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Variation in the Nutritional Value of Primate Foods: Among Trees, Time Periods, and Areas

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*The study of nutritional ecology has proven to be useful for understanding many aspects of primate behavior and ecology and is a valuable tool in primate conservation. However, to date this approach has had limited application since chemical analyses of food items is very time-consuming and collections of perishable food material are often made in remote field locations. Such logistic difficulties have led to plant material being collected in a variety of fashions, and it is not known how variation in collection method might influence our understanding of the chemical basis of dietary selection. A standardization of collection methods is greatly needed to allow for direct comparison among studies. To develop an appropriate standardized method and to evaluate past research, it is necessary to understand along what dimensions plant chemistry varies. We evaluated variation in nutritional value—protein, fiber, digestibility, alkaloids, saponins, cyanogenic glycocides, and minerals—of leaf material from species eaten by red colobus (*Piliocolobus tephrosceles*) and black-and-white colobus (*Colobus guereza*) of Kibale National Park, Uganda. We consider variation at 3-levels: among trees, time periods, and areas. While there was considerable variation among species with respect to protein, digestibility, and saponins, there was also variation among individuals of the same species; in fact, individuals may vary by as much as 20%. The average coefficients of variation (CV) among individuals of the same species are 13.4 for protein, 12 for digestibility, and 43 for saponins, while the average CV among species are 35, 31.3, and 82.4, respectively. No species showed a variable response*

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with respect to testing for the presence or absence of cyanogenic glycocides, while 2 of 11 species tested for alkaloids showed a variable response. Over 2 years there was evidence of variation among time periods in the chemical composition of the same food items. The protein-to-fiber ratio of mature leaves of the same species collected from 4 sites separated by 12 km within Kibale was also variable and in some cases the variation among sites was greater than the differences among species. For example, while Funtumia latifolia *had little variation in protein-to-fiber ratio at 3 sites (0.44 at all sites), the remaining site was 28% greater. Because temporal variation is less than variation among individuals, it is likely more important to sample from multiple trees at a single point in time than to sample across time. However, the most accurate assessment of nutrient intake is obtained by collecting plant material from the specific trees selected for consumption.*

KEY WORDS: nutritional ecology; colobus; foraging; dietary selection; Kibale; minerals.

INTRODUCTION

Investigating the chemical basis of dietary selection in primates has provided a unique understanding of their foraging strategies (Whiten *et al.*, 1991), facilitated evaluations of socioecological explanations of social organization (Byrne *et al.*, 1993), and provided means to explore determinants of abundance (Chapman *et al.*, 2002; Oates *et al.*, 1990). Understanding determinants of primate abundance is becoming increasingly important as ecologists are asked to apply their knowledge to assist conservation biologists to construct informed management plans for endangered species. The importance of these theoretical issues has become critical because most primates live in tropical forests which are increasingly being impacted by human modification (National Research Council, 1992). Cumulatively, countries with primate populations are losing 125, 140 km^2 of forest annually, resulting in the annual loss of 32 million primates (Chapman and Peres, 2001). These populations are also being seriously harmed by forest degradation, particularly logging and fire, and hunting. Understanding the nutritional requirements of an endangered species can assist in the development of sound conservation and management policies. For example, if important tree species could be left standing in selective logging operations, population declines following logging might be lower and/or the speed of recovery might be more rapid for those species negatively affected by logging.

Unfortunately, the application of information from primate nutritional ecological studies to conservation issues has been limited by the difficulties of collecting, transporting, and analyzing plant material. Multiple trees or multiple samples of a specific plant part are not always collected and analyzed. Samples are often collected at remote field sites where drying and

transporting to laboratories can be difficult and chemical analyses are time-consuming. Such logistic difficulties have led to plant material being collected in a variety of fashions. For example, Waterman *et al.* (1988) collected leaf samples opportunistically, avoiding saplings and low coppiced branches. Byrne *et al.* (1993) collected samples over the same period as they observed their subjects eating them, and processed the items in as close a fashion as possible to that used by the baboons. Often authors do not report details about sample collection, making it impossible to assess whether samples were collected from the tree in which the animals fed or in different trees in the same area, or if they were collected at the same general time the animals fed on the item or at a different time. If there is little variation among individual trees of the same species, time periods, or areas, fewer samples could be collected than if there was marked variation. Thus, to conduct primate nutritional studies efficiently and to provide new tools for primate conservation, it is necessary to develop collection methods that increase the precision and accuracy of estimating nutrient intake and selection by primate groups.

We evaluated variation in the nutritional value—protein, fiber, digestibility, minerals, alkaloids, cyanogenic glycocides, and saponins—of leaf material of tree species eaten by red colobus (*Piliocolobus tephrosceles*) and black-and-white colobus (*Colobus guereza*) of Kibale National Park, Uganda. First, to quantify variation among trees of the same species, we considered the nutritional value of food items collected from a minimum of 10 trees of 11 species at one time. Second, for 9 species that are commonly eaten by the colobus, we evaluated variation among time periods over 2 yr. Third, we considered how the protein to fiber ratio of mature leaves from 9 species varies among 4 sites separated by an average of 12 km. We make this last comparison because previous studies have shown the potential importance of the protein-to-fiber ratio of mature leaves in accounting for variation in the blomass of folivorous monkeys (Chapman *et al.*, 2002; Davies, 1994; McKey, 1978; Milton, 1979; Oates *et al.*, 1990; Waterman *et al.*, 1988).

MATERIALS AND METHODS

Study Site

Kibale National Park (766 km^2) is located in western Uganda (0 13′–0 41′ N and 30 19′–30 32′ E) near the foothills of the Ruwenzori Mountains (Chapman *et al.*, 1997; Struhsaker, 1975, 1997). The park comprises mature, mid-altitude, moist semi-deciduous and evergreen forest (57%), grassland (15%), woodland (4%), lakes and wetlands (2%), colonizing forest (19%), and plantations of exotic trees (1%; primarily *Cupressus lusitanica*, *Pinus*

patula, *P. caribaea*, and *Eucalyptus* spp.; Chapman and Lambert, 2000). Mean annual rainfall in the region is 1749 mm (1990–2001, or 1547 mm from 1903–2001); the mean daily minimum temperature is 15.5°C ; and the mean daily maximum temperature is 23.8°C (1990–2001, Chapman and Chapman, unpublished data). Rainfall is bimodal, with two rainy seasons generally occurring from March to May and September to November.

We evaluated variation in nutritional value of leaf material among individuals and among time periods of a food item from the same species at the Kanyawara site (forestry compartments K30 and K14) and compared mature leaf nutritional value among spatially separated sites at Sebatoli, Kanyawara, Dura River, and Mainaro (Chapman *et al.*, 1997; Chapman and Lambert, 2000).

Plant Collections

We collected samples using a tree-pruning pole to cut down tree limbs, typically from the middle of the tree's canopy. The sample trees were in the same general areas as colobus groups foraged, but were not necessarily the same tree as a group fed in. We made no collection from trees growing in unusual situations, such as tree fall gaps or forest edges, except for species typically only found in such habitats, e.g., *Prunus africana* on edges. We processed food items in a fashion that closely mimicked the feeding behavior of the colobus, and collected only parts eaten by them. For example, if the monkeys ate leaf petioles, we collected the length of petiole typically consumed. We dried samples in the field either by sun-drying, via a dehydrator that circulated warm air past them, or via a light-bulb to heat a box containing a series of racks. We stored dried samples in sealed plastic bags until they could be transported from Uganda to the University of Florida for analysis.

In June 1999, we collected food items from a minimum of 10 trees (mean = 11.7, range = 10–16 trees per species) of 11 species from which colobus often fed. Collections were made during this month, since many trees bore young leaves; a preferred food item of Kibale colobus (Struhsaker, 1975; Oates, 1977; Chapman *et al.*, 2002). The samples were all dried via the dehydrator.

To obtain samples to evaluate variation among time periods in the chemical composition of the same species/part when it was eaten by the colobus, we observed 2 groups of red colobus and 2 groups of black-and-white colobus from dawn to dusk for 5 days each mo from August 1998 until June 1999 in unlogged forest adjacent to Makerere University Biological Field Station (forestry compartment K30). This produced *ca.* 800 h of observations for each species (red colobus = 3264 feeding scores, black-and-white colobus = 2281). From July 1999 to May 2000 we observed 2 other

groups in an adjoining moderately logged area (Mikana area in forestry compartment K14) for *ca*. 480 h each (red colobus = 2163 feeding scores, black-and-white colobus = 1558). Each group had several recognizable individuals allowing verification of group identity. During each half-hour an observer was with the group he or she made 5 point samples of different individuals. If the subject was feeding, the species and plant part, e.g., fruit, young leaf, leaf petiole, were recorded. We tried to avoid repeatedly sampling particularly conspicuous individuals by moving throughout the group when selecting subjects and by sampling ones that were in clear view and ones that were more hidden. Often the observer had to wait for a number of minutes to determine what a less observable colobus was doing. The behavioral observations were conducted by Lauren Chapman, Colin Chapman, and 3 Ugandan field assistants. The field assistants have worked with us since 1990 and know the tree species, the observational technique, and monkey age classes. At the end of each week of observation, we collected the 5 most frequently eaten food items for both species. Some foods were eaten repeatedly over the study period, and we report on temporal variation in their composition.

Nutritional Analyses

We ground dried samples to pass through a 1-mm mesh screen in a Wiley mill (stainless steel). Dry matter was determined by drying a portion of each sample overnight at 105°C. We analyzed samples in duplicate, and considered replicates acceptable if the relative error was 2%. We used the 2% criterion for dry matter, organic matter, fiber, protein, saponins, and digestibility.

We assessed the protein (nitrogen) content of the plant parts via Kjeldahl procedures (Horwitz, 1970). Samples were digested via a modification of the aluminum block digestion procedure of Gallaher *et al.* (1975). The digestive mix contained 1.5 g of 9:1 K_2SO_4:$CuSO_4$, and digestion proceeded for $\geq$4 h at 375°C in 6 ml of H_2SO_4 and 2 ml H_2O_2. We determined the nitrogen in the digestate by semiautomated colorimetry (Hambleton, 1977). Measuring total nitrogen provides an estimate of crude protein (protein levels = N $\times$ 6.25; Maynard and Loosli, 1969). A better conversion factor for tropical foliage may be *ca.* 4.3 (Conklin-Brittain, *et al.*, 1999) or 4.4 (Milton and Dintzis, 1981); however, we used the 6.25 factor so that our results would be comparable to those of previous studies (Davies, 1994; Gartlan *et al.*, 1980; Oates *et al.*, 1990; Waterman *et al.*, 1988).

We measured fiber (acid detergent fiber, ADF) via methods outlined by van Soest (1963) and modified by Goering and van Soest (1970) and

Robertson and van Soest (1980). ADF is a measure of cell wall cellulose and lignin. ADF has a strong negative correlation with food selection by some primates (Glander, 1982; Oates *et al.*, 1990). However, ADF is somewhat fermentable, while lignin is not (van Soest, 1982).

We assessed digestibility via a procedure that is commonly used in ruminant forage analysis (Moore and Mott, 1974) and assumed that the efficiency of digestion via cattle rumen fluid will be correlated with the efficiency of red colobus digestion. The sample is first incubated with rumen microorganisms for 48 h and then it is incubated with an acid-pepsin solution.

Many alkaloids are bitter tasting and perhaps play a role as a feeding deterrent or damage the microbial community of colobine stomachs (Harborne, 1993; Roberts and Wink, 1998); however, most studies have not demostrated that colobines avoid foods high in alkaloids (Waterman, 1993). We tested for the presence of alkaloids via a spot test with Dragendorff's reagent, which sometimes produces false positive results (Waterman, 1993).

Saponins are surfactants, and have a soaplike foam-forming property in aqueous solutions. They are bitter-tasting and occur in >70 plant families. Most importantly, saponins can cause bloat in ruminants and have been implicated in dietary selection of cattle. Given the ruminant-like digestive system of colobines (Lambert, 1998), it is intriguing to consider whether saponins are important in colobine dietary selection. They also can hemolyse red blood cells when injected into the bloodstream, irritate the digestive tract, and serve as a steriod hormone precursor (Phillips-Conroy, 1986). The role of saponins in colobine dietary selection had not been investigated. We indexed the quantity of saponins in a sample via the Forth Test (Fong *et al.*, unpublished guide) using 60-sec and 1800-sec criteria. This involves shaking the sample in a specific fashion and measuring the height of the foam at set intervals.

Cyanogenic glycosides can release toxic hydrogen cyanide, but their role in determining herbivory is questionable (Jones, 1998). We determined the presence or absence of hydrogen cyanide by the Feigl-Anger test (Feigl and Anger, 1966; Glander *et al.*, 1989).

To assess mineral content, we dried, weighed, ashed, and solubilized samples with hydrochloric acid (Miles *et al.*, 2001). Additionally, we ran a sample of known mineral concentration (Certified National Bureau of Standards Citrus leaves SRM-1572) with each set of plant samples to ensure that values obtained from the AAS were accurate (NIST, 1982). We tested 8 minerals for in each sample: iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) by atomic absorption spectrophotometry via a Perkin Elmer AAS 5000 (Perkin-Elmer, 1980)

We considered the variance between individuals and over time separately, and not all chemical procedures were conducted on all samples. When considering variation in chemical propeties of plant parts from different trees collected at one time we consider protein, digestibility, alkaloids, cyanogenic glycosides, and saponins. When evaluating variation among time periods in the same species/part, we consider each of the same variables plus minerals. When evaluating variation among the widely separated sites within Kibale we only assessed the protein and fiber content of mature leaves.

Statistical Evaluation of Variance

When there are appreciable differences in the mean values of a parameter, variation can be evaluated via the coefficient of variation (CV; Sokal and Rohlf, 1981). We use the CV to contrast the variation among individuals and time periods in the nutritional quality of colobus foods. We calculated CV as the standard deviation among groups or time periods for a given dietary component divided by the mean.

To determine whether the variances in the measurements among samples collected from different individuals at one time were significantly different from the variance among time periods for the same species, we used an F-max test for homogeneity of variance.

We contrast $\leq$11 different chemical characteristics of colobus food items when addressing specific questions, e.g., whether there evidence for differences in leaf chemistry between wet and dry season, and did not adjust for multiple comparisons. We did not make adjustments, as we view our comparisons as attempts to identify patterns that can be evaluated in future studies. The number of comparisons are clearly presented for each test, thus readers can consider adjusting for multiple comparisons themselves, i.e., Bonferonni corrections.

RESULTS

Intraspecific Variation

For 11 species, we collected food items from a minimum of 10 trees (mean = 11.7, range = 10 to 16 trees per species) to consider intraspecific variation with respect to protein (Fig. 1), digestibility (Fig. 2), and saponin content (Fig. 3). While there was considerable variation among species for all 3 parameters, there was also variation among individuals of the same species. In fact individuals of the same species could vary by as much as 20% from one another. The average CV among individuals of the same species are 13.4

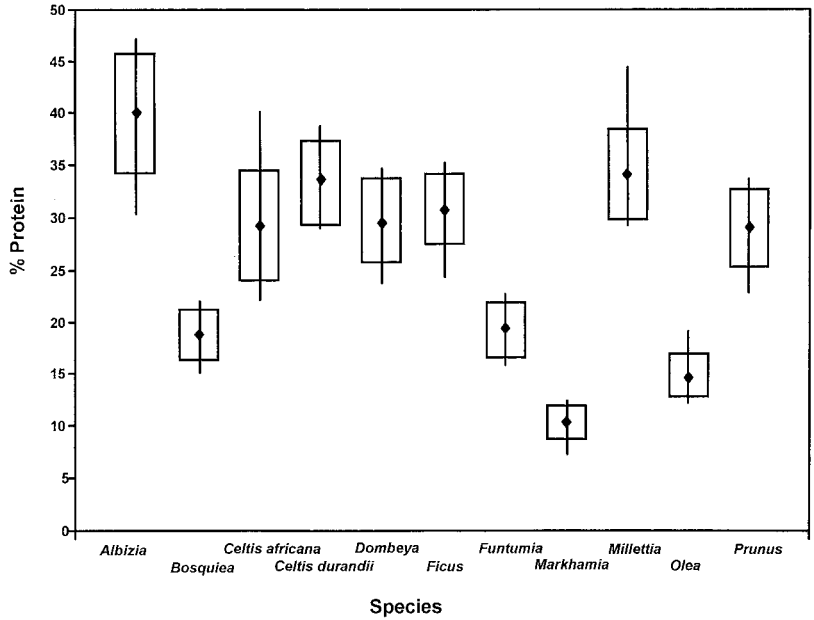

Fig. 1. Intraspecific (the same food item collected from a minimum of 10 individual trees, mean = 11.7, range = 10–16 trees) and interspecific variation in protein content of food items commonly eaten by the colobus of Kibale National Park. All samples were collected in June 1999. Indicated in the figure are the mean (diamond), the standard deviation (box), and range (ends of the lines). The species and food items are the following; *Albizia grandibracteaeta*, young leaves; *Bosqueia phoberos*, young leaves; *Celtis africana*, *young leaves*; *Celtis durandii*, young leaves; *Dombeya mukole*, young leaves; *Ficus exasperata*, young leaves; *Funtumia latifolia*, young leaves; *Markhamia platycalyx*, leaf petioles; *Millettia dura*, young leaves; *Olea welwitschii*, young leaves; *Prunus africana*, young leaves.

for protein, 12 for digestibility, and 43 for saponins, while the average CV among species are 35, 31.3, and 82.4, respectively.

No species showed a variable response with respect to testing for the presence or absence of cyanogenic glycocides, while 2 of 11 species tested for alkaloids showed a variable response.

Variation Among Time Periods

There is evidence of variation in the chemical compositon of the same food items eaten by the colobus in different months (Table I). Figure 4 illustrates the variation in our estimation of the protein content of the young leaves for 3 species for which we have the greatest number of collections.

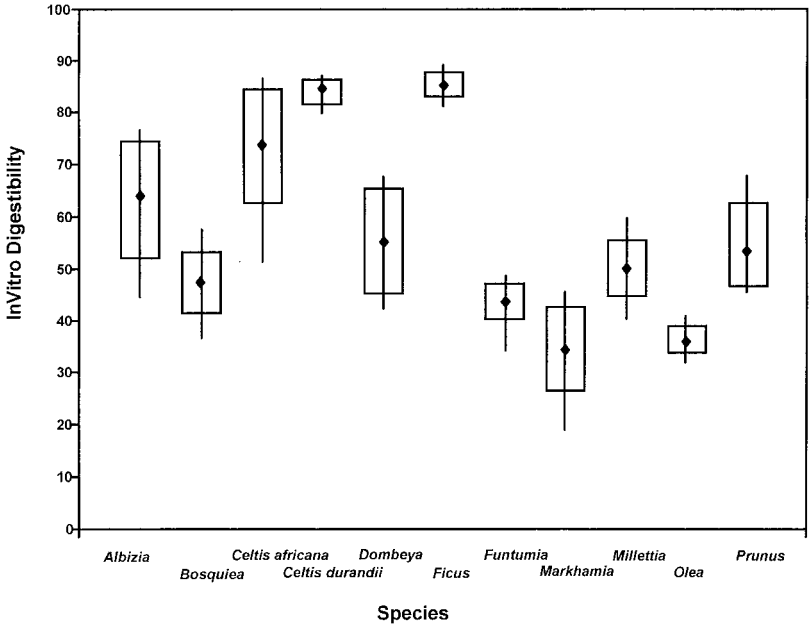

Fig. 2. Intraspecific (the same food item collected from a minimum of 10 individual trees, mean = 11.7, range = 10–16 trees) and interspecific variation in digestibility of food items commonly eaten by the colobus of Kibale National Park. All samples were collected in June 1999. Indicated in the figure are the mean (diamond), the standard deviation (box), and range (ends of the lines). Specific names and plant parts are in fig. 1.

We categorized each sample according to its season of collection and found that of the 11 variables only one showed a significant difference between seasons: Ca was more abundant in samples collected in the wet season ($t = 2.37$, $P = 0.02$).

We have estimates of the variation among individuals of the same species at one time and of the same food item among time periods for 6 species with respect to protein, digestibility, and saponin content (Table I). Of the 24 comparisons, variance differed significantly in 4 cases; in one instance variation among individuals is larger, while in 3 instances variation over time is larger than variation among individuals (Table I).

Spatial Variation in Protein to Fiber

We contrasted the protein-to-fiber ratio of the mature leaves collected from 3 or 4 sites within Kibale among 9 species (Fig. 5). The average CV

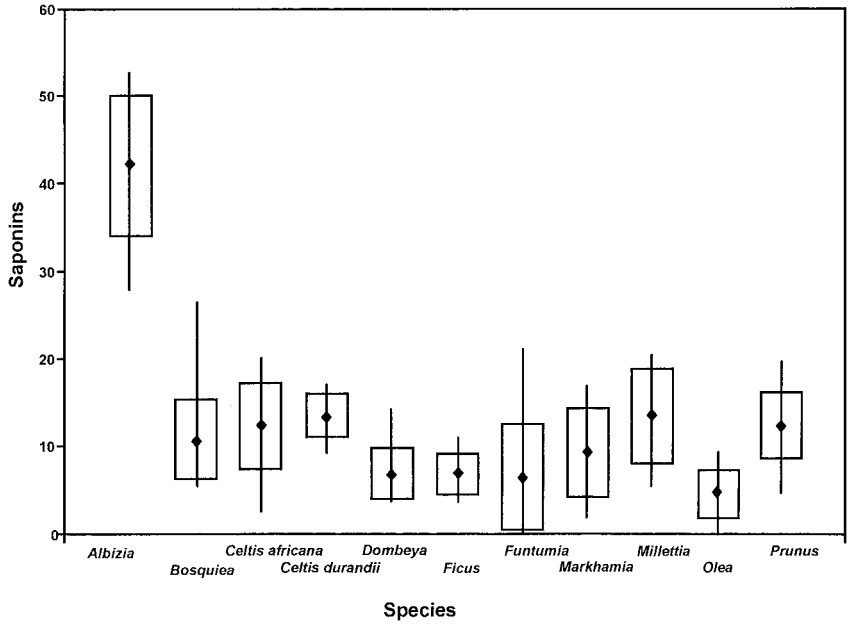

Fig. 3. Intraspecific (the same food item collected from a minimum of 10 individual trees, mean = 11.7, range = 10–16 trees) and interspecific variation in saponin content of food items commonly eaten by the colobus monkeys of Kibale National Park. All samples were collected in June 1999. Indicated in the figure are the mean (diamond), the standard deviation (box), and range (ends of the lines). Species names and plant parts are in fig. 1.

among species is 11.7. In some cases the variation among sites is greater than the differences among species. For example, the protein-to-fiber ratio of *Markhamia platycalyx* (mature leaves) varied little among sites (0.3 to 0.35) and while *Funtumia latifolia* showed little variation at 3 sites (0.44 at all sites), the remaining site was 28% greater.

DISCUSSION

Our results suggest that there is considerable variation in nutrient content of colobus food items among trees, time periods, and areas in a tropical forest. The variation documented among time periods is of a similar magnitude to interindividual variation. Since variation among time periods includes variation among time periods and individuals, the main source of variation is likely to be among individuals. Variation among distantly separated areas—12–15 km—appears to be less than variation among trees, as indicated by smaller CV, but is substantial for some species. Despite this

Table I. Details (average, standard deviation, coefficient of variation, and sample size) about the chemical composition of foods that were repeatedly sampled in different months in Kibale National Park, Uganda

Species	Part/Sample size	Protein	Digestibility	Saponin	Cu ppm	Mn ppm	Zn ppm	Fe ppm	Na ppm	Mg ppm	K ppm	Ca ppm
Albizia grandibracteata	Young leaves (13)											
	Average	43.37	77.02	33.77	12.75	35.26	39.84	137.30	177.83	1925.60	16669.58	4271.77
	StDev	1.77	6.62	16.33	9.18	12.18	12.75	57.81	76.53	719.52	4273.42	6366.97
	CV	4.08 (14.20)	8.60 (17.17)	48.34 (18.30)	71.98	34.54	32.01	42.11	43.03	37.37	25.64	149.05
Celtis africana	Young leaves (20)											
	Average	33.59	78.82	16.63	8.07	72.39	34.33	141.88	124.18	3333.92	12685.61	27031.22
	StDev	3.80	5.22	6.28	2.52	37.60	13.32	65.93	58.69	761.99	3616.92	6705.90
	CV	11.32 (17.55)	6.63 (15.13)	37.77 (40.01)	31.18	51.94	38.81	46.47	47.27	22.86	28.51	24.81
Celtis durandii	Young leaves (28)											
	Average	38.64	82.67	16.93	9.39	64.60	35.27	206.02	142.02	2173.18	17812.30	7098.26
	StDev	3.23	6.73	5.52	3.78	15.80	12.17	162.58	58.15	401.02	4112.13	3624.57
	CV	8.36 (10.75)	8.15 (2.80)	32.59 (19.43)	40.23	24.46	34.50	78.92	40.95	18.45	23.09	51.06
Diospyros abyssinica	Young leaves (5)											
	Average	23.51	47.78	21.67	6.60	157.18	29.02	118.32	86.48	2159.69	16084.63	7477.97
	StDev	5.73	9.75	10.54	4.56	67.94	6.85	53.01	47.23	626.98	3628.19	7074.16
	CV	24.36	20.40	48.64	69.04	43.23	23.61	44.80	54.61	29.03	22.56	94.60
Dombeya mukole	Young leaves (6)											
	Average	26.62	48.91	8.57	11.68	74.26	40.44	125.50	665.48	3314.66	24471.56	12082.69
	StDev	2.10	14.29	6.13	5.89	77.59	12.48	56.45	1115.10	907.34	5974.55	10131.99
	CV	7.91 (13.51)	29.21 (18.53)	71.56 (43.97)	50.43	104.48	30.85	44.98	167.56	27.37	24.41	83.86
Millettia dura	Young leaves (7)											
	Average	36.92	52.63	19.00	10.77	101.17	30.04	141.97	142.10	1999.15	15618.28	2677.85
	StDev	5.65	9.13	10.23	5.27	16.99	7.12	37.03	60.62	416.32	5449.86	1287.31
	CV	15.30 (12.73)	17.34 (10.67)	53.85 (39.26)	48.90	16.79	23.70	26.08	42.66	20.82	34.89	48.07
Olea welwitschii	Young leaves (7)											
	Average	16.17	32.24	4.21	6.47	21.57	18.19	79.87	133.26	1238.69	13146.89	5557.99
	StDev	1.22	4.77	2.80	1.58	3.12	2.99	12.17	104.03	245.84	3339.89	1004.47
	CV	7.52	14.79	66.36	24.41	14.48	16.43	15.23	78.06	19.85	25.40	18.07
Parinari exceisa	Young leaves (5)											
	Average	13.22	16.22	10.20	9.90	42.02	22.61	117.59	109.24	2108.19	10740.96	6401.18
	StDev	2.42	3.33	8.14	1.76	11.92	15.12	31.98	45.76	392.06	2587.46	8773.82
	CV	18.29	20.51	79.77	17.74	28.37	66.89	27.19	41.88	18.60	24.09	137.07
Prunus africana	Young leaves (5)											
	Average	26.25	54.46	4.20	9.45	27.16	39.15	263.83	146.05	2685.62	11505.39	6041.67
	StDev	6.88	7.36	3.19	3.69	5.94	22.63	309.26	70.87	1470.32	6012.96	3523.89
	CV	26.20 (12.25)	13.51 (14.22)	76.04 (29.97)	39.01	21.86	57.82	117.22	48.53	54.75	52.26	58.33
	Average CV	12.83 (13.50)	12.21 (13.09)	49.26 (31.82)	38.06	26.19	32.64	44.23	44.11	24.64	26.27	64.56

Note. The coefficient of variation among individuals is presented in brackets for the first the variables considered.

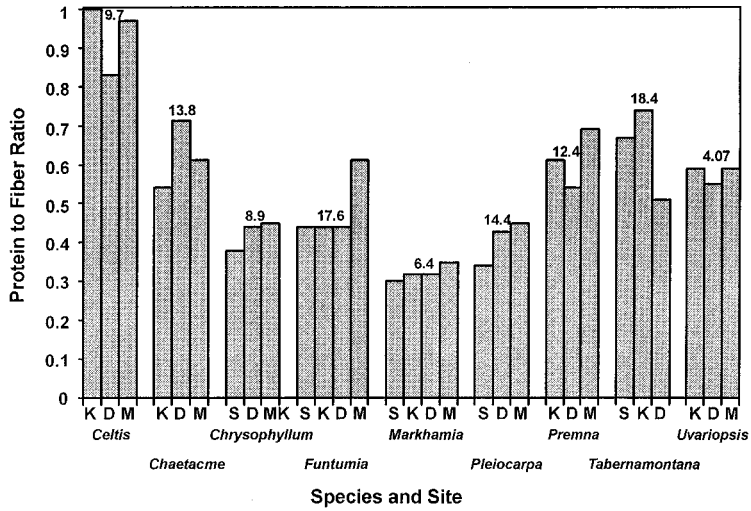

Fig. 4. Variation in protein content of the young leaves for 3 species on which the colobus of Kibale National Park, Uganda, repeatedly fed between October 1998 and May 2000.

variation, there are significant differences and specific ranges of nutrients for different tree species.

The relatively high levels of leaf nutrient variation among individuals of the same species raises the intriguing question about what leads trees in the same general area to be so chemically variable. Sunlight, soil composition, tree phenology, and local rates of microbial activity can vary locally and can have significant impacts on leaf nutrient concentration. It is well-established that increased irradiance results in increased leaf N content, and several researchers have concluded that irradiance is the single greatest factor affecting variation in N within trees of a single forest canopy (Marenco *et al.*, 2001; Schlesinger, 1997; Weinbaum *et al.*, 1994). A study of *Olea europaea* showed that leaf N varied 11.8% from the south side to the north side of a single tree (Perica, 2001). Thus, leaf protein content not only appears to differ between individuals of a single tree species but also varies within the canopy of a single tree. Tree phenology, including fruit production and leaf abscission is variable among individuals, can also affect leaf N and mineral concentrations (Fernandez-Escobar *et al.*, 1999; Vemmos, 1999; Weinbaum *et al.*, 1994). Vemmos (1999) found that leaf N, K, Mg, Mn, Ca, Phosphorus, Zn, and Fe differs significantly between fruiting and nonfruiting pistachio trees (*Pistachia vera*). Because fruiting synchrony occurs in only 64% of species at Kanyawara and Ngogo

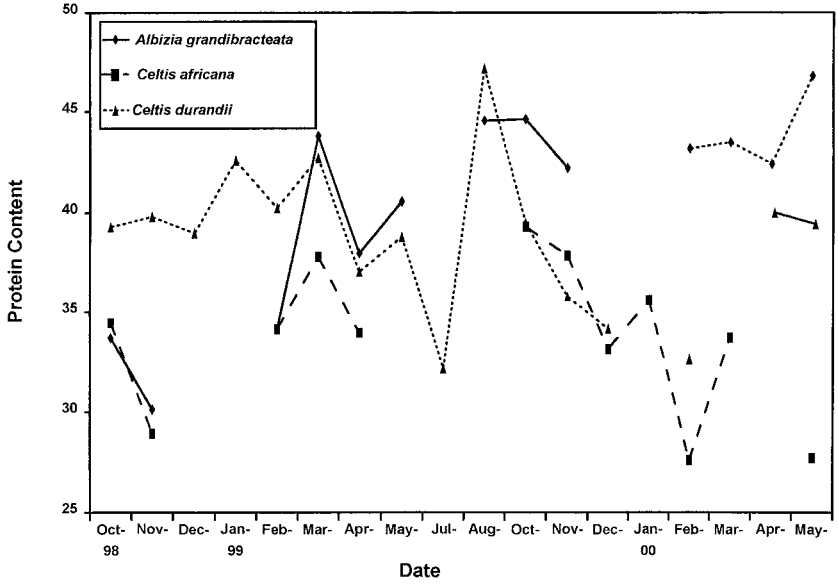

Fig. 5. Variation in the protein-to-fiber ratio of mature tree leaves among 4 sites in Kibale National Park, Uganda, each separated by *ca.* 12 km (S = Sebatoli, K = Kanyawara, D = Dura River, M = Mainaro). The Coefficient of Variation for each species in indicated above the bars for that species. The species considered are *Celtis durandii*, *Chaetacme aristata*, *Chrysophyllum sp*., *Funtumia latifolia*, *Markhamia platycalyx*, *Pleiocarpa pycnantha*, *Premna angolensis*, *Tabernamntana holstii*, and *Uvariopsis congensis*.

in Kibale National Park (Chapman *et al.*, 1999) variation in nutrient levels between individuals of the same species may be a result of variation in phenological stage. Because leaf N, P, and K levels often decline before leaf abscission (Killingbeck, 1996; Woodwell, 1974) variation in timing of senescence could also result in differences in nutrient levels between trees.

Nitrogen and mineral concentrations within a tree can be affected by local soil mineral availability and interactions between minerals both within the soil and within the plant (Beeson and Matrone, 1976; McDowell, 1992; Zeng *et al.*, 2001). For example, increased soil salinity results in higher leaf levels of Na and lower leaf levels of Ca, Mg, and K (Ferreira *et al.*, 2001). Vitousek and Sanford (1986) found that among tropical forests, major leaf nutrients are higher on more fertile soils. Higher soil N levels or increased N availability due to microbial activity can lead not only to higher leaf N levels but also to lower concentrations of P, Ca, and Mg in the leaves (Olff, 1992). Additionally, microbial activity can directly affectly soil mineral availability (Beeson and Matrone, 1976). Although the factors affecting

local leaf nutrient content are well-documented, they have typically been ignored when considering sampling methods for primate nutritional studies.

The magnitude of the plant variation can have significant impacts on the interpretation of primate nutritional ecological studies. For example, the protein-to-fiber ratio of red colobus foods has previously been documented as a significant predictor of their foraging effort after controlling for the effects of food tree density (Chapman *et al.*, 2002). The relationship is primarily driven by selection for young leaves of *Celtis durandii*, which, based on 28 separate plant collections, have the highest protein-to-fiber ratio of any plant part eaten (35% higher than the second highest species/part). However, if instead of using data from these 28 collections, we used only a single sample of *Celtis durandii*, selection for high protein, low fiber foods would not have been shown for 14% of the samples tested.

High intraspecific variation in nutritional content may also affect studies of seasonality in nutrient content. For example, Baranga (1983) monitored 10 individuals of *Celtis durandii* and *Markhamia platycalyx* over a year to examine seasonal variation in protein and mineral content. Based on our results, a lack of clear seasonality could have been masked by intraspecific variation. Seasonal differences may best be examined by monitoring individual trees.

Based on our results, the most accurate method to determine primate nutrient intakes is to collect samples from the exact trees used by the primate groups. Unfortunately, this can be difficult as primates often dramatically reduce the number of food items in a tree during a feeding bout (Chapman, 1988; Chapman and Chapman, unpub for colobus; Gillespie and Chapman, 2001; Whitten, 1988). If it is not possible to collect samples from the tree the animals used, then samples should be collected from several trees. Since variation over time appears to be less than variation among individuals, samples from different individuals should provide a more accurate estimate of leaf nutrient content than replicate samples of the same plant species/part at different times. Fortunately, obtaining samples from several trees at one time is logistically much easier than obtaining samples of the same species/part at different times. Samples from different trees can be mixed, dried, and processed together to provide an average nutrient content for a single species and part. Ultimately, how much one has to be concerned about different sources of variance will depend on the question being asked. For studies with very specific questions, such as selection of specific plant parts, it is likely very important to obtain items from the exact tree in which the animals are feeding.

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