How does diet quality affect the parasite ecology of mountain gorillas?

JESSICA M. ROTHMAN, ALICE N. PELL, AND DWIGHT D. BOWMAN

Introduction

Host nutritional status affects host–parasite dynamics, but is little is known about this relationship in free-ranging primate populations. Nutrition and parasitic infections interact in three important ways. Firstly, since nutritional deficits...
compromise immune function, the ability of the host’s immune response to resist parasitic infections and cope with the negative consequences of parasites depends on adequate nutritional supplies (Coop & Kyriazakis, 2001). Secondly, some parasites, gastrointestinal parasites in particular, may reduce the ability of the host to absorb nutrients, thereby altering digestive efficiency and further compromising the host’s nutritional status (Koski & Scott, 2001). Third, because parasites have their own nutritional demands, they compete for the host’s food supply and further intensify the effects of malnutrition. As a consequence, concurrent malnutrition and parasitic infection may initiate a downward spiral where each intensifies the effect of the other, leading to increased nutritional stress, reduced immunocompetence, and increased infection (Koski & Scott, 2001). Alternatively, good nutrition can increase the ability of the host to overcome parasitism by improving immune response, affecting parasite establishment, and decreasing the chance for reinfection (Coop & Kyriazakis, 2001).

Some dietary items may possess antiparasitic properties. Janzen (1978) speculated that folivorous primates lack the protozoan parasites seen in frugivores because foliage contains higher concentrations of secondary compounds than fruit. More recent studies have demonstrated that protozoan parasites are indeed found in folivorous species (Stuart et al., 1998; Gillespie et al., 2005), but there is support for the idea that secondary compounds may help to limit parasite infections in wild primate populations (reviewed in Huffman, 1997; Nunn & Altizer, 2006). Condensed tannins are among the most widespread secondary compounds in tropical foliage, and have been a focus of primate feeding ecology studies for decades (Milton, 1979; Oates et al., 1980; Wrangham & Waterman, 1981; Glander, 1982). They have long been considered as a feeding deterrent because they reduce palatability and bind protein, minerals, and other macromolecules (Mueller-Harvey, 2006). In domestic animals, they decrease productivity by lowering digestibility, protein availability, and food intake, affecting weight gain and milk yield. However, tannins also possess properties that positively affect health. For example, they suppress intestinal parasites in domestic ruminants (Hoste et al., 2006). One study suggested that sifakas (Propithecus verreauxi verreauxi) consume tannins intentionally for medicinal purposes (Carrai et al., 2003). These authors proposed that female sifakas increased their tannin intake during the birthing season, and suggested that the tannins consumed may provide anti-abortive and anti-hemorrhagic effects, and stimulate milk secretion. To our knowledge, no studies have quantified the effects of tannin intake on the parasite infections of primates.

The objective of this study was to understand how diet quality affected parasite infections of a group of mountain gorillas (Gorilla beringei) inhabiting Bwindi Impenetrable National Park, Uganda. We predicted that if diet quality decreased, this would affect parasite infections. We considered two indices of
Does diet quality affect the parasite ecology of mountain gorillas?

Diet quality: concentrations of protein and condensed tannins. Since the Bwindi gorillas eat diets that contain condensed tannins (Rothman et al., 2006), we predicted that increased dietary concentrations of condensed tannins would decrease parasite infection. Since varying protein and tannin concentrations may not necessarily have an immediate effect on parasite infections, we predicted that weeks when the individuals consumed lower quality diets would be followed by weeks with more severe parasite infections. Accordingly, we explored this question on two time scales. We considered the diet eaten by the gorillas, and the parasite infections 2 and 4 weeks after ingestion of a particular diet. We chose these two time scales because experimental studies on livestock have demonstrated that changes in parasite infections are associated with the diet that an animal has obtained in the prior 2 weeks (Coop & Kyriazakis, 2001). Our data collection was facilitated by our ability to systematically observe identifiable individuals, determine their nutrient intake, and monitor their parasite infections almost daily for one year.

Methods

Study site

Located in southwestern Uganda, Bwindi Impenetrable National Park (BINP) (0° 53′–1° 08′S, 29° 35′–29° 50′E) is a 331 km² rugged mountainous rain forest at 1160–2600 m asl characterized by steep-sided hills and narrow valleys (Butynski & Kalina, 1993; McNeilage et al., 2001). The study area is dominated by forest gaps (67%; Nkurunungi et al., 2004), which are both natural and a result of pit sawing prior to 1991 (Babassa et al., 2004). Other habitats include: mixed forest (29%), mature forest (2%), and swamps (2%) (Nkurunungi et al., 2004). The annual rainfall during the study was 1646 mm and the mean annual rainfall at the study site is 1440 mm. Annual rainfall is bimodal: the dry months are typically December to February and June to August, while the wet months are September to November and March to May.

Study group

The Kyagurilo group of gorillas has been habituated to human observers since the mid-1990s through daily monitoring by park rangers and field assistants of the Uganda Wildlife Authority and the Institute of Tropical Forest Conservation. Each individual is identifiable based on facial characteristics. The group ranges in a 40 km² area of the interior of BINP at high altitudes of the park,
between 2100–2500 m above sea level (Robbins & McNeilage, 2003). At the time of the study, August 2002–2003, the group included 12 independent individuals (two silverbacks, six females, and four juveniles) and two infants. One additional infant was born during the study. To our knowledge, the group does not range in areas inhabited by humans, and group members only encounter humans when park staff and/or researchers observe them from a distance of >5 m for four hours daily. Tourists do not have access to this group.

Estimation of diet quality

To evaluate the quality of the diets consumed by each independent gorilla (adults and juveniles; infants were not studied), focal sampling was used to determine the concentrations of protein and tannin in the diet (detailed in Rothman, 2006). We observed the animals on 319 days during August 2002–2003. We observed each independent animal in 30 min intervals. Although all animals were habituated to observers, the dense terrestrial vegetation often interfered with observations of specific animals. Therefore, focal individuals were followed to the extent possible until they were out of view. If the focal individual was out of view for more than 5 min, another animal was chosen. Since the goal of our focal sampling was to estimate nutrient intake, if the animal was out of view before the 30 min focal sample was complete, data on food intake rates were considered if they continued for more than five minutes. Missing intervals where the animal was out of view while feeding were not used in calculations of nutrient intake. When several animals could be observed simultaneously, opportunistic focal observations were made of the visible individuals. Our focal follows totaled 1318 hours.

During focal follows, we counted all food items ingested during timed feeding bouts. A feeding bout began whenever an animal first made contact with a food item and ended when the animal stopped feeding on any food item for ten seconds. Multiple food items were frequently eaten during these feeding bouts. Observer biases were minimized by standardizing observational techniques by having all researchers observe the same animal on 3 days per month.

Because of variation in the weight and size of food items and how they are processed by different-sized gorillas (i.e. a leaf in the dry season may be smaller and lighter than a leaf in the wet season, and a juvenile may take smaller bites than an adult), specific food units were defined for each food item. A unit could be considered a single plant item (e.g. a leaf, or fruit), the approximate dimensions of the food item (for peel, bark, pith, wood) or in the case of small leaflets or fruits, the average number of item in a cluster (Chivers, 1998). To account for variation across time and space, we calibrated these units
Does diet quality affect the parasite ecology of mountain gorillas?

by weighing them immediately after collection (n = 50) to calculate the mean unit wet and dry weight for each food item regularly (at least once per month for each food that represented > 1% of the wet weight dietary intake).

Food items eaten during our focal observations were collected from the exact plant eaten by the gorillas when possible or from several adjacent plants of the same species. We attempted to process the selected food items similarly to the gorillas. For example, if the gorilla removed the outer peel of herbaceous stems, but ate the interior stem core, we selected the inner stem core for analysis. After collection, plants were immediately weighed in the field using a portable balance to the nearest 0.1 g. The samples were dried in a dark area of the field station (≤25°C). When the samples achieved a constant weight, they were ground in a Wiley Mill through a 1-mm screen. Food species that were eaten often during the study were regularly sampled and reanalyzed to adjust for seasonal and environmental variation in nutritional composition (Chapman et al., 2003). In total, we analyzed 336 plant samples.

Foods were analyzed by JMR in the Animal Nutrition laboratory at Cornell University. The amount of nitrogen in each food items was estimated using a Leco FP-528 combustion analyzer using standard procedures (AOAC, 1990). The measurement of total nitrogen provides an estimate of crude protein (protein levels = n × 6.25). However, a substantial portion of the nitrogen may be bound to the lignin in tropical plant parts and therefore unavailable to animals as a protein source (Milton & Dintzis, 1981; Conklin-Brittain et al., 1999). For example, in many food items, the nitrogen bound to acid detergent fiber (mainly comprised of cellulose and lignin) exceeded 10% of the total nitrogen. We estimated this bound nitrogen by analyzing the nitrogen bound to the acid detergent fiber (cellulose + lignin) following the methods by Licitra et al. (1996).

To account for this indigestible nitrogen in our estimates of protein intake by gorillas, we calculated digestible protein in each plant part in the following way. First, we subtracted the amount of nitrogen bound to the acid detergent residue (ADIN) from the nitrogen to calculate the available nitrogen (National Research Council, 2003). Then we multiplied the amount of available nitrogen by 6.25 to estimate the amount of protein available to the gorillas (e.g. (N – ADIN) × 6.25 = available protein).

To estimate the amounts of condensed tannins in food items (% dry matter), we followed the methods of Hagerman & Butler (1994). The dried, ground plant samples were extracted in 70% (v/v) aqueous acetone in a sonicator containing ice water bath for 20 min. After 20 min, extracts were centrifuged at 2500 g for 10 min. The supernatant was collected and stored covered in the dark at 4°C. These extractions were repeated four times, and the supernatants were combined to reach a final concentration of 10 mg of crude plant material per
ml of acetone extract. The acid butanol assay was used to estimate the amount of condensed tannin in each crude extract (Porter et al., 1986) with controls for pigments in the crude plant extract (Watterson & Butler, 1983). Using Sephadex LH-20, internal standards were generated for each plant part representing at least 0.5% by weight of the diet (Hagerman & Butler, 1994). These purified tannins were used to develop standard curves which were used to estimate the amounts of tannin in the plant extracts of unknown quantities of condensed tannin.

Purifying tannin from the plant species of interest is important because there is a great deal of variation in the structure of tannins, which affects their reactivity in spectrophotometric assays. Many studies use a single standard to estimate tannin quantity in primate foods (e.g. quebracho: Remis et al., 2002; Powzyk & Mowry, 2003; Norconk & Conklin-Brittain, 2004). However, this approach can cause ambiguous results (Giner-Chavez et al., 1997; Schofield et al., 2001; del Pino et al., 2005). For example, a fruit commonly eaten by gorillas could misleadingly be estimated as anywhere between 0.3–11% DM in condensed tannin content, solely based on which of four external standards was chosen (Rothman et al., 2006), when its actual tannin content is 8.9% DM.

To estimate the concentrations of protein and tannins in the diets of each individual, we multiplied the number of units consumed during a focal follow by the mean weight of that unit and then by the percent dry weight protein or tannin content of each food item. We summed this for all units consumed during each focal follow and multiplied this by the number of minutes spent feeding during that day. This result was divided by the total dry weight consumed during the day. Although we were only able to watch the gorillas for 4 hours, we considered this 4-hour period to be representative of the entire day and scaled the data accordingly. On 7 days additional observation time was permitted and the gorillas were followed from first contact until they built night nests (16:30–17:30 hrs). We confirmed that our 4-hour observations were representative of the remainder of the day (Rothman, 2006). The mean nutrient intake per follow per individual was used to calculate nutrient intake over a 2-week period.

Parasite analysis

Like other great apes, gorillas typically sleep each night in a newly built nest; infants nest with their mothers (Schaller, 1963), and before leaving its nest in the morning, a gorilla typically defecates on the nest’s outer rim. Fecal samples were collected from the night nest of each group member and were assigned to
Does diet quality affect the parasite ecology of mountain gorillas?

age-sex classes by the diameter of the central bolus of the dung (infant < 2 cm; juvenile 2–4 cm; adult female 5–7 cm; silverback > 7 cm; Schaller, 1963); a technique commonly used in censuses (McNeilage et al., 2001). The ability to differentiate fecal samples by age and sex was confirmed by opportunistically collecting samples when defecation was observed by identifiable animals for verification: males (n = 27, mean 7.1 ± 0.4 cm), females (n = 33, 6.0 ± 0.4 cm), and juveniles (n = 16, 3.9 ± 1.3 cm), and there was no overlap in size between any of the age-sex categories including juvenile males and adult females without infants. Since the gorillas did not have diarrhea or soft stool in their night nests during the study, the diameter of dung was considered to reliably correspond to a specific age-sex class.

In addition to classifying samples by age and sex, some fecal samples could be assigned to identifiable individuals including a female, a juvenile, and the two silverbacks. Because one female and her juvenile nested together, their nest was the only one in the group that contained feces of both a juvenile and female. The two silverback nests were differentiated on the basis of distance from the rest of the group. As in an earlier study (Schaller, 1963), the dominant silverback slept in the middle of the group and the subordinate silverback slept > 10 m from other group members (JMR, unpubl. observation). Therefore, all dung samples collected from the nest > 10 m from the center of the group were considered to be from the subordinate silverback and those samples collected from the silverback nest in the center of the group were considered to be from the dominant silverback male.

Fecal samples were collected once weekly from all group members and assigned to a particular age-sex category based on their dung diameter. For at least 4 of 7 days of each week, fecal samples from the dung that could be assigned to the four individually identifiable gorillas were collected: the dominant silverback (n = 306), the subordinate silverback (n = 256), a female (n = 239), and a juvenile (n = 239). Though every attempt was made to collect samples daily for these four animals, sometimes the gorilla nests were not located or the dung samples had been tampered with by the gorillas. This did not happen often and was not biased towards any one month or season, so we do not feel this is significant. A sample (∼3 g) was taken from the interior of the fecal bolus and preserved in pre-weighed tubes containing 10% formalin for later examination.

At the College of Veterinary Medicine at Cornell University, feces were processed using the formalin-ethyl acetate sedimentation concentration technique (Garcia, 1999). Because specific identification of eggs in the feces would have required coproculture (in the case of the strongylid eggs) or details of worms present in these gorillas (based on necropsies), familial or generic determinations based on the appearance of the eggs or larvae are presented.
Data analysis

We explored the possibility that diet quality is related to indices of intestinal parasitism. We considered the percentage of available protein content of the diet, and the percentage of the diet comprised of condensed tannins (% dry matter basis) as indicators of diet quality, and the percentage of fecal samples positive for a particular parasite, and mean infection intensity (eggs per gram (epg) or larvae per gram (lpg) in all samples) as indices of parasitic infection. We considered these indices biweekly (every two weeks), so the results of all samples collected in a specific week for each age-sex class and identifiable individuals were pooled and the mean was taken. Since the amount of food eaten did not vary over the study period for each individual (Rothman, 2006), using diet concentrations is a valid estimation of diet quality.

Because we did not expect to see an effect on parasite infections immediately (Coop & Kyriazakis, 2001), we used a time lag of 2 weeks and 4 weeks to consider whether diet quality affected indices of parasite infections. Since our data were not normally distributed, we used Spearman rank correlations to assess relationships between diet quality and parasitic infection. We consider our analysis preliminary and exploratory, and so we did not correct for multiple comparisons using Bonferroni corrections (Nakagawa, 2004).

Results

Diet quality

Our assessment of diet quality revealed that intake of protein varied throughout the year, but diets were always well in excess of the protein requirements of humans, and never fell below a mean of 8% available protein (Table 22.1). This suggests that protein was not limiting and therefore may not have an effect on parasite infections.

Tannin content in the gorilla diet also varied throughout the year. During some weeks of the year, the gorillas ate a diet that contained minute concentrations of tannins (0.1% of the dry matter) and other weeks the diet contained appreciable concentrations of tannins (8.8%) (Figure 22.1).

Parasite community

The parasite community described from the 1408 fecal samples included the following: unidentified strongyle eggs, Anoplocephala gorillae (Superfamily
Table 22.1. The diet quality (biweekly mean ± SD) and parasite infections (biweekly mean and range) of a group of mountain gorillas in Bwindi Impenetrable National Park, Uganda

<table>
<thead>
<tr>
<th>Infection index</th>
<th>Dominant silverback</th>
<th>Subordinate silverback</th>
<th>All females</th>
<th>All juveniles</th>
<th>Known female</th>
<th>Known juvenile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of samples positive for</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strongyle eggs</td>
<td>87.5 (67–100)</td>
<td>77.8 (36–100)</td>
<td>75.9 (16–100)</td>
<td>67.9 (25–100)</td>
<td>71.1 (0–100)</td>
<td>62.4 (0–100)</td>
</tr>
<tr>
<td>Probstmayria sp.</td>
<td>13.9 (0–43)</td>
<td>7.6 (0–38)</td>
<td>12.2 (0–37)</td>
<td>9.8 (0–38)</td>
<td>10.6 (0–71)</td>
<td>10.0 (0–86)</td>
</tr>
<tr>
<td>Anoplocephala gorillae</td>
<td>8.0 (0–21)</td>
<td>8.5 (0–33)</td>
<td>8.3 (0–33)</td>
<td>7.2 (0–50)</td>
<td>6.3 (0–43)</td>
<td>10.5 (0–50)</td>
</tr>
<tr>
<td>Strongyloides fuelleborni</td>
<td>2.0 (0–22)</td>
<td>0.83 (0–12.5)</td>
<td>0 (0)</td>
<td>0.7 (0–38)</td>
<td>0.38 (0–9.0)</td>
<td>0.33 (0–7.7)</td>
</tr>
<tr>
<td>Parasite burden</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strongyles (egg/g)</td>
<td>89.0 (21–267)</td>
<td>29.9 (5–76)</td>
<td>40.1 (2–184)</td>
<td>35.8 (3–132)</td>
<td>27.0 (0–105)</td>
<td>19.7 (0–61)</td>
</tr>
<tr>
<td>Probstmayria sp. (larvae/g)</td>
<td>0.75 (0–4)</td>
<td>0.28 (0–1)</td>
<td>1.0 (0–12)</td>
<td>0.80 (0–10)</td>
<td>0.66 (0–9)</td>
<td>0.73 (0–12)</td>
</tr>
<tr>
<td>Anoplocephala gorillae (egg/g)</td>
<td>0.72 (0–4.9)</td>
<td>1.29 (0–12.1)</td>
<td>0.7 (0–10)</td>
<td>1.0 (0–16)</td>
<td>0.70 (0–13)</td>
<td>1.4 (0–19)</td>
</tr>
<tr>
<td>Strongyloides fuelleborni (egg/g)</td>
<td>0.15 (0–1.2)</td>
<td>0.04 (0–0.75)</td>
<td>0 (0)</td>
<td>0.7 (0–17)</td>
<td>0.01 (0–0.27)</td>
<td>0.01(0–0.23)</td>
</tr>
<tr>
<td>Diet quality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (% dry matter basis)</td>
<td>14.5 ± 3.3</td>
<td>13.7 ± 4.9</td>
<td>12.9 ± 2.7</td>
<td>12.9 ± 3.0</td>
<td>12.8 ± 5.5</td>
<td>11.5 ± 4.2</td>
</tr>
<tr>
<td>Tannin intake (% dry matter basis)</td>
<td>3.3 ± 2.6</td>
<td>3.0 ± 2.8</td>
<td>3.6 ± 2.7</td>
<td>3.8 ± 2.8</td>
<td>3.0 ± 3.7</td>
<td>3.2 ± 3.1</td>
</tr>
</tbody>
</table>
Figure 22.1. The relationship between the concentration of tannins in the diet of mountain gorillas and the (a) percentage of fecal samples positive for *Probstmayria* after a 2-week lag, and (b) mean number of *Probstmayria* larvae per gram (lpg) of feces after a 2-week lag. The data represent the mean infection levels and tannin intake of the four identifiable individuals monitored.
Does diet quality affect the parasite ecology of mountain gorillas?

Anoplocephalidae), Probstmayria sp. (Superfamily Cosmocercoidea), Strongyloides fuelleborni (Superfamily Rhabditoidea). In addition, on three occasions an unidentified trematode egg was found in the feces of three different individuals (the dominant silverback and two different juveniles). This trematode egg was not considered further. Based on coprocultures and a necropsy of a female gorilla in Bwindi (Durette-Dusset et al., 1992; Ashford et al., 1996), the strongyle eggs present in the majority of fecal samples could be: Oesophagostomum sp., Murshidia devians, Paralibyostrongylus kalinae and/or Hyostrongylus kigezensis. Paralibyostrongylus kalinae and H. kigezensis have only been described from gorillas in Bwindi (Durett-Dusset et al., 1992). Since all of these parasites have similar eggs and the gorillas host multiple strongylid species, it would be difficult to differentiate the strongylid eggs found in the feces to genera or species without necropsy.

In weekly samples collected from all group members categorized by age-sex class and fecal samples attributed to specific individuals, we found all of the above parasites during the year (Table 22.1). During some weeks the samples from all individuals were positive for strongyles, A. gorilla, and Probstmayria, indicating that all animals were infected and the prevalence of these parasites in the group was 100%. Nematodes and tapeworms can live for a long time inside their hosts, which may result in chronic infection. Since we do not know anything about the lifespan of these worms in particular, it is unclear whether the variation in egg output and percentage of positive samples represents the acquisition of a new infection, or heterogeneity in egg output. Since we did not repeatedly examine the same dung sample, we do not know whether variation could be due to methods of examination, but our techniques to examine fecal samples are standard practice in parasitology (Garcia, 1999).

Relationships between diet quality and parasite infections

We compared the biweekly mean diet quality with the mean biweekly infection levels (at 2 and 4 weeks after consumption of a particular diet quality) of each age-sex class (females and juveniles) and the known individuals (two silverbacks, female and juvenile). We contrasted two indices of parasite infection with protein and tannin concentrations in the diet: parasite burden (eggs or larvae per gram) and percentage of positive fecal samples.

Following a 2- and 4-week time lag, we found no correlations between protein intake and indices of parasite infection for any animal or age-sex class for all parasites found (P > 0.20). However, if individuals in the group had a higher tannin intake during the previous 2 weeks, then indices of parasitic infection were affected in some animals. Specifically, we found correlations between
tannin intake and indices of one parasite, *Probstmayria* sp. (Figure 22.1), a pinworm that is likely of little clinical importance and is found in many species of mammals. When tannins were consumed, the percentage of positive samples for *Probstmayria* sp. infections decreased in the following 2-week period for females (Spearman rank coefficient: $-0.42, P = 0.05$) and juveniles ($-0.54, P < 0.01$), and the known juvenile ($-0.72, P < 0.01$). However, this relationship was not found for the dominant silverback ($-0.28, P = 0.20$), the subordinate silverback ($0.03, P = 0.89$), or the known female ($-0.29, P = 0.19$). Similarly, following periods of tannin consumption, the number of *Probstmayria* larvae per gram of feces decreased for the females ($-0.48, P = 0.02$) and juveniles ($-0.55, P = 0.01$), the dominant silverback ($-0.48, P = 0.02$) and the known juvenile ($-0.50, P = 0.02$). This relationship was not found in the subordinate silverback ($0.03, P = 0.89$) or the known female ($-0.11, P = 0.61$). We found no significant correlations for any other indices of infection for any of the other parasites with a 2-week time lag. Following a 4-week time lag, we found no correlations between tannin intake and indices of parasitic infection for any members of the gorilla group ($P > 0.20$).

**Discussion**

Our results support predictions that the intake of condensed tannins could be affecting one parasite that infects the Bwindi mountain gorillas. We found trends suggesting that *Probstmayria* sp., a pinworm, was negatively affected by the level of condensed tannins in the diet, suggesting that the level of tannins may impact this parasite of the gorillas. Further long-term work, complementary experimental studies and a larger sample size are needed to explore these trends further. The majority of tannins in the diet of the gorillas were provided by the staple foods eaten by the gorillas. The variation in dietary composition throughout the year, combined with the intra-specific variation in tannin content in staple foods accounted for the varying concentrations of tannin intake. For example, *Urera hypselodendron* leaves, one of the most frequently eaten foods, was highly variable in tannin content. According to the season and growing conditions, tannin concentrations in the leaves varied from 0% to 15% dry weight in areas where the gorillas fed (JMR, unpublished data). The gorillas did not appear to alter their diet based on this intraspecific variation in the quality of this food item. Thus, it is our view that the gorillas do not intentionally adjust their diet to reduce their parasite loads (i.e. intentionally consume tannins for medicinal purposes), but that variation in tannin concentration in their diets is a consequence of the intra-specific and inter-specific variation in nutritional content of their diets. Over the year, the gorillas ate diets that
Does diet quality affect the parasite ecology of mountain gorillas?

Figure 22.2. A female Probstmayria sp. giving birth. She was found in the fecal sample of an adult female mountain gorilla. (a) head (40 × magnification) (b) body (10×).
Primate Parasite Ecology

contained between 0.1% and 8.8% tannin on a dry weight basis (Figure 22.1). Hoste et al. (2006) reviewed experimental studies on domestic ruminants and suggested that threshold levels of condensed tannin should reach at least 3–4% tannin for biological activity against parasites to be observed.

The mechanism by which condensed tannins may affect Probstmayria sp. is not known, but two main hypotheses have been proposed with respect to the effects of tannins on parasitic nematodes of ruminant livestock (Hoste et al., 2006). The first hypothesis is that condensed tannins act as a pharmaceutical agent and have anthelmintic properties that affect biological processes of the parasite. Based on a series of in vitro and in vivo studies on ruminants and their parasites, it has been demonstrated that tannins influence the development and establishment of early nematode stages (Hoste et al., 2006). In vitro studies have consistently shown that crude tanniferous plant extracts affect the mobility and viability of infective stage larvae, or hatching of eggs (Molan et al., 2000, 2002, 2003; Max et al., 2005). When polyethylene glycol (a tannin binding agent) was added to these assays, the effects of tannins disappeared and nematode populations recovered to match control levels, suggesting that tannins play a key role. Condensed tannins have a high affinity for proline and hydroxyproline, two amino acids that are found on the cuticular coating of nematodes (Bahuaud et al., 2006; Hoste et al., 2006). The cuticular coating is not only found on the outside of the worms, but also covers internal reproductive and digestive anatomy. The number of free hydroxyl groups on condensed tannin is directly related to the inability of trichostrongyle larvae to remove its outer coating or sheath (exsheath) (Brunet & Hoste, 2006), which is a critical step in their development from the free-living to the parasitic stage. Lesions are evident on the internal structures of the digestive and reproductive tract of larvae exposed to tannins. Therefore, tannins may damage the biological integrity of cuticular membranes and affect parasite viability. The wide variation in tannin chemistry suggests that tannin structure and concentration modulate these effects on gastrointestinal parasites. Although there are only a few studies that have looked at the effects of tannins on non-ruminant animals, the results of these studies support that tannins effect gastrointestinal parasites (e.g. swine: Salajpal et al., 2004; rodents: Rojas et al., 2006).

The second hypothesis suggests that condensed tannins could improve host condition in ruminant livestock indirectly by binding high-quality dietary protein, allowing it to bypass the rumen and be absorbed in the small intestine. At low levels, condensed tannins could be beneficial in this way to ruminants because they could protect important amino acids from being used by ruminal microbes (Wang et al., 1994). Instead, this high-quality protein is absorbed in the small intestine, improving nutritional status and alleviating any parasite-induced protein deficiency (Bown et al., 1991). While this may be important
Does diet quality affect the parasite ecology of mountain gorillas?

for ruminants, it would not apply to gorillas because they are simple stomached animals with hindgut fermentation. Therefore, this is not a plausible hypothesis as to why indices of Probstmayria sp. infections were reduced in these gorillas. Additionally, improving host resilience to parasitic infection would take time as nutritional condition improved; reduction of parasite infections has been fairly rapid after tannin intake in most studies (about 2 weeks), suggesting a direct anthelmintic effect (Coop & Kyriazakis, 2001).

Probstmayria sp. is a pinworm found in a variety of species including chimpanzees, baboons, domestic horses, zebra, tapirs, and swine. It has been reported as a parasite of gorillas at several research sites (Sleeman et al., 2000; Lilly et al., 2002; Rothman & Bowman, 2003; Freeman et al., 2004). Despite its widespread prevalence, Probstmayria spp. are little studied. This is probably because they are of minimal clinical importance; pinworms in humans are more of a nuisance than a problem. They cause anal itching and restlessness, which could lead to insomnia and stress. Transmission is direct and occurs when infective larvae in the feces are ingested; coprophagy, which was observed rarely (three times in > 1300 hours of observation), may facilitate transmission (Graczyk & Cranfield, 2003). Probstmayria are viviparous and their entire life cycle takes place internally so that large numbers in the large bowel can accumulate rapidly. As a result, since gorillas are hindgut fermenters and food is probably retained in the colon for a longer time relative to other segments of their gastrointestinal tract, exposure of the worms to condensed tannins may be facilitated.

No other parasites appeared to be affected by condensed tannins. Heavy infections of strongylids are associated with dysentery, mucosal inflammation, ulceration, weight loss, and death, so these worms could cause substantial damage, severe pain, and pose a serious threat to the gorillas. Post-mortems of mountain gorillas in the nearby Virunga region revealed nodular worm disease in three of eight gorillas (Mudakikwa et al., 2001). Strongyle eggs were routinely found in the gorilla feces, and did not fluctuate in response to increased dietary tannin intake. It is not known whether eggs found in the feces represent more than one species, which could confound the results because different nematodes may have different responses to tannins. The results of several mountain gorilla necropsies suggest that multiple strongyle infections are common (Fossey, 1983; Durette-Dussette et al., 1992; Ashford et al., 1996; Mudakikwa et al., 2001). Alternatively, the fact that we saw no variation in egg output with tannin intake could be a consequence of the location of the worms. Most of the strongyles identified from the Bwindi gorillas live in the small intestine and stomach. Since food likely moves more rapidly through these segments, reduced tannin-parasite interaction may reduce the impact of tannins. There was no evidence that tannins affected the tapeworm
Anoplocephala gorillae, which has been recovered from the small intestine of the necropsied gorillas (Mudakikwa et al., 2001). It may be that the location of tapeworms does not allow for adequate exposure to tannin, or it is possible that cestodes are not affected by tannins in the same manner as nematodes. In a study of growing goats, increasing levels of a high tannin feed affected nematode eggs and coccidian oocysts in the feces, but had no effect on the number of cestode eggs (Dung et al., 2005). Very few studies have investigated the effects of tannins on tapeworms and therefore our knowledge is minimal.

It is well-established that protein and energy malnutrition with accompanying parasitic infection affect susceptibility to parasites, and that parasitic infection further exacerbates nutritional stress due to the competition for resources (Smith et al., 2005). The interactive effect of concomitant nutritional stress and parasitic infection has demonstrably led to population crashes in the wild (Gulland, 1992; Milton, 1996; Newey et al., 2005). The synergy between nutritional stress and parasitism has been suggested to be the mechanism driving population declines in endangered red colobus monkey populations living in fragments of an African rain forest (Chapman et al., 2006); within contiguous forest, where nutritional supplies were abundant, these interactive effects were not observed (Chapman et al., in press). Fluctuations in the dietary protein content did not affect the parasite ecology of the gorillas, likely because the gorillas did not appear to have a limiting amount of protein in their diets and many of their staple foods eaten daily contain >20% crude protein (Rothman et al., 2006a). Energy intake of these gorillas did not fluctuate seasonally and also did not appear to be limiting in the diet of the gorillas (Rothman et al., in revision). With sufficient amounts of dietary energy and protein, the effects of parasitism may be negligible (Munger & Karasov, 1989), and since these gorillas did not show evidence of nutritional stress, it is not surprising that fluctuations in protein content did not affect parasite infections (Murray et al., 1998).

While the gorillas appeared to be in good clinical health throughout the study, illnesses may prompt self-medication (Huffman et al., 1997). Huffman (1997) outlined the conditions required as evidence for intentional medicinal plant use: (1) The intake of plant species which are not normally consumed as part of the regular diet and do not provide a nutritional benefit. (2) The presence of an illness or parasite infection. (3) A positive change in the illness or parasite infection after ingestion of the proposed medicinal plant. Although the gorillas harbored parasite infections throughout the study, we did not see any signs of intestinal disease, such as diarrhea or blood in the stool, nor did we observe any evidence of self-medicative behaviors, such as leaf swallowing, which has been suggested to assist in the expulsion of parasites from the intestinal tract of chimpanzees, bonobos, and lowland gorillas (Huffman et al., 1996; Dupain...
Does diet quality affect the parasite ecology of mountain gorillas?

et al., 2002; Huffman, 2003; Dupain et al., Chapter 14, this volume). During the year, the gorillas ate a diverse diet including at least 158 food items from 107 food species; many are used by humans in surrounding villages for their medicinal properties (Rothman, unpublished data) (Cousins & Huffman, 2002). Minute quantities of a diversity of foods may provide important prophylactic health benefits (Sherman & Billing, 1999; Arimond & Ruel, 2004). Although it did not appear that the gorillas were intentionally consuming plants for their medicinal value, we suggest that properties of their diet affect parasite infections.

Our study permitted an exploration of the effects of diet on the parasite infections of endangered mountain gorillas. While gorillas in Bwindi are protected and healthy, many other populations are being decimated by commercial hunting, habitat destruction through mechanized logging, and Ebola (Walsh et al., 2003; Bermejo et al., 2006). As humans invade the habitats of gorillas, the unfortunate reality is that we are even more pressed to understand baseline patterns of host–parasite relationships (Chapman et al., 2005; Nunn & Altizer, 2006). This knowledge will allow us to more clearly identify the consequences of our actions and contribute to informed management plans that mitigate disease risks in endangered apes.

Acknowledgements

We thank Moses Akatorana and the assistants at the Institute of Tropical Forest Conservation for their hard work in the field. We are grateful to Kathy Duisenberre, James Robertson, Debbie Ross, Mike Van Amburgh, Peter Schofield, Susanne Pelton, Ralph Obendorf, and Ron Butler for assistance with analyses, technical laboratory training and use of equipment. We thank Peter Van Soest, Debbie Cherney, Linda D’Anna, Ellen Dierenfeld, Skip Hintz, Araceli Lucio-Forster, Jan Liotta, Colleen McCann, John Bosco Nkurunungi, John Bosco Nizeyi, Eloy Rodriguez, and Colin Chapman for insightful discussions related to this project. We thank Tamaini Snaith for helpful comments that improved the quality of this chapter. Alastair McNeilage, William Olupot, Dennis Babaasa, Robert Bitariho, John Makombo, and Aventino Kasangaki provided logistical support in the field. Jane Engel and the Robert G. Engel Family Foundation, the Department of Animal Science at Cornell University, Mario Einaudi Foundation, Cornell University Graduate School, and the Institute of African Development provided funding for this research. We thank the Uganda Wildlife Authority and the Uganda Council for Science and Technology for permission to conduct this research. Finally, we thank Mike Huffman and Colin Chapman for inviting us to contribute to this volume.
References


Does diet quality affect the parasite ecology of mountain gorillas?


Primate Parasite Ecology


Does diet quality affect the parasite ecology of mountain gorillas?


Primate Parasite Ecology


