Research Article

What is the predictive power of the colobine protein-to-fiber model and its conservation value?

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Abstract
Predicting variation in animal abundance across time and space has proven very difficult; however, a model exists to predict the biomass of small folivorous primates that has considerable correlative support. This model suggests that the protein-to-fiber ratio of leaves in a habitat can predict folivore biomass. Here we present an experimental test of this protein-to-fiber model to assess if the number of infant monkeys per female and group size can be predicted based on the leaf chemistry of a habitat. We expected regenerating forest in Kibale National Park, Uganda to have leaves with higher concentrations of crude protein and lower concentrations of fiber than old-growth forest trees, and consequently, we expected a greater number of infants per female in the folivorous red colobus (*Procolobus rufomitratus*) with access to this area. As predicted, regenerating forests did have trees with leaves with high concentrations of protein and low concentrations of fiber, but there was no corresponding change in the demographic structure of red colobus groups. We also tested whether energy was a potential determinant of these parameters, but found no evidence for its importance. Our findings support recent studies that are critical of the protein-to-fiber model, which lead us to question the model's generality, particularly for conservation and management.

Keywords: red colobus, Kibale National Park, restoration, population regulation

Resumen
Predecir la variación en la abundancia animal a través del tiempo y espacio ha sido muy difícil; sin embargo, existe un modelo para explicar la biomasa de pequeños primates folívoros que tiene un considerable soporte de correlación. Este modelo sugiere que el radio de proteína-a-fibra en hojas en un hábitat determinado puede predecir la biomasa de primates. Presentamos aquí una prueba experimental de este modelo de proteína-a-fibra para estimar si el número de crías por hembra y el tamaño de grupo pueden predecirse basándose en la química de las hojas de un hábitat. Nosotros esperamos que la selva en regeneración en el Parque Nacional de Kibale, en Uganda tenga hojas con mayores concentraciones de proteína cruda y menores concentraciones de fibra que el bosque maduro y en consecuencia, esperamos un mayor número de infantes por hembra en el colobo rojo (Procolobus rufomitratus) un mono folívoro que habita esta área. Como se predijo, las selvas en regeneración tuvieron más arboles con hojas con altas concentraciones de proteínas y bajas concentraciones de fibra, pero contrario a lo esperado no hubo cambios en la estructura demográfica de los grupos de colobos rojos. También probamos si la energía era un determinante potencial de esos parámetros, pero no encontramos evidencia de su importancia. Nuestros resultados apoyan estudios recientes que critican el modelo de proteína-a-fibra, lo que nos permite cuestionar la generalidad del modelo, en particular para la conservación y gestión.

Palabras claves: colobo rojo, Parque Nacional Kibale, regeneración, regulación poblacional.

Introduction

Since animal populations are not distributed evenly across the landscape, a fundamental issue in ecology is determining what factors affect their density and distribution over space and time [1]. The importance of this issue has recently increased because of human-induced changes to the environment due to increased consumption and population growth [2-4]. Ultimately, a clear understanding of the factors influencing animal abundance and distributions is critical for the development of informed conservation and management plans. However, understanding and predicting factors that affect the abundance of particular species have proven extremely difficult. Yet despite the need for this understanding in conservation, there are few direct tests of general hypotheses purporting to account for the variation in populations across a landscape. A notable exception deals with folivorous primates.

Milton [5] proposed that the protein-to-fiber ratio of leaves was an important criterion for leaf selection by primates, whereby leaves with higher protein-to-fiber ratios were selected over those with lower ratios. Fiber is often considered an antifeedant because it requires fermentation by symbiotic microbes, and insoluble fiber (cellulose, hemicellulose, and lignin) is only partially digestible by microbes [6]. Furthermore, since nitrogen is a limiting nutrient in many food webs and is predominantly found in protein, herbivores should compensate for this limitation by choosing foods with higher protein-to-fiber ratios [7]. This seminal idea proposed by Milton has since been applied at the population level by others [8-11]. Waterman et al. [10] suggested that the weighted contributions of the protein-to-fiber ratios of the mature leaves of the most abundant trees in a particular habitat could predict the biomass of folivorous colobines. Subsequently, this index of dietary quality has been successfully applied to predict the biomass of small-bodied folivorous monkeys at local [12, 13] and regional scales [9, 10, 14-16]. The mechanism by which this index operates to determine folivore biomass is not clearly understood. Davies [14] suggested that the year-round availability of digestible mature leaves with high protein-to-fiber ratios, which are used by colobus species when other, more preferred foods are unavailable, serves to limit the size of colobine populations (i.e., high protein to fiber mature leaves are important fallback foods). However, some colobines rarely eat mature leaves since young leaves are always available, yet their biomass is still predicted well by this index [15]. One possible explanation is that the protein-to-fiber ratio of mature leaves in an area is correlated with the protein-to-fiber ratio of foods in general. This idea is supported by the fact that in a sample of leaves from Kibale National Park, Uganda, we found that the protein-to-fiber ratios of mature and young leaves were strongly correlated (r=0.837, P<0.001) [15]. Thus, measuring the protein-to-fiber ratio of mature leaves may be useful because it correlates

with the general availability of high-protein, low-fiber foods, and thus is a useful index of habitat quality for colobus monkeys.

While these studies suggest that the ratio of protein to fiber of leaves is a critical factor limiting populations of folivorous primates, available data are insufficient to confidently use this model to construct management plans. This skepticism is based on four main factors. First, studies attempting to empirically verify the protein-to-fiber model are correlative, and it is quite possible that this ratio is related to another factor that is truly driving the population density of small folivorous monkeys [17-19] (See [19] for statistical issues concerning use of a ratio). Second, protein concentrations of ingested leaves are higher than species' estimated requirements [20, 21]. Third, we should use available nitrogen to evaluate this nutritional model, but total nitrogen is what is typically measured, and tannins and fiber-bound nitrogen may reduce the availability of nitrogen [19, 22, 23]. Finally, new empirical data draw into question the generality of this model.

For example, while gorillas (*Gorilla beringei*) and colobines have different digestive physiology, it has been found that instead of selecting high protein foods, folivorous gorillas over-ingested protein when eating a leaf-based diet instead of a fruit-based diet [20], suggesting that they prioritize energy, not protein. It could be that small-bodied folivorous monkeys are using a similar strategy. A second example deals with the red colobus (*Procolobus rufomitratus*) of Kibale National Park, Uganda, the species and site of the study reported here. Here, changes in the density of red colobus over 36 years could not be predicted by changes in the protein-to-fiber ratio of mature leaves that occurred as a result of changes in the composition of the forest [17, 18]. These methodological issues, combined with the recent studies not providing empirical support, suggest that it is extremely premature to use this predictive model in conservation (e.g., recommending that restoration efforts grow trees with high protein and low fiber leaves, which might have unforeseen impacts on ecosystem stability and health).

In Kibale National Park, Uganda, after episodes of deforestation and subsequent land abandonment, forest regeneration has occurred as a consequence of managed replantation of native species, harvest of introduced pine plantations, , and natural regeneration, which successfully broke the arrested succession [24]. The regenerating areas have many colonizing tree species, the leaves of which are expected to have higher concentrations of protein and lower concentrations of fiber than old growth tree species [25]. Kibale is a stronghold for endangered red colobus monkeys (*Procolobus rufomitratus*) that prefer leaves of this type [11]. Previous research concerning the plant chemistry of tree species found in regenerating forests elsewhere in the tropics suggests that regenerating forest initially have plants with a higher protein-to-fiber ratio [25]. We take advantage of the increased access of red colobus to these newly available habitats to provide a new test of the protein-to-fiber model.

Based on the protein-to-fiber model, we expected red colobus groups using regenerated areas to have higher birth rates than groups that were limited to old growth forests, because they have access to higher quality foods. Variation in the nutritional content of plants, particularly plant secondary metabolites, has been demonstrated to impact reproductive success of the common brushtail possum (*Trichosurus vulpecula* Kerr [26]), and enhanced nutrition has been documented to increase reproduction and decrease infant mortality in several primate species [27-29]. Thus, if improved nutrition impacts reproductive rates, higher birth rates will be represented as higher infant to female ratios (i.e., adult females have shorter interbirth interval and infants have a greater probability of surviving in groups that have a nutrient rich diet), which will ultimately result in higher colobus biomass. Groups with access to regenerating areas were also expected to be larger for three reasons: first,

because there is a long delay between when infants are born and when they disperse, the number of immature animals will be high; second, because red colobus males tend to remain in their natal group, the increased birth rate will result in more males which remain as residents; and third, since nutritionally good foods will be abundant in regenerating areas, the home range of the group can be small and thus biomass will be high (Chapman et al. unpublished data).

To test these predictions, we first verified if the regenerating areas had higher leaf quality than old growth forest (indicated by the protein-to-fiber ratio). Subsequently, we examined the difference in the number of infants per female among groups that had access to regenerating forest and groups that did not. Given past interest in energy as a determining factor of folivorous primate abundance [30, but see 31] and that black and white colobus (*Colobus guereza*) population sizes in Kibale appear to be limited by the availability of energy [32], we also tested if differences in demographic variables might be correlated with indices of energy; total energy, non-protein energy, and non-structural carbohydrates, which are easily digestible energy sources [23].

Methods

Study site

Kibale National Park (795 km^2), Uganda is a moist, evergreen forest that receives 1696 mm of rainfall annually (1990-2011; Chapman unpublished data; [33, 34]. Pine plantations (*Pinus caribaea, P. patula, Cupressus lusitanica*) were established in Kibale between 1953 and 1977 on grasslands that were previously forested lands that had been cleared, cultivated, and abandoned by agriculturalists shortly after 1900 [35-37]. Active fire exclusion was initially important to protect young pine seedlings, but became less important as the plantation matured. Similar areas not planted with exotic trees are still largely fire-maintained grassland [38, 39]. Once the plantations matured, native tree species and shrubs invaded the understory and were not removed by the plantation managers [38, 40]. Management plans changed when Kibale became a national park in 1993: plantations started to be harvested and the areas were left to regenerate to native forest from the few remaining indigenous trees, resprouts from trees damaged by the harvest, seeds in the soil seed bank, or newly arrived seeds deposited in the area by animal or wind dispersal. The sites were surrounded by natural forest with abundant bird and mammal seed dispersers; the interiors of former pine plantations were consistently less than 250 m from natural forest. We compare demographic variables in groups with access to the former pine plantations and those without (see definitions below). Groups without access to the former pine plantations were found in areas of forest in the Kanyawara area. These forests include areas that were old growth forest (known as forestry compartment K30) and forests that had been selectively logged in the late 1960s (K14, K15, K31; [41]. Hereafter, all of these forest types are called old growth as they are old relative to the forest of the regenerating pine plantation. To consider variation in these old growth forests, analyses were conducted with all of these forestry compartments and also just considering the compartment that never experienced mechanical logging (K30).

Field Methods

We conducted group counts from July 2006 to January 2011 (*n*=42 groups). When groups are large and animals are widely dispersed, obtaining an accurate count of the group is very difficult, particularly if they are in the dense foliage of a tropical forest. Our strategy was to census red colobus groups in a slightly different area of the forest during eight days each month (two consecutive days, followed by a day off, followed by 6 consecutive days). When a primate group was encountered along the way or in the designated search area, three skilled observers would record the GPS location and try to make an accurate count. To obtain reliable estimates of group size, identity, and composition, observers would

frequently stay with a group for up to 10 hrs, often needing to return to the group the next day, and wait for members to make a coordinated movement across an opening in the canopy, such as a large canopy gap or a road. Repeat counts were made of the same group to ensure accuracy. Groups were identified either by individually recognizable members or by matching the group count to those made previously, and matching the area in which the group was seen to the next count and the typical home range for the species [42]. For groups that were counted repeatedly over the 5 years, we used the average female to infant ratio for analysis.

In May 2002, two 200 x 100 m (2 ha) plots were established in the harvested pine plantation and each plot was divided into fifty 20 x 20 m sub-plots to facilitate sampling (see [24] for details). The plots were adjacent to one another and separated by approximately 20 m. Plots were dominated by a variety of grasses, primarily *Pennisetum purpureum* and *Hyparrhenia* spp., and no seedlings/saplings could be seen emerging from the grass. These grasses are flammable during the dry season, thus the area needed to be protected from fire and fire lanes were maintained in the regenerating area for five years, after which time it was viewed that the tree community had developed sufficiently so that the area would not easily burn. Dispersed throughout the grasses were small stands of native trees that had grown up under the pines and had not been harvested. In May 2006, we identified all the woody tree species of any size [43]. For the nutritional analysis we considered 35 of the 40 tree species found in these plots (representing 99.4% of the stems) and 51 of 60 tree species found to occur on 26 plots (200 by 10 m) in the old-growth forested area (representing 98 % of the stems; details of the sample of the plots in the old-growth forest can be found in [17]).

Nutritional Methods
We collected multiple samples of mature and young leaves of each species during 2007-2011 and immediately dried them using a plant dehydrator at < 50°C. We milled the leaves through a 1 mm sieve using a Wiley Mill in Kibale and transported them to Hunter College, where we followed standard nutritional methods for crude protein using combustion analysis, crude fat using ether extract, ash by burning the sample at $550C^o$, and fiber via detergent methods (neutral and acid detergent fiber; hereafter NDF and ADF; as per [23]). We estimated total non-structural carbohydrate concentration using equations whereby we subtracted neutral detergent fiber, fat, ash, crude protein from 100. We estimated metabolizable energy and non-protein energy via the methods outlined in Rothman et al. [23]. We used lignin as an indigestible marker to estimate NDF digestibility in energy equations [22]. A portion of the samples were analyzed using near infrared reflectance spectroscopy (NIRS; [44]). Nutrient concentrations are reported on a dry matter basis.

Analysis
We positioned the monkey groups using GIS and found the nearest distance to a regenerating forest for each group. To separate groups considered having access to the regenerating forest from those without access, we used three cut-off points (300 m, 400 m, 600 m), as well as a cut-off set to include the 50% of groups that were closest to the regenerating forest. We examined group demographics and the infant to female ratio, considering either 1) infants only, or 2) infants and juveniles (with age/class delineations following Struhsaker [45] and personal training he conducted with our team). We performed a Shapiro-Wilk test to confirm if group counts and female to infant ratios were normally distributed. If the variables were normally distributed, we compared the regenerating forest and old forest groups with a one-sided t test where the hypothesis was that higher infant to female ratios and larger groups would be found in regenerating forest than in the old growth forest. If the groups were not normally distributed we used a non-parametric one-sided Mann-Whitney test. Because of the multiple statistical tests

conducted on total group size and the female to infant ratio, which could arguably be partially non-independent, we also applied a sequential Bonferroni test with a table wide α = 0.0125 for each of the variables, although none of these results were significant with an α = 0.05, either [46, 47]. We also tested for a relationship between the distance to regenerating forest and group size and female to infant ratio using simple linear regression.

To assess differences in nutrient concentrations between regenerating and old-growth forest, we identified and counted all the tree species found in each area and collected samples that were analyzed using the methods described above. We collected multiple samples over time to account for intraspecific variability, and used average nutrient concentrations. We measured total energy, total non-protein energy (NPE), total non-structural carbohydrates (TNC) and the protein-to-fiber ratio. For each of these, we conducted three sets of analyses, comparing 1) all the trees present in each forest, 2) the top ten most abundant plants, and 3) the plant parts of species known from long-term observations to be eaten by the red colobus [48]. Furthermore, we structured our analysis by both combining and separating young and mature leaves for each species. The reason to separate them is that red colobus preferentially feed on young leaves, but many past evaluations of the protein-to-fiber model have tested the model with mature leaves, with the understanding that mature leaves may serve as a proxy for the nutritional quality of the forest in general. Finally, we compared the total species diversity in the two parts of the forest using the Shannon's diversity index and used a t-test to determine if the diversities were significantly different [49]. Based on Ideal-Free Distribution Theory [50], we would expect that because it has been almost 40 years since the logging, birth rates would be the same between the logged and unlogged forests used to contrast to the regenerating forest. However, to validate that this assumption was not influencing our interpretation, we ran all analyses comparing just the groups in the unlogged forest (K30) to the regenerating forest. This produced identical trends (i.e., significance or not) and both sets of results are reported below. All statistical analysis was conducted in R, version 2.14.2 (R-Development-Core-Team 2012).

Results

Nutritionally the regenerating pine and old growth forest areas were different. As predicted, we detected a difference in protein-to-fiber ratio between the two areas for all trees, as well as for the trees consumed by red colobus, with a higher protein-to-fiber ratio in the regenerating forest (Table 1). Similarly, the regenerating forest had leaves with higher total energy (kcal/100g) for the all tree comparison and for the comparison of red colobus foods (Table 1). The red colobus foods were higher in total non-structural carbohydrate in the regenerating forest (Table 1).

We found no differences in the female to infant ratio, regardless of whether or not juveniles were included in the infant class (Table 2). Similarly, group size did not differ among groups with access to regenerating forest and those without access (Table 2). There was no significant relationship between the distance to regenerating forest and either group size (R^2=0.0040, $F_{[1,40]}$=0.1583, p=0.693; Figure 1A) or infant to female ratio when juveniles were included in the infant class (R^2=0.0008, $F_{[1,40]}$=0.0033, p=0.954; Figure 1B), or when examining just clinging infants (R^2=0.0036, $F_{[1,40]}$=0.0014, p=0.905). A similar set of non-significant results were obtained when we considered only those groups encountered in the old growth forest (forestry compartment K30). Within K30, there was no relationship between the distance to regenerating forest and either group size (R^2=0.0006, F[1,14]=0.00878, p=0.927) or infant to female ratio when juveniles were included in the infant class (R^2=0.0005, F[1,14]=0.00682, p=0.935), or when just considering clinging infants (R^2=0.007, F[1,14]=0.0967, p=0.761). Using a threshold of <300 m from a regenerating forest on the K30 subset also did not detect a significant differences in group size

(mean<300 m=47.4, mean>300 m=41.7, t=0.7117, df=13.741, p=.756) or the number of infants per female (mean<300 m=0.306, mean>300 m=0.301, t=0.103, df=8.174, p=0.540) or infants and juveniles per female (mean<300 m=0.542, mean>300 m=0.535, t=0.0994, df=11.494, p=0.461).

The tree species community in the regenerating forests was different from the old growth forest, with regenerating forests exhibiting lower diversity (Shannon's index regenerating forest = 2.968, Shannon's index K30 = 3.401, t= 8.376, $p<0.001$).

Table 1. T-test or Mann-Whitney test for the different nutritional components comparing the regenerating forest and old growth forest by all the plants, the most abundant plants and only the plants known to be eaten by the red colobus.

Tree Parts	Non Protein Energy (NPE; kcal/100grams)				Total Energy (kcal/100grams)				Total Non-Structural Carbohydrate (TNC; %)				Protein-to-fiber ratio			
	$\bar{x}$ RF	$\bar{x}$ OGF	Test statistic [df]	P	$\bar{x}$ RF	$\bar{x}$ OGF	Test statistic [df]	P	$\bar{x}$ RF	$\bar{x}$ OGF	Test statistic [df]	P	$\bar{x}$ RF	$\bar{x}$ OGF	Test statistic [df]	P
All Trees																
YL+ML	152.6	147.9	3893.0 [200]#	NS	244	234	1.9 [132.6]	**0.031**	20.5	18.2	4033.5 [200]#	NS	0.842	0.743	4233 [200]#	**0.030**
YL	138.7	135.8	923.5 [99]#	NS	241	232	1.1 [61.1]	NS	18.0	15.8	964.5 [99]#	NS	0.937	0.812	1029 [99]#	NS
ML	166.1	159.7	0.8 [64.2]	NS	246	236	1.6 [70.5]	NS	22.9	20.5	1.0 [60.6]	NS	0.749	0.674	1114.5 [99]#	NS
10 most abundant trees																
YL+ML	137.4	140.2	-0.2 [15.3]	NS	237	239	-0.1 [16.9]	NS	15.0	16.4	-0.3 [16.6]	NS	0.745	0.915	-1.0 [18.0]	NS
YL	120.8	134.2	-0.6 [7.6]	NS	229	247	-0.8 [5.8]	NS	11.5	16.3	-0.7 [7.7]	NS	0.819	1.117	-1.0 [7.7]	NS
ML	153.9	146.2	0.3 [6.5]	NS	246	231	0.9 [6.7]	NS	18.6	16.5	0.3 [7.4]	NS	0.670	0.713	-0.3 [8.0]	NS
Plant parts eaten by red colobus																
	151.6	143.0	2148.5 [130]#	NS	246	234	2.1 [105.7]	**0.021**	20.7	17.0	2235.5 [130]#	**0.045**	0.881	0.759	2266.5 [130]#	**0.032**

# indicates a Mann Whitney test were performed (see text for details), all other comparisons without a # were T-tests. Results highlighted in bold are significant at the P < 0.05. NS indicates a non-significant result. YL indicates young leaves and ML indicates mature leaves. RF indicates regenerating forest while OGF indicates old-growth forest.

Discussion

As predicted, there was a nutritional difference between the regenerating forest and the old-growth forest, with trees in the regenerating area having leaves with higher protein-to-fiber ratio. The differences in nutrition were consistent with our predictions that early successional plants in regenerating forests invest more heavily in growth and less in defenses against herbivory [25, 51, 52]. However, counter to what was predicted by the protein-to-fiber model, the red colobus groups that had access to this type of food were not different in composition or size when compared to groups that did not have this access. This suggests that the nutritional difference in the forests is not reflected in the actual birth rate of the red colobus, which would be expected to result in greater biomass. This may be due to the fact that the groups having access to these regenerating areas are not actually using these areas to a degree that it represents a significant supplement. On the other hand, the correlations found between the protein-to-fiber ratio and the biomass of different primate groups might not be a causal relationship, but be due to other underlying mechanisms, such as other important aspects of nutrient quality (e.g., plant defensive compounds such as tannins or phytoestrogens: [9, 53]. If this interpretation is correct, researchers should attempt to find the factors actually driving the variation in biomass of these folivores. However, our analysis suggests that the driver is not energy, which the literature would suggest is another likely nutritional driver to explain variation in folivore biomass [30, 32].

Table 2: T-test value or Mann-Whitney test for different estimates of birth rates comparing the groups at the generating forest and old growth forest at different distances from the regenerating forest.

Cut off	Total group counts				Infant to Female ratio				Infant to Female ratio			
	Mean RF	Mean OGF	Test statistic [df]	P	Mean RF	Mean OGF	Test statistic [df]	P	Mean RF	Mean OGF	Test statistic [df]	P
300m	44.0	48.9	-0.863 [38.7]	NS	0.535	0.564	190.5 [40]#	NS	0.263	0.300	172.5 [40]#	NS
400m	45.2	48.8	-0.669 [30.3]	NS	0.542	0.564	160.5 [40]#	NS	0.272	0.300	160.5 [40]#	NS
600m	47.2	40.0	1.212 [9.7]	NS	0.551	0.532	115.0 [40]#	NS	0.281	0.275	124.0 [40]#	NS
Half[k]	43.4	49.0	-1.005 [39.8]	NS	0.523	0.574	167.5 [40]#	NS	0.264	0.296	188.5 [40]#	NS

# indicates a Mann Whitney test was performed because variables were non-normally distributed (see text for details).
t indicates a T-tests was performed because variables were normally distributed.
NS indicates a non-significant result.
j Includes juveniles in the infant class.
k Threshold set so that the 50% of groups closest to the regenerating forest were included in the regenerating forest category.
RF indicates regenerating forest while OGF indicates old-growth forest.

At small spatial scales there has been some indication that the abundance of high protein-to-fiber ratio leaves in forests can constrain folivore biomass [11, 13], but our study adds to growing concern that the ratio may not consistently explain large amounts of variation in folivore biomass on this scale. At broader spatial scales [9, 10, 14, 15] there has also been support for the predictive power of the protein-to-fiber model, although again the evidence has been mixed. For example, long temporal variation in the protein-to-fiber of colobus foods does not exhibit a strong relationship with folivore biomass [17, 18]. A numerical response by primates may only be observed on certain scales. At small spatial scales, animals may be able to compensate or move to areas with higher protein-to-fiber ratios. Although studies have encompassed relatively long temporal spans [17, 18], the numerical response of folivores may be at a larger temporal scale than observed. It has recently been suggested that frugivore biomass can be predicted by the degree of environmental seasonality, particularly in resource availability [54, 55]. While seasonality of fruits is thought to be more extreme than availability of leaves, seasonal variation in food availability and quality might play a role in determining folivore abundance and warrants future study [56].
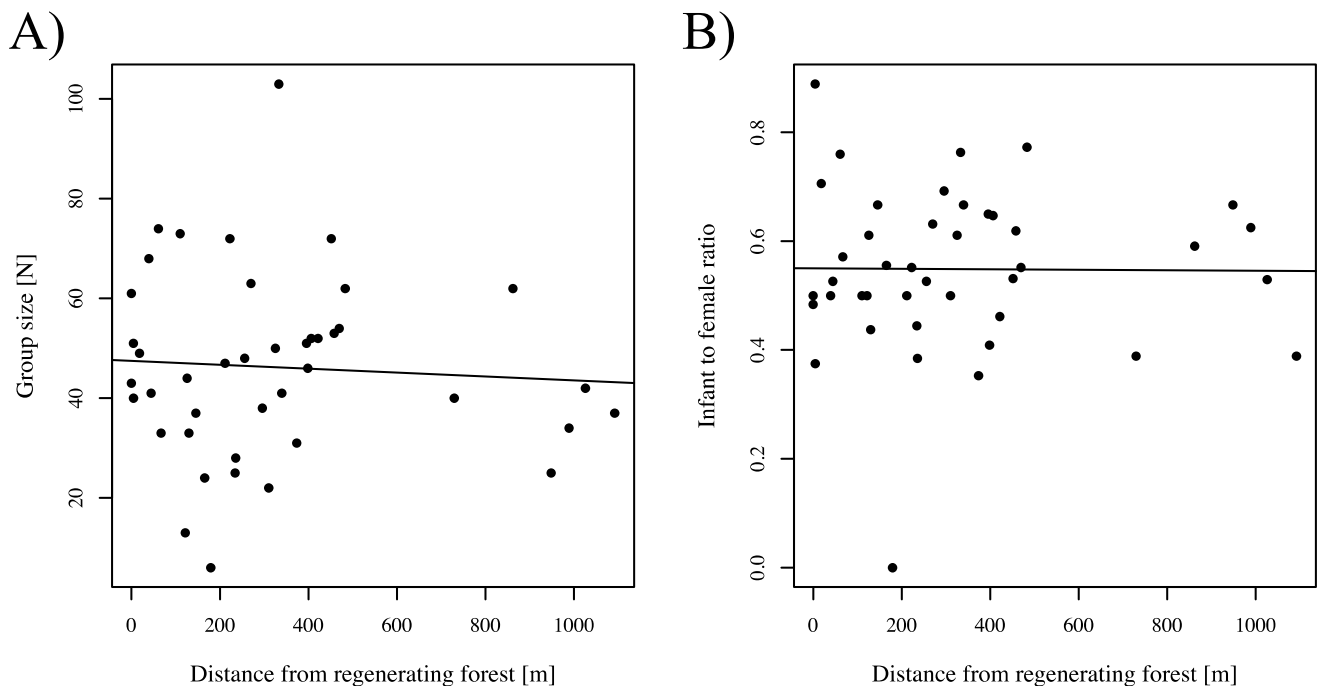

Fig. 1. Demonstrates the non-significant relationship between birth rate (as group size and infant to female ratio) and the distance from the regenerating forest.

Mortality and reproduction are influenced by a number of factors, such as disease [57, 58], predation [59, 60], injury [61], and resource availability [62]. The interaction of these factors is likely habitat- and species-specific [63], and the predictive power of the protein-to-fiber model may be obscured by such factors. Another possible confounding factor in our analysis is that we examined only the numerical response of a single species of colobine, although black and white colobus (*Colobus guereza*) are present and common in the area and have high dietary overlap with red colobus [64]. Future studies may profit from examining the impact of the protein-to-fiber ratio on the entire community of primates, including frugivores that can feed extensively on leaves. In addition, to refine our conclusions it may be beneficial to measure density and the percentage of time folivorous primate groups spend in the regenerating forest, rather than using proxies such as group size and female to infant ratio.

Implications for Conservation

The result documented here supports other recent studies that have not found a relationship between primate biomass and the protein-to-fiber ratio [17, 18], further questioning the generality of this ratio in predicting folivore biomass [19]. It also raises the question about what really is the main driver of red colobus abundance in Kibale and of folivore abundance in general. While it is clear that information about determinants of animal abundance is needed to construct informed management plans, it is also clear that this is a complex area of research.

Acknowledgments

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