

UNCORRECTED PROOF

Leaf choice in black-and-white snub-nosed monkeys *Rhinopithecus bieti* is related to the physical and chemical properties of leaves

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Abstract: To understand the effects of the chemical and physical properties of plant leaves on food choice in *Rhinopithecus bieti*, we collected mature leaves of nine food and five non-food plant species at the southernmost part of the species's range. Chemical properties such as fat, ash, crude protein (CP), total phenolics (TP), and fiber content including neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, celluloses and hemicelluloses, and physical toughness were measured. *R. bieti* tended to choose leaves with lower fiber content, higher ash, a higher ratio of CP/ADF, and lower toughness. No difference was found for fat, crude protein, total phenolics, hemicelluloses and lignin between food and non-food leaves. Even though the ratio of CP/ADF is generally regarded as a good indicator for colobine food choice, the difference in the ratio of CP/ADF between food and non-food leaves was only the result of differences in ADF. Since positive correlations were found between ADF and toughness from all leaves (both food and non-food species), and toughness of leaves was likely easier for *R. bieti* to sense than fiber content via mastication, the toughness of leaves may function as a predictor of food choice in this species [*Current Zoology* 56 (6): – 2010].

Keywords *Rhinopithecus bieti*, diet selection, protein-to-fiber ratio, toughness, Mt. Longma, Yunnan

The food choices of primates can be examined using five major nutritional hypotheses: (i) energy maximization (Schoener, 1971): a high energy intake equates with a high quality diet (Leighton, 1993; Cowlshaw and Dunbar, 2000; Lambert, 2007); (ii) nitrogen (protein) maximization: nitrogen is a limiting factor for growth,

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health, reproduction and survival (Mattson, 1980); (iii) avoidance or regulation of plant secondary metabolites (PSMs) (Freeland and Janzen, 1974): plant secondary metabolites interrupt protein digestion and the activity of digestive enzymes, and are considered as inhibitors of digestion (Rhoades and Cates, 1976; Swain, 1979; Haslam, 1989); (iv) limitations of dietary fiber (Milton, 1979): food items high in fiber (NDF, ADF, lignin) are difficult to digest and are avoided (Milton, 1979; Yeager et al., 1997); and (v) nutrient balancing: the fundamental aim of wild primates is to balance nutrient intake (Davies et al., 1988; Whiten et al., 1991; Raubenheimer and Simpson, 2004). These hypotheses have been reviewed in detail by Felton et al. (2009).

It has been proposed that many folivorous primates deal with digestive limitations by selecting leaves that are rich in protein and low in dietary fiber (Davies et al., 1988; Waterman et al., 1988). Plant fiber consists predominantly of cellulose and hemicelluloses contained within plant cell walls and digestible through bacterial fermentation, and lignin, which cannot be digested by mammals or their microbes (Van Soest, 1994). Amongst primates, colobines are widely known for their ability to digest cellulose using a multi-chambered stomach (Fashing, 2007; Kirkpatrick, 2007). Fiber content is considered a major indicator of food quality, and data on several primate species have shown that fiber content, as a percentage of dry matter, is inversely related to food choice (Milton, 1979; Waterman et al., 1988; Whiten et al., 1991). Examinations of primate food choice and protein content further suggest that some primates prefer foods high in protein (Fashing et al., 2007), choose protein-rich parts and supplement their diet with insects (Oftedal, 1991; Chapman et al., 2004; Xiang, 2005)¹.

The influence of nutrition on food choice in primates has been studied most extensively in colobine monkeys (Chapman and Chapman, 2002; Davies et al., 1988; Kool, 1992; McKey et al., 1981; Mowry et al., 1996; Yeager et al., 1997). This group possesses an enlarged, sacculated forestomach containing large quantities of anaerobic bacteria, protozoa, and fungi that ferment ingested food (Kay and Davies, 1994). This capacity for microbial fermentation in the forestomach allows colobines to extract nutrients contained within the fibrous cell walls of plants and to detoxify plant secondary compounds such as phenolics and tannins (Kay and Davies, 1994). Food choice in colobines is believed to be primarily dependent on protein and fiber content (Oates et al., 1980) and they appear to select rare but protein-rich resources during critical periods of growth and reproduction (Cords, 1986; Soued and Gautier-Hion, 1986; Butynski, 1988. but see Lambert 2007). McKey et al. (1981) and Waterman and Choo (1981) posited that the protein-to-fiber ratio (CP/ADF) as a solid predictor of food suitability for colobines. This ratio also explains variation in colobine biomass across different study sites (Oates et al., 1980; Davies, 1994;

¹ Xiang ZF, 2005. The ecology and behavior of black-and white snub-nosed monkey *Rhinopithecus bieti* in Honglaxueshan National Reserve, Tibet. Unpublished Ph.D. Dissertation, Kunming Institute of zoology, Chinese Academic of Science, Kunming, China.

Chapman et al., 2002; Chapman et al., 2004; Fashing et al., 2007).

Minerals function as structural components of organs and tissues, as cofactors or activators in enzyme and hormone systems, as constituents of body fluids and tissues, and as regulators of cell replication and differentiation (Underwood and Suttle, 1999). Some primates travel long distances to reach rare items rich in particular minerals, impacting both their food choices and behavior (Fashing et al., 2007). However, factors other than the chemical properties of plant foods may influence primate food choice, such as the physical properties (toughness). Fracture toughness is defined as resistance to crack propagation, and it plays a critical role in the ability of leaves to resist physical damage (Lucas et al., 2000). A clear relationship was found between the toughness of plant parts and whether or not they were eaten by Japanese macaques (*Macaca fuscata yakui*) (Hill and Lucas, 1996). Thus, both physical and chemical properties of food should be considered in assessments of food choice in primates.

R. bieti is found only in conifer forests of the eastern Himalayas (Long et al., 1994), in an area (<25, 000 km²) between the Mekong River (to the west) and Yangtze River (to the east), and latitudes of 26.14° N and 29.20° N (Long et al., 1996, Xiao et al., 2003). The diet of *R. bieti* in their natural habitat has been studied by several researchers at different sites of this range. These studies have suggested that the major dietary components of *R. bieti* are lichen and leaves, and that it has a more diverse diet in its southern range than northern (Li et al., 1981; Wu, 1991; Kirkpatrick, 1996; Yang, 2000²; Ding and Zhao, 2004; Huo, 2005³; Xiang, 2005). Food choice and the chemical properties of leaves was examined by Kirkpatrick (1996) in *R. bieti*'s middle range, and suggested that contrary to the pattern of food choice for other colobines, *R. bieti* tends to choose mature leaves with a low CP/ADF ratio. However, no studies have been done at other sites to test the generality of these findings.

To understand which properties of leaves have an impact on food choice for *R. bieti*, we selected a group living in Mt. Longma located in the southern most extreme part of *R. bieti*'s range and that has the highest dietary diversity of the species (Huo, 2005). Toughness was included as an index as well as chemical properties. Only mature leaves used by *R. bieti* in autumn were sampled to minimize the impact of seasonal variation.

² Yang SJ, 2000. Habitat, diet, range use and social organization of *Rhinopithecus bieti* at Jingsichang. Ph.D. dissertation, Kunming Institute of Zoology, CAS, Kunming, China.

³ Huo S, 2005. Diet and habitat use of *Rhinopithecus bieti* at Mt. Longmashan, Yunnan. Unpublished Ph.D. Dissertation, Kunming Institute of zoology, Chinese Academic of Science, Kunming, China.

1 Materials and Methods

1.1 Samples collection and analysis

We collected mature leaves of nine food species and five non-food species from Mt. Longma (26.23°N, 99.25°E; Fig. 1) in November 2005, based on dietary data collected by Huo (2005). Nine food species were all autumn food ones that can be found during study period. Non-food species were those occurred in monkey habitat but never reported as food species in former studies (Li et al., 1981; Wu, 1991; Kirkpatrick, 1996; Yang, 2000⁴; Ding and Zhao, 2004; Xiang, 2005). Mature leaves were chosen from the mid–upper portion of tree crown and on the outside of the tree trunk for each individual. Three samples were collected from different elevations and data was averaged for analysis for each species (Appendix 1).

The toughness of mature leaves was measured in our field station as soon as possible after samples were collected. The scissor-cut method was used to measure the toughness of mature leaves (Lucas et al., 2003). The leaves were cut at quarter length, and the mean of three measurements of toughness was used in our analysis.

In the field, we prepared extractions from a subsample of fresh leaves using 50 % methanol; 50 g all fresh leaves of each specimen was used for extraction. We assayed total phenolics following Lucas et al. (2003).

Remaining leaf samples were dried at room temperature in a ventilated room, and transported to the Kunming Institute of Zoology, Chinese Academy of Science (KIZ) for further analysis after one week. There, samples were dried at 60 °C to constant weight, and stored in desiccators at room temperature. We assayed macronutrients and ash in the nutritional laboratory at KIZ. All macronutrient data are presented on a 100 % dry matter (DM) basis. We analyzed samples for fat, crude protein (CP), neutral detergent fiber (NDF), hemicelluloses (HC), acid detergent fiber (ADF), cellulose (CEL), sulfuric acid lignin, and ash content. We calculated CP content by multiplying total nitrogen by 6.25 using a macro-Kjeldahl method with a Cu catalyst (Cunniff, 1996). We conducted analyses of the fibrous components of plant cell walls (NDF, HC, ADF, CEL, and lignin) using detergent methods (Van Soest et al., 1991).

1.2 Data analysis

All data were analyzed by SPSS 15.0 (SPSS Inc., Chicago, USA) and tested for normality. We used a combination of parametric and non-parametric tests. All tests are two-tailed, unless noted. We compared the macronutrient, ash, total phenol, and toughness of food leaves versus non-food leaves using a Mann-Whitey *U*

⁴ Yang SJ, 2000. Habitat, diet, range use and social organization of *Rhinopithecus bieti* at Jingsichang. Ph.D. dissertation, Kunming Institute of Zoology, CAS, Kunming, China.

test. We also used Spearman Rank correlations to examine the relationship between fiber content (ADF), ash content, ratio of CP/ADF, and toughness. A stepwise linear regression was used to examine the effects of toughness and ash content on ADF content including food and non-food species.

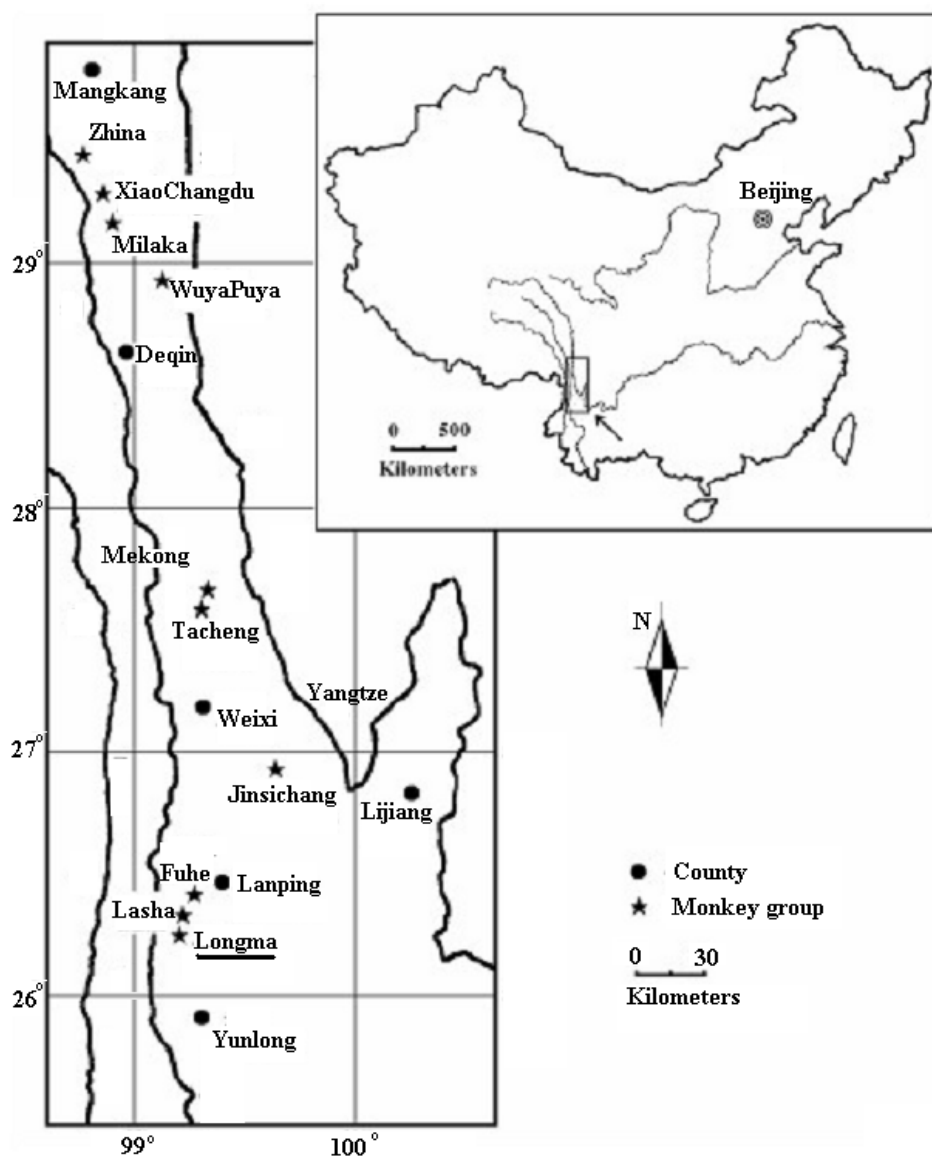


Fig. 1 Distribution of black-and-white snub-nosed monkey

The study group at Mt. Longma is the southmost group of its range.

2 Results

Food leaves had lower NDF, ADF, CEL content and lower toughness compared with non-food leaves (Mann-Whitey U test, NDF: $U = 2.739$, $P = 0.006$; ADF: $U = 2.867$, $P = 0.004$, CEL: $U = 2.739$, $P = 0.006$, toughness: $U = 3.00$, $P = 0.003$). Food leaves were also found to have a higher ash content, and a higher ratio of CP/ADF than non-food leaves (Ash: $U = 2.467$, $P = 0.014$, CP/ADF: $U = 2.867$, $P = 0.004$). No difference was found for fat, crude protein (CP), total phenolics (TP), hemicelluloses and lignin (Fat: $U = 0.801$, $P = 0.423$,

CP: $U = 1.402$, $P = 0.161$, TP: $U = 0.467$, $P = 0.641$, HC: $U = 1.80$, $P = 0.072$, Lignin: $U = 0.868$, $P = 0.386$).

The CP/ADF ratio was higher in food leaves than non-food leaves. *R. bieti* tended to chose leaves with higher protein and lower ADF content (Fig. 2). Because CP content was relatively constant ($U = 1.402$, $P = 0.161$), differences in the CP/ADF ratio resulted primarily from the differences in ADF content. *R. bieti* tended to choose mature leaves high in protein and low in ADF.

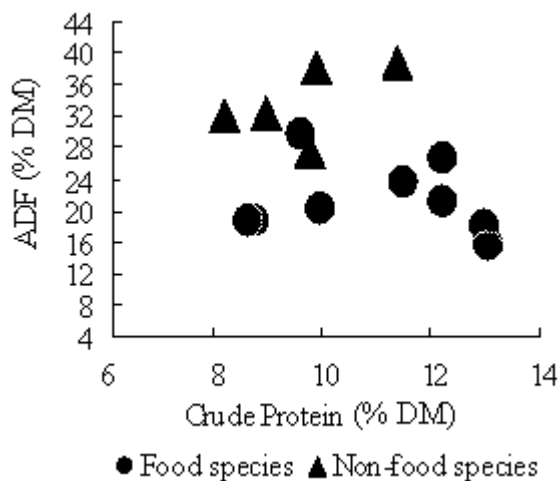


Fig. 2 Acid detergent fiber and nitrogen ($\times 6.25$) in mature leaves selected and rejected by *R. bieti* at Mt. Longma

A positive correlation was found between toughness and ADF content ($r_s = 0.719$, $P = 0.004$, $n = 14$) and a negative correlation was found between ash and ADF content ($r_s = -0.688$, $P = 0.007$, $n = 14$). A negative correlation was found between ash content and toughness ($r_s = -0.556$, $P = 0.039$, $n = 14$) in all leaves, including both food and non-food species. Our stepwise linear regression test indicated that toughness influenced ADF content and that it may be a predictor of ADF content in plant leaves including food and non-food species ($F_{1,12} = 18.723$, $r = 0.781$, $P = 0.001$).

3 Discussion

R. bieti at Mt. Longma prefer leaves with high CP/ADF, which agrees with previous studies of colobine nutritional goals which appear to maximize nitrogen and limit dietary fiber (Mattson, 1980; Milton, 1979; Felton et al., 2009). This pattern of food choice is contrary to that of the Wuyapuya group at Mt. Baima (Kirkpatrick, 1996) and we posit two reasons to explain this difference. First, more food species are found at Mt. Longma than at Wuyapuya (Huo, 2005), and because we selected the main food species the results of our study may be a more comprehensive assessment of food choice for *R. bieti* than former studies. Second, plant specimens were collected across different seasons at Wuyapuya and mature leaves samples were grouped together regardless of season potentially biasing the results of Kirkpatrick (1996). Seasonal variation in CP/ADF occurs because of

variations in the chemical composition of leaf material across months and seasons (Chapman et al., 2003; Jaman et al., 2010).

R. bieti tended to choose mature leaves with lower fiber content (NDF, ADF, and CEL). This phenomenon has been demonstrated in several other colobine species (*C. guereza*: Chapman et al., 2004; *C. guereza*: Bocian, 1997; *C. angolensis*: Maisels et al., 1994; *C. polykomos*: Dasilva, 1994). This pattern might initially seem surprising in light of the well-known ability of colobines to extract nutrients from the fibrous cell walls of plants by forestomach fermentation (Bauchop, 1978; Bauchop and Martucci, 1968; Watkins et al., 1985); however, extensive reliance on the extraction of nutrients from high-fiber foodstuffs requires long gut retention times, a luxury probably unavailable to colobines owing to their relatively small body and gut-size relative to other foregut fermenters such as cattle (Kay and Davies, 1994). Unlike large-bodied ruminants such as cows and buffaloes which have the capacious guts necessary to focus on fibrous, low energy foodstuffs, colobines appear to adopt a more selective feeding strategy that limits their reliance on foregut fermentation for nutrition (Fashing, 2007).

What sensory cues are available to primates to assess the nutritional value of food? Sensations within the gut are difficult to attribute to a particular food, and visceral sensory pathways are poorly developed in vertebrates as a whole (Romer and Parsons, 1986). The most efficient time for an animal to assess a food would be just before or during ingestion. As fiber is odorless, tasteless, and colorless, the only rapid means of assessment available via oral sensation likely arises from mechanical properties (Hill and Lucas, 1996). In this study, toughness was another predictor of leaf choice by *R. bieti* in addition to fiber content. Toughness was positively correlated with ADF content and therefore relative digestibility. Therefore, *R. bieti* may be choosing food plant leaves with lower fiber content by sensing toughness.

To confirm our result, further studies are needed across different sites. A comparative study is also required for analysis of all diet components and selected major food species. The role of minerals on dietary selection of *R. bieti* should also be emphasized, as minerals can be as important as protein to primates (Lambert, 2007).

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Appendix 1 Mature leaves of food and non-food species and their chemical and physical properties from Mt. Longma, Nov 2005

Mature leaves of species (elevation, m)	Food sp.	Fat	CP	Ash*	NDF*	HC	ADF*	CEL*	Lignin	TP	Toughness*	CP/ADF*
<i>Acero liverianum</i> (3000,3220, 3260)	Y	7.9	10.0	8.1	31.1	11.1	20.1	9.2	10.6	0.139	0.181	0.692
<i>Schisandra rubrifloa</i> (3100,3210, 3260)	Y	5.7	13.0	9.9	28.6	10.6	18.0	10.5	7.3	0.234	0.233	0.842
<i>Sorbus thibetica</i> (3000,3100, 3250)	Y	12.6	8.6	6.3	30.9	12.3	18.6	16.7	1.8	0.122	0.180	0.669
<i>Holboellia angustifolia</i> (3000,3100, 3200)	Y	5.8	11.5	8.9	42.9	19.3	23.6	19.1	4.5	0.050	0.252	0.682
<i>Malus asiatica</i> Nakai (2900,3000, 3100)	Y	23.4	8.8	5.1	31.4	12.7	18.7	7.9	10.6	0.248	0.133	0.672
<i>Lonicera japonica</i> (3050,3100, 3200)	Y	6.0	12.3	8.4	35.1	14.0	21.0	8.9	11.8	0.082	0.122	0.751
<i>Betula alnoides</i> (2900,3000, 3100)	Y	10.7	9.6	4.3	45.0	15.4	29.6	14.3	14.9	0.176	0.139	0.548
<i>Smilax glaucophylla</i> (2800,2830,2850)	Y	8.8	13.1	8.0	25.1	9.6	15.5	10.6	4.7	0.144	0.075	0.915
<i>Nothopanax delavayi</i> (2720,2760,2780)	Y	5.5	12.3	10.1	43.8	17.2	26.6	10.2	16.0	0.042	0.123	0.660
Mean		9.6	11.01	7.66	34.88	13.6	21.3	11.93	9.14	0.14	0.16	0.72
<i>Tsuga dumosa</i> (3150,3200,3250)	N	7.6	9.0	4.4	48.5	16.2	32.3	23.2	8.8	0.173	0.736	0.504
<i>Pinus yunnanensis</i> (2920,2950,3000)	N	6.0	11.4	3.2	57.4	18.7	38.7	25.3	12.9	0.033	1.181	0.513
<i>Quercus semecarpifolia</i> (3000,2950,2900)	N	13.8	9.9	3.3	60.2	22.1	38.1	24.0	13.8	0.223	0.598	0.481
<i>Picea crassifolia</i> (3200,3250,3300)	N	9.3	8.2	4.6	45.0	13.1	32.0	18.3	13.2	0.168	1.169	0.484
<i>Rhododendron delavayi</i> (3100,3150,3250)	N	11.4	9.8	5.4	43.8	16.5	27.4	16.9	10.2	0.204	0.529	0.578
Mean		9.64	9.66	4.17	50.99	17.2 9	33.69	21.53	11.77	0.16	0.84	0.51

*Significant difference found between mature leaves of food and non-food species. CP is crude protein; NDF is neutral detergent fiber; HC is hemicelluloses; ADF is acid detergent fiber;

CEL is celluloses; TP is total phenolics; CP/ADF is crude protein/acid detergent fiber content. Fat, CP, Ash, NDF, HC, ADF, CEL and Lignin are expressed on a 100% DM basis. TP expressed on Gallic acid equivalents ($\mu\text{g/mL}$). Unit of toughness is KJ/m^2 . Bold values indicate significant differences between food and non-food species.