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Rapid Assessment of the Nutritional Value of Foods Eaten by Mountain Gorillas: Applying Near-Infrared Reflectance Spectroscopy to Primatology

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Abstract The increasing recognition of the considerable intraspecific spatial and temporal variability in the nutritional contents of primate foods has necessitated development of fast and cost-effective analytical methods. Used widely for agricultural products, near-infrared reflectance spectroscopy (NIRS) is a quick, inexpensive means of assessing nutritional chemistry. The general principle of NIRS is that when the sample is irradiated with near-infrared light, the reflectance

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spectrum is characteristic of the mixture of chemical bonds present in the sample. These spectra, when calibrated against reference values—determined via traditional nutritional analysis—to develop regression equations, can be used to estimate nutritional values of similar samples without doing traditional nutritional analysis. We validated the use of NIRS for estimating the nutritional attributes of African herbs and trees, which were foods eaten by mountain gorillas (*Gorilla beringei*) collected as part of a larger study on gorilla nutritional ecology. We determined the near-infrared spectra (1100–2400 nm) of 241 dried samples of 13 species of tropical herbs and trees that formed the staple diet of the gorillas. We used modified partial least-squares regression to develop calibration equations that could predict nutritional attributes of gorilla foods, and we performed an independent validation of the calibrations. The equations had robust predictive power similar to those used in agricultural and ecology, and we found no differences between samples measured via NIRS and traditional nutritional analysis. Our analysis indicates that NIRS offers a rapid and cost-effective means of analysis of tropical leaves and herbs, and has the potential to transform primate feeding ecology studies by allowing us to evaluate the importance of intraspecific variation in nutritional value.

Keywords Bwindi · intraspecific variation · plant chemistry · primate nutrition · spectroscopy · tropical ecology

Introduction

Determining the ecological factors that influence primate abundance, diversity, and social behavior are central questions to primate behavioral ecology and conservation. To address these questions, we often need nutritional data collected under varied environmental conditions. It has been known for some time that the nutrient content of plants within a single species can vary over different spatial and temporal scales. Depending on location, young leaves of the same species eaten by monkeys in Kibale National Park, Uganda varied in protein content from 22% to 47% (Chapman *et al.* 2003) and the fat content of a single species of ripe fruit in this forest varied seasonally from 0.3% to 30.0% (Worman and Chapman 2005). In Madagascar, sun-exposed leaves had more protein than shaded leaves (Ganzhorn 1995), and ring-tailed lemurs (*Lemur catta*) ate tamarind leaves and fruits that varied in nutritional value according to forest type (Mertl-Millhollen *et al.* 2003).

Striking variation among plant samples of the same species means that we cannot use a species-specific nutritional profile to estimate food quality; spatial and temporal variation in nutritional quality must be taken into consideration to address many questions of interest to primatologists. Unfortunately, this requires the analysis of thousands of samples and traditional chemical analysis is time-consuming and expensive when dealing with sample sizes of this magnitude. For example, using the latest technology for analyzing fiber, it is possible to analyze *ca.* 48 samples in duplicate for 1 fiber component in a day without consideration of weighing and drying time. A similar time commitment is needed for evaluating each nutritional attribute, such as protein, sugars, fat, etc., and so analyzing 50 samples takes ≥1–2 wk. The cost of chemicals and time are high when using these traditional approaches, and many of

the analyses require large quantities of hazardous chemicals, such as concentrated sulfuric acid (Van Soest *et al.* 1991). Consequently, traditional nutritional analyses are unsuitable to address many of the nutritional questions that have become of interest because they are too laborious and expensive.

Near-infrared reflectance spectroscopy (NIRS) is a quick, nondestructive, and economical technology that can be used to assess nutritional content of primate foods. The general principle of NIRS is that spectra are reflected based on the number and type of chemical bonds (C–H, N–H, etc.) in a sample when it is irradiated with near-infrared light. These spectra are then statistically calibrated against reference values, which are determined via traditional nutritional analysis, to develop multivariate regression equations that can then be used to estimate nutritional values for samples similar to those in the calibration set. With NIRS, up to 150 plant samples per day can be analyzed for multiple nutrients simultaneously once the spectrometer is calibrated. This offers a powerful alternative method to address better many questions of interest to primate nutritional ecologists. Researchers have widely used near-infrared spectroscopy to estimate the nutritional composition of foodstuffs in agriculture and food science since the 1970s (Norris *et al.* 1976; Shenk *et al.* 1979), but its value has only recently been realized for ecological studies (Foley *et al.* 1998; McIlwee *et al.* 2001; Moore and Foley 2005). Excellent reviews of NIRS, which include ecological applications, are provided in Foley *et al.* (1998) and Batten (1998).

To our knowledge, aside from this study, there have only been 2 applications of NIRS to primatology. In a preliminary study, Ortmann *et al.* (2006) used NIRS to predict the nitrogen contents of bamboo eaten by lemurs, and in a recent study, Felton *et al.* (2009) used it to investigate nutrient regulation in spider monkeys. The potential of NIRS to transform primatology can be illustrated by recent ecological studies that employed this technique to study plant-animal interactions at larger scales than has been possible in the past. For example, Moore and Foley (2005) used NIRS to provide a snapshot of the nutritional composition of the leaves of every tree in a koala habitat and recorded tree use over 10 yr to reveal the intricacies of koala tree choice. Other researchers demonstrated the influence of chemistry on foraging behavior in captive studies and used NIRS to consider how the relevant chemical parameters of foliage varied in natural plant communities (Lawler *et al.* 2000; Wallis *et al.* 2002) and the effects of intraspecific variation of plant chemistry on feeding and habitat quality of marsupial folivores (Dury *et al.* 2001; Lawler *et al.* 1998; Moore *et al.* 2004; Woolnough and Foley 2002). In a subarctic community, Stolter *et al.* (2006) demonstrated the feasibility of NIRS to assess aspects of large mammal nutritional ecology. In comparisons between allometry of birds and mammals, van Gils *et al.* (2007) used NIRS to examine nutrients in swan diets and Veraart *et al.* (2006) used it to quantify the nutrients and secondary compounds in willow trees to determine their susceptibility of browsing by beavers. In savanna systems, Ezenwa (2004) used NIRS to evaluate ungulate diet quality to understand interactions between diet and host gastrointestinal parasite status and Woolnough and du Toit (2001) used it to determine differences in feeding heights by browsers. All of these studies required extensive sampling and nutritional analysis of a large number of plant samples. Without the application of NIRS, the methodological demands would have rendered them unfeasible.

We aimed to evaluate whether NIRS could be used to predict the nutritional attributes of tropical herbs and trees, which are primate foods. Using 241 samples of 13 species of herb parts and tree leaves eaten by mountain gorillas (*Gorilla beringei*) as a case in point, we assessed whether NIRS could successfully predict protein and fiber contents of these primate foods. We collected plant samples from different areas in Bwindi Impenetrable National Park, Uganda to study the nutritional ecology of gorillas (Rothman *et al.* 2006, 2007, 2008b). Estimating the nutrient composition of herbs and tree leaves across a landscape contributes to our understanding of gorilla nutrition, habitat use, ranging patterns, and habitat quality.

Methods

Sample Collection

We collected plant samples from different areas in Bwindi Impenetrable National Park. Uganda (0°53′–1°08′S, 29°35′–29°50′E) from June 2002 to June 2003 to study the nutritional ecology of gorillas. Samples for NIRS equation development (n=241) comprised 13 species of 14 food items, and 4 different plant parts: 1) herbaceous leaves of *Basella alba*, *Urera hypselodendron*, *Momordica foetida*, *Momordica pterocarpa*, *Ipomoea involucrata*, *Mimulopsis solmsii*, *Triumfetta tomentosa*, *Smilax anceps*, *Carduus* sp. (probably all of the same species), *Gouania longispicata*, 2) tree leaves of *Olea capensis* and *Myrianthus holstii*, 3) inner stem core of *Mimulopsis arborescens*, and 4) outer herb peel of *U. hypselodendron* (Table I). Aside from wood, all species that represented >1% of total intake of the nonfruit portion of the gorilla diet were included, which represented >80% of their diet on a mass basis (Rothman *et al.* 2007). These herbs and trees are common in the gorilla habitat across various areas of the park (Nkurunungi *et al.* 2004). We omitted fruits because we did not have an adequate sample size, but researchers have used NIRS to estimate fruit qualities (Butz *et al.* 2005; Felton *et al.* 2009). We collected multiple samples of each species (Table I). Each of these samples was from a different location or time-point when we observed the gorillas feeding on them. We processed samples in the same way as they were eaten by the gorillas based on observations of feeding behavior (Rothman *et al.* 2008b). For example, when the gorillas ate the inner stem core, they discarded the hard outside peel of the stem, so we did the same. We dried plant parts on newspaper in a cool, dark area (<22°C) of the field station until they achieved a constant weight. We then transported them to Makerere University, Uganda, where they were milled in a Wiley Mill through a 1-mm screen. After milling, we transported the samples to Cornell University for nutritional analysis.

Standard Nutritional Analysis (Wet Chemistry)

We analyzed all of the samples using both NIRS and wet chemistry. Protein and fiber are important predictors of the nutritional quality of primate foods for species that eat a leafy diet (Chapman *et al.* 2002; Milton 1979). We analyzed the samples sequentially for neutral detergent fiber (NDF), acid detergent fiber (ADF; Van Soest

Table I Protein and fiber composition of gorilla plant foods (n=191 samples from different locations and seasons) used to construct calibration equations

Plant species	Family	Part	n	NDF	ADF	ADL	CP
Basella alba	Basellaceae	HL	13	24.3±2.7 (20.2–29.5)	14.0±2.4 (10.7–19.7)	4.6±3.0 (1.8–11.3)	29.0±4.0 (21.4–34.0)
Carduus sp.	Asteraceae	HL	6	32.4±4.9 (26.9–38.3)	24.1±2.9 (21.0–27.2)	6.8±1.5 (4.5–9.0)	19.2±3.8 (15.2–23.1)
Gouania longispicata	Rhamnaceae	HL	11	28.4±2.5 (25.2–32.0)	15.8±2.0 (12.7–19.7)	5.5±1.0 (6.8–19.5)	24.7±3.5 (19.5–31.6)
Ipomoea involucrata	Convolvulaceae	HL	23	27.8±11.4 (18.9–45.0)	19.3±7.6 (13.8–31.4)	8.8±5.3 (5.2–20.3)	25.1±2.6 (19.8–30.6)
Mimulopsis arborescens	Acanthaceae	PT	10	41.4±7.3 (36.2–59.6)	31.8±5.3 (15.1–23.8)	3.5±1.3 (1.8–5.9)	6.0±0.9 (4.8–7.5)
Mimulopsis solmsii	Acanthaceae	HL	12	33.8±4.3 (28.0–39.4)	18.7±3.1 (15.1–23.8)	7.7±2.9 (4.4–13.3)	28.9±2.1 (25.4–31.0)
Momordica foetida	Cucubitaceae	HL	26	23.9±10.0 (16.4–45.4)	16.3±7.7 (11.1–33.0)	5.0±3.7 (2.3–13.6)	27.5±6.9 (13.3–38.2)
Momordica pterocarpa	Cucubitaceae	HL	24	19.9±3.4 (13.0–27.3)	13.3±3.1 (2.5–17.2)	4.3±1.9 (1.3–9.2)	26.4±3.2 (20.4–32.6)
Myrianthus holstii	Moraceae	TL	13	50.1±2.7 (46.6–54.7)	37.1±2.1 (32.5–40.2)	14.7±2.4 (10.1–19.6)	16.2±1.4 (12.8–18.5)
Olea capensis	Oleaceae	TL	10	51.9±3.7 (45.5–56.1)	37.2±3.3 (32.5–42.7)	19.8±3.6 (14.8–27.1)	12.3±1.7 (9.3–14.1)
Smilax anceps	Smilacaceae	HL	11	39.6±2.2 (35.9–43.1)	26.9±2.2 (23.7–30.3)	10.7±1.6 (8.4–13.4)	14.5±0.8 (13.4–15.6)
Triumfetta tomentosa	Tilaceae	HL	10	36.2±4.4 (28.9–44.5)	20.1±2.9 (17.1–27.8)	8.7±1.7 (6.7–12.0)	25.6±2.0 (22.5–27.8)
Urera hypselodendron	Urticaceae	PL	12	64.4±4.9 (56.2–72.8)	57.0±4.7 (49.9–65.6)	13.0±4.0 (7.6–19.7)	11.4±2.3 (9.4–17.3)
U. hypselodendron	Urticaceae	HL	10	31.7±4.4 (23.1–36.8)	21.5±3.9 (14.6–27.1)	12.6±4.7 (5.4–20.0)	24.1±3.8 (19.2–31.1)

All values are expressed as percent dry matter, and means are presented ± standard deviation (range). HL = herb leaves; PT = inner stem core; PL = herb peel; TL = tree leaves (mature); NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein

et al. 1991), and for acid detergent lignin (ADL; Goering and Van Soest 1970) via an Ankom 200 Fiber Analyzer (Ankom, Inc., Macedon, NY). We estimated total nitrogen (N) using a Leco FP-528 combustion analyzer (Leco Co., St. Joseph, MI). We calculated crude protein (CP) by multiplying N by 6.25; *cf.* Conklin-Brittain *et al.* (1999) and Milton and Dintzis (1981) for discussion of the use of this index for tropical plant parts. We provide details of the wet chemistry analysis in Rothman *et al.* (2008b).

Collection of Spectral Data

We followed the standard methods of NIRS calibration described in Hruschka (2001) using WinISI (Version 2, Foss NIRSystem, Silver Spring, MD). Before scanning, we placed each milled sample in a sample quarter cup and took care to ensure the samples were spread evenly (Foley *et al.* 1998). We scanned the milled

samples of the plant parts of herbs/trees (n=241) with a near-infrared reflectance spectrometer (Model 5000, Foss NIRSystems, Silver Spring, MD) at Cornell University, Ithaca, NY, and collected spectra between 1100 nm and 2498 nm. We scanned the samples once. To minimize sample and instrument variation, we housed the NIRS instrument and samples in a room maintained at constant temperature and humidity. We chose a selection of 50 samples that included all food species and parts for our independent validation set.

Calibration Set and Equation Development

The purpose of the calibration process is to develop the equations with high predictive power that can be used generally for all gorilla foods. We used a trial-and-error approach to develop calibration equations (Foley *et al.* 1998; Woolnough and Foley 2002). We chose the equations with the lowest standard error of calibration (SEC), and highest coefficient of determination (r^2) for use after trying a number of math treatments and regression techniques as follows.

We followed the methods of Shenk and Westerhaus (1996). We used the algorithms CENTER and SELECT (Shenk and Westerhaus 1991) to select samples for equation development. We included all species/part combinations within this set. The SELECT algorithm uses principal component analysis and Mahalanobis distances (H statistic) to rank spectra in relation to the average of the population and identifies spectra that represent the full spectral variability of the data set. The H statistic is based on the distances measured from a set of multivariate data, whereby an ellipse is fitted to the data, and the Euclidean distances measured from the center of the ellipse to the edge, which varies through space (Mark 2001). We considered spectra to be outliers if they were >3H values from the mean (Williams and Norris 2001).

After trying a number of regression methods, we used modified partial-least squares regression (MPLS) to develop calibration equations. The MPLS technique combines principal components analysis (PCA) and multiple linear regression. The PCA reduces the spectral data to a few combinations of absorbances that describe most of the spectral information, and the regression relates those principle components to the reference values obtained via traditional nutritional analysis. The MPLS approach uses cross-validation to prevent over-fitting of the equation. In addition, we tried a variety of mathematical treatments (following treatments listed in Table I of Woolnough and Foley 2002), and we used the formula 2, 4, 4, 1 for all equations. Mathematical treatments are described by 4 components. The first number indicates which derivative (or rate of change) is used (0 = underivatized, 1 = 1st derivative, 2 = 2nd derivative, etc.). The second number indicates the gap (in nm) over which the derivative was calculated. The third and fourth numbers indicate the degree of primary and secondary smoothing. The use of mathematical treatments with MPLS reduces the variation and multicollinearity caused by developing calibrations based on >1 wavelength, and the potential variation in spectra due to differences in sample particle size and moisture (Batten 1998). We used the detrend and standard normal variate (SNV) transformation, which is a scatter correction algorithm included in WinISI. The use of scatter algorithms such as SNV is a mathematical strategy for dealing with potential variation in particle size (Foley *et al.*

1998). Although we milled samples to 1 mm, because they contained many different species and plant parts, variation in particle size could still be present.

Validation Set

We used 2 methods of equation validation as outlined in Shenk and Westerhaus (1994). The first method uses cross-validation during the development of the equation using samples that are in the initial data set. This cross-validation procedure involves separating the sample set into groups and performing calibration on all but 1 of the groups. The remaining group is used as an independent validation set. The procedure is repeated such that all groups of samples have been cross-validated. The pooled residuals of each prediction provide a SECV (standard error of cross-validation) and the coefficient of determination of cross-validation (1 – VR). The 1 – VR term is the explained variance divided by the total variance, which can be thought of as an r^2 of the cross-validation.

Second, we validated the equations through the use of an independent sample set comprising 50 gorilla foods that represented the same food species and parts as those in the calibration set. We first identified the outlier spectra that fell outside of the population means using the *H* statistic, which is important because if the spectra are outliers, they may not be well-predicted by the equation. After removing the outliers from the data set, we compared the predicted results using our equation to the wet chemistry analyses, using the PREDICT algorithm. We compared the NIRS predicted values versus the laboratory reference values via linear regressions, and computed the standard error of prediction (SEP) for each nutritional attribute. We compared the SEP to the standard error of the laboratory (SEL). The SEL reflects laboratory precision, where $SEL=\sqrt{(\sum (y_2-y_1)^2/n)}$, where y_1 and y_2 are the replicates of the analyzed nutrient (Woolnough and Foley 2002).

For both the calibration and the cross-validation set, we computed descriptive statistics to evaluate the predictive power and precision of the chosen equations for each nutritional attribute, including the SEC, SECV, SEP, coefficient of determination (r^2), and the coefficient of cross-validation (1 – VR). Because the nutritional values and the differences between the NIRS predicted and laboratory chemistry in the data sets for each nutrient were not normally distributed (as assessed by an Anderson-Darling test), we used the nonparametric Wilcoxon paired signed rank test to assess the difference between wet chemistry values and NIRS predicted values. We performed all statistical tests with SPSS Version 10.0.0 (Chicago, IL).

Results

We compared the descriptive statistics of the best calibration equations with the equations developed for other ecological and agricultural applications. Aside from ADL, the equations for each nutritional attribute had strong predictive power (r^2= 0.95–0.99; Table II).

In the cross-validation procedure the predictive power for NDF, ADF, and CP was high (1 – VR=0.94–0.98), but ADL was relatively low (1 – VR=0.75; Table II). We scanned the independent set of samples for validating the calibration equations, and

Table II Predictive power and error associated with calibration equations used to estimate attributes of plant foods

	Component	SEC	r^2	SECV	1 – VR	SECV/ SD
Herbs and trees eaten by gorillas[a]	NDF	2.62	0.96	3.27	0.94	0.252
	ADF	2.46	0.95	2.77	0.94	0.254
	ADL	2.29	0.78	2.48	0.75	0.506
	CP	0.89	0.99	1.16	0.98	0.153
Rice[b]	NDF	1.93	0.85	2.13	0.81	0.435
	ADF	0.69	0.96	0.88	0.95	0.232
	ADL	0.38	0.90	0.47	0.84	0.427
Oat hulls[c]	NDF	1.75	0.92	2.47	0.87	0.323
	ADF	1.29	0.95	1.56	0.93	0.260
	ADL	0.49	0.94	0.59	0.91	0.303
	CP	0.47	0.94	0.77	0.83	0.379
Tropical seagrass[d]	NDF	2.14	0.99	2.79	na	na
	ADF	1.89	0.91	2.15	na	na
	ADL	3.11	0.73	3.35	na	na
	N	0.07	0.99	0.08	na	na
Acacia and *Boscia* eaten by African ungulates[e]	NDF	na	0.96	2.67	na	na
	N	na	0.98	0.59	na	na
Eucalyptus eaten by possums[f]	NDF	na	0.98	3.17	na	na
	N	na	0.96	0.76	na	na
Fruits, leaves, and seeds eaten by spider monkeys[g]	NDF	5.5	0.91	5.8	na	na
	N	0.1	0.99	0.3	na	na
Grasses, sedges, and forbs eaten by wombats[h]	NDF	1.49	0.98	2.59	0.99	0.242
	ADF	1.92	0.89	2.85	0.76	0.448
	ADL	1.82	0.77	2.28	0.63	0.449
	N	0.04	0.99	0.07	0.99	0.123

All values are expressed as % dry matter; na indicates not available; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein; SEC = standard error of calibration; SECV = standard error of cross-validation; 1 – VR = coefficient of determination of cross-validation; SECV/ SD (standard deviation) = an index of bias wherein the higher the number, the greater the bias; N = nitrogen

[a] This study; [b] Kong *et al.* (2005); [c] Redaelli and Berardo (2007); [d] Lawler *et al.* (2006); [e] Woolnough and du Toit (2001); [f] Lawler *et al.* (2000); [g] Felton *et al.* 2009; [h] Woolnough and Foley (2002)

2 samples had spectra with H values >3. These 2 outliers were *Momordica foetida* and *Triumfetta tomentosa* leaves, and we removed them from the validation set. With the exception of ADL, the predictive power of the equations for nutrients remained high with this independent validation data set ($r^2 \geq 0.95$; Table III, Fig. 1).

Through the use of an independent set of samples for validation of the procedure, we found no differences in NIRS predicted values versus the laboratory values for any nutrients (Wilcoxon paired signed rank test; p values were between 0.22 and 0.82), indicating that the NIRS calibration equations predicted laboratory values successfully.

Table III Performance of the NIRS calibration equations of gorilla foods using an independent set of 48 samples of the same parts and species

Component	Mean	Slope	Intercept	SEP	SEL	r^2	p-value
CP	25.2±8.5	0.938	1.83	2.01	na	0.95	<0.001
NDF	31.1±12.9	1.04	0.27	3.45	1.75	0.95	<0.001
ADF	21.5±11.4	1.01	0.09	1.94	2.42	0.97	<0.001
ADL	7.3±4.5	0.74	2.62	2.27	2.39	0.77	<0.001

SEP = standard error of prediction; SEL = standard error in the laboratory based on duplicate or triplicate analyses of the same sample

Discussion

Our results indicate that robust equations using NIRS can be generated for tropical herbs and leaves. These equations have similar predictive power to broad-based equations used in applications in the agricultural industry and ecology (Table II). Most studies in agriculture and ecology use NIRS to generate predictive equations for a single species, such as domestic oats, rice (Kong *et al.* 2005; Redaelli and Berardo 2007), or individual species of *Eucalyptus* (DeGabriel *et al.* 2008, 2009;

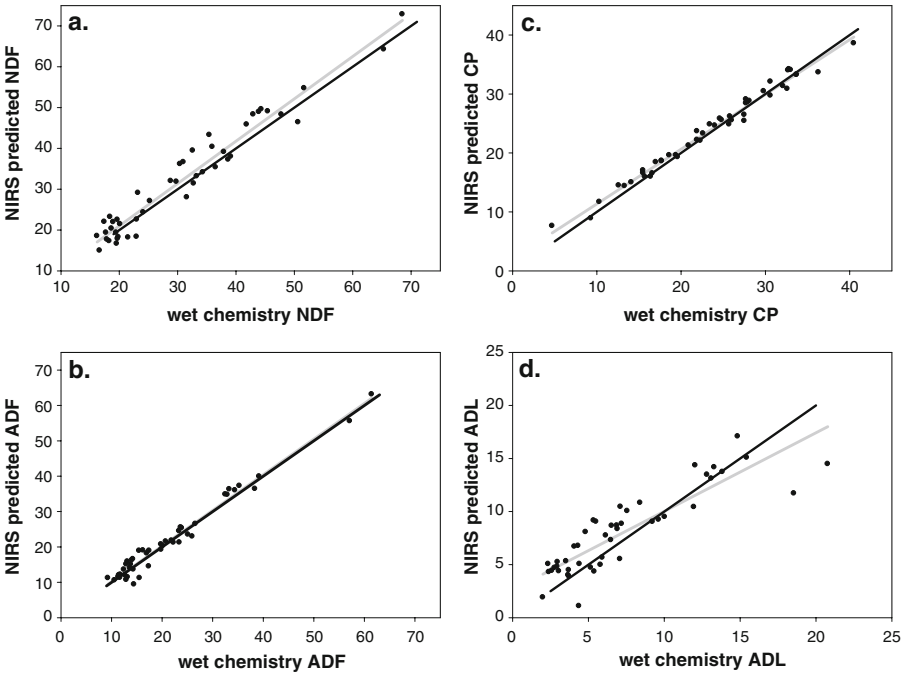

Fig. 1 Relationships among the (**a**) neutral detergent fiber, (**b**) acid detergent fiber, (**c**) crude protein, and (**d**) acid detergent lignin of gorilla foods using wet chemistry and near-infrared reflectance spectroscopy. The black regression line represents a slope of 1, and the gray regression line represents the slope of the relationship between wet chemistry and NIRS predicted values. All values are expressed as % dry matter. For statistics, see Table III.

Moore *et al.* 2004). However, a handful of recent studies including this one demonstrate that NIRS can be applied broadly, and can include a number of different plant species in a single equation that predicts nutritional attributes (da Costa and Volery 2005; Lawler *et al.* 2006; Locher *et al.* 2005; Woolnough and Foley 2002), which is important in studies of primates, whose diets can be very diverse.

The equations that we generated for gorilla foods indicated good prediction of an external data set of the same species. The predictive nature of the NIRS equations is usually affected by the accuracy of the standard laboratory analyses. However, if unbiased error occurs around laboratory measurements, it may not have an impact on the accuracy of the predictions, and the coefficient of determination may not be affected (Coates 2002). One can assess the precision of estimates by making predictions using multiple repeated scans, or multiple samples from the same plant. In addition, it is important to monitor the performance of equations continuously as they are used to predict new sample sets and expand the calibration sets where necessary by adding new spectra and wet chemistry values, particularly so that variation due to growing season and changing environmental conditions is captured (Foley *et al.* 1998; Stuth *et al.* 2003). Fibrous components present a challenge because the wet chemistry analyses are based on gravimetric determination of plant materials after exposure to different solutions with varying acidity, and the residues from each sequential step are not homogeneous (Van Soest *et al.* 1991). Neutral detergent fiber is mainly structural carbohydrates, but may also include tannins, tannin-protein complexes, and cell wall protein, all of which are present in the diets of these gorillas (Rothman *et al.* 2008a, 2009). This may affect NIRS prediction, as illustrated by the higher SECV of NDF and ADL (Table II); however, the statistics are acceptable for analyses where the standard deviation of the data set is high and the plant material is variable, as in this study (Stuth *et al.* 2003). The predictive power of our equation for ADL was similar to those computed in other studies (Table II). Because ADL is a complex mixture whose subcomponents are not clearly known (Van Soest 1994), it is difficult to quantify both in the laboratory and via NIRS (Lawler *et al.* 2006; Woolnough and Foley 2002).

We found that the plant parts consumed by gorillas had remarkable intraspecific variability in nutritional composition. For example, CP in *Urera* leaves varied from 19% to 31% and ADL varied from 5% to 20% (Table I). Often, habitat quality measurements rely on the availability of specific resources based on the plant species composition in these areas (Koenig *et al.* 1998; Watts 1998; Worman and Chapman 2006), but the variations in nutritional quality of these resources are not considered. With the documented intraspecific variation in primate diets (Baranga 1983; Chapman *et al.* 2003; Ganzhorn 2002; Glander 1982; Mertl-Millhollen *et al.* 2003; Worman and Chapman 2005; Yamashita 2008), we know that this is inadequate. Because initial costs of an NIR spectrometer are high (*ca.* 40–80,000 US$), primatologists may wish to consider first forming collaborations with existing users.

The prediction of nutritional composition of primate foods may not be the only application of NIRS. Ecological studies used NIRS to predict biological response to dietary properties, such as food intake, feeding rates (McIlwee *et al.* 2001), and tannin-binding ability (DeGabriel *et al.* 2008). Studies in the animal science industry have developed NIRS to predict botanical composition of the diets using the spectra of feces (Glasser *et al.* 2008; Parveen *et al.* 2008), which could be particularly useful

for determining the diet contents of elusive primates, and others have used NIRS to examine measures of digestibility (Steen *et al.* 1998). Researchers have produced fecal NIRS models to predict the diet quality of donkeys (Kidane *et al.* 2008), deer (Showers *et al.* 2006), ostrich (Landau *et al.* 2006), and free-ranging cattle (Lyons and Stuth 1992). In addition, portable NIRS have been developed for use in the field (Ventura *et al.* 1998) and have great promise for ecological studies. We foresee that with further development to improve its generality for use in primate diets, NIRS will be of important use in primatology.

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