of additional studies is unlikely to significantly alter the result.

### Acid detergent fiber

A meta-analysis of 26 effect sizes revealed a significant, negative effect of acid detergent fiber content on selectivity by primates (mean  $g = -0.72$ ,  $n = 26$ , 95%CI:  $-1.04$  to  $-0.40$ ,  $P < 0.0001$ ) (Table I, Fig. 1c). There was considerable and significant between-study heterogeneity ( $I^2 = 84.3\%$ ,  $Q_{25} = 305.0$ ,  $P < 0.0001$ ). Inclusion of the acid detergent fiber

concentration of a representative sample as a moderator variable was non-significant (coefficient =  $-0.16$ ,  $n = 26$ , 95%CI:  $-0.048$  to  $0.015$ ,  $P = 0.31$ ). Significant residual heterogeneity remained, suggesting that additional moderator variables are required to explain the between-study variation in effects ( $QE_{24} = 300.8$ ,  $P < 0.0001$ ). A leave-one-out analysis produced a range of mean  $g$  of  $-0.76$  to  $-0.59$ . The removal of any individual effect had no significant impact on the model, suggesting that additional studies are unlikely to influence this result.

## 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., Ganzhorn et al., 2009; Mowry et al., 1996; Simmen et al., 2014; Yeager et al., 1997] and that, once average protein concentrations are above requirements, selection could be based on other components and criteria, such as the availability within the environment [e.g., Fashing et al., 2007; Oftedal, 1991] or secondary plant chemicals [Moore & Foley, 2005]. While this idea has been around for some time, it has rarely been tested in folivores [Jensen et al., 2015; Marsh et al., 2014]. Instead, studies started to focus on long-term nutrient budgets and nutrient balancing using the conceptual approach of geometric frameworks [e.g., DeGabriel et al., 2014; Felton et al., 2009; Irwin et al., 2014; Johnson et al., 2013; Rothman et al., 2011], 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 indicate that primates select for high protein leaves mainly 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 an inverse relationship between protein and fiber concentrations in food. As such, primates discriminated against fiber, yet contrary to the situation with protein, the fiber concentrations in the representative samples of leaves had no effect on fiber discrimination. We



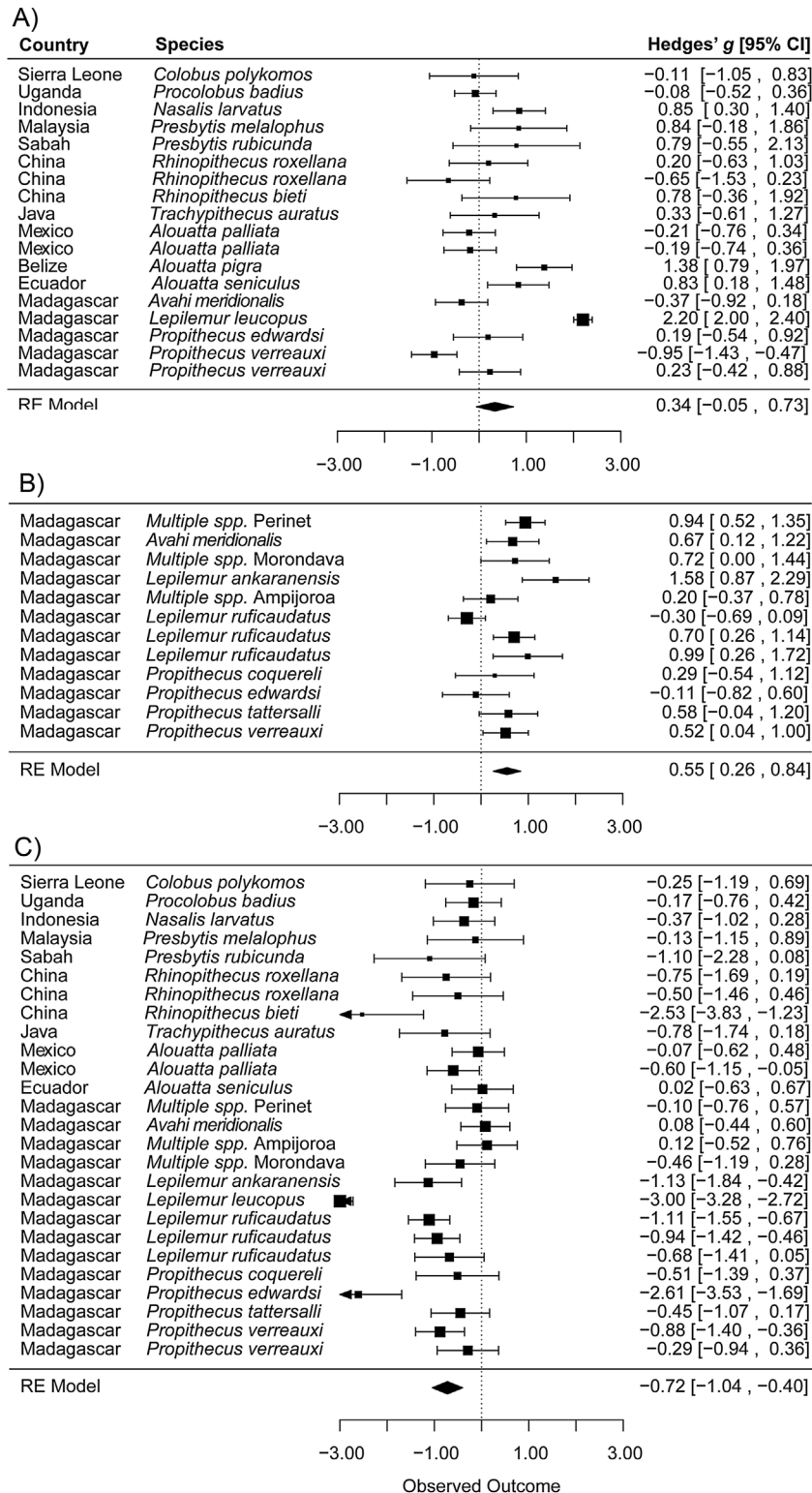


Fig. 1. Forest plots of standardized effect sizes (Hedges' *g*)  $\pm$  95% confidence intervals of leaf selection in relation to (A) nitrogen, (B) soluble protein, and (C) acid detergent fiber (ADF). Random effect meta-analytic mean effect size  $\pm$  95% confidence interval (RE Model) is shown at the bottom of each panel. Vertical dotted lines indicate zero (no effect). "Multiple spp." include *Avahi laniger*, *Eulemur fulvus*, *Indri indri*, and *Lepilemur microdon* for Perinet, *Avahi occidentalis*, *Lepilemur edwardsi*, and *Propithecus coquereli* for the forest of Ampijoroa, and *Eulemur rufifrons* and *Propithecus verreauxi* for Morondava (Site CN5).

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 et al., 2014].

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